Mexican origins for the Texas green turtle foraging aggregation: A cautionary tale of incomplete baselines and poor marker resolution

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The green turtle (*Chelonia mydas*) foraging aggregation along the Texas coast has increased dramatically in recent years, but the source populations for these turtles have not been adequately resolved. Previous mixed stock analysis (MSA) based on 490 base pair (bp) mitochondrial control region haplotypes suggested a large Florida contribution, but widespread sharing of common haplotypes among potential source populations and incomplete source population baseline data precluded precise assessment. To test the hypothesis that Texas turtles may represent proximal western Gulf of Mexico (GoM) nesting populations, we analyzed novel rookery samples from Rancho Nuevo, Tamaulipas, Mexico (RNMX) and conducted oceanic connectivity simulations. The RNMX samples yielded haplotypes CM-A1.1 and CM-A3.1 in frequencies not significantly different from those of the central eastern Florida nesting population. However, mitogenomic sequencing identified a diagnostic mitochondrial SNP (mtSNP) variant that is fixed in RNMX relative to the Florida CM-A1.1 lineage. Pairwise comparisons indicate that the Tamaulipas rookery represents a discrete population relative to those previously described in the northern Greater Caribbean, warranting recognition of a western GoM management unit (MU). Contrary to previous findings, the Florida populations were ruled out as major contributors to the Texas aggregation through screening of the mtSNP. Mixed stock analysis incorporating the mtSNP data suggested a western GoM origin for approximately 70% of the Texas foraging aggregation, with Quintana Roo contributing the majority of the remainder. Backtracking

simulations within an ocean circulation model were broadly congruent with genetic results in indicating substantial probability of oceanic transport from Mexican rookeries to the Texas coast (68%) while also dismissing the possibility of transport from the eastern Florida rookeries (0%). The mixed stock analyses and backtracking simulations are consistent with previous hypotheses implicating oceanic dispersal followed by natal homing by neritic juveniles to explain juvenile green turtle distributions. In contrast to a pattern of stepping stone connectivity across the remaining northern Greater Caribbean, the Texas foraging aggregation was distinct from all others analyzed in the region, including one in the eastern GoM. This isolation highlights the significance of Texas as developmental habitat for the proposed western GoM MU and reiterates the importance of continued international cooperation to facilitate recovery of this stock. This study also underscores the importance of satisfying underlying assumptions of mixed stock analysis in order to make robust inferences of connectivity.

Keywords: *Chelonia mydas,* stock structure, Tamaulipas, mixed stock analysis, migratory connectivity

1. Introduction

Assessing connectivity is an important consideration for management of migratory marine species with complex life histories. Despite potential for considerable dispersal and migration, many marine and anadromous taxa have discrete mating sites that create genetic structure among populations (Quinn and Dittman, 1990; Hoelzel, 1998; Hueter et al., 2005; Jensen et al., 2013). However, individuals from these distinct populations often mix during other parts of their life cycle, obscuring their population identity. Green turtles (*Chelonia mydas*) exhibit this complex life history. Female turtles home to natal regions to nest (Meylan et al., 1990). Hatchlings disperse from their natal beaches and spend the first few years of their lives in an epipelagic, oceanic stage (Reich et al., 2007). Juveniles recruit to neritic foraging sites, where they transition through multiple developmental habitats before ultimately selecting a foraging area to which they show high fidelity, at least in the Atlantic basin (Meylan et al., 2011; Moncada et al., 2006). Given this dispersal and migratory behavior, monitoring and management efforts require knowledge of the number of discrete nesting populations and the distribution of all life history stages from each of these populations. Because marine turtle nesting populations are structured through female natal philopatry, they are often designated as management units (MUs) on the basis of significant differentiation of maternally inherited mitochondrial DNA (mtDNA) haplotypes as outlined by Moritz (1994). Assessing stock structure and migratory connectivity have been highlighted as global research priorities for marine turtle conservation (Hamann et al., 2010).

Mixed stock analyses (MSA) based on mtDNA haplotypes provide critical linkages between foraging sites and source rookeries for juvenile green turtles. The first study in the Atlantic Ocean detected considerable Costa Rican contributions to a Bahamian foraging site, suggesting that relative rookery size might be an important predictor of foraging aggregation composition (Lahanas et al., 1998). A later study of a foraging aggregation on the east coast of Florida suggested that proximity of nesting and foraging

sites might play a larger role than relative rookery sizes in determining the distribution of juveniles (Bass and Witzell, 2000). These investigators also hypothesized that the distribution of juvenile green turtles was likely influenced by dispersal away from natal rookeries via currents followed by regional natal homing as turtles migrate through neritic developmental habitats. Several subsequent studies have supported this hypothesis (Bass et al., 2006; Luke et al., 2004; Naro-Maciel et al., 2012). Increasingly sophisticated ocean circulation models have permitted more direct tests of the influence of ocean currents on dispersal of epipelagic juveniles (Putman and Naro-Maciel, 2013). However, recent research has demonstrated that even small oceanic juveniles actively affect their distribution through directed swimming behavior (Putman and Mansfield, 2015). The potential for natal homing by larger juveniles, often against prevailing currents, following recruitment to initial neritic foraging sites could contribute to incongruence between biophysical model predictions assuming passive drift and genetic MSA results. Analysis of the Barbados foraging aggregation highlights this discrepancy as backwards tracking of virtual particles from the foraging aggregation indicated contributions solely from eastern Caribbean and South Atlantic rookeries (Putman and Naro-Maciel, 2013), whereas the genetic results highlighted substantial contributions (~ 50%) from western and northern Caribbean rookeries (Luke et al., 2004). Ocean currents are undoubtedly critical in dispersing small juveniles. However, gaps remain in determining the effects of swimming behavior by oceanic juveniles and how mechanisms driving juvenile turtle habitat selection following initial neritic recruitment shape their distributions.

Resolving migratory connectivity for Greater Caribbean green turtles is particularly important given their conservation status. Nesting populations and foraging aggregations across the region were severely depleted by centuries of systematic harvest that expanded with European exploration and colonization in the region, resulting in extirpation of some of the largest rookeries (McClenachan et al., 2006). The foraging aggregation along the Texas coast was no exception. Green turtles were historically abundant in the state, but turtle numbers became so low that the turtle fishery and related processing industry collapsed in Texas by the dawn of the 20th century, most likely as a result of overharvest and hypothermic stunning events (Doughty, 1984; Hildebrand, 1982). After decades of protection under the U.S. Endangered Species Act, the aggregation of green turtles inhabiting Texas waters has increased in recent years (Shaver, 2000; Shaver et al., 2013). Green turtle catch per unit effort increased exponentially in the Lower Laguna Madre from 1991- 2010 (Metz and Landry, Jr., 2013), suggesting a rapidly growing juvenile green turtle foraging aggregation. Based on documentation of large numbers of individuals stranded and captured during netting studies (Metz and Landry, Jr., 2013; Shaver, 1994, 2000; Shaver et al., 2013), the Laguna Madre, Mansfield Channel, and Brazos Santiago Pass in south Texas are likely among the most important developmental habitats for green turtles in the western Gulf of Mexico (GoM).

The source populations of the Texas foraging aggregation remain unresolved. A recent MSA suggested northern Greater Caribbean origins, dominated by Florida contributions (Anderson et al., 2013). However, as highlighted by the authors of that study, the findings should be interpreted with caution for two important reasons. First, one critical assumption of most mixture analysis methods is that all potentially contributing source

populations have been sampled (Manel et al., 2005). This assumption is violated with respect to northern Greater Caribbean green turtles. Although the largest Mexican rookeries are represented by genetic data from Isla Cozumel and X'cacel, Quintana Roo (Encalada et al., 1996), genetic data are unavailable from regionally significant rookeries along the entire GoM coast of Mexico (NMFS and USFWS, 2015). Second, extensive marker overlap among potential source populations can introduce considerable uncertainty around their estimated contributions to mixed aggregations (Okayama and Bolker, 2005). The Texas MSA results reflect this as Florida and Quintana Roo contributions had extremely wide credible intervals that severely limited the utility of fine scale results (FL contribution: 0.8, 0.2-1.0; QR contribution: 0.2, 0-0.8; Anderson et al., 2013).

Two common 490 base pair (bp) haplotypes (CM-A1 and CM-A3) dominate the rookery profiles of Mexico, Cuba, and Florida (Encalada et al., 1996; Ruiz-Urquiola et al., 2010; Shamblin et al., 2015a), and the scale of demographic and migratory connectivity are unresolved in many cases because of this extensive marker overlap. Recent studies incorporating additional mitochondrial markers have demonstrated increased resolution of stock structure among nesting assemblages of marine turtles. Expanding standard control region sequences to ~ 800 bp resulted in geographically informative subdivision of common 400 – 500 bp haplotypes for loggerhead turtles (*Caretta caretta*), hawksbill turtles (*Eretmochelys imbricata*), and green turtles (Dutton et al., 2014a, 2014b; LeRoux et al., 2012; Shamblin et al., 2014). Beyond the control region, mitogenomic sequencing of green turtles carrying the common 490 bp haplotype in the eastern Caribbean (CM-A5) yielded mitochondrial single nucleotide polymorphisms (mtSNPs) that were highly informative regionally (Shamblin et al., 2012). Incorporating mitogenomic sequencing to identify informative mtDNA polymorphism, we assessed the potential rookery sources of foraging green turtles from the Texas coast using novel baseline data from a Tamaulipas, Mexico rookery.

2. Methods

2.1 Sample collection and laboratory analysis

Tissue samples were collected from nests in Rancho Nuevo, Tamaulipas Mexico (RNMX) and from juvenile green turtles that stranded along the southern Texas coast in 1998-2002 (Table 1). Tissue was sampled from dead embryos salvaged from nests after hatchling emergence at Rancho Nuevo, taking care to only collect one sample from each nest and avoiding sampling more than one clutch from same female. Stranded turtles ranged in size from 14.0 to 81.3 cm straight carapace length (SCL) (Supplemental Figure 1). Because sample sizes were small for oceanic and subadult turtles, all individuals were treated as a single juvenile cohort for analyses. Samples were stored in 95% ethanol prior to DNA extraction. These samples were originally analyzed through amplification and sequencing of a 490 bp fragment of the mitochondrial control region using primers LTCM2 and HDCM2 (Allard et al., 1994). The available samples were subsequently characterized for the 817 bp control region sequence as previously described (Shamblin et al., 2015a). A subset of the Rancho Nuevo CM-A1 samples (n = 22) was consumed

through 490 bp analysis, so only six of the original 24 CM-A1 samples and four of the original eight CM-A3 samples were available for additional sequencing.

Novel samples from Archie Carr National Wildlife Refuge, Melbourne Beach, Florida were obtained using 4-mm biopsy punches from the rear flipper of females following oviposition from 2011 and 2012 (Table 1). Individuals were tagged using external tags in each front flipper and passive integrated transponder tags to prevent replicate sampling (Balazs, 1999). Samples were stored in 95% ethanol prior to DNA extraction. These samples were processed as previously described, and the resulting 817 bp haplotype data were combined with published data from the central eastern Florida (CEFL) MU (Shamblin et al., 2015a). Sample metadata and haplotype data are provided in Supplemental Table 1.

To determine if informative variation occurred outside of the standard control region sequence, the majority of the mitogenomes (positions 72 through 16421) of one Florida-nesting and four Florida-foraging CM-A1.1 individuals were sequenced as previously described for CM-A5 turtles using the same primers and reaction conditions (Shamblin et al., 2012). All CM-A1.1 individuals identified from this study and from Shamblin et al. (2015a) were screened at the informative mitochondrial SNP (mtSNP) identified by mitogenomic sequencing (Table 1). The ~ 1.6 kilobase ND5 fragment failed to amplify in some degraded stranding samples, so a primer pair that amplified a shorter product of approximately 300 bp was designed: CM12751F-GCCAACTGGGCCTCATAATA and CM13064R-TGTCAGGAGTAGGGCTCAGG. Amplification and sequencing was completed using reaction conditions previously described with sequencing primer CM12781-GCCTAAATCAACCACAA (Shamblin et al., 2012). Beyond haplotype CM-A1.1, all Texas foraging individuals and at least one individual representing each haplotype from the Florida nesting aggregation (Shamblin et al., 2015a) were screened for this mtSNP to provide phylogeographic context for the mutation. Florida-foraging individuals representing CM-A1.3 and CM-A48 variants (Bagley, 2003; unpublished data) were also characterized for the mtSNP for phylogeographic context.

2.2 Data analyses

Sequences were aligned, edited, and compared to previously described haplotypes using the program Sequencher 5.0 (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/resources/mtdna-sequences/). Haplotypes representing 490 bp sequences are designated based on numerical codes without suffixes, eg. CM-A1. Haplotypes based on 817 bp sequences retain their original 490 bp designations but were given suffixes to reflect variation in the novel sequences outside of the internal 490 bp fragment, eg. CM-A1.1 and CM-A1.2. Finally, variation uncovered using the mtSNP was applied as a second suffix to the 817 bp haplotype names, eg. CM-A1.1.1 and CM-A1.1.2. Novel data from RNMX were compared to published rookery data from the Greater Caribbean region using 490 bp and 817 bp plus the mtSNP data where available. Population structure among rookeries and among foraging aggregations were tested using frequency-based pairwise F_{ST} comparisons and

analysis of molecular variance (AMOVA) as implemented in Arlequin version 3.5 (Excoffier and Lischer 2010). 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 (Raymond and Rousset, 1995). P values were corrected for multiple tests using a false discovery rate approach (Benjamini and Yekutieli, 2001).

Rookery contributions were tested through Bayesian many-to-one MSA as implemented by the program BAYES (Pella and Masuda, 2001). Greater Caribbean rookeries for which genetic data were available were included as potential source populations (Bjorndal et al., 2005; Encalada et al., 1996; Ruiz-Urquiola et al., 2010; Shamblin et al. 2012; Shamblin et al., 2015a). Rookery contributions were estimated using three different models: MSA1 with uniform priors and MSA2 and MSA3 with relative rookery sizes as priors. Because the boundaries of the population to which RNMX belongs are unknown, we considered two extreme scenarios that reflect the possible range in rookery sizes: MSA2 assumed that the western GoM (WGMX) population was limited to Tamaulipas state only, and MSA3 assumed that the western GoM population encompassed all Atlantic Mexican beaches except the Caribbean coast (Quintana Roo state). Relative rookery sizes based on estimated nester abundance from the Green Turtle Status Review (NMFS and USFWS, 2015) were used to weight contributions. To compare across the same time series, updated nest counts from Veracruz state from 2010-2012 (Red de Campamentos Tortugueros en el Estado de Veracruz unpublished data, Raúl de Jesús González Díaz Mirón, personal communication) were used to generate estimated female abundance (Supplemental Table 2) using the same approach and parameters as in the Status Review (((total nest count over years divided by number of years monitored) divided by mean clutch frequency) multiplied by mean remigration interval).

Each of the three MSA models was run considering three different baselines: A) 490 bp haplotype data only, B) 490 bp frequencies but also incorporating 817 bp and mtSNP data for CM-A1 turtles and 817 bp frequencies for CM-A5 and CM-A18 individuals and using only real data for reanalyzed RNMX samples (6 CM-A1.1.1 and 2 CM-A3), and C) the same haplotype data as B except that the RNMX baseline was assumed to be fixed for CM-A1.1.1 (therefore 24 CM-A1.1.1 and 7 CM-A3). The number of CM-A3 individuals in the RNMX baseline for B analyses was reduced in order to preserve the original relative frequencies of CM-A1 and CM-A3 in the complete sample to the extent possible. In order to include QRMX and southwest Cuba (SWCB) CM-A1 individuals from the literature in the 817 bp and mtSNP analysis, hypothetical CM-A1 subhaplotypes were assigned because these samples were unavailable for reanalysis. Subhaplotypes were assigned based on phylogeographic relationships among haplotypes (see Results 3.1 for more detailed rationale). QRMX and SWCB both contain a large percentage of population informative 490 bp haplotypes with CM-A1 accounting for 35% and 11% of these samples, respectively (Encalada et al., 1996; Ruiz-Urquiola et al., 2010). Several iterations given different assumed distributions for these CM-A1 haplotypes did not affect the overall contributions. Similarly, a single CM-A5 individual and three CM-A18 individuals sampled at QRMX had to be assigned hypothetical 817 bp haplotypes because these samples were unavailable for re-sequencing. A total of 300,000 Markov

Chain Monte Carlo steps were run for eight chains to ensure convergence, as indicated by Gelman-Rubin shrink factors of less than 1.2.

Oceanic connectivity between the Texas foraging ground and major green turtle rookeries was estimated using "backtracking" simulations within the surface layer of the Global Hybrid Coordinate Ocean Model (HYCOM; Chassingnet et al., 2007). HYCOM is forced using wind stress, wind speed, heat flux, precipitation, and river discharge. This model assimilates satellite altimetry data, sea surface temperature and in situ measurements from a global array of expendable bathythermographs, Argo floats, and moored buoys to produce hindcast model output. Thus, HYCOM accurately resolves mesoscale processes such as meandering currents, fronts, filaments and oceanic eddies (Chassignet et al., 2007). The HYCOM output used here was from the newly released Global Reanalysis (http://hycom.org/dataserver/glb-reanalysis), and output is a daily snapshot of current velocity at 00:00 h (GMT) at a spatial resolution of 0.08° (approx. 6–9 km grid spacing) – sufficiently high resolution to characterize ocean circulation processes at scales important for depicting the movement of animals at the ocean surface (Putman and He, 2013).

Following methods previously described (Putman and Naro-Maciel, 2013; Putman et al., 2015), the movement of virtual particles was simulated using ICHTHYOP (v2) particle-tracking software (Lett et al., 2008). In accordance with the period when DNA samples were collected from turtles caught along the Texas coast, we backtracked particles within the model years of 1998, 1999, 2000, 2001, and 2002. A release zone for virtual particles was defined between latitudes 28.5°N and 26°N and west of longitude 98°W. Within this zone, 150 particles were released each day between the 10m and 50m isobaths and tracked backwards through time for 5 years, as this is thought to be the maximum duration of the green turtle oceanic-stage. Particles were advected using a Runge–Kutta fourth-order, time-stepping method whereby particle position was calculated each half an hour. Thus, ICHTHYOP determined where a total of 273,750 particles came from to reach their final location in the along the Texas coast.

The percentage of particles entering 25 major green turtle rookeries throughout the Atlantic was recorded (rookeries defined as 2.5° latitude x 2.5° longitude zones; Putman and Naro-Maciel, 2013, Putman et al., 2015). In contrast to previous simulations (e.g., Putman and Naro-Maciel, 2013, Naro-Maciel et al., 2014, 2016; Putman et al., 2015), results were not weighted by rookery size as records for the time period modeled were not available for a number of beaches now known to host large nesting populations. Regardless, the present implementation allowed us to determine from which rookeries transport to Texas via ocean currents was most likely.

3. Results

3.1 Haplotypes

Variable positions in the 490 bp control region sequences yielded nine haplotypes in the Texas foraging aggregation and the RNMX rookery samples (Table 2). All but one

haplotype from the foraging aggregation belonged to clade A (Encalada et al., 1996, Figure 2). These haplotypes have previously been described from northern Greater Caribbean rookeries except for CM-A22 (Encalada et al., 1996; Ruiz-Urquiola et al., 2010; Shamblin et al., 2015a), which is of unknown origin. RNMX individuals carried the two most common haplotypes in the region, CM-A1 (24) and CM-A3 (7). Analysis of 817 bp sequences for the RNMX individuals available for reanalysis yielded CM-A1.1 (6) and CM-A3.1 (4), the two most common haplotypes detected in the Florida nesting aggregation (Shamblin et al., 2015a). A single CM-A1.4 female was detected in the novel Melbourne Beach samples, marking the first time this haplotype has been recorded from a rookery. This variant of CM-A1 contains the CM-A1.2 diagnostic insertion with an additional insertion at position 16255 (Supplemental Table 3). All Texas foraging juvenile 817 bp haplotypes represented the conserved ".1" variants of their respective 490 bp haplotypes with the exception of three CM-A1.2 individuals (Supplemental Table 3).

Mitogenomic sequencing yielded a single informative mtSNP in the ND5 gene. The Florida-nesting CM-A1.1 individual was A at mitogenomic position 12958, whereas one of the Florida-foraging turtles carried a G at this position (Supplemental Table 3). All Florida nesting, Rancho Nuevo nesting, and Texas foraging individuals carrying haplotypes other than CM-A1.1 were G at this position (Supplemental Table 1), indicating that the Florida-nesting variant is derived. The conserved and derived variants were designated CM-A1.1.1 and CM-A1.1.2, respectively. The six RNMX CM-A1.1 samples available for reanalysis were CM-A1.1.1, whereas all 315 Florida-nesting CM-A1.1 females were CM-A1.1.2. The Texas (TX) foraging aggregation was dominated by CM-A1.1.1 (Table 2), and all oceanic juveniles and subadults sampled were CM-A1.1.1.

In order to have complete baseline data for the mtSNP MSA, some assumptions were necessary. First, RNMX CM-A1 samples unavailable for reanalysis (n = 18) were assumed fixed for CM-A1.1.1 in order to preserve relative frequencies of CM-A1 and CM-A3 from the original 490 bp sequencing. QRMX and SWCB CM-A1 samples unavailable for reanalysis were assigned subhaplotypes based on relationships to 817 bp and mtSNP defined haplotypes. CM-A48 is considered to be endemic to Cuban rookeries (Ruiz-Urquiola et al., 2010). This haplotype is equivalent to CM-A1 but with a derived six bp insertion. Three different variants of CM-A48 have been reported to the ACCSTR haplotype database, equivalent to CM-A1.1, CM-A1.2, and CM-A1.4. Therefore, it was assumed likely that these three variants of CM-A1 were also present in SWCB. Neither of the CM-A48 variants tested carried the Florida variant of the mtSNP (Supplemental Table 3). Similarly, CM-A18 is a common haplotype in QRMX (Encalada et al., 1996). CM-A18.1 foraging individuals from Texas did not carry the derived (Florida) mtSNP at position 12958 (Supplemental Table 3). Moreover, CM-A18.2 individuals nesting in Florida did not carry this derived position, either, suggesting that they colonized from elsewhere rather than arising via mutation from Florida CM-A1 types *in situ*. Therefore, for the purposes of this MSA, CM-A1.1.1 and CM-A1.2 were assumed to be present in the SWCB and QRMX rookeries along with CM-A1.4 in SWCB. Alternate runs considering different CM-A1 subhaplotype assignments for QRMX and SWCB demonstrated that primary MSA results were insensitive to these assumptions (results not shown).

3.2 490 bp haplotype analyses

There was significant structure among northern Greater Caribbean green turtle rookeries (F_{ST} = 0.232, $p < 0.001$) despite dominance of CM-A1 and CM-A3 in their haplotype profiles. However, RNMX was not significantly different from CEFL (Table 3). There was also significant genetic structure across Greater Caribbean foraging aggregations (F_{ST} = 0.087, $p < 0.001$). The Texas foraging aggregation was distinct from all others previously characterized in the Greater Caribbean region, including NWFL in the eastern GoM (Supplemental Table 4). Of all Greater Caribbean foraging aggregations considered, only Texas and Barbados were distinct from all others with respect to both pairwise F_{ST} values and pairwise exact tests following false discovery rate correction (Supplemental Table 4). The lack of differentiation between RNMX and CEFL was reflected in considerable uncertainty around contribution point estimates for these two rookeries (Supplemental Table 5). Although point estimates for RNMX were similar across the three MSA schemes considered, credible intervals ranged from zero to 87% for RNMX and zero to 73% for CEFL (Figure 3), highlighting sensitivity to the rookery size priors. This uncertainty also affected estimates from QRMX, with credible intervals ranging from 16 to 79%.

3.3 817 bp plus mtSNP CM-A1 analyses

Incorporation of the mtSNP data clearly indicated that RNMX and CEFL are distinct nesting populations (Table 3). The Texas foraging aggregation was dominated by Mexican contributions, with approximately 70% of individuals assigned to RNMX and 20% assigned to QRMX, with the remainder from TORT (Figure 3, Supplemental Table 5). Florida was excluded as a major source, but a small contribution from southern Florida (SOFL) rookeries could not be definitively ruled out based on the assumption that CM-A1.1.2 is a Florida endemic haplotype. Despite nearly identical contribution estimates across priors, credible intervals for RNMX and QRMX were sensitive to priors, with RNMX ranging from zero to 87% and QRMX from eight to 89% (Figure 3). However, MSA-C results that relied on the assumption that RNMX was fixed for CM-A1.1.1 had the smallest credible intervals that were comparatively insensitive to prior assumptions (Supplemental Table 5).

3.4 Oceanic connectivity analyses

Transport to the Texas coast via ocean currents was most likely from Mexican rookeries. Of the particles backtracked from Texas that passed within the vicinity of at least one rookery, 42% arrived from Gulf of Mexico populations (Tamaulipas = 9.2%, 95% CI = 1.3%; Veracruz = 6.6%, 95% CI =1.5%; Campeche = 6.6%, 95% CI = 1.9%; Yucatan = 19.6%, 95% CI =2.7%). Approximately 26% (95% CI = 1.5%) of particles arrived from Quintana Roo (Supplemental Table 6). Transport was also possible from the southern Greater Caribbean rookeries, particularly from along the continental coast of Venezuela (17%, 95% CI = 5.0%) and Costa Rica (5.8%, 95% CI = 1.8%). In contrast, no possibility

of transport from the east coast of Florida to Texas was predicted for any of the years that were modeled (Figure 4).

4. Discussion

4.1 Oceanic dispersal and neritic juvenile natal homing

The differentiation of the Texas foraging aggregation strongly contrasted with the pattern of broad scale, stepping stone connectivity linking the remaining northern Greater Caribbean foraging aggregations (NWFL to NC in Figure 1). Based on MSA analyses, the proximal western GoM population was by far the major contributor to the Texas aggregation with most of the remaining juveniles representing QRMX. The backtracking simulations also supported the GoM Mexican rookeries as those most likely to reach the Texas foraging aggregation given the currents in the region. The primary current in the GoM is the Yucatan/Loop Current that originates at the Yucatan Channel between the Yucatan Peninsula and Cuba and flows northward before turning clockwise and exiting the GoM via the Straits of Florida. The Loop Current is an extension of the Caribbean Current that originates in the southeastern Caribbean. These strong surface currents likely facilitate transport of oceanic juveniles from QRMX (and the portion of TORT turtles that escape the Colombia-Panama Gyre) into the eastern GoM and along the Atlantic coast of the United States, and may explain why large numbers of juveniles from these massive nesting aggregations do not occur in the western GoM. However, the Loop Current is known to irregularly shed anticyclonic rings that detach and drift westward from the main flow (Sturges and Leben, 2000). The lifespan of these rings is often several months (Oey et al., 2005), sufficient time to transport some oceanic juveniles from the QRMX rookeries into the western GoM, also consistent with the backtracking simulations. Despite this potential for connectivity between QRMX and the Texas foraging aggregation, the genetic evidence suggests that most Texas turtles originate in nearby western GoM rookeries. What is less certain is the extent to which the concentration of RNMX turtles is a consequence of retention of oceanic juveniles in the western GoM or results from natal homing by neritic juveniles initially dispersed to more distant habitats. The size-frequency distribution of strandings in this study was skewed slightly towards smaller individuals than those sampled from NWFL in the eastern GoM (Foley et al., 2007, Supplemental Figure 1), consistent with a majority representing new neritic recruits that had not previously settled elsewhere.

Fully characterizing the foraging distribution of juveniles from the western GoM population will require application of the mtSNP in other aggregations in the northern Greater Caribbean region. Given the lack of differentiation between RNMX and CEFL without consideration of the mtSNP, reanalysis of published 490 bp foraging aggregation data with the new RNMX baseline was not attempted. In the absence of genetic reanalysis, forward-tracking dispersal simulations from the GoM rookeries and comparisons with Kemp's ridley turtles (*Lepidochelys kempii*), which nest primarily in Tamaulipas, suggest that juvenile green turtles originating from the western GoM likely occur throughout the GoM region and along the Atlantic coast of the United States (Putman et al., 2015, 2013, 2010). Models indicate considerable annual variation in the

distribution of oceanic juvenile Kemp's ridley turtles based on ocean current dynamics, with most oceanic turtles retained in the western GoM (Putman et al., 2013). Nonetheless, some proportion of most cohorts were also distributed into the eastern GoM (Putman et al., 2013), consistent with one to two year old oceanic juveniles associated with *Sargassum* floats on the Southwest Florida shelf (Witherington et al., 2012). The presence of neritic juvenile Kemp's ridleys along the Atlantic coast of the United States lends support for dispersal of oceanic juveniles via the Loop Current and Florida Straits (Carr, 1980). Of all regions considered as possible initial neritic recruitment sites for oceanic juveniles originating at Rancho Nuevo (the Campeche basin and the continental coast of North America from Texas to Novia Scotia), recruitment rates were highest for Texas and declined eastwards (Putman et al., 2010). These results confer the possibility that a significant portion of oceanic juvenile green turtles might be retained within the western GoM. However, recent research has indicated behavioral differences between the species that might promote relatively quick transit through the eastern GoM by most green turtles but retention of Kemp's ridley turtles in the GoM (Putman and Mansfield, 2015). Caveats include that only turtles caught in the eastern GoM were tracked, and the origins of the green turtles were unknown. Differences in directional swimming among marine turtle populations seem likely, but this remains to be demonstrated. An additional caveat in comparing across species is that the Kemp's ridley oceanic stage was modeled to last 1.5 to 2 years (Putman et al., 2013, 2010) but in green turtles may be slightly longer, 3 to 5 years (Reich et al., 2007). An extended oceanic stage along with different orientation and swimming behavior could permit a larger proportion of western GoM green turtles to initially recruit to more distant neritic foraging habitats in the eastern GoM or along the Atlantic coast of the United States.

Despite the likelihood that juveniles representing the western GoM population occur to some degree in the eastern GoM and Atlantic basin, juvenile green turtles tagged and satellite-tracked from Texas have been documented in Tamaulipas, but not in Florida (Shaver, 2000; Shaver et al., 2013). Two additional juveniles originally tagged in Texas during stranding events were subsequently recovered in Veracruz (Donna Shaver and Raúl de Jesús González Díaz Mirón, unpublished data). Tag returns from juvenile and subadult green turtles in the Greater Caribbean region have generally documented broad scale northeastern to southwestern movements (Bjorndal et al., 2003; Meylan et al., 2011; Moncada et al., 2006), consistent with the hypothesis that oceanic juveniles in the Atlantic basin dispersed by currents initially recruit to distant neritic nursery habitats but ultimately move closer to home as they transition through developmental habitats (Bass et al., 2006; Meylan et al., 2011; Monzón-Argüello et al. 2012, Naro-Maciel et al., 2012). Apparent isolation of western GoM turtles based on the Texas tagging data does not preclude the possibility of migratory connectivity across the entire GoM and beyond. Because tags were applied to neritic juveniles in Texas, any turtles that dispersed outside the western GoM as oceanic juveniles could have already returned from initial recruitment sites via natal homing prior to tagging. Indeed, one of 24 oceanic juvenile green turtles tracked from waters off the Louisiana coast entered the Atlantic during the tracking period (Putman and Mansfield, 2015), and a neritic juvenile tagged during a hypothermic stunning event in NWFL was recaptured offshore of South Padre Island, Texas five years later (Foley et al., 2007). Migratory connectivity between Texas and

Tamaulipas inferred from the tagging data is congruent with our genetics results linking these regions. Although additional genetic data are required to map the distribution of RNMX juveniles, the available tagging and genetic data conform to the oceanic dispersal followed by regional natal homing hypothesis that has been proposed to explain juvenile green turtle distribution in the Atlantic (Bass et al., 2006).

4.2 Improving resolution of structure and connectivity

RNMX was distinguished from all other northern Greater Caribbean green turtle rookeries, warranting recognition of a western GoM MU for green turtles. It is evident that Tamaulipas and Quintana Roo rookeries represent distinct nesting populations based on 490 bp haplotype frequency differences, but resolving their boundaries requires additional sampling. Several previous studies failed to find mtDNA differentiation in green turtle rookeries separated by 150 km or less (Bowen and Karl, 2007). However structure was detected across a very narrow transition zone (~ 1 km) along the east coast of Florida, despite essentially contiguous nesting habitat on continental barrier island beaches (Shamblin et al., 2015a). This raises the possibility that similar structure may occur along the more than 2,000 km of GoM coastline in Mexico. Stock structure of green turtle rookeries in the region should be ascertained through additional sample collection from Veracruz, Tabasco, Campeche, and Yucatan to better resolve the number of MUs and their boundaries.

The mtSNP identified through mitogenomic sequencing improved resolution of phylogeography, stock structure, and the MSA. The mtSNP differentiated western GoM and Florida CM-A1.1 lineages and clearly eliminated Florida as a major contributor to the Texas foraging aggregation. Based on haplotype sharing with other rookeries in the Greater Caribbean region, the Florida nesting aggregation was hypothesized to have arisen through colonization from more tropical nesting populations following the Younger Dryas Event (Encalada et al., 1996). The finding that the Florida CM-A1.1 turtles carry a derived mutation provides additional support for this hypothesis. The mtSNP also highlighted a phylogeographic anomaly. CM-A2.1 has been considered endemic to Florida nesting populations because it has not been sampled elsewhere (Encalada et al., 1996; Shamblin et al., 2015a). However CM-A2.1 individuals from the Florida rookery and Texas foraging aggregation were conserved at the mtSNP, suggesting that the CM-A2.1 lineage nesting in Florida colonized from elsewhere rather than arising through *in situ* mutation from CM-A1.1.2. Given lack of measurable SWCB contributions and highly consistent QRMX contribution estimates across all models, the hypothetical CM-A1 subhaplotype assignments assumed for the MSA baseline appear to have yielded reasonable results. Nonetheless, whether CM-A1.1.1 and CM-A1.2 are both present in these rookeries and their relative frequencies should be determined. The assumption that CM-A1.1.2 is endemic to Florida MUs should also be tested through application of the mtSNP in QRMX and SWCB samples. Our study rules out Texas as an important foraging site for juveniles from the Florida nesting populations. Incorporating the mtSNP marker in analyses of CM-A1.1 individuals representing foraging aggregations elsewhere in the Greater Caribbean region should be useful in identifying key foraging areas for juveniles from Florida nesting populations.

Given the clarified stock structure achieved through inclusion of a novel genetic marker, additional genetic data should also be explored. CM-A3 is the most geographically widespread haplotype in the Greater Caribbean region (Encalada et al., 1996), and its high frequency in TORT (Bjorndal et al., 2005) also makes it the most common haplotype in the region numerically. Mitogenomic sequencing of CM-A3 individuals representing the major Greater Caribbean rookeries should be a priority to determine if similar informative variation occurs for this haplotype. The mitochondrial short tandem repeat (mtSTR) present in the 3' end of the control region provides another possibility for improved resolution. Researchers identified 33 different mtSTR haplotypes in Mediterranean green turtles despite nearly complete fixation of an 817 bp haplotype in the nesting aggregation (Tikochinski et al., 2012). Analysis of Brazilian green turtle rookeries using mtSTR haplotypes also resolved fine scale structure that was not apparent using the traditional 490 bp haplotypes (Shamblin et al., 2015b). It is likely that mtSNPs will compliment the mtSTR loci in subdividing common shared haplotypes, so use of a combination of the mtSTR loci and mitogenomic screening to identify additional informative variation offers the best approach for resolving matrilineal structure. Nuclear markers may also improve assessments of connectivity. Western Atlantic green turtle rookeries were all significantly different with respect to microsatellite allele frequencies (Naro-Maciel et al., 2014). However, Florida was the only northern Greater Caribbean rookery represented in that analysis as samples from Cuba and Mexico were not available. F_{ST} values for Florida comparisons ranged from 0.006 to 0.030, more than an order of magnitude weaker than those detected for leatherback turtles over similar geographical scales (Dutton et al., 2013). Nonetheless, the utility of nuclear markers in improving resolution of demographic and migratory connectivity of northern Greater Caribbean green turtles should be explored.

4.3 Satisfying assumptions of MSA

Increased resolution from inclusion of the mtSNP highlighted the potential confounding effects of applying an ecological covariate as a prior to weight rookery contributions in the face of poor marker resolution. Marine turtle MSA often employ the use of informative priors, the most common of which is weighting contributions based on relative rookery sizes (Okayama and Bolker, 2005). The assumption that larger rookeries are contributing more juveniles than smaller ones is intuitive and may be appropriate in a many-to-many MSA context where foraging aggregations have been well sampled across broad distributions (Bolker et al., 2007). However in the case of the Texas foraging aggregation, where marker resolution was poor prior to implementation of the mtSNP analysis, rookery contribution estimates were highly sensitive to this weighting. This suggests that assumptions should be carefully considered to determine if they are appropriate in each case, rather than universally incorporating these ecological data as priors. The CEFL MU is roughly the same size as that of the western GoM MU, if not much larger depending on the latter's boundaries, but this population was not represented in the Texas foraging aggregation. Similarly, TORT is at least an order of magnitude larger than all other nesting populations in the Greater Caribbean region (Troëng and Rankin, 2005), but likely contributed only a small percentage of juveniles to the Texas

foraging aggregation. In this study, relative rookery sizes were clearly less informative than ocean current dynamics in explaining the distribution of juvenile turtles. Therefore results from MSA that have incorporated rookery scaling should be interpreted with caution when marker resolution is poor, which is often when the rookery size priors are employed.

Comparing MSA results from this study with those from Anderson et al. (2013) illustrates potential pitfalls of drawing inferences from incomplete baselines. In some cases, significant contributions from unsampled populations are apparent via the presence of "orphan" haplotypes that have been characterized from foraging aggregations but not from nesting populations. For example, the hawksbill turtle foraging aggregation in Cape Verde was dominated by haplotypes of unknown origin that were later discovered in the Príncipe rookery (Monzón-Argüello et al., 2011, 2010). However, orphan haplotypes comprised only 3% and 1% of the Texas foraging individuals from the previous and present studies, respectively (Anderson et al., 2013; present study). This apparently low level of orphan haplotypes might lead investigators to assume that potentially contributing source populations have been adequately sampled, but the widespread sharing of haplotypes at regional and even ocean basin scales in marine turtles (Jensen et al., 2013) could make this an erroneous assumption. Backtracking simulation results from the present study indicated significant probability of transport from Campeche and Yucatan. These results are consistent with MSA of oceanic juvenile hawksbill turtles stranded along the Texas coast, which indicate an almost exclusive Mexican composition (Bowen et al., 2007), implicating substantial contributions from the major hawksbill rookeries in Campeche and Yucatan (Garduño-Andrade et al., 1999). The small percentage of orphan haplotypes described from Texas suggests that Campeche and Yucatan green turtle rookeries share haplotypes with the western GoM and QRMX MUs at high frequency and further highlights the need to collect baseline haplotype data from these nesting areas. In addition to ensuring that all potential source populations have been sampled, it is critical that sample sizes are sufficiently large to provide a representative baseline (Bolker et al., 2007). As previously noted, the presence of several haplotypes at low frequency in the QRMX rookery sample has led to large sampling errors that are reflected in the credible intervals for its contributions (Anderson et al., 2013). Deeper sampling of RNMX, QRMX, and SWCB rookeries should further reduce uncertainty in future MSA.

4.4 Conservation implications

The combination of novel RNMX samples and mtSNP data indicate that Texas is important foraging habitat for turtles of Mexican origin. The dominance of local western GoM turtles in the Texas aggregation contrasts sharply with more admixed juvenile aggregations elsewhere in the northern Greater Caribbean region and confers unique conservation challenges and opportunities in this region. If significant numbers of western GoM MU oceanic juveniles are retained in western GoM waters and neritic juveniles are homing back to the region to establish permanent foraging sites as subadults, several life history stages may be concentrated off the coasts of Texas to Veracruz. On the positive side, conservation actions in the region may directly benefit a

significant proportion of turtles representing the western GoM MU. However, this concentration of turtles from a single nesting population could magnify risks from localized threats and make the western GoM MU particularly vulnerable to catastrophic events such as oil spills. This vulnerability was highlighted by oceanic circulation modeling indicating that approximately 75% of oceanic juvenile marine turtles affected by the Deepwater Horizon spill originated from Mexican rookeries (Putman et al., 2015). During 2015, the United States Bureau of Ocean Energy Management sold leases for the final available 21 million acres of the Western Gulf of Mexico Planning Area (Bureau of Ocean Energy Management, 2015), opening the waters offshore of Texas to increased oil and natural gas exploration.

In addition to concerns related to fossil fuel exploration and extraction, the proposed western GoM MU faces other natural and anthropogenic threats. Hypothermic stunning is the largest cause of juvenile green turtle stranding in Texas, affecting hundreds to more than 1,500 turtles in recent winters (Shaver 2000, unpublished data). It is important to rapidly locate and rescue green turtles during these events so that they can be rehabilitated and released. Incidental capture in fishing gear is another source of mortality. Although gillnets have been banned in Texas, they are still legal in Mexico (NMFS and USFWS, 2015). While data on green turtle bycatch from the Gulf coast of Mexico are unavailable, artisanal gillnet fisheries contribute to significant green turtle mortality in Baja California (Mancini et al., 2012), and interactions have been documented broadly in the western Atlantic where gillnet fisheries are permitted (López-Barrera et al., 2012; McClellan and Read, 2010). Given the threats facing this species in the western GoM and connectivity across the international border demonstrated by the MSA results, continued recovery of this stock will benefit from cooperation and partnership between managers in Mexico and Texas.

Acknowledgments

This work was conducted under state and federal permits to DJS, PHD, and LJP and state permits to LME, KLM, and CJN. We gratefully acknowledge the participants of the Sea Turtle Stranding and Salvage Network in Texas who documented and sampled the stranded turtles and to members of the US-Mexico binational Rancho Nuevo field crew, the Gladys Porter Zoo and SEMARNAT for facilitating collection of samples from Rancho Nuevo. Thanks to J.S. Walker for collating stranding size data and E. LaCasella for coordinating access to samples from Texas accessioned in the NOAA-Southwest Fisheries Science Center Marine Mammal and Turtle Molecular Research Sample Collection. We are grateful to Raúl de Jesús González Díaz Mirón and the Red de Campamentos Tortugueros en el Estado de Veracruz for sharing unpublished nest counts. We thank J. Stiner, N. Desjardin, E. Martin, C. Johnson and D. Sobel for providing Florida green turtle nest samples for a previous project and the University of Central Florida Marine Turtle Research Group for collecting the new Melbourne Beach samples. Thanks to K. Kichler for contributing to an earlier version of the manuscript. NFP and KLM acknowledge support from the Gulf Research Program of the National Academy of Sciences under award number 2000006434. The content is the sole responsibility of the authors and does not necessarily reflect the views of the Gulf Research Program or the

National Academy of Sciences. NFP acknowledges funding support from NOAA's Atlantic Oceanographic & Meteorological Laboratory. The 1/12 deg global HYCOM+NCODA Ocean Reanalysis was funded by the U.S. Navy and the Modeling and Simulation Coordination Office. Computer time was made available by the DoD High Performance Computing Modernization Program. The output is publicly available at http://hycom.org. The rookery and foraging area map was produced using the Maptool at www.seaturtle.org. We thank three anonymous reviewers for improving the quality of the manuscript. This research was funded in part by grants awarded from the Sea Turtle Grants Program. The Sea Turtle Grants Program is funded from proceeds from the sale of the Florida Sea Turtle License Plate. Learn more at www.helpingseaturtles.org. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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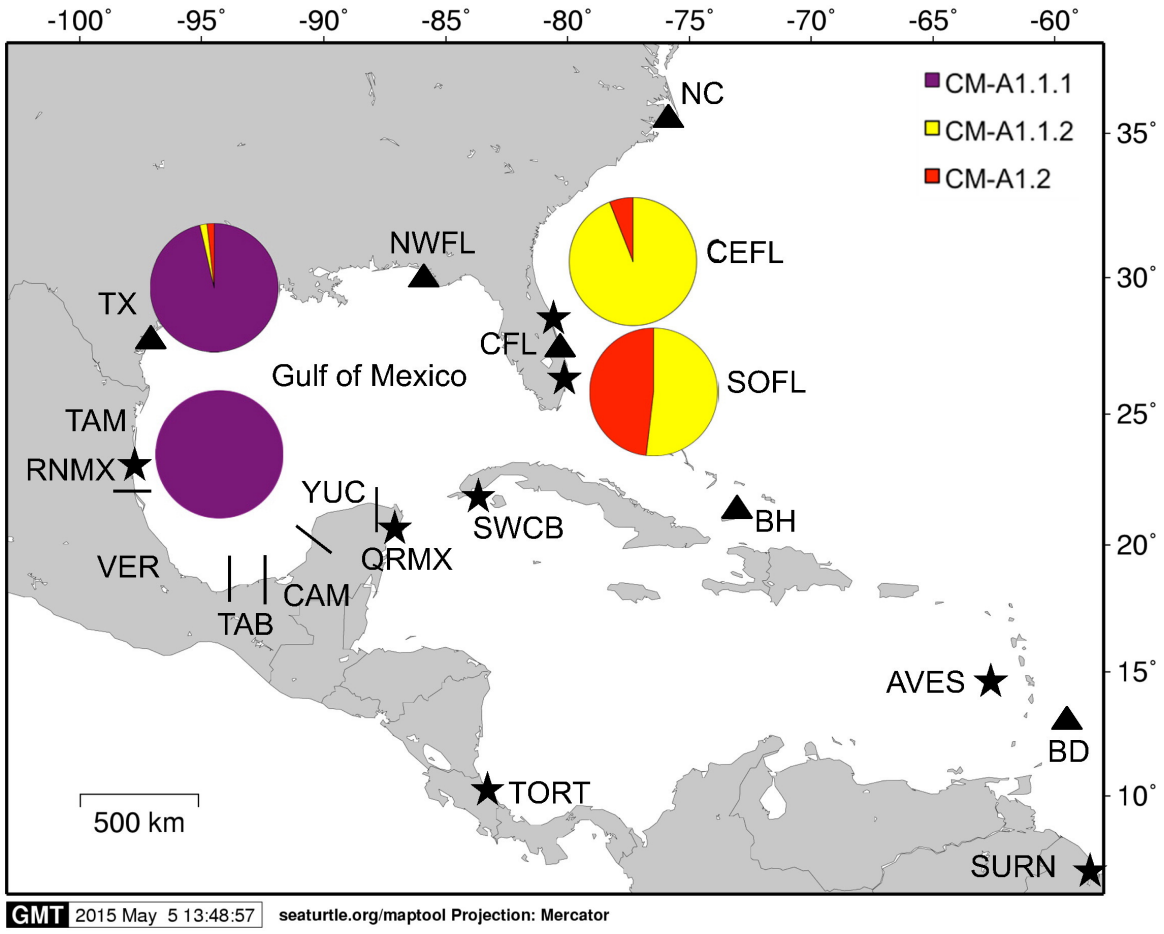

Figure 1. Distribution of CM-A1 haplotypes among northern Greater Caribbean rookeries and the Texas foraging aggregation. Stars indicate rookeries considered as sources for the mixed stock analysis: TORT, Tortuguero, Costa Rica; RNMX, Rancho Nuevo, Tamaulipas, Mexico; QRMX, Quintana Roo, Mexico; SWCB, southwest Cuba; SOFL, southern Florida; CEFL, central eastern Florida; AVES, Aves Island, Venezuela; and SURN, Suriname. Triangles indicate Greater Caribbean juvenile foraging aggregations: TX, Texas; NWFL, Northwest Florida; BH, Inagua, Bahamas; CFL, central Florida; NC, North Carolina; and BD, Barbados. Abbreviations for the Mexican states are: TAM, Tamaulipas; VER, Veracruz; TAB, Tabasco; CAM, Campeche; and YUC, Yucatan.

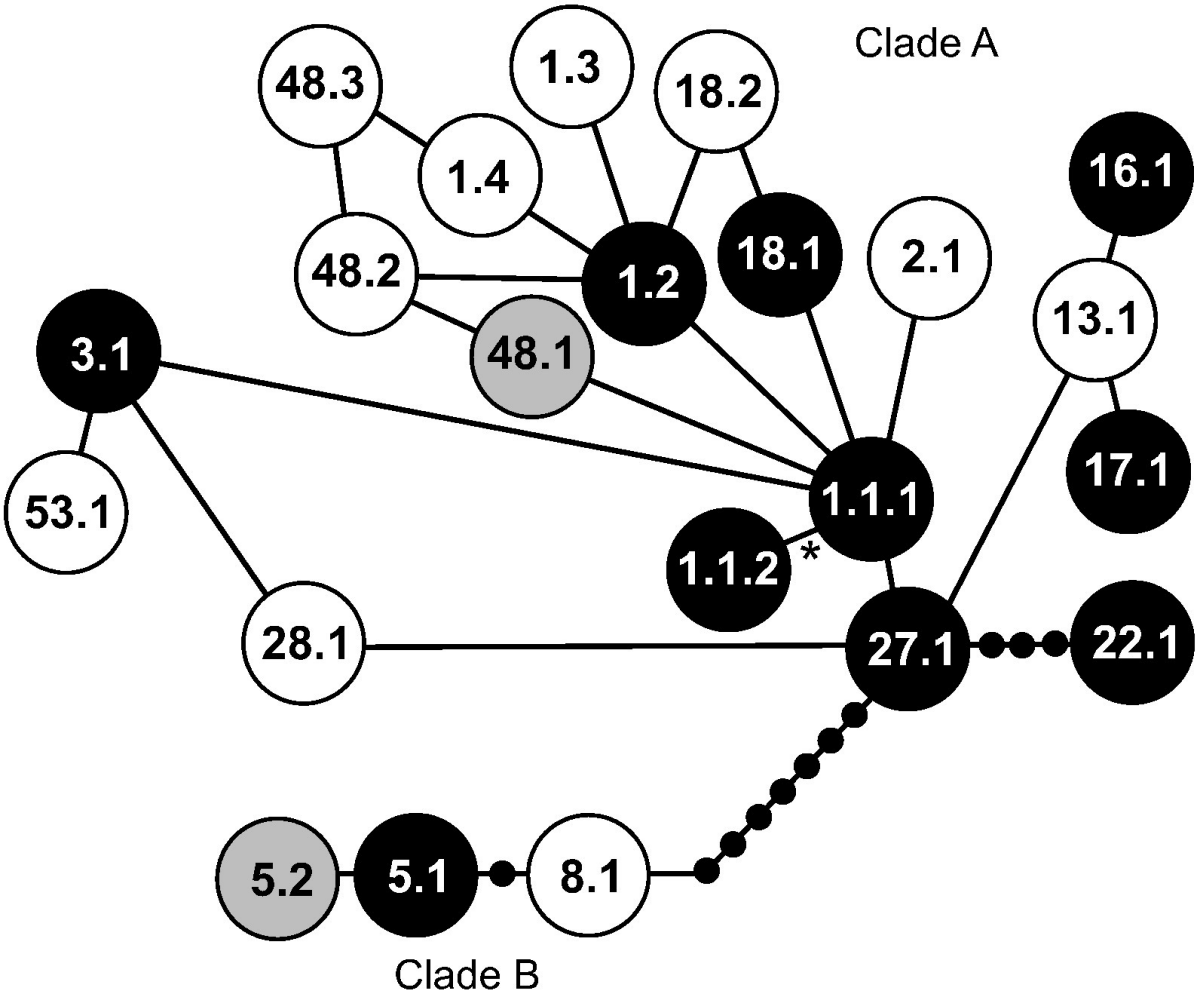

Figure 2. Greater Caribbean green turtle haplotypes defined by 817 base pair mitochondrial control region sequences and the single nucleotide polymorphism at mitogenomic position 12958. Haplotypes shaded in gray were not characterized for position 12958. Haplotypes identified from the Texas foraging aggregation in the present study are shaded in black. Additional related haplotypes from the Archie Carr Center for Sea Turtle Research sequence database are included for context. * indicates the mutation identified in the Florida CM-A1.1 population and not detected in any other haplotypes. Small filled circles indicate hypothetical haplotypes.

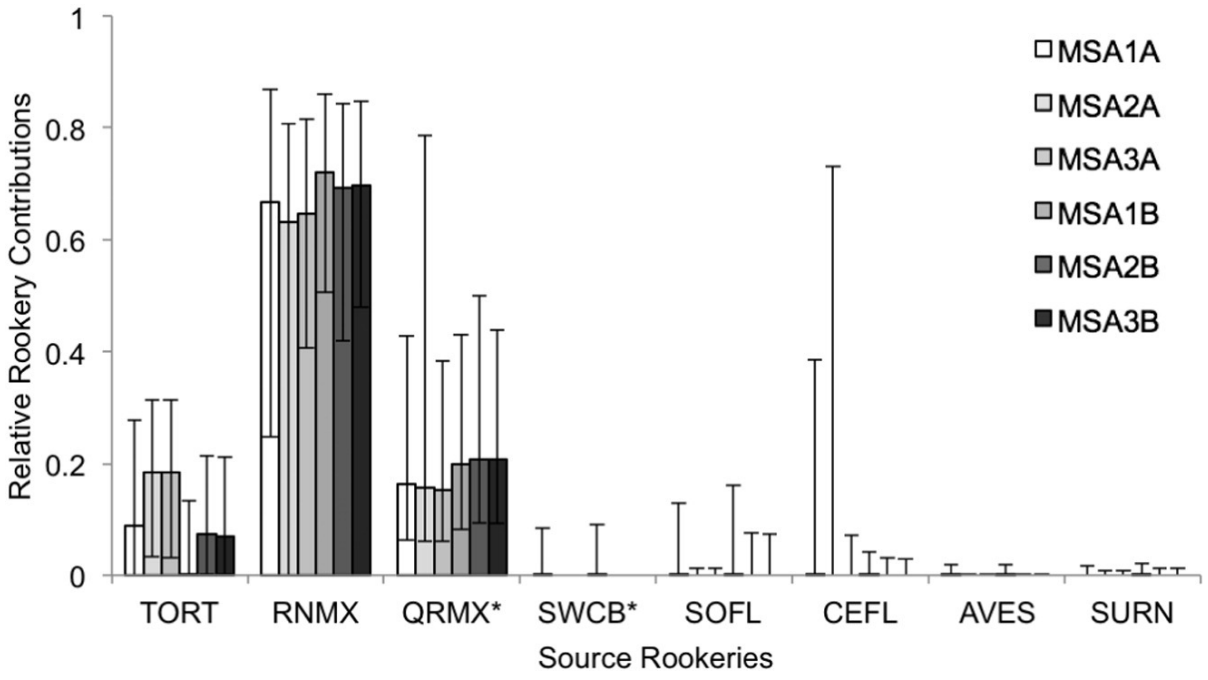

Figure 3. Many-to-one mixed stock analysis results for the Texas green turtle foraging aggregation. Potential source rookeries are defined in Figure 1. The three priors included 1) uniform distributions (MSA1), 2) the assumption that the western Gulf of Mexico (WGMX) nesting population was limited to Tamaulipas (MSA2), and 3) the assumption that the WGMX nesting population encompassed the entire Gulf of Mexico coast of Mexico (MSA3). Datasets considered were 490 bp control region haplotypes only (A) and 490 bp haplotypes with the addition of 817 bp and mtSNP haplotypes for CM-A1 turtles and 817 bp haplotypes for CM-A5 and CM-A18 turtles (B). Asterisks indicate that CM-A1, CM-A5, and CM-A18 haplotype assignments were hypothetical for QRMX and SWCB for the latter three (version B) analyses.

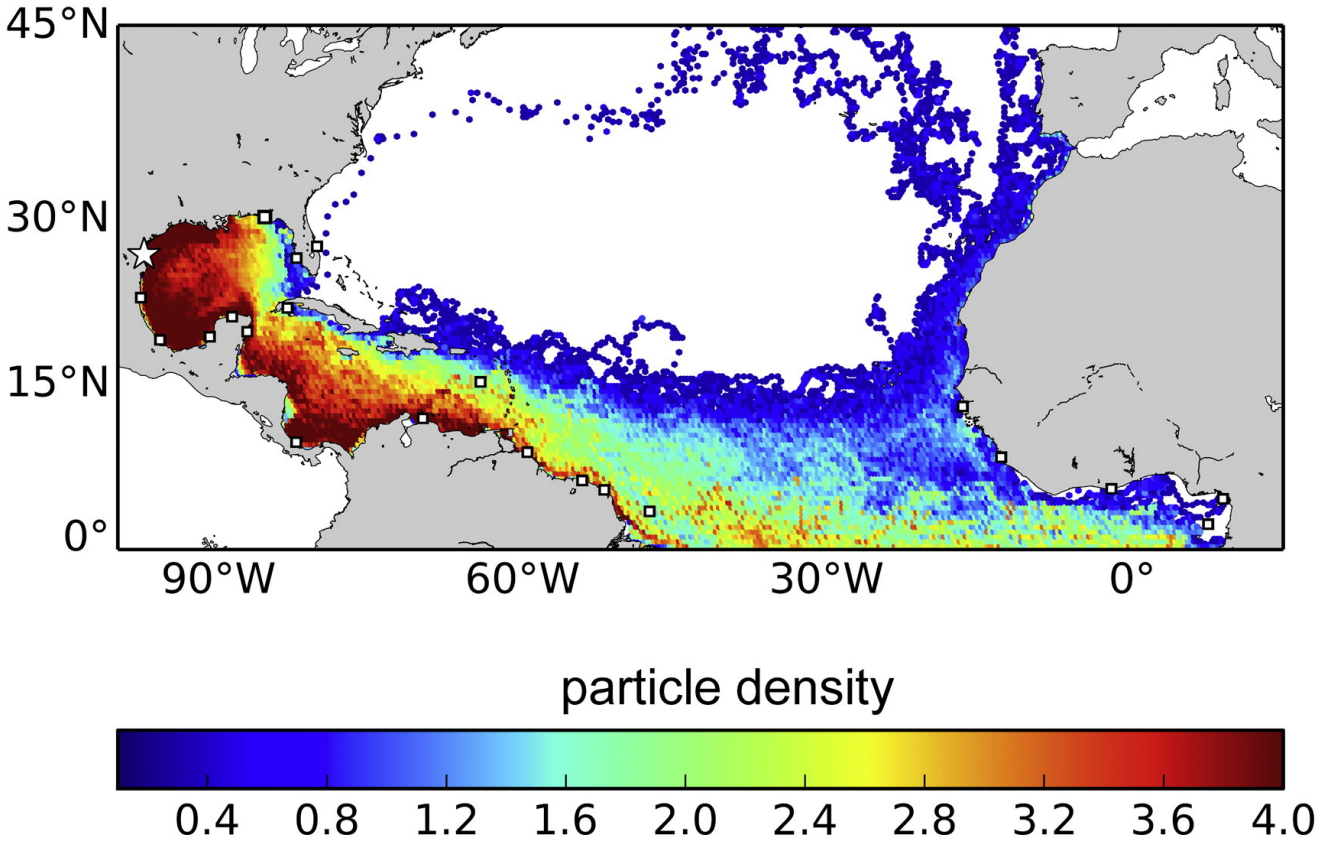

Figure 4. Map of predicted green turtle distribution based on five-year backtracking simulations from the Texas foraging grounds, indicated by the white star, relative to major green turtle nesting sites (small white squares). Colors indicate particle density within a grid cell throughout the simulations (counted every 48 h, logarithmic scale), highlighting connectivity between the Texas foraging aggregation and rookeries in the Gulf of Mexico and western Caribbean.

Table 1. Sample metadata for green turtles in the northern Greater Caribbean region. Sample numbers in parentheses indicate CM-A1.1 individuals that were screened for mitogenomic position 12958. N indicates nesting samples. FJ indicates foraging juvenile samples.

Code	Location	Sample Years	N	Type	Sample reference
RNMX	Rancho Nuevo, Mexico	1995	31 (6)	N	this study
SOFL	southern Florida, USA	2007-2012	174 (14)	N	Shamblin et al., 2015
CEFL	central eastern Florida, USA	2007-2010	311 (186)	N	Shamblin et al., 2015
CEFL	Melbourne Beach, FL , USA	2011-2012	222 (129)	N	this study
TX	southern Texas coast, USA	1998-2002	167 (99)	FJ	this study

Table 2. Green turtle mitochondrial haplotypes for Greater Caribbean green turtle rookeries and the Texas foraging aggregation used for mixed stock analysis. Site codes are explained in Figure 1. Haplotye names without suffixes indicate 490 bp sequences. Haplotype names with single suffixes represent 817 bp sequences. Haplotype names with two suffixes represent 817 bp plus mtSNP sequences. * indicates the assumption that all 24 of the original CM-A1 samples were fixed for the same haplotype found in the six individuals available for re-sequencing in order to preserve the original haplotype frequencies. ? indicates hypothetical expanded haplotype assignments for samples where only 490 bp haplotypes are available from the literature for the mixed stock analysis.

	TORT	RNMX	QRMX	SWCB	SOFL	CEFL	AVES	SURN	TX
CM-A1		24	7	3	27	335			102
CM-A1.1.1		6 (24*)	6?	1?					97
CM-A1.1.2					14	315			2
CM-A1.2			1?	1?	13	19			3
CM-A1.4				1?		1			
CM-A2					4	8			
CM-A3	395	7	5	16	127	170	5	1	47
CM-A4	1								
CM-A5	32		1		4	2	62	55	3
CM-A5.1	32		1?		4	2	48	55	3
CM-A5.2							14		
CM-A6								2	
CM-A8						1			
CM-A13					2	10			
CM-A15			1						1
CM-A16			1		1	3			4
CM-A17			2		2				1
CM-A18			3		1	1			5
CM-A18.1			2?						5
CM-A18.2			1?		1	1			
CM-A20	2								
CM-A21	3								
CM-A22									2
CM-A27				1					2
CM-A28				1	3	3			
CM-A48				5					
CM-A53					3				
CM-A56				1					
CM-A57				1					
Data	A, F	B	C	D	B, E	B, E	F	F	B

Table 3. Pairwise comparisons of genetic differentiation among northern Greater Caribbean green turtle rookeries that are known to host CM-A1 nesting lineages. Comparisons using 490 base pair (bp) haplotypes appear above the diagonal. Comparisons using 817 bp plus the CM-A1.1 mitochondrial SNP are below the diagonal. Pairwise F_{ST} values are without parentheses. P values from exact tests of population differentiation are enclosed in parentheses. ND indicates tests that were not done due to lack of availability expanded sequence data. Gray shading indicates the non-significant F_{ST} comparisons following correction.

	RNMX	QRMX	SWCB	SOFL	CEFL
RNMX		0.141 (0.002)	0.363 (< 0.001)	0.419 (< 0.001)	0.014 (0.758)
QRMX	ND		0.110 (0.001)	0.223 (< 0.001)	0.084 (< 0.001)
SWCB	ND	ND		0.046 (< 0.001)	0.257 (< 0.001)
SOFL	0.451 (< 0.001)	ND	ND		0.285 (< 0.001)
CEFL	0.437 (< 0.001)	ND	ND	0.289 (< 0.001)	