

The Interplay of Homing and Dispersal in Green Turtles: A Focus on the Southwestern Atlantic

EUGENIA NARO-MACIEL, ANA CRISTINA VIGLIAR BONDIOLI, MEREDITH MARTIN, ANTÔNIO DE PÁDUA ALMEIDA, CECÍLIA BAPTISTOTTE, CLAUDIO BELLINI, MARIA ÂNGELA MARCOVALDI, ARMANDO JOSÉ BARSANTE SANTOS, AND GEORGE AMATO

From the Department of Biology, College of Staten Island, City University of New York, Staten Island, New York, 10314, USA; Eugenia.NaroMaciel@csi.cuny.edu (Naro-Maciel); Instituto de Pesquisas Cananéia – IPeC, Cananéia, SP, 11990-000, Brasil; anabondioli@yahoo.com.br (Bondioli); Yale School of Forestry and Environmental Studies, Yale University, New Haven, CT, 06511, USA; meredith.martin@yale.edu (Martin); Projeto TAMAR-ICMBio, Vitória, Espírito Santo, 29900, Brasil; tonim@tamar.org.br; cecilia@tamar.org.br (Almeida and Baptistotte); Base Projeto Tamar – RN, Avenida Joaquim Patrício, 4000, Distrito Litoral - Pium, Parnamirim, RN - 59160-530; claudio@tamar.org.br; armando@tamar.org.br (Bellini and Santos); Projeto TAMAR-ICMBio, Praia do Forte, Bahia, 40210, Brasil; neca@tamar.org.br (Marcovaldi); and Sackler Institute for Comparative Genomics, American Museum of Natural History, New York, New York, 10024, USA; gamato@amnh.org (Amato).

Address correspondence to Eugenia Naro-Maciel at the address above, or e-mail: Eugenia.NaroMaciel@csi.cuny.edu.

Abstract

Current understanding of spatial ecology is insufficient in many threatened marine species, failing to provide a solid basis for conservation and management. To address this issue for globally endangered green turtles, we investigated their population distribution by sequencing a mitochondrial control region segment from the Rocas Atoll courtship area ($n = 30$ males) and four feeding grounds (FGs) in Brazil ($n = 397$), and compared our findings to published data ($n_{\text{nesting}} = 1205$; $n_{\text{feeding}} = 1587$). At Rocas Atoll, the first Atlantic courtship area sequenced to date, we found males were differentiated from local juveniles but not from nesting females. In combination with tag data, this indicates possible male philopatry. The most common haplotypes detected at the study sites were CMA-08 and CMA-05, and significant temporal variation was not revealed. Although feeding grounds were differentiated overall, intra-regional structure was less pronounced. Ascension was the primary natal source of the study FGs, with Surinam and Trindade as secondary sources. The study clarified the primary connectivity between Trindade and Brazil. Possible linkages to African populations were considered, but there was insufficient resolution to conclusively determine this connection. The distribution of FG haplotype lineages was nonrandom and indicative of regional clustering. The study investigated impacts of population size, geographic distance, ocean currents, and juvenile natal homing on connectivity, addressed calls for increased genetic sampling in the southwestern Atlantic, and provided data important for conservation of globally endangered green turtles.

Key Words: *Chelonia mydas*, mtDNA, control region, mixed stock analysis, connectivity

Population connectivity and spatial distribution are fundamentally related to the ecology, evolution, and behavior of many species. In the marine realm, the life histories of diverse taxa are shaped by movements that vary among stages, such as passive dispersal of younger individuals and directed migration later in life. Inadequate understanding of such key life cycle aspects in threatened marine taxa hinders effective conservation and management. Highly migratory and globally endangered green sea turtles (IUCN 2011; *Chelonia mydas*),

for example, are important elements of the diverse and often distant ecosystems occupied during their life cycles. All sea turtles hatch from eggs on nesting beaches, also referred to as rookeries, then enter the ocean. As juveniles, green turtles leave the pelagic habitat for coastal feeding grounds (FGs, Musick and Limpus 1997). These FGs are usually “mixed stocks” drawn from different natal sources (Bowen and Karl 2007). Adult sea turtles undergo breeding migrations between feeding and nesting habitats potentially separated by

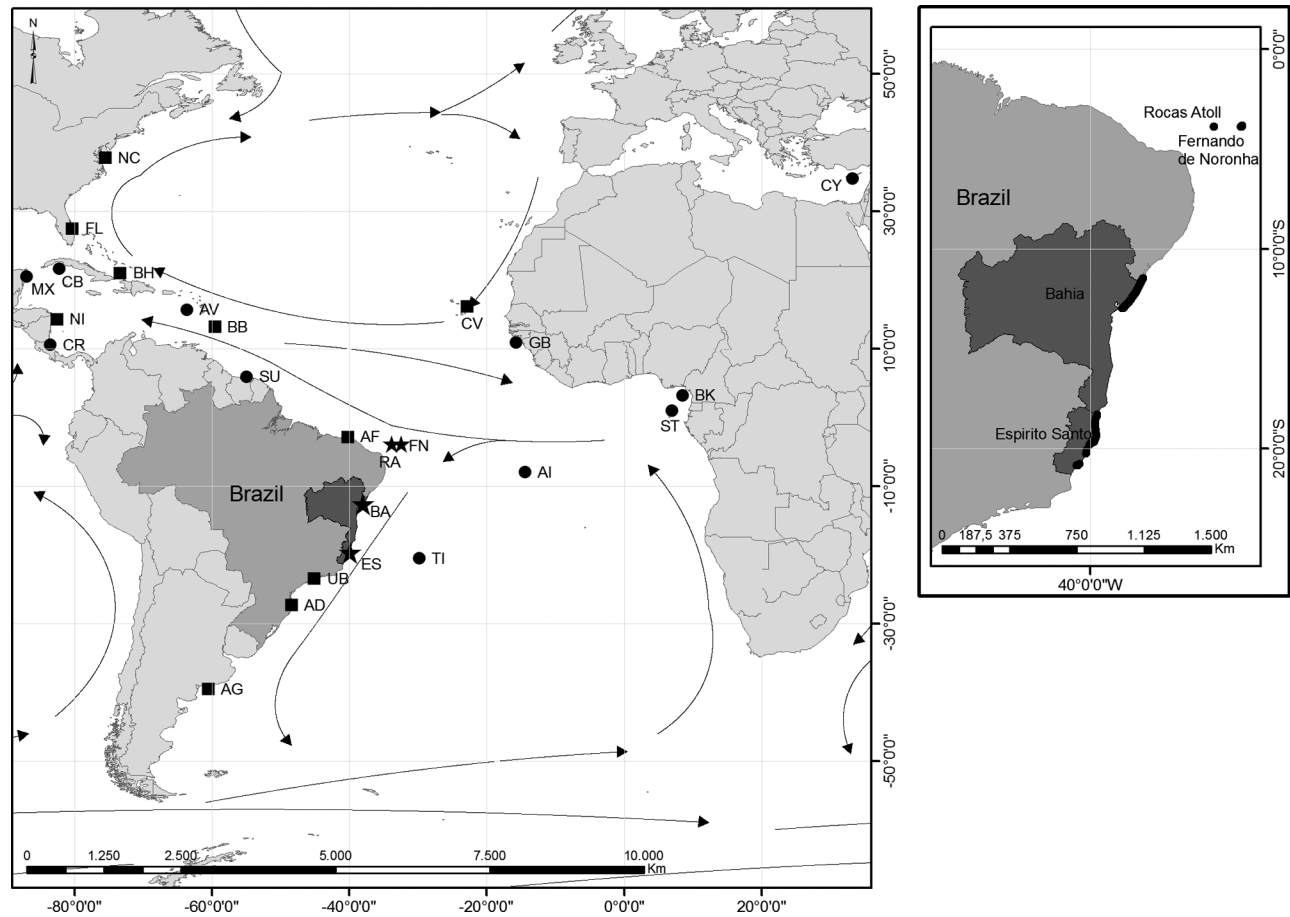


Figure 1. Map of the Rocas Atoll (RA), Fernando de Noronha (FN), Bahia (BA), and Espirito Santo (ES) study sites (symbolized by stars) with respect to general oceanic circulation patterns shown as arrows, and other *C. mydas* groups previously subject to genetic analysis. References and abbreviations for other FGs (symbolized by squares) are as follows: Almofala (AF, Naro-Maciel et al. 2007); Argentina (AG, Prosdocimi et al. 2011); Arvoredo (AD, Proietti et al. 2009); Bahamas (BH, Lahanas et al. 1998; Bolker et al. 2007); Barbados (BB, Luke et al. 2004); Cape Verde (CV, Monzon-Arguello et al. 2010); Florida (FL, Bass and Witzell 2000); Nicaragua (NI, Bass et al. 1998); North Carolina (NC, Bass et al. 2006); Rocas Atoll (RA, Bjorndal et al. 2006); and Ubatuba (UB, Naro-Maciel et al. 2007). FGs with fewer than 20 samples and rookeries with fewer than 10 samples or 20 females nesting annually were not included in the analyses. Rookeries that were assessed (symbolized by circles) were: Hutchinson Island, Florida, USA (FL); Quintana Roo, Mexico (MX); Aves Island, Venezuela (AV); Matapica, Surinam (SU; Encalada et al. 1996); Lara Bay, Cyprus (CY; Encalada et al. 1996; Kaska 2000); Cuba (CB; Ruíz-Urquiola et al. 2010); Tortuguero, Costa Rica (CR; Encalada et al. 1996; Bjorndal et al. 2005); Ascension Island, UK (AI); Poilao, Guinea Bissau (GB; Encalada et al. 1996; Formia et al. 2006); Bioko Island, Equatorial Guinea (BK); Sao Tome (ST; Formia et al. 2006); Trindade Island, Brazil (TI; Bjorndal et al. 2006); and Rocas Atoll, Brazil (RA; Encalada et al. 1996; Bjorndal et al. 2006). The inset map shows the sample collection sites.

hundreds to thousands of kilometers. The breeding migration to Ascension Island, a remote site on the mid-Atlantic ridge (Figure 1), requires precise island-finding abilities, can involve movements spanning ~2000 km, and is recognized as one of the most spectacular marine migrations (Papi and Luschi 1996; Luschi et al. 1998). Breeding occurs offshore of the nesting beach at nearby in-water courtship areas (CAs) and also during overlapping reproductive migrations (FitzSimmons et al. 1997a,b). Many females return to nest in the vicinity of their natal beach, a process known as natal homing (Carr 1967).

Elucidating patterns and processes of dispersal and migration is challenging in highly migratory and cryptic

organisms such as sea turtles. Genetic analysis is a powerful tool for investigating marine turtle connectivity (Bowen et al. 1995; Bowen and Karl 2007). MtDNA control region analyses reveal green turtle rookeries to be generally significantly differentiated, supporting the natal homing hypothesis (Bowen et al. 1992; Encalada et al. 1996; Bjorndal et al. 2005, 2006; Formia et al. 2006; Wallace et al. 2010). Less is known about males as their cryptic marine habitat makes them less accessible than females nesting on land. In contrast to the significant mitochondrial structure of rookeries, investigations of nuclear loci revealed less differentiation (Karl et al. 1992; FitzSimmons et al. 1997b; Roberts et al.

2004). The distinct mitochondrial and nuclear patterns were attributed to either mating during overlapping breeding migrations or male-mediated gene flow, which could involve non-philopatric males dispersing to mate away from their natal colonies. However, a unique study in Australia compared control region sequences of males and females at and among three courtship areas. The research demonstrated that both sexes were equally philopatric and supported the hypothesis of mating during overlapping migrations (FitzSimmons et al. 1997a).

Genetic analysis also provided key insights into FG population distribution. The mtDNA differentiation among rookeries enables use of Mixed Stock Analysis (MSA), a method borrowed from fisheries research, to determine natal origins of FGs. The traditional “one-to-many” (o2m) MSA examined origins of a single mixture using Bayesian methods (Pella and Masuda 2001). This approach was expanded to include metapopulation structure, and the new “many-to-many” (m2m) MSA method allows for analysis of multiple FGs and rookeries simultaneously (Bolker et al. 2007). This single program uniquely provides “rookery-centric” MSAs, which address the question of where turtles from nesting areas are going, to complement its “feeding ground-centric” analyses, which ask where turtles at an FG are coming from (Bolker et al. 2007). M2m MSAs require all key sources be included but allow for unsampled FGs, which are identified as “unknown” destinations in the rookery-centric MSA. MSAs have revealed relationships between Atlantic green turtle FG composition and rookery size (Bass et al. 1998; Lahanas et al. 1998), geographic distance (Bass and Witzell 2000), ocean currents, and/or juvenile natal homing (Luke et al. 2004; Bass et al. 2006; Naro-Maciel et al. 2007; Monzon-Arguello et al. 2010; Prosdocimi et al. 2011; Proietti et al. 2012). In the latter process, older juveniles move closer to their birthplace to forage (Laurent et al. 1998; Engstrom et al. 2002; Bowen et al. 2004 and references therein). Ocean currents may impact dispersal by influencing movements of drifting post-hatchlings, which may also be increasingly affected by severe weather (Monzon-Arguello et al. 2012).

Despite these advances, a large gap in genetic sampling of Atlantic green turtle FGs has left an incomplete understanding of their spatial ecology (Bjorndal et al. 2006; Bolker et al. 2007; Godley et al. 2010). MSAs indicated contributions to SW Atlantic (SWA) FGs from the Trindade Island rookery were lower than expected based on tag returns (Marcovaldi et al. 2000; TAMAR ICMBio, unpublished data) and favorable currents flowing toward Brazil (Naro-Maciel et al. 2007; Proietti et al. 2009; Prosdocimi et al. 2011; Figure 1). In another example, long-distance dispersal from the Guinea Bissau (Africa) colony was evaluated in a recent study that found limited connectivity to the SWA (Godley et al. 2010). That study hypothesizes substantial connections instead to a known but as yet genetically uncharacterized FG area north of Guinea Bissau. Their oceanographic modeling shows most drifting hatchlings would be restricted to local gyres. Additionally, all satellite movements from Guinea Bissau were local, and no tag returns connecting Guinea Bissau and the SWA have been recovered (Godley et al. 2010; TAMAR

ICMBio, unpublished data). Yet Lagrangian drifters revealed possible trans-Atlantic linkages, such as between Surinam and Cape Verde, and Guinea Bissau and Brazil (Monzon-Arguello et al. 2010; Proietti et al. 2012). Further, genetic analyses (Naro-Maciel et al. 2007; Bolker et al. 2007; Monzon-Arguello et al. 2010) included distant Guinea Bissau among potential top sources of Brazil FGs. However, Guinea Bissau is fixed for the CMA-08 haplotype common throughout the South Atlantic (Formia et al. 2006; Godley et al. 2010), and historic rather than current gene flow could be affecting outcomes (Naro-Maciel et al. 2007; Proietti et al. 2009). Indeed, a historical analysis of FGs based on the two distinct and previously reported (Encalada et al. 1996; Bjorndal et al. 2005) Atlantic/Mediterranean green turtle lineages is lacking.

To investigate these unknowns, characterization of Brazil and Africa within the Atlantic context is needed (Godley et al. 2010). Temporal variation has been investigated at only two sites in this region (Naro-Maciel et al. 2007), and further research is needed into this issue that can have substantial impacts on MSAs assuming temporal constancy (Bjorndal and Bolten 2008). Further, while published data are available for northern and southern Brazilian FGs through Argentina (Bjorndal et al. 2006; Naro-Maciel et al. 2007; Proietti et al. 2009; Prosdocimi et al. 2011; Proietti et al. 2012), the intervening vast area spanning ~3000 km of coastline had not been characterized genetically. Notably there have been repeated caveats regarding the small sample sizes analyzed from the oceanic island FGs of Fernando de Noronha (FN; $n = 9$) and Rocas Atoll (RA; $n = 23$; Bjorndal et al. 2006). RA is particularly interesting because it is a courtship area that includes adult males of unknown affiliation, as well as a nesting area and a juvenile feeding ground. Indeed the entire study area encompasses a striking range of human impacts, from the remote oceanic islands and World Heritage Sites of RA and FN, through an increasingly developed but protected area in Bahia (BA), to a highly urbanized site in Espirito Santo (ES; Figure 1). The latter FG, which spans the effluent discharge channel of a steel plant, is of special concern due to high levels of contagious and tumor-causing fibropapillomatosis disease (~34%; Torezani et al. 2010), starkly contrasting with the oceanic islands where tumors have not been observed.

Quantifying connectivity is important for conservation planning. In the SWA, sea turtles are exposed to myriad threats including disease, fisheries bycatch, and industrial or coastal development but protected by effective conservation organizations such as Brazil's Projeto TAMAR ICMBio, Karumbe in Uruguay, and the Programa Regional de Investigación y Conservación de Tortugas Marinas en la Argentina (PRICTMA). Together these organizations form the Tortugas Marinas del Atlántico Sur Occidental/Tartarugas Marinhas do Oceano Atlântico Sul Ocidental (ASO) Network (<http://www.tortugasaso.org/portal.htm>). With population increases, green turtles have been downgraded from Endangered to Vulnerable in Brazil (Almeida et al. 2011a). However future trajectories may well be impacted by threats during movements between protected or remote areas and those increasingly affected by human activities. In this study genetic methods were applied to address the

knowledge gaps discussed above, and to advance the research and conservation of green sea turtles with a focus on Brazil. Our objectives were to: (1) determine the partial genetic composition of the mtDNA control region at the study sites; (2) assess genetic differentiation between these FGs, the RA courtship area, and other Atlantic populations; (3) investigate temporal variation, as well differences among juveniles and adults, and tumored and tumor-free turtles where applicable; (4) elucidate the connectivity of FGs and rookeries using mixed stock analysis; and (5) consider effects of population size, geographic distance, juvenile natal homing, and ocean currents on genetic composition.

Materials and Methods

Sample Collection

Projeto TAMAR-ICMBio biologists and veterinarians obtained samples from live or stranded turtles at four FGs in Brazil (Figure 1): Espirito Santo (ES; $n = 157$ plus five recaptures), Bahia (BA; $n = 45$), Fernando de Noronha (FN; $n = 117$ plus two recaptures), and Rocas Atoll (RA; $n = 78$). These turtles were visually examined, measured, and their blood or tissue was sampled for genetic analysis following standard protocols (Dutton 1996). Blood was stored in a lysis buffer and frozen, while tissue samples were stored in ~90% ethanol and frozen. Samples were collected at ES from July 2004 to November 2005, at BA from August 2003 to October 2005, and at FN from July 2004 to December 2005. All of the turtles sequenced from these sites were juveniles of unknown gender. ES is an exclusively developmental habitat where Curved Carapace Length (CCL) ranges from 25.2 to 77.5 cm (To rezani et al. 2010). CCL of sequenced turtles ranged from 34 to 83 cm at FN, and 30 to 76 cm at BA. The larger animals occasionally observed in BA were too infrequent to be included in this study. As the largest turtles sequenced were well under the minimum nesting female size in Brazil of ~90 cm CCL (P. Barata, personal communication; Hirth 1997), there was no reason to believe that transient adults migrating through the area to breed might be confused with resident foraging turtles. However, at RA, samples were collected from adult males (RA males; $n = 30$; CCL: 97–112 cm) present during the breeding season (December 2004 to January 2005; December 2005 to January 2006). These samples were analyzed separately from juveniles (RA juveniles; $n = 78$; CCL: 31–69 cm) collected throughout the year (December 2004 to May 2006).

Laboratory Analysis

DNA extractions were performed using a DNeasy Kit following manufacturer's instructions (QIAGEN Inc.). Primers LCM15382 and H950 (Abreu et al. 2006) were used to amplify a ~857-bp fragment of the mtDNA control region. Standard conditions and negative controls were employed for PCRs, using an annealing temperature of 51 °C, and sequencing was

carried out in both directions following previously described protocols (Naro-Maciel et al. 2007). Sequences were aligned using SEQUENCHER v4.6 (Gene Codes Corporation) and named according to the standardized Archie Carr Center for Sea Turtle Research (ACCSTR) designations.

Genetic Diversity and Differentiation

Calculations of genetic diversity and differentiation were conducted using sequences truncated to ~481 bp for comparison to previous studies (Figure 1; Supplementary Table 1 online). Arlequin v3.11 (Excoffier et al. 2005) was employed to calculate number of haplotypes (a) as well as haplotype (h) and nucleotide (π) diversities (Nei 1987). Arlequin was also used to carry out pairwise and global exact tests of population differentiation (Raymond and Rousset 1995), as well as pairwise tests and Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) using F -statistics based on haplotype frequencies only (Weir and Cockerham 1984). F -statistics, rather than Φ -statistics, were used because Shamblin et al. (2012) reported that using haplotype frequencies only was more accurate for assessing Atlantic green turtle population structure. The study found that the presence of haplotypes from the two divergent lineages inaccurately lowered green turtle population structure estimates based on Φ -statistics (Shamblin et al. 2012). These tests were also applied to analyses at each site. In temporal comparisons samples were grouped into tropical winter (April–September) and summer (October–March) seasons, which were then paired for testing among years. Recaptures were included in temporal analyses, although they were not double-counted in the overall sample. At ES, where sample sizes were sufficient, turtles with fibropapilloma tumors were compared to those without tumors to investigate the possibility of differing natal origins, FGs, or migratory pathways of diseased versus healthy turtles. At RA juveniles were compared to males. Prior to carrying out the MSAs it was necessary to determine whether the areas could be considered mixed stocks (Chapman 1996). To test the possibility of single origins, the pairwise tests described above were used to compare FGs and the RA males to rookeries (Supplementary Table 1 online and references therein). Significance values were obtained from at least 10 000 permutations. Two different correction factors were applied to unadjusted P values: (1) the more conservative sequential Bonferroni procedure (Rice 1989) widely used in the sea turtle literature, and (2) the linear step-up procedure to control the false discovery rate (Benjamini and Hochberg 1995).

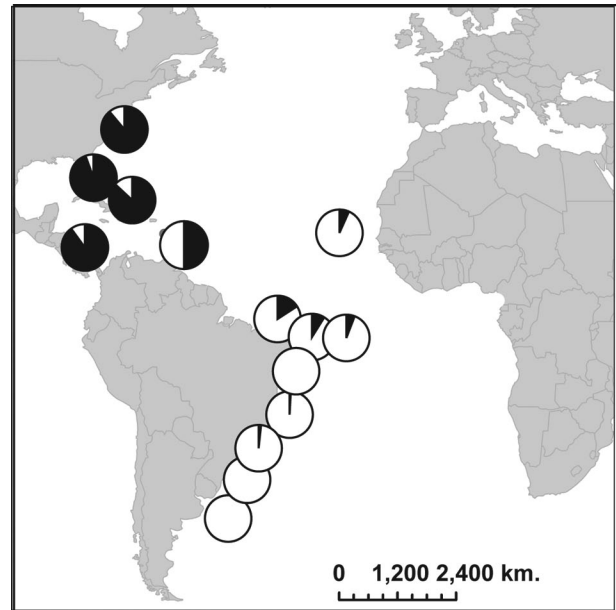
Mixed Stock Analysis

To investigate connectivity of the study sites and Atlantic nesting and feeding areas, many-to-many MSAs (m2ms; Bolker et al. 2007) were carried out utilizing new and published data and incorporating rookery population size as prior information (Supplementary Table 1 online, Figure 1). All FGs subjected to MSA were developmental areas, since

Table 1 Green sea turtle mtDNA control region haplotypes and relative frequencies detected at the study CA (Courtship Area) and FGs, with respect to total sample size (n)

Haplotype	CA		FGs		
	RA males	RA juv	FN	BA	ES
CM-A1		0.01	0.02		
CM-A3	0.03	0.10	0.03		0.01
CM-A5	0.07	0.27	0.44	0.31	0.30
CM-A6		0.03	0.03	0.04	0.04
CM-A8	0.77	0.46	0.39	0.51	0.56
CM-A9	0.07	0.09		0.07	0.04
CM-A10		0.04	0.03		0.02
CM-A12	0.03				
CM-A17			0.01		
CM-A23			0.01	0.04	0.01
CM-A24			0.01	0.02	0.01
CM-A25	0.03				
CM-A32			0.01		0.01
CM-A42			0.01		
CM-A46					
CMA-66			0.01		
Total n	30	78	117	45	157

the two sites that were not significantly differentiated from single rookeries (Nicaragua and RA males, samples composed exclusively of adults) were not suitable for MSA. Also, Cyprus was not included as a source following initial MSAs revealing it did not contribute to Atlantic FGs (data not shown; Naro-Maciel et al. 2007; Monzon-Arguello et al. 2010; Proietti et al. 2012), with which it shares haplotypes with three individuals in Florida constituting 0.2% of the total FG sample (Supplementary Table 1 online). The first MSA included Atlantic sources and FGs that could be considered mixed stocks (Supplementary Table 1 online, Figure 1). In MSA2 prior knowledge was used to exclude a source following Godley et al. (2010): Guinea Bissau was not included in accordance with the hypothesis that it constitutes a local population connected to an as yet unsampled proximate FG (Godley et al. 2010). In MSAs 3–5, Guinea Bissau was included as a source along with a simulated local FG fixed for CMA-08. Since Guinea Bissau is fixed for CMA-08, this would be the hypothesized genetic composition of the unsampled local FG. To span the range of sample sizes normally included in FG studies, three MSAs were run with different sample sizes for this simulated FG ($n_{MSA3} = 40$, $n_{MSA4} = 80$, $n_{MSA5} = 120$). All MSAs were run until diagnostic tests indicated convergence of all chains with a Gelman Rubin criterion below 1.2 (Gelman et al. 1996), and both rookery- and feeding ground-centric outputs were examined. Pearson's correlation tests and linear regression were used in comparative analyses of MSA estimates through the StatPlus program version 2009 (AnalystSoft Inc). MSA1 results were compared to MSA2 and MSA4. In light of broad convergence, MSA4 was used to represent MSAs 3–5 since its simulated sample size

**Figure 2.** Distribution of Atlantic control region lineages among FGs. Lineage A (Cluster A; Encalada et al. 1996) is shown in black and Lineage B (Cluster B; Encalada et al. 1996) is shown in white. The lineage of each haplotype is given in Supplementary Table 1 online, along with the sample size for each FG. Locations and references as in Figure 1.

was closer to the average FG sample size (Supplementary Table 1 online). Comparisons were also made between the FG-centric output and results from o2m SWA MSAs that also incorporated rookery population size (Naro-Maciel et al. 2007; Prodocimi 2011; Proietti et al. 2012).

Results

Genetic Diversity and Differentiation

Most haplotypes found at the study sites belonged to Lineage B (Cluster B, Encalada et al. 1996; Figure 2; Supplementary Table 1 online), with CMA-05 and CMA-08 being the most common (Table 1). One previously undescribed haplotype was found at FN and assigned the standardized ACCSTR designation CMA-66 (GenBank: JF308463.1). The sequence differed by a single transition from CMA-08. Molecular diversities of the study FGs in comparison to other juvenile FGs are given in Table 2. In RA males haplotype diversity was 0.414 and nucleotide diversity was 0.003. Genetic differentiation of the four study FGs and the RA courtship area is shown in Table 3. Global tests revealed highly significant differentiation among 13 Atlantic juvenile FGs ($F_{ST} = 0.328$, $P < 0.001$, exact $P < 0.001$) as well as the eight SWA FGs ($F_{ST} = 0.035$, $P < 0.001$, exact $P < 0.001$), and the four study FGs ($F_{ST} = 0.016$, $P = 0.011$, exact $P = 0.004$). Pairwise comparisons revealed less pronounced intra-regional structure (Table 4). Intra-FG tests found no consistent temporal variation (Table 3), and no differentiation among turtles

Table 2 Mitochondrial control region diversity at the four study FGs (in bold), as compared to other Atlantic juvenile FGs from the published literature (references in Figure 1)

FG	# Haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)	Sample size
North Carolina	12	0.729 ± 0.030	0.005 ± 0.003	106
Florida	16	0.626 ± 0.018	0.004 ± 0.002	362
Bahamas	23	0.612 ± 0.021	0.006 ± 0.003	560
Barbados	8	0.773 ± 0.028	0.010 ± 0.005	60
Almofala	13	0.717 ± 0.031	0.007 ± 0.004	117
RA_{juv}	8	0.688 ± 0.036	0.005 ± 0.003	101
FN	12	0.650 ± 0.028	0.004 ± 0.003	117
BA	6	0.648 ± 0.053	0.002 ± 0.002	45
ES	9	0.595 ± 0.031	0.003 ± 0.002	157
Ubatuba	10	0.446 ± 0.056	0.002 ± 0.002	113
Arvoreda	8	0.557 ± 0.070	0.002 ± 0.002	49
Argentina	9	0.553 ± 0.051	0.002 ± 0.002	93
Cape Verde	5	0.588 ± 0.045	0.004 ± 0.003	44

For standardization with other studies, these measures were based on ~481 bp long mtDNA segments and recalculated for FGs described in the literature (Figure 1 and references therein).

Table 3 Genetic differentiation at the study courtship area (CA; RAmales) and FGs

Study site	N	Genetic differentiation		
		Seasons	Years	Rookeries
RA_{males}	30	N/A	$n_{\text{year1}} = 12, n_{\text{year2}} = 19$ $F_{\text{ST}} = -0.022$, $P > 0.795$, exact $P > 0.781$	$F_{\text{ST}} > 0.027, P < 0.042$, exact $P < 0.006$, but RA _{males} vs. RA _{females} ($F_{\text{ST}} = -0.006, P = 0.598$, exact $P = 0.329$); RA _{males} vs ST ($F_{\text{ST}} = 0.006, P = 0.312$, exact $P = 0.127$)
RA_{juv}	78	$n_{\text{season1}} = 24, n_{\text{season2}} = 21, n_{\text{season3}} = 12, n_{\text{season4}} = 20$ $F_{\text{ST}} < 0.086, P > 0.067$, exact $P > 0.206$	$n_{\text{year1}} = 43, n_{\text{year2}} = 29$ $F_{\text{ST}} = 0.046$, $P > 0.056$, exact $P > 0.308$ vs. 2000 (Bjorndal et al. 2006): $F_{\text{ST}} = 0.006$, $P > 0.483$, exact $P > 0.240$	$F_{\text{ST}} > 0.045, P < 0.043$, exact $P < 0.001$
FN	117	$n_{\text{season1}} = 31, n_{\text{season2}} = 34, n_{\text{season3}} = 33, n_{\text{season4}} = 21$ $F_{\text{ST}} < 0.087, P > 0.128$, but season 1 vs. 4: $P = 0.034$ (ns after sequential Bonferroni correction), exact $P > 0.051$ (season 1 vs. 4)	$n_{\text{year1}} = 65, n_{\text{year2}} = 53$ $F_{\text{ST}} = 0.019$; $P > 0.092$, exact $P > 0.059$ vs. 2000 (Bjorndal et al. 2006): $F_{\text{ST}} = 0.187, P = 0.007$, exact $P = 0.033$	$F_{\text{ST}} > 0.138, P < 0.001$, exact $P < 0.001$
BA	45	$n_{\text{season1}} = 5, n_{\text{season2}} = 25, n_{\text{season3}} = 10$ $F_{\text{ST}} < -0.024, P > 0.546$, exact $P > 0.317$	$n_{\text{year1}} = 30, n_{\text{year2}} = 10$ $F_{\text{ST}} = -0.024$, $P > 0.570$, exact $P > 0.353$	exact $P < 0.012$, but BA vs. ST: $F_{\text{ST}} = 0.050, P = 0.061$
ES	157	$n_{\text{season1}} = 54, n_{\text{season2}} = 52, n_{\text{season3}} = 45$ $F_{\text{ST}} < 0.015, P > 0.154$, exact $P > 0.181$	$n_{\text{year1}} = 104, n_{\text{year2}} = 49$ $F_{\text{ST}} = -0.009; P > 0.730$, exact $P > 0.886$	exact $P = 0.000$, but ES vs. ST: $F_{\text{ST}} = 0.046, P = 0.059$

Sample size (n) is given along with results of genetic differentiation tests among years and seasons at the study sites, and between the study sites and regional rookeries. Statistically significant comparisons ($P < 0.05$) are shown in bold. Abbreviations as in Figure 1.

with tumors ($n = 27$) and those without ($n = 126$) at ES ($F_{\text{ST}} = -0.022, P = 1.000$, exact $P > 0.979$). There was significant differentiation between RA males and juveniles ($F_{\text{ST}} = 0.085, P < 0.004$, exact $P < 0.035$) but not among males and females ($F_{\text{ST}} = -0.006, P = 0.598$, exact $P = 0.329$; Table 3). The Nicaraguan adult FG was significantly differentiated from all rookeries ($F_{\text{ST}} > 0.185, P < 0.001$, exact

$P < 0.001$) except for Tortuguero ($F_{\text{ST}} = -0.007, P = 0.650$, exact $P = 0.760$).

Mixed Stock Analysis

The main difference among MSAs, all of which included population size as a prior, centered on Ascension and Guinea

Table 4 Control region pairwise exact test *P*-values (above diagonal) and pairwise F_{ST} values (below diagonal) among *C. mydas* juvenile FGs

FG	NC (106)	FL (362)	BH (560)	BB (60)	RA (101)	FN (117)	AF (117)	BA (45)	ES (157)	UB (113)	AD (49)	AG (93)	CV (44)
North Carolina	–	0.012*	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
Florida	0.006	–	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
Bahamas	0.045***	0.029***	–	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
Barbados	0.055***	0.100***	0.081***	–	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
Rocas Atoll _{juv}	0.230***	0.307***	0.299***	0.088***	–	0.001**	0.206	0.145	0.068	0.000***	0.009**	0.021*	0.036*
Fernando de Noronha	0.262***	0.334***	0.323***	0.110**	0.030*	–	0.006**	0.077	0.005**	0.000***	0.023*	0.000***	0.982
Almofala	0.193***	0.267***	0.252***	0.054***	–0.001	0.035**	–	0.016*	0.000***	0.000***	0.033*	0.000***	0.064
Bahia	0.271***	0.355***	0.349***	0.124***	–0.006	0.016	0.012	–	0.60300	0.010**	0.267	0.376	0.039*
Espirito Santo	0.302***	0.371***	0.364***	0.151***	0.003	0.034**	0.020*	–0.010	–	0.002**	0.115	0.295	0.033*
Ubatuba	0.376***	0.431***	0.425***	0.249***	0.066***	0.162**	0.081**	0.070**	0.050**	–	0.449	0.173	0.000***
Arvoredo	0.310***	0.388***	0.385***	0.169***	0.015	0.069**	0.029*	0.004	0.000	0.009	–	0.719	0.005**
Argentina	0.322***	0.393***	0.388***	0.182***	0.016	0.079**	0.035**	0.008	0.004	0.011	–0.012	–	0.001***
Cape Verde	0.278***	0.351***	0.337***	0.122***	0.050*	–0.009	0.054**	0.033	0.054*	0.217***	0.103**	0.113***	–

The study sites are in bold in the FG column, sample size is given in parentheses, and abbreviations follow those in Figure 1. Asterisks indicate statistically significant comparisons prior to corrections (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). All values remained significant after the linear step-up procedure was used to control the false discovery rate (Benjamini and Hochberg 1995). Values that remained significant at or below the $P = 0.05$ level after sequential Bonferroni corrections are shown in bold. Estimates differing in significance between the two tests or correction procedures are highlighted in gray.

Bissau (FG-centric: Figure 3; Rookery-centric: Figure 4). MSA1 estimates were highly correlated with MSAs 2–5 ($R > 0.278$; $P < 0.002$), as well as o2m values incorporating population size (Naro-Maciel et al. 2007; Prosdocimi et al. 2011; Proietti et al. 2012; $R > 0.811$; $P < 0.002$). In comparison to MSA1, the o2ms estimated lower Guinea Bissau and higher Ascension contributions to SWA FGs, which additionally had higher contributions of Trindade at Arvoredo (Proietti et al. 2009) and Ubatuba, and of Surinam at Almofala (Naro-Maciel et al. 2007). Besides having no contributions from Guinea Bissau, MSAs 1 and 2 were $>5\%$ different in that Ascension contributed more to the SWA and Cape Verde in the second analysis. Results of MSA4 differing by $>5\%$ from MSA1 were: (1) higher Ascension contributions in southern Brazil; (2) lower Guinea Bissau contributions to the SWA, and (3) high connectivity between Guinea Bissau and the simulated FG in MSA4 (~80% of the simulated FG originated at Guinea Bissau, and ~40% of Guinea Bissau turtles went to the simulated FG, with the remainder heading primarily for the SWA). Also, there were $>5\%$ increases in turtles from the Bioko, Sao Tome, and Rocas Atoll rookeries going to the simulated FG in MSA4.

In all MSAs Ascension was the main source for SWA FGs, and ~85% of its turtles went to SWA FGs with contributions peaking in southern Brazil (but also Almofala). About ~78% of Trindade turtles went to the SWA with peaks in BA and Argentina. Surinam and Aves had fewer turtles in southern Brazil than elsewhere in the region, and a regional peak in FN. In sum, ~74% of Surinam and ~67% of Aves turtles were present in the SWA. Rocas Atoll and Sao Tome turtles were evenly distributed in the region, which was also a frequent destination for Bioko turtles. Turtles from Costa Rica

were rare in the SWA except for at Almofala. The remaining rookeries did not usually constitute over ~4% of the SWA juvenile FGs (but see Bioko). The FG-centric m2m (Figure 3) with the lowest confidence intervals was MSA4 (~13.8%), followed by MSA2 (~14.1%), MSA1 (~14.5%), and the o2ms (~17.2%). Several lower CI boundaries included zero. Linear regression showed a significant relationship between number of nesting females and average MSA1 rookery contributions from central and southern Atlantic rookeries to SWA FGs ($R^2 = 0.980$; $P = 0.000$), although no relationship was detected if northwestern Atlantic rookeries were included ($R^2 = 0.001$; $P = 0.912$).

Discussion

Connectivity of FGs and Rookeries

This genetic study of green turtles foraging in the SWA addresses requests for increased spatial and temporal sampling and provides information necessary for conservation of globally endangered green turtles. With new data from Brazil, and MSAs that simultaneously included multiple FGs as well as rookery- and FG-centric approaches (Bolker et al. 2007), the study enhanced understanding of green turtle juvenile dispersal but also underscored the need for greater resolution particularly with respect to Africa. Overall, with some possible exceptions rooted in Africa, our results matched expectations under juvenile natal homing (Bowen et al. 2004): (1) rookeries with larger contributions from closer FGs; (2) correspondence between proximate rookeries and FGs; and (3) differentiation among FGs, as previously reported in the region (Bolker et al. 2007; Naro-Maciel et al. 2007; Proietti

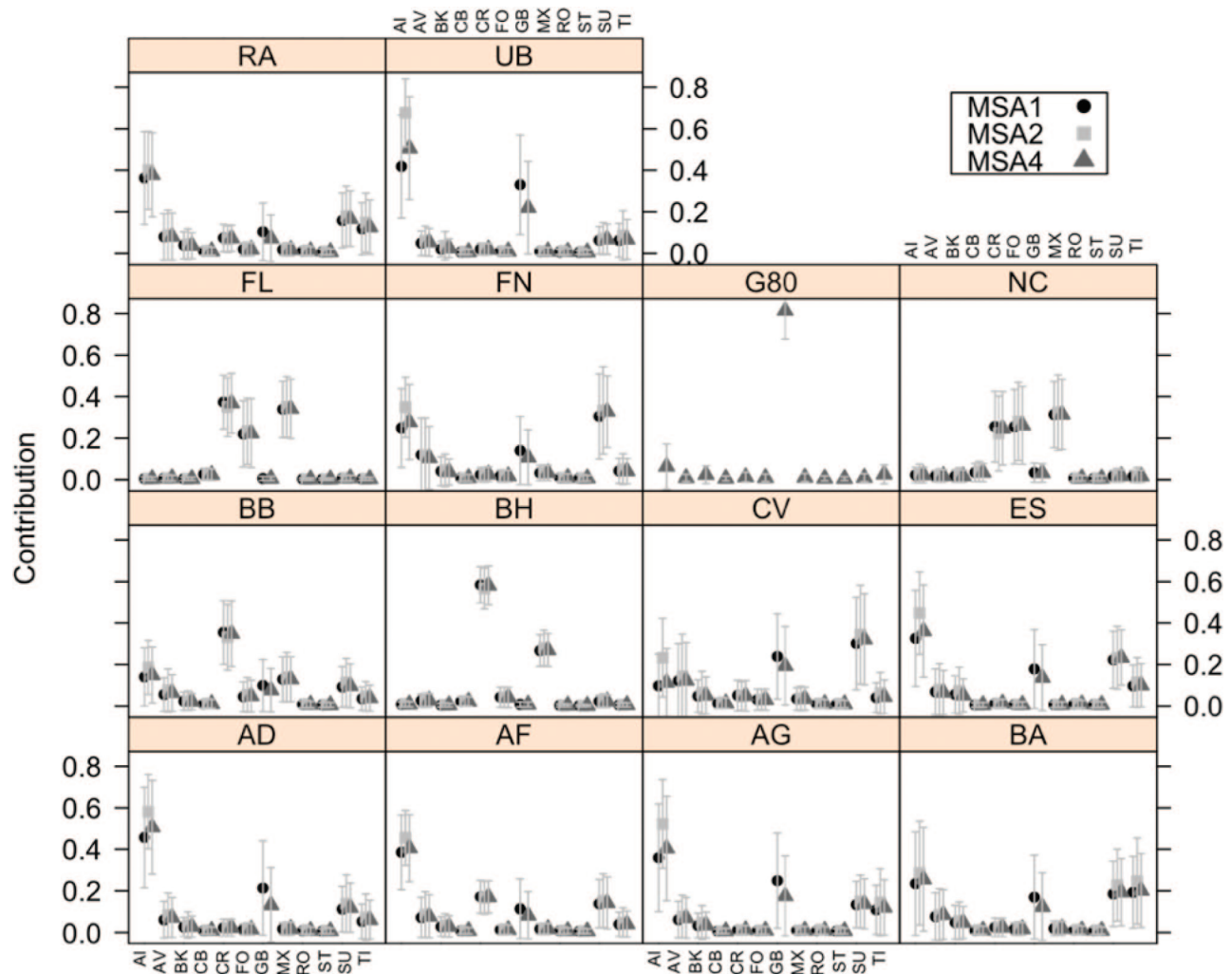


Figure 3. FG-centric many-to-many-mixed stock analysis results showing mean estimates and 95% confidence intervals. MSA1 includes Atlantic sources and mixed stock FGs (Supplementary Table 1 online, Figure 1). MSA2 differs from MSA1 in that Guinea Bissau was excluded to explore the hypothesis that it constitutes a local population connected to an as yet unsampled proximate FG (Godley et al. 2010). MSA4 differs from MSA1 in that a simulated local FG fixed for CMA-08, the hypothesized genetic composition of the unsampled local FG, is included. The abbreviation G80 represents the simulated FG north of Guinea Bissau ($n = 80$), while FO and RO stand for the Florida and Rocas rookeries, respectively. Other abbreviations as in Figure 1.

et al. 2009; Prosdocimi et al. 2011). The contributions to SWA FGs of central and southern Atlantic rookeries (South Caribbean, and East, South Central, and Southwest Atlantic Regional Management Units, RMUs; Wallace et al. 2010) were correlated to nesting female population sizes, although the degree to which sampling or ongoing versus past gene flow affected estimates from distant African sites remains to be conclusively determined. We emphasize that the relationship did not hold when northwest Atlantic rookeries were included, highlighting the importance of geographic proximity. While distance is difficult to estimate precisely because turtle movements cannot usually be measured in straight lines (Naro-Maciel et al. 2007), there was a general pattern of larger contributions from closer rookeries, with some possible long-distance dispersal requiring further

investigation. Although broadly in line with MSA results, our study also indicates that population distribution is not fully explained in terms of drifting hatchlings guided by ocean currents.

Our findings of substantive connectivity between Ascension and the SWA coast were consistent with satellite (Luschi et al. 1998), tag (Mortimer and Carr 1987), and previous genetic research (Bolker et al. 2007; Naro-Maciel et al. 2007; Monzon-Arguello et al. 2010; Prosdocimi et al. 2011; Proietti et al. 2012). Past genetic studies also indicated secondary connectivity to Surinam supported by tag data (Schulz 1975; Pritchard 1976; Meylan 1995). Among our novel contributions was clarification of the regionally important Trindade rookery's connectivity over the entire SWA rather than at single FGs. Studies not utilizing the rookery-centric

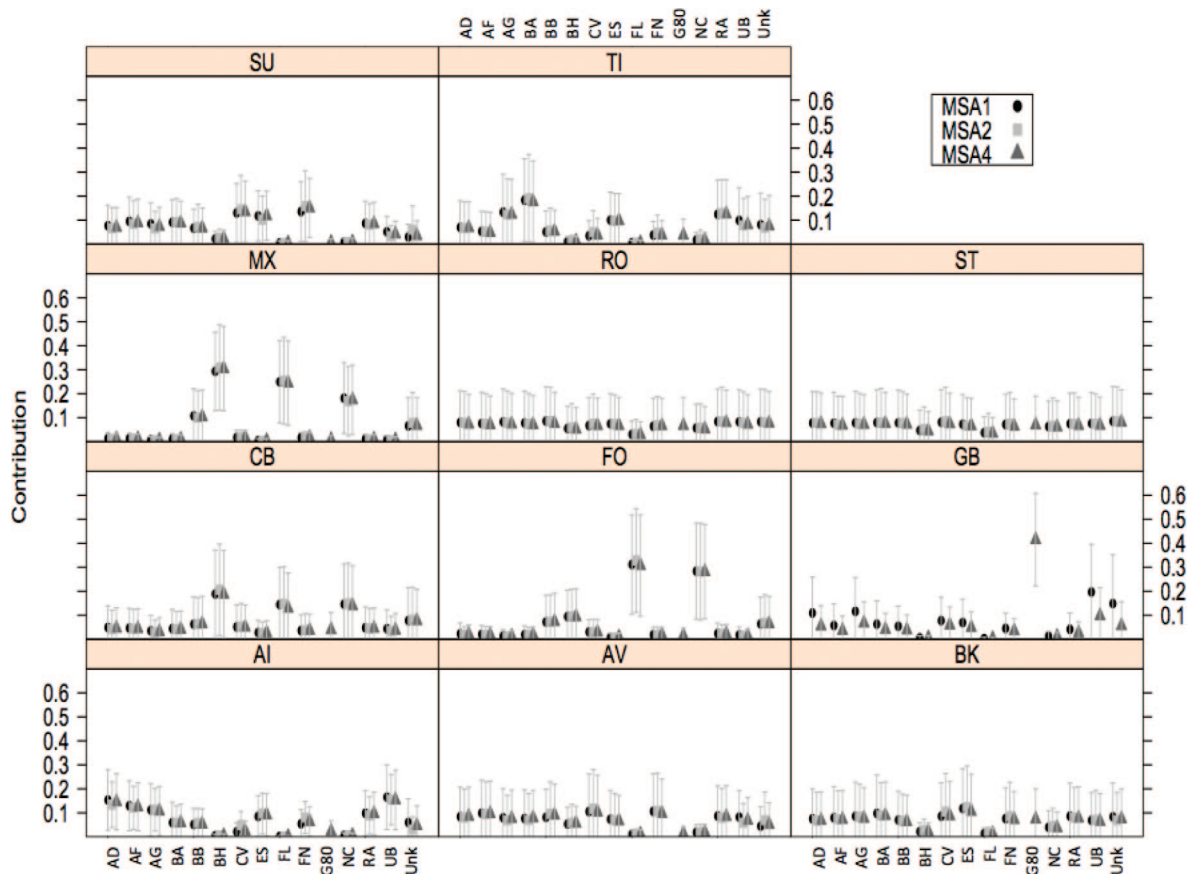


Figure 4. Rookery-centric many-to-many-mixed stock analysis results showing mean estimates and 95% confidence intervals. Costa Rica is not shown. MSA1 includes Atlantic sources and mixed stock FGs (Supplementary Table 1 online, Figure 1). MSA2 differs from MSA1 in that Guinea Bissau was excluded to explore the hypothesis that it constitutes a local population connected to an as yet unsampled proximate FG (Godley et al. 2010). MSA4 differs from MSA1 in that a simulated local FG fixed for CMA-08, the hypothesized genetic composition of the unsampled local FG, is included. The abbreviation G80 represents the simulated FG north of Guinea Bissau ($n = 80$), Unk stands for “Unknown” FGs, and FO and RO represent the Florida and Rocas rookeries, respectively. Other abbreviations as in Figure 1.

approach reported that Trindade contributions to SWA FGs were consistently lower than expected based on tag returns, population size, favorable ocean currents flowing directly to Brazil, and geographic proximity (Figure 1; Naro-Maciel et al. 2007; Proietti et al. 2009; Prosdocimi et al. 2011; but see Proietti et al. 2012). Further, in the absence of new SWA data (this study; Prosdocimi et al. 2011), Monzon-Arguello et al. (2010) found that ~30% of Trindade turtles were going to unsampled FGs, as reflected in the “unknown” category of the rookery-centric output (Bolker et al. 2007). Our analysis addressed these issues and showed that in aggregate ~78% of Trindade’s turtles foraged along the coast and oceanic islands of the SWA. On average ~10% of each SWA FG was drawn from Trindade, and about ~9% of Trindade turtles went to each of these FGs. We note that, although average rookery-centric estimates of the five closest rookeries were close to 10% per SWA FG (Figure 4), these represented very different numbers of turtles considering the variation in rookery sizes; Ascension has almost four times as many nesting turtles as

Trindade, and Rocas Atoll is a small rookery (Supplementary Table 1 online).

The m2m method was useful in highlighting the uneven distributions of several rookeries among SWA FGs (Figures 3 and 4). For example, although a peak in the distribution of Trindade turtles might have been expected at the closest ES FG, more of these turtles were found not only in neighboring BA, but also in the Argentina developmental habitat. In contrast, the lowest coastal estimates for this rookery were at the northern Almofala and southern Arvoredo FGs. Ascension turtles, ~85% of which went to SWA FGs, were also bimodally distributed. However, their distribution was roughly the inverse of Trindade’s, with coastal peaks in southern Brazil and Almofala, and lows in BA and ES. Barata et al. (2011) reported that the size of SWA green turtles increased northwards along the coast. The southern peaks may reflect initial post-pelagic recruitment to juvenile developmental habitats such as ES and Argentina, depending on the source rookery, while the peaks further north could be from larger turtles

swimming closer to their natal habitats. Larger turtles are found in both Almofala and BA (Marcovaldi and Marcovaldi 1999; Lima et al. 2003) but not usually in Argentina or ES (Torezani et al. 2010; Barata et al. 2011). Rocas Atoll rookery turtles however appeared to be evenly distributed in the SWA (Figure 4). Monzon-Arguello et al. (2010) noted decreased MSA resolution for the smallest rookeries, which could also have affected estimates for Sao Tome, another small nesting area with an even distribution among FGs (Figure 4).

Trans-Atlantic crossings between Africa and the SWA would be less consistent with juvenile natal homing than more localized movements. However it is possible that some small turtles drift long distances with the currents but eventually make their way back toward their natal areas, while others continue to forage at sites distant from their birthplaces. The study confirmed that there were few long-distance movements between the SWA and the Northwestern Atlantic or Mediterranean, as previously noted (Bolker et al. 2007; Monzon-Arguello et al. 2010). However, as a centrally located FG, Barbados is a moderate destination for turtles from diverse rookeries (Figures 3 and 4). While the different MSAs were broadly consistent with respect to the trans-Atlantic connectivity of Cape Verde and Surinam highlighted by Monzon-Arguello et al. (2010), our estimates were somewhat lower with ~30% of Cape Verde turtles born in Surinam and ~14% of Surinam turtles going to Cape Verde. The difference was likely due to the new FG data from Brazil (this study) and Argentina (Prosdocimi et al. 2011). All MSAs indicated that Bioko turtles fanned out among FGs, including those in distant areas. The small Sao Tome rookery was indistinguishable from some of our study sites (Table 3); however given its rookery size and distance the island was not considered a realistic source. Indeed MSAs indicated that the small Gulf of Guinea rookeries were sources of only small percentages of turtles at individual FGs (Figure 3; Naro-Maciel et al. 2007; Proietti et al. 2009; Prosdocimi et al. 2011).

Our exploratory MSAs (2–5) proved to be useful tools for further investigating long-distance dispersal in the absence of complete sampling in Africa. Besides the unsampled North African FG described by Godley et al. (2010), data on Corisco Bay and the Gulf of Guinea FGs (Formia 2002), as well as from Liberia to Benin (Godley et al. 2010), remain publicly unavailable. In addition, there is no published information about potentially important rookeries such as those in Angola or the Congo. Although these data may alter our findings, excluding Guinea Bissau in MSA2 had little effect on Northwest Atlantic (or Mediterranean) estimates, and turtles from Ascension primarily made up the balance in the SWA and Cape Verde FGs (Figure 3). Of note, similar results were obtained in o2m analyses, which generally found few Guinea Bissau turtles at individual SWA FGs corresponding with greater contributions mainly from Ascension (Figure 3; Naro-Maciel et al. 2007; Proietti et al. 2009; Prosdocimi et al. 2011; Proietti et al. 2012). However the o2m analyses did not take into account relationships among multiple FGs or offer a rookery-centric perspective (Bolker et al. 2007), and the o2ms also had the highest confidence intervals.

While implausible sources had been excluded based on prior knowledge (Engstrom et al. 2002; Godley et al. 2010; Monzon-Arguello et al. 2010), MSAs 3–5 could only be used to explore hypotheses because simulated data for the local Guinea Bissau FG needs to be corroborated with real samples, and sampling gaps in Africa need to be filled. However these simulations did suggest that even if the as yet unsampled local FG were made up mostly of Guinea Bissau turtles as predicted by Godley et al. (2010), more than half of the Guinea Bissau turtles would still disperse to other FGs including in the distant SWA (Figure 4). This would not be inconsistent with drifter trajectories (Monzon-Arguello et al. 2010; Proietti et al. 2012). If the turtles going to the SWA were small post-hatchlings, this dispersal would not necessarily contradict the lack of satellite-tracked adult movements or tag returns linking the two areas noted by Godley et al. (2010). Larger turtles tagged feeding in the SWA might not yet have been recaptured reproducing in Africa since tagging efforts in Brazil FGs started in the 1990s (Marcovaldi et al. 1998). However, even with the known limits to tagging such as extensive tag loss, insufficient monitoring and reporting, and mortality, there was one turtle tagged nesting at Trindade recaptured dead in Senegal (Marcovaldi et al. 2000).

Our findings also suggest that population distribution is not fully explained in terms of drifting hatchlings guided by ocean currents. The evidence of bidirectional mixing among SWA FGs is consistent with swimming and movements not determined by currents. Similarly, while the northward-flowing Guiana Current would influence hatchlings from Surinam and Aves, larger turtles capable of swimming against the currents may be the ones feeding particularly in northern Brazil (although younger animals could be guided south by the counter current; Prosdocimi et al. 2011; Figure 1). Alternately, additional FG sampling closer to Aves, for which mark-recapture or satellite tag confirmation of linkages to Brazil is lacking, may revise this hypothesis, even though the m2m MSA did not indicate the presence of any significant unknown FG (Figure 4). Some turtles from the Costa Rican rookery, where major currents also flow northwards, and whose turtles are primarily distributed in Nicaragua (Bolker et al. 2007; this study) but connected to a lesser extent to northeastern Brazil (Marcovaldi et al. 2000; Lima and Troeng 2001; Naro-Maciel et al. 2007; Lima et al. 2008), may also be swimming to Brazil. On the other hand, the major Ascension and Trindade rookeries are bathed by currents that flow toward Brazil and likely guide young turtles in that direction (Figure 1).

Regional Clustering of FGs

Providing additional support for the juvenile natal homing hypothesis, the correspondence between proximate rookeries and FGs was apparent in similar northern, central, and southern/eastern clusters of FG lineages (Figure 2) and rookery groups (Bass et al. 2006; Monzon-Arguello et al. 2010). Indeed the lineage distribution map partially illuminated the genetic diversity measures, revealing general regional patterns

of lower diversity at higher latitudes and higher diversity at more central locations (Table 2) where source contributions are more diverse (Prodocimi et al. 2011). The split composition of mtDNA lineages at the Barbados FG was particularly striking (Figure 2) and was consistent with its high diversity measures in comparison to other FGs (Table 2). Exceptions to this pattern may be attributed in part to higher diversity observed in areas at the confluence of major currents (Bass et al. 2006), and effects of sample size, population history, and other factors.

Supporting the third line of evidence for juvenile natal homing, Atlantic FGs overall were indeed distinct with some pairwise exceptions, as reported previously (Naro-Maciel et al. 2007; Monzon-Arguello et al. 2010; Proietti et al. 2012). While in broad agreement, the exact tests uncovered more significant pairwise differences than the F_{ST} -based tests and were generally more consistent with the published literature (Table 4). The pairwise comparisons whose results were in agreement across tests can be considered the most robust; for example, the islands of The Bahamas and Barbados were unambiguously differentiated from all other juvenile FGs. Florida and North Carolina were as well, except possibly from each other (Table 4). Results that were not as consistent should be interpreted with caution, keeping confounding effects of population history and sample size issues in mind, for example, in the case of Cape Verde. Along the SWA coast, mixing was evident and was consistent with tag returns showing diverse northwards and southwards movements there (Marcovaldi et al. 2000; Gallo et al. 2006). However the Ubatuba and Almofala FGs continued to be distinct from each other and from many other Brazil FGs (Naro-Maciel et al. 2007; Proietti et al. 2012) despite the addition of the new FG data in between. Ubatuba did tend to group with other southern Arvoredo and Argentina FGs, and Almofala with RA and possibly FN (Table 4). As might be expected given its central location on the Brazilian coast, the BA FG was less distinct than others in pairwise comparisons (Table 4). Mixing at ES indicates that turtles severely afflicted with fibropapillomatosis disease at this site are indeed in contact with those from other areas, potentially spreading the affliction. However, no record of fibropapillomatosis has been found at FN and RA, which were not significantly differentiated from several coastal areas or from each other in all tests. The disease may be absent at FN and RA due to mortality of afflicted turtles, increased adult resistance to the disease, demographic independence, environmental characteristics or, alternately, the disease may be expected to appear in due time even at these remote World Heritage Sites.

Temporal Variation

The previous study of RA provided a useful test of temporal variation. The RA sample from 2000 ($n = 23$; Bjorndal et al. 2006) was not significantly differentiated from our RA samples collected between 2004 and 2006, indicating temporal constancy. Temporal constancy was found overall both between seasons and years at all Brazil study sites sampled over two- to three-year periods (this study; Naro-Maciel

et al. 2007). This supports the use of MSA, which assumes temporal constancy. In contrast, haplotype frequencies in the Bahamas did vary significantly over a 10-year period, underscoring the importance of further temporal sampling (Bjorndal and Bolten 2008).

Courtship Areas and Male Philopatry

RA is the first courtship area to be characterized genetically in the Atlantic, providing an important baseline for comparison to future CAs. In their rare study of three Australian CAs, FitzSimmons et al. (1997a) found no significant differences in haplotype frequencies between males and females at each site, and similar levels of divergence among sites for both males and females, concluding that both sexes were philopatric to the CAs (FitzSimmons et al. 1997a). Similarly, at the RA courtship area we detected no significant differentiation between males (this study) and nesting females (Bjorndal et al. 2006), and mark-recapture data revealed repeated returns to RA over the years (Longo and Grossman 2010). However RA males were significantly different from the resident juveniles. We offer the hypothesis that mixing revealed by nuclear loci at RA (Roberts et al. 2004) most likely occurred during overlapping migrations with both sexes being philopatric.

Conservation Applications

The finding of more localized green turtle clusters is an emerging, if not ubiquitous, theme in the biology of these highly migratory animals (Bolker et al. 2007; Monzon-Arguello et al. 2010). Proximity of FGs or rookeries may simplify international and regional management. Indeed the SWA countries are already linked through the ASO network. However even within this area there are greatly differing levels of protection and threat. Particularly striking contrasts are evident between the remote World Heritage Sites of RA and FN, or the isolated Trindade naval base, and the highly urbanized ES site and increasingly developed BA site. Turtles moving between these sites on breeding or developmental migrations may face heightened threats outside of protected areas, underscoring the importance of understanding their population distribution. The connectivity within the SWA does indicate that special attention should be paid to sites with high prevalence of fibropapillomatosis disease, and efforts should be made to avoid the spread of this affliction to remote and healthy populations if possible. The study was useful for clarifying the population distribution of these sites, particularly with respect to Trindade. It also pointed to the importance of improving resolution to discern connections between Africa, Ascension, and the SWA to enable coordinated science-based management. The study highlights the importance of the cooperation already achieved by ASO countries and suggests possible extension of the network to include Africa and Ascension as is already being carried out through the South Atlantic Sea Turtle Network (http://oceanecology.org/?page_id=426). Further, an important next step in sea turtle conservation will be to incorporate FGs such as the study sites into RMU

definitions (Wallace et al. 2010), and our research provides data needed for these and other regional management initiatives. The work highlights the local and possibly distant impacts of conservation efforts in the region and provides data necessary for conservation and management of endangered green turtles in protected and threatened areas.

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Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>.

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