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Dispersal of green turtles from Africa's largest rookery assessed through genetic markers

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ABSTRACT: Marine turtles are highly migratory species that establish multiple connections among distant areas, through oceanic migration corridors. To improve the knowledge on the connectivity of Atlantic green turtles *Chelonia mydas*, we analysed the genetic composition and contribution to juvenile aggregations of one of the world's largest rookeries at Poilão Island, Guinea-Bissau. We amplified 856 bp mitochondrial DNA (mtDNA) control region sequences of this population (n = 171) containing the ~490 bp haplotypes used in previous studies. Haplotype CM-A8 was dominant (99.4%), but it divided in 2 variants when the whole 856 bp was considered: CM-A8.1 (98.8%) and CM-A8.3 (0.6%). We further identified the haplotype CM-A42.1 (0.6%), found previously only in juvenile foraging grounds at Argentina, Brazil and Equatorial Guinea. The Poilão breeding population was genetically different from all others in the Atlantic (F_{ST} range: 0.016–0.961, $p < 0.001$). An extensive 'many-to-many' mixed-stock analysis (MSA) including 14 nesting populations (1815 samples) and 17 foraging grounds (1686 samples) supported a strong contribution of Poilão to West Africa (51%) but also to the Southwest Atlantic (36%). These findings, in particular the strong connectivity within West Africa, where illegal harvesting is still common, should motivate conservation partnerships, so that population protection can be effectively extended through all life stages. Our study expands the knowledge on migration patterns and connectivity of green turtles in the Atlantic, evidences the importance of larger sample sizes and emphasizes the need to include more finely resolved markers in MSAs and more genetic sampling from West African foraging grounds to further resolve the connectivity puzzle for this species.

KEY WORDS: Connectivity · Dispersal · Green turtle · Migration · Mitochondrial DNA · mtDNA · Mixed-stock analysis · MSA · Population genetics · West Africa

INTRODUCTION

Many marine species undertake migratory movements among distant geographic areas and across distinct habitats, for feeding, reproduction or development. As a result, they may be subject to a diverse range of threats during their extensive movements. Sea birds (Catry et al. 2011), marine mammals (Rasmussen et al. 2007), large fish (Bonfil et al. 2005, Rooker et al. 2014) and sea turtles (Hays & Scott 2013) undertake such movements and are known to play important ecological roles. Understanding their

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dispersal patterns and the links they establish among different areas is critical to contextualize threats and inform effective management strategies (Rees et al. 2016).

Marine turtles are long-lived organisms and their life histories are marked by ontogenic habitat shifts and large-scale migrations (Bowen & Karl 2007). Green turtles *Chelonia mydas* L. associate with oceanic currents after hatching and undergo an oceanic pelagic stage, which is thought to last ca. 3–5 yr (Reich et al. 2007). After this period, often referred to as 'the lost years', as the whereabouts of the turtles at this phase are poorly known, they generally recruit to coastal habitats, which may change seasonally (Fukuoka et al. 2015), and shift into benthic foraging at a straight carapace length of 25–35 cm (Bolten 2003). These neritic zones are used as developmental habitats and turtles may spend several years foraging in the same area until reaching a size or maturity stage that triggers them to migrate to additional foraging areas (Patrício et al. 2011, 2014, Shimada et al. 2014). Upon reaching maturity, adults make periodic migrations between their neritic foraging areas and natal rookeries (Bowen & Karl 2007). This complex migratory behaviour creates multiple connections among distant coastal areas through oceanic migration corridors (Velez-Zuazo et al. 2008). Genetic studies have been critical in enlightening such connectivity (Encalada et al. 1996, Naro-Maciel et al. 2007, Prosdocimi et al. 2012).

Most studies have used sequences of the control region of mitochondrial DNA (mtDNA), a maternally inherited genetic marker (Bowen & Karl 2007). This marker shows generally high levels of genetic structuring among marine turtle nesting populations worldwide, supporting the natal homing hypothesis, in which the females of marine turtles return to the beaches where they were born to reproduce as a consequence of philopatry (Meylan et al. 1990). In contrast, foraging aggregations are usually mixed stocks composed of individuals from different rookeries (Bowen & Karl 2007). The high genetic structuring of nesting populations allows the use of mixed-stock analysis (MSA; Millar 1987), to estimate contributions of rookeries (stocks) to mixed foraging grounds (mixed stocks). A Bayesian MSA (Pella & Masuda 2001) has been widely applied, allowing the incorporation of informative priors, such as rookery size or geographic distance. Bolker et al. (2007) subsequently developed a 'many-to-many' (m2m) MSA, aiming to simultaneously answer the questions: (1) Where do the individuals from a given source population go? and (2) Where do individuals from a given mixed foraging ground originate from? Limitations of MSAs have been pointed out, however, in particular the assumption that all source populations and mixed aggregations have been adequately sampled (Proietti et al. 2012). The existence of orphan haplotypes at juvenile foraging grounds indicates that some stocks still lack genetic assessment or have not yet been adequately sampled; hence estimates should be interpreted cautiously and along with meaningful ecological data.

One controversial result of recent MSAs of the Atlantic green turtles is the suggested potential connectivity between Guinea-Bissau, West Africa, and the Southwest Atlantic. Although MSAs have supported this migration (Bolker et al. 2007, Monzón-Argüello et al. 2010, Naro-Maciel et al. 2012), the fact that the population at Poilão, Guinea-Bissau, was found to be fixed for the common South Atlantic haplotype (CMA-8; Encalada et al. 1996, Formia et al. 2006, Godley et al. 2010) has limited the interpretations of these results. Notably, the discovery of exclusive haplotypes at low frequency is highly dependent on sample size. This putative migration seems to involve movements greater than expected, according to the 'closest to home' hypothesis, where immature turtles tend to move to and settle in foraging grounds closest to their natal beach after recruiting to neritic habitats (Bolker et al. 2007). Additionally, studies using particle dispersal modelling with major oceanic currents did not support this connectivity (Godley et al. 2010, Putman & Naro-Maciel 2013). However, when Putman & Naro-Maciel (2013) estimated the origins of the green turtle Atlantic mixed stocks, tracking particles back through time, this crossing seemed feasible, albeit at low incidence. Lagrangian drifter data have further shown this route to be possible with particle drift (Monzón-Argüello et al. 2010, Proietti et al. 2012). Finally, a similarly large-scale migration of post-hatchling green turtles from Suriname to Cape Verde was supported using mtDNA (Monzón-Argüello et al. 2010).

With this is mind we investigate 2 questions: (1) Where do the post-hatchlings from Poilão disperse to? and (2) Do some of the juveniles found at Southwest Atlantic foraging grounds originate in Poilão? To answer these questions, we greatly increased the available sample to characterize the genetic composition of Poilão's nesting population, in an attempt to detect rare haplotypes. We then sought to improve our understanding of the migration patterns and connectivity among Atlantic green turtle populations by comparing our results with molecular data (n = 3501 sequences) from 14 nesting populations and 17 foraging grounds, resulting in the most extensive analysis thus far for this species in the Atlantic.

MATERIALS AND METHODS

Study site and sampling

Poilão Island (10° 52′ N, 15° 43′ W) is part of the João Vieira and Poilão Marine National Park (PNMJVP), in the Bijagós Archipelago, Guinea-Bissau. It hosts one of the major green turtle nesting populations worldwide (Catry et al. 2002, 2009). This population has been monitored yearly around the peak of the nesting season (August–September) since 2004. In 2013 and 2014, we collected skin samples from 171 nesting females. Samples were taken from the shoulder area using a 6 mm sterile biopsy punch as the females laid their eggs, and were stored in 96% ethanol at room temperature. All sampled individuals were identified with unique tags on both front flippers to avoid sample duplication. Furthermore, the loss of a metal tag leaves scar marks easily recognized within, so we were certain that no previously tagged individual was mistakenly identified as 'new'. Sampling protocols were approved by the research ethics committee of the University of Exeter and the government of the Republic of Guinea-Bissau.

Sequencing and haplotype assignment

We extracted DNA using the QIAGEN DNeasy blood and tissue kit, according to the manufacturer's instructions. A fragment of ~860 bp of the mtDNA control region was amplified using PCR with the primers LCM15382 (5′-GCT TAA CCC TAA AGC ATT GG-3′) and H950 (5′-TCT CGG ATT TAG GGG TTT-3′) (Abreu-Grobois et al. 2006), including the short region (~486 bp) historically surveyed for green turtle genetic studies (Encalada et al. 1996, Lahanas et al. 1998, Bjorndal et al. 2006, Formia et al. 2007). Amplifications were performed in a total volume of 25 µl, containing 2.5 µl of *Taq* buffer, 3 µl of dNTPs, 1 µl of $MgCl_2$, 0.5 µM of each primer at 10 µM, and 0.2 µl of *Taq* DNA polymerase. Cycling conditions were 94°C for 5 min, followed 35 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min with a final extension step at 72°C for 10 min. Desired PCR products were purified with a combined exonuclease I and shrimp alkaline phosphatase solution (ExoSAP®). The reaction was incubated for 15 min at 37°C, followed by 15 min incubation at 80°C to inactivate the 2 enzymes. Sequences of forward and reverse DNA strands were performed at Macrogen (Netherlands). Sequences were assembled and aligned manually using BioEdit 7.2.5 (Hall 1999). Unique haplotypes were identified using the basic local alignment search tool (BLAST) from the National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov), following the nomenclature of the Archie Carr Center for Sea Turtle Research (ACCSTR; https://accstr.ufl.edu/resources/mtdna-sequences/).

Population structure

To assess the genetic diversity of the nesting population at Poilão compared with the other Atlantic nesting populations, we truncated the mtDNA fragments to 490 bp length, the fragment historically explored and for which most genetic information of other locations is currently available. We used Arlequin 3.5.1.3 (Excoffier & Lischer 2010) to estimate the haplotype (h) and nucleotide (π) diversity of nesting populations, to estimate the genetic distances among population pairs (ϕ_{ST}) and to test the significance of differentiations with exact tests based on haplotype frequencies. A false discovery rate (FDR) correction (Narum 2006) was applied to calculate the most fitting threshold for the p-value significance considering the number of comparisons involved in the analysis and under an expected original threshold of $p < 0.05$. To contextualize our sampling location within the Atlantic region, the genetic distances were used to perform a principal coordinate analysis (PCoA) using the package GenAlEX 6.5.0.1 (Peakall & Smouse 2012). We tested the significance of the PCoA grouping with an analysis of molecular variance (AMOVA), using Arlequin 3.5.1.3 (Excoffier & Lischer 2010).

'Many-to-many' mixed-stock analysis

We generated a dataset of 14 nesting populations (n = 1815) and 17 foraging grounds (n = 1686) when including our new mtDNA data for Poilão and the previously existing data for Atlantic nesting populations and foraging grounds (see Fig. 1 & Table 1 for sites included in this study and literature sources). We used only sequences generated by this study to characterize the genetic composition of Poilão in order to avoid potential pseudoreplication with datasets obtained in previous years. Relative contributions to foraging areas from nesting populations (mixed stock-centric

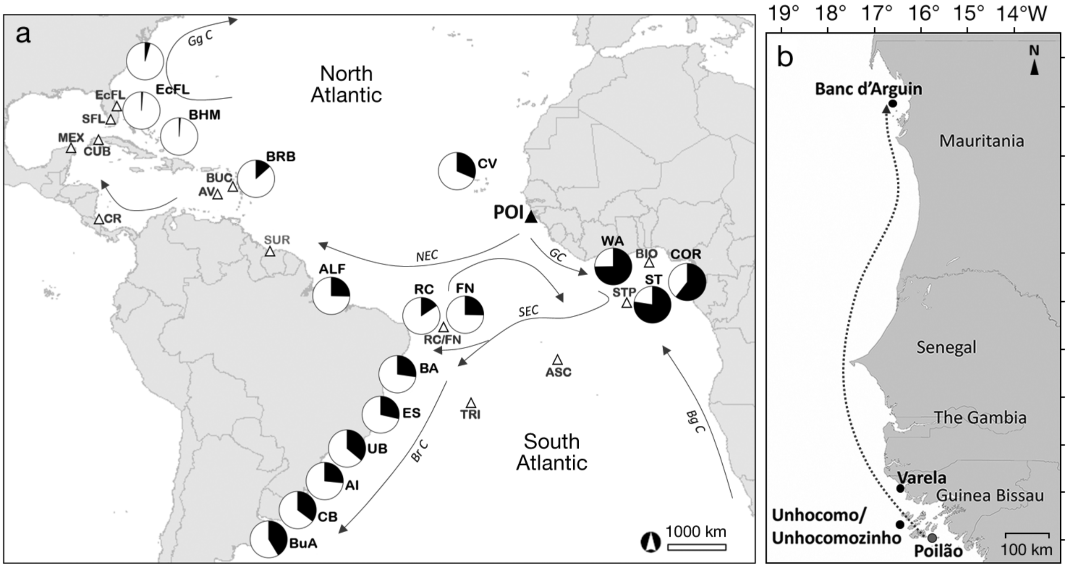

Fig. 1. (a) Atlantic green turtle *Chelonia mydas* nesting populations (Δ; n = 14) and foraging grounds (n = 17) used in the many-to-many mixed-stock analysis (m2m MSA), and results of foraging ground-centric MSA (pie charts: proportion of each foraging site that originates from the study population in **bold**; see Table 1 for abbrevations and data sources. Arrows indicate general direction of major currents. GfC: Gulf Current, NEC: North Equatorial Current, SEC: South Equatorial Current, BrC: Brazil Current, GC: Guinea Current, BgC: Benguela Current. (b) Region map with study site, Poilão, and 3 juvenile foraging grounds likely to partly originate at Poilão, but to date are genetically uncharacterized: Unhocomo/Unhocomozinho and Varela (Guinea-Bissau) and Banc d'Arguin (Mauritania). Dashed arrow illustrates the direction of 4 green turtle females tracked from Poilão to Banc d'Arguin (Godley et al. 2010). (Maps created using www.seaturtle.org/maptool)

approach), and probable use of foraging grounds from nesting populations (source-centric approach) were estimated with m2m MSA, using the R package mixstock (Bolker et al. 2007) and WinBUGS (Lunn et al. 2000). We conducted the MSA including the number of nesting females in each population (Seminoff et al. 2015) as a weighting factor (Prosdocimi et al. 2012). We used the Gelman–Rubin diagnostic to assess convergence of the chains to the posterior distribution, assuming that there was no evidence of non-convergence at values <1.2 (Pella & Masuda 2001). As it is reasonable to assume that other African juvenile aggregations remain to be identified, we simulated a juvenile foraging ground fixed for haplotype CM-A8 (similar to Naro-Maciel et al. 2012), with a sample size equal to the mean of the foraging grounds sample sizes (n = 99), and added this sample to the dataset to conduct another m2m MSA.

RESULTS

Genetic composition of Poilão nesting population

Genetic variability of the Poilão nesting population was the lowest of all Atlantic populations (mean ± SD: $h = 0.012 \pm 0.011$, $\pi = 0.0001 \pm 0.0003$; Table 2). The haplotype CM-A8 was dominant, as suggested by previous studies (Formia et al. 2006). However, the use of longer sequences (856 bp sequences) distinguished 2 variants of this haplotype: CM-A8.1 (98.8%) and CM-A8.3 (0.6%). We also identified the haplotype CM-A42.1 (0.6%), a previously orphan haplotype found to date only in juveniles from West Africa and South American foraging aggregations (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m569p215_supp.pdf for haplotype frequencies of nesting populations). Because this is a rare haplotype and not previously detected in the population, we performed 2 independent PCRs, and sequenced the amplified fragment on 2 independent occasions, to confirm that this result was not a product of genotyping error.

Population structure

The nesting population at Poilão was significantly different from all other Atlantic green turtle rookeries (Table S2 in the Supplement). All other nesting populations were distinct from each other, except when comparing Ascension Island with Bioko Island, Aves with Suriname, and Aves with Buck Island. The comparisons between Suriname and Buck Island,

Table 1. Nesting populations (n = 14) and foraging grounds (n = 17) for Atlantic green turtles *Chelonia mydas* included in a many-to-many mixed-stock analysis, using the control region of mtDNA as a marker (490 bp)

Site name	Abbreviation	Reference
Nesting populations		
East central Florida	EcFL	Shamblin et al. (2015a)
South Florida	SFL	Shamblin et al. (2015a)
Southwest Cuba	CUB	Ruiz-Urquiola et al. (2010)
Quintana Roo, Mexico	MEX	Encalada et al. (1996)
Tortuguero, Costa Rica	CR	Encalada et al. (1996), Bjorndal et al. (2005)
Matapica/Galibi, Suriname	SUR	Encalada et al. (1996), Shamblin et al. (2012)
Buck Island	BUC	Shamblin et al. (2012)
Aves Island	AV	Lahanas et al. (1998, 1994), Shamblin et al. (2012)
Rocas/Fernando Noronha	RC/FN	Encalada et al. (1996), Bjorndal et al. (2006)
Trindade Island	TRI	Bjorndal et al. (2006)
Ascension Island	ASC	Encalada et al. (1996), Formia et al. (2007)
Poilão, Guinea-Bissau	POI	This study
Bioko Island, Eq. Guinea	BIO	Formia et al. (2006)
Sao Tome and Principe	STP	Formia et al. (2006)
Foraging grounds		
North Carolina, USA	NC	Bass et al. (2006)
East central Florida, USA	EcFL	Bagley (2003), Bass & Witzell (2000)
Bahamas	BHM	Lahanas et al. (1998)
Barbados	BRB	Luke et al. (2004)
Almofala, Brazil	ALF	Naro-Maciel et al. (2007)
Rocas Atoll, Brazil	RC	Naro-Maciel et al. (2012)
Fernando de Noronha, Brazil	FN	Naro-Maciel et al. (2012)
Bahia, Brazil	BA	Naro-Maciel et al. (2012)
Espirito Santo, Brazil	ES	Naro-Maciel et al. (2012)
Ubatuba, Brazil	UB	Naro-Maciel et al. (2007)
Arvoredo Island, Brazil	AI	Proietti et al. (2012)
Cassino Beach, Brazil	CB	Proietti et al. (2012)
Buenos Aires, Argentina	BuA	Prosdocimi et al. (2012)
Cape Verde	CV	Monzón-Argüello et al. (2010)
Corisco Bay, Equatorial Guinea	COR	Formia et al. (2006)
'West Africa': Liberia to Benin	WA	Formia et al. (2006)
Sao Tome, Sao Tome and Principe	ST	Formia et al. (2006)

and between Sao Tome and Principe and Bioko, became non-significant after FDR correction. Populations pairs where genetic differentiation was not detected were kept as discrete sources for the m2m MSA, based on their divergence in population size and geographic position (Monzón-Argüello et al. 2010, Putman & Naro-maciel 2013). The PCoA separated rookeries by region and evidenced 3 major groups: South Atlantic, Southeast Caribbean and Northwest Caribbean (Fig. 2), each group defined by a major haplotype(s): CM-A8, CM-A5 and CM-A3/A1, respectively. An accumulated 87.6% of the genetic variability was explained by the 2 principal coordinates of the PCoA. Although located in the North

Table 2. Haplotypic and genetic diversity (means ± SD) of Atlantic green turtle *Chelonia mydas* nesting populations (n = 14) included in a many-to-many mixed-stock analysis (Bolker et al. 2007), using the control region of mtDNA as a marker (490 bp). Number of females refers to total number of reproductive females in each population (Seminoff et al. 2015). The present study population is in **bold**. Site abbreviations as in Table 1

Nesting population	Sample size	No. of females	No. of haplotypes	Haplotypic diversity (h)	Nucleotide diversity (π)
EcFL	311	4490	9	0.512 ± 0.020	0.0016 ± 0.0013
SFL	174	3302	10	0.444 ± 0.043	0.0022 ± 0.0016
CUB	26	2226	7	0.648 ± 0.089	0.0053 ± 0.0033
MEX	20	18257	7	0.816 ± 0.058	0.0051 ± 0.0032
CR	433	131751	5	0.163 ± 0.023	0.0033 ± 0.0022
SUR	46	13067	4	0.132 ± 0.053	0.0013 ± 0.0011
BUC	61	63	2	0.153 ± 0.065	0.0030 ± 0.0020
AV	55	2833	2	0.140 ± 0.055	0.0029 ± 0.0020
RC/FN	69	345	7	0.463 ± 0.071	0.0026 ± 0.0018
TRI	99	2016	7	0.505 ± 0.052	0.0012 ± 0.0011
ASC	245	1417	13	0.303 ± 0.038	0.0008 ± 0.0008
POI	**171**	**29016**	**2**	**0.012 ± 0.011**	**0.0001 ± 0.0003**
BIO	50	850	2	0.184 ± 0.068	0.0004 ± 0.0006
STP	26	376	7	0.569 ± 0.110	0.0026 ± 0.0019

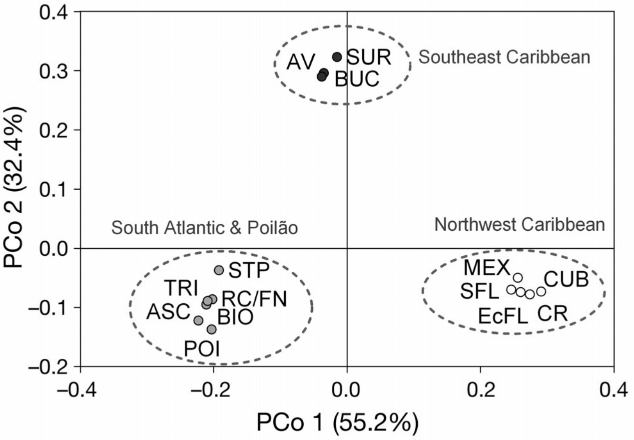

Fig. 2. Principal coordinate analysis (PCoA) of 14 Atlantic green turtle *Chelonia mydas* populations using ϕ_{ST} and considering the 490 bp mtDNA fragment. Rookeries were grouped in 3 clusters: the South Atlantic & Poilão (light grey), the Southeast Caribbean (dark grey), and the Northwest Caribbean (open). Percentage of variability explained by each coordinate is shown in brackets. See Table 1 for site abbreviations

Atlantic, Poilão clustered within the South Atlantic group. Using this *a priori* grouping in the AMOVA, highly significant structure was observed among the 3 groups (F_{ST} = 0.691, p < 0.001), with 55.9% of the variation found among groups.

'Many-to-many' mixed-stock analysis

The source-centric m2m MSA indicated that most of Poilão's hatchlings recruit to African foraging grounds (51.4%), but 36.2% would reach juvenile aggregations in the Southwest Atlantic and 8.6% reached North Atlantic aggregations (Fig. 3). A small proportion of the Poilão rookery was attributed to an 'unknown' foraging area (3.7%). The foraging ground-centric m2m MSA estimated that at Sao Tome, Corisco Bay and 'West Africa' (Liberia to Benin) foraging grounds, over 60% of the juveniles originate at Poilão, as do 31% of the green turtles foraging at Cape Verde (Fig. 1a). Notably, at the Southwest Atlantic foraging aggregations, proportions ranging from 16–41% were attributed to Poilão (Fig. 1b). Adding the simulated West African foraging ground did not change contributions at a regional scale, but the relative contributions to the Gulf of Guinea were significantly lower (8–14% lower; Fig. S1 in the Supplement), to accommodate a large contribution to this putative aggregation. Because CM-A42 is a rare haplotype and therefore difficult to detect when sampling a population, we decided to run 2 additional MSAs using simulated datasets, each of these including haplotype CM-A42 in 1 of the other 2 major green turtle rookeries in the Atlantic (i.e. Costa Rica and Ascension Island), and observed no significant changes (Fig. S1 in the Supplement).

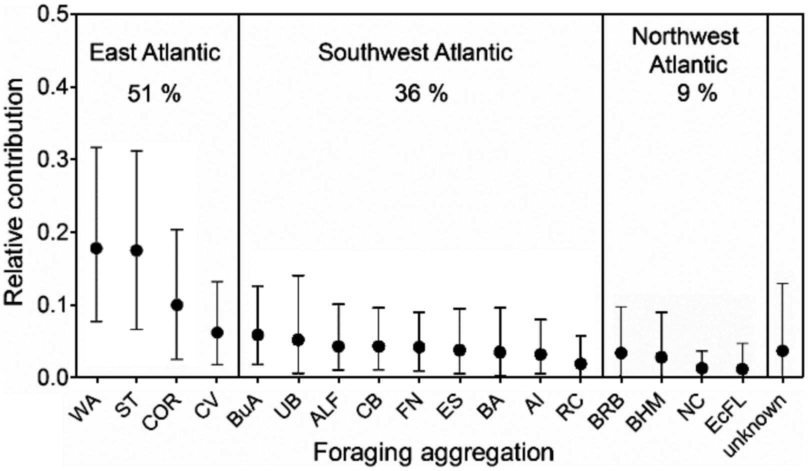

Fig. 3. Mean relative contribution of the Poilão nesting population of Atlantic green turtles *Chelonia mydas* to 17 foraging grounds, estimated by a many-to-many mixed-stock analysis (Bolker et al. 2007). Error bars show 95% CIs. See Table 1 for site abbreviations. Dashed lines separate geographic regions

DISCUSSION

Green turtle connectivity in the Atlantic

One of the principal techniques that can offer insight into the migratory connectivity of species with complex life cycles is genetics. The robustness of subsequent inferences, however, are highly dependent on the amount of information available, including the number of populations and foraging grounds analysed, and the strength of the signal, including sample sizes at each site and length of the genetic sequence and number of genetic markers analysed. Here we substantially increased the sampling effort at one of the largest Atlantic green turtle rookeries, in Poilão, Guinea-Bissau, in order to resolve the uncertainties surrounding the connectivity between this nesting population and distant juvenile aggregations. We successfully found the origin of a previously orphan haplotype, present in West Africa but also in South American foraging grounds, giving strength to the hypothesis of east-to-west connectivity.

Post-hatchling dispersal to east and west

The contributions estimated by the m2m MSA confirm a strong connectivity within West Africa, as previously hypothesized (Godley et al. 2010), particularly with foraging grounds in the Gulf of Guinea (i.e. 'Sao Tome', 'West Africa' and 'Corisco'). This dispersal was also predicted under an ocean circulation model and through passive drifting associated with the Guinea current (Putman & Naro-Maciel 2013). Due to the large size of the nesting population at Poilão, it is likely, however, that significant proportions of other African juvenile aggregations originate there. In Guinea-Bissau, there are at least 2 known aggregations of immature green turtles: (1) at Unhocomo and Unhocomozinho Islands, in the Bijagós Archipelago, ca. 100 km NE from Poilão Island, and (2) at Varela beach, ca. 200 km NE from Poilão, that have not been genetically described. The same is true for a foraging ground in Mauritania, mentioned in Godley et al. (2010), and in Congo. We have shown that the estimated proportions of post-hatchlings distributed among West African foraging grounds depend on the inclusion of new juvenile aggregations. To fully understand the connectivity of the large nesting population at Poilão, it is essential that investigation into identifying and genetically characterizing these aggregations is undertaken. The MSA also suggests the existence of a transatlantic developmental migration for the green turtle, from east to west, potentially associated with the Equatorial currents, and continuing south, reaching foraging grounds in the south of Brazil and in Argentina.

Studies using estimations of passive drift with major oceanic currents to predict the movements of post-hatchlings have suggested that dispersal from Guinea-Bissau to Southwest Atlantic is unlikely (Godley et al. 2010, Putman & Naro-Maciel 2013). However, marine turtle hatchlings are capable of oriented swimming significantly impacting trajectories (Putman et al. 2012a, 2012b, Scott et al. 2012), and able to swim against currents (Booth 2014). Indeed, recent research has shown that drifter tracks can diverge substantially from those of young turtles (Putman & Mansfield 2015), and it is likely that this process is contributing to observed divergence between genetic- and drift-based predictions (Naro-Maciel et al. in press). Because CM-A42 is a rare haplotype and therefore difficult to detect, we ran additional MSAs using simulated datasets, including this haplotype in each of the 2 other major green turtle rookeries in the Atlantic (i.e. Costa Rica and Ascension Island), and observed no significant changes (Fig. S1 in the Supplementf).

Expanded sample size and geographic coverage

Formia et al. (2006) assessed the genetic composition of Poilão nesting females (n = 51) and found it was fixed for the South Atlantic dominant mtDNA haplotype CM-A8. By extending this previous sample size, we were able to detect a rare haplotype, CM-A42, which to date had only been reported from juvenile green turtles foraging in South America, and in West Africa. This enabled the differentiation of Poilão from other Atlantic rookeries, agreeing with the high philopatry, characteristic of the green turtle, and the fine-scale differentiation existent in other places. Increasing sample size has previously been shown to improve the statistical power of the detection of structure among populations, through the finding of rare haplotypes (Formia et al. 2007).

The existence of non-significant comparisons among certain population pairs could result from (1) recent isolation, such that haplotype frequencies did not have time to differentiate, or (2) current gene flow, mediated by incidental deviations from natal homing. Lack of differentiation between Bioko and Ascension Island has been attributed to recent colonization of the former (Formia et al. 2006). Likewise, Aves and Buck Island may be more recent than the more diverse population in Suriname. Alternatively, the proximity between Aves and Buck Island (<300 km), and between Bioko and Sao Tome (<400 km), may be more likely to result in occasional migrants preventing substantial differentiation at an evolutionary timescale (Formia et al. 2006).

Our study further expands the geographic coverage of previous MSAs of the green turtle in the Atlantic, incorporating 14 nesting populations and 17 foraging grounds in our dataset. In particular, the inclusion of African foraging grounds (i.e. Corisco Bay, Sao Tome and 'West Africa') improved the estimates for the distribution of hatchlings from Poilão, significantly reducing the estimate of the putative 'unknown' foraging site (here, 3.7%) compared to a recent MSA (14.3% in Putman & Naro-Maciel 2013), as well as substantially reducing the confidence intervals. In a previous m2m MSA, a high contribution of Ascension Island to Corisco Bay was estimated (ca. 40%; Bolker et al. 2007). Here, that contribution drops to 9.2%, and we predict a much stronger connectivity between Poilão and Corisco. By including more foraging grounds in our analyses, we show that the Ascension rookery contributes primarily to juvenile aggregations along the Southwest Atlantic (71.6%), also seen in Putman & Naro-Maciel (2013). Analogously, the foraging ground-centric MSA in Bolker et al. (2007) attributes most of the Corisco Bay foraging ground to Ascension Island (>70%), while we estimate that 60.5% of the aggregation originates at Poilão, and only 27.7% would come from Ascension. Additionally, the contributions of Aves Island and NE Brazil to Corisco Bay estimated before (ca. 15% each; Bolker et al. 2007) were considerably lower in our study (2.7% and 4.8%, respectively), and these populations also seem to contribute more to the Southwest Atlantic. See Tables S3 & S4 in the Supplement for m2m MSA summary results.

Limitations of MSA and future directions

Although increasing the available sample size at Poilão and expanding the dataset for Atlantic green turtles has improved MSA estimates, this analysis is based on a single marker and on a short fragment of the mtDNA. To further unveil the green turtle connectivity puzzle in the Atlantic (and elsewhere), the strength of the genetic signal can be enhanced, at a lesser cost than substantially increasing sample sizes. Data from the longer mtDNA sequences should be obtained from existing samples and made available, to be incorporated in MSAs. Additionally, a new marker consisting of 4 AT short tandem repeats (STRs) in the 3′ end of the mtDNA, the mtSTR, had been shown to add information on the genetic variability within unique mtDNA haplotype classes and to contribute to improve the knowledge on population connectivity and evolutionary relationships (Tikochinski et al. 2012, Shamblin et al. 2015b). Recent research using nuclear markers found significant structure among sea turtle rookeries, supportive of male philopatry (Carreras et al. 2011, Naro-Maciel et al. 2012, 2014, Roden et al. 2013). Finally, new genomic approaches have the potential to greatly increase signal resolution and detect fine-scale population structure (Funk et al. 2012, Milano et al. 2014, Benestan et al. 2015).

Some of the above information is now becoming available at local scales. Hopefully, future collaborations among research groups at wider scales will lead to significant advances in our understanding of the dispersal and distribution of marine turtles.

Adult linkage

Godley et al. (2010) recorded the trajectories of 8 post-nesting females from Poilão using satellite transmitters, finding that they foraged either locally, at the Bijagós Archipelago (n = 4), or regionally (n = 4), at the Banc d'Arguin National Park, Mauritania (>1000 km distant). This aspect of investigation would clearly benefit from enhanced sampling effort, preferably across multiple seasons, at different points of the season and across a range of size classes, to avoid inter-annual (Witt et al. 2011), seasonal (Rees et al. 2010) and phenotypic (Hawkes et al. 2006) biases in dispersal. Future satellite tracking should be conducted in tandem with stable isotope analysis to facilitate the posterior assignment of turtles to these areas, facilitating the analyses of larger sample sizes, more relevant for population studies (Zbinden et al. 2011).

If nesting females from Poilão are limited to the East Atlantic, it does not necessarily contradict our suggestion of transatlantic dispersal as post-hatchlings. Post-hatchling turtles forage during their developmental migration (Reich et al. 2007), which allows them to travel much longer distances than adults that typically fast during their reproductive migrations (Hays & Scott 2013, Scott et al. 2014). According to Scott et al. (2014), if the developmental foraging area is so far as to be too costly to be repeatable during the cyclic reproductive migrations, adults may forage locally, as observed at the Bijagós, instead of returning to the sites experienced when younger. This mechanism reduces the consumption of reproductive energy utilized, potentially increasing fecundity; however, it is dependent on the availability of foraging areas.

Conservation implications

In this study, we show the importance of the Poilão rookery for the recruitment of juvenile green turtles in West Africa, and also that the link with the Southwest Atlantic is very likely. In Guinea-Bissau, despite marine turtles being fully protected by the national fisheries law, illegal take continues to occur without much law-enforcement effort (Catry et al. 2009), particularly at the Bijagós Archipelago, where turtles are frequently harvested at the nesting beaches, mostly for local consumption (Catry et al. 2009). The nesting population at Poilão is one exception, thanks to the Bijagós traditional 'law' (reinforced by state authorities), restricting access to the island on very rare ceremonies of social and religious significance (Catry et al. 2009). Off Guinea-Bissau and along the coast of West Africa, however, vast artisanal fleets and many industrial fishing fleets operate, using trawlers without turtle-excluder devices (Zeeberg et al. 2006, Catry et al. 2009), and longlining (Moore et al. 2010). Unfortunately, there is a scarcity of quantitative data in the region, either on bycatch or on targeted harvesting of marine turtles, particularly from artisanal fisheries (Moore et al. 2010). The foraging grounds in the Southwest Atlantic to which Poilão seems to contribute to, on the other hand, are mostly protected from illegal harvesting (Marcovaldi & dei Marcovaldi 1999), although bycatch may be a problem (Wallace et al. 2010). Despite the existing threats, major green turtle populations are recovering globally following decades of conservation efforts (Broderick et al. 2006, Catry et al. 2009, Bourjea et al. 2015). It may be that the long-term enhanced protection in South America and the efforts in Poilão itself are the principal factors involved in the recovery of this population.

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