

Green turtle (*Chelonia mydas*) mixed stocks in the southwestern Atlantic, as revealed by mtDNA haplotypes and drifter trajectories.

Maíra Carneiro Proietti¹; Júlia Wiener Reisser¹; Paul Gerhard Kinas²; Rodrigo Kerr³;
Danielle Monteiro⁴; Luis Fernando Marins⁵ and Eduardo Resende Secchi¹

¹*Laboratório de Tartarugas e Mamíferos Marinhos, Instituto de Oceanografia, Universidade Federal do Rio Grande, Avenida Itália km8, CEP 96201-900, Rio Grande, RS, Brazil; mairaproietti@gmail.com, jroceano@hotmail.com, edu.secchi@furg.br*

²*Laboratório de Estatística Ambiental, Instituto de Matemática, Estatística e Física, Universidade Federal do Rio Grande, Avenida Itália km8, CEP 96201-900, Rio Grande, RS, Brazil; paulkinas@furg.br*

³*Laboratório de Estudos dos Oceanos e Clima, Instituto de Oceanografia, Universidade Federal do Rio Grande, Avenida Itália km8, CEP 96201-900, Rio Grande, RS, Brazil; rodrigokerr@furg.br*

⁴*Núcleo de Educação e Monitoramento Ambiental (NEMA), Rua Maria Araújo 450, CEP 96207-480, Rio Grande, RS, Brazil; danismonteiro@yahoo.com.br*

⁵*Laboratório de Biologia Molecular, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Avenida Itália km8, CEP 96201-900, Rio Grande, RS, Brazil; dqmluf@furg.br*

Keywords: green turtle, foraging grounds, Southwestern Atlantic, mtDNA, mixed stock analysis, dispersal patterns

Short running title: Green turtle mixed stocks in Southern Brazil.

Abstract

Genetic analyses have the potential to elucidate many aspects of juvenile green turtle (*Chelonia mydas*) biology and ecology, such as foraging ground composition, hatchling dispersal and migrations. To evaluate genetic structure and assess natal origins of mixed stocks in Southern Brazil, we analyzed mitochondrial DNA control region sequences from Arvoredo Island (n = 115) and Cassino Beach (n = 101), comparing them to other mixed stocks and examining their composition in terms of Atlantic Ocean stocks (nesting areas). In order to compare natal origin estimates (obtained through Bayesian Mixed Stock Analysis) with oceanographic data and develop novel informative priors for this analysis, surface drifter trajectories in the Atlantic Ocean were analyzed. Each study area presented twelve haplotypes, of which ten were shared at extremely similar frequencies. Haplotypes CM-A8 and CM-A5 were most frequent, representing respectively around 60% and 20% of samples from both areas, and remaining haplotypes were present in less than 5% of samples. Genetic structuring was not observed between the study areas. Arvoredo Island and Cassino Beach also did not present structuring in relation to Ubatuba and Rocas/Noronha, in the southwestern Atlantic, but were structured when compared to farther feeding areas in Brazil, the Caribbean, and North America. Analysis of drifter trajectories revealed that drifters from Ascension and Trindade Islands are dominant at the eastern coast of Brazil. Informative priors developed for Mixed Stock Analysis did not greatly alter stock estimates; we do, however, consider them to be ecologically more realistic. According to the Bayesian mixed stock analyses applied here, Ascension, Aves and Trindade Islands, as well as Gulf of Guinea, were the main contributors to the Southern Brazil mixed stock. This analysis has important implications for the conservation of this species, since impacts on mixed stocks along

the coast may affect some reproductive stocks which are frequently thousands of kilometers away.

Introduction

The globally-distributed and endangered green turtle (*Chelonia mydas*) occupies various ecological niches throughout its life cycle (Meylan & Meylan 1999, Bolten 2003, Godley 2003). A general life pattern encompasses a juvenile oceanic phase, in which it is believed that young turtles drift with ocean currents, a subsequent neritic phase, when animals reach a certain size and recruit to coastal foraging grounds, and large-scale migrations between foraging and breeding areas upon sexual maturity (Bolten 2003). Movements between foraging grounds, often long-range, are also observed (Godley et al. 2003, Reisser et al. 2008). The complexity of this life cycle and the large geographical and temporal distances involved make direct studies of these animals difficult. Indirect approaches through molecular analyses, preferably using more than one marker, can help elucidate many aspects of their biology and behavior (Bowen 1995, Bowen & Karl 1997, Bowen & Karl 2007, Avise 2007), such as paternity, mating systems, population structure, inter-rookery gene flow, phylogeography, systematic, natal origins and homing (Avise 2007).

Natal homing, in which female green turtles return to their birth site to reproduce, was first hypothesized by Carr (1967), based on the observation that female green turtles are phylopatriotic, that is, they return to nesting sites (rookeries) at varying degrees of precision throughout subsequent nesting cycles (Carr 1967, Miller 1997, Formia et al. 2007, Lee et al. 2007). Despite difficult to test, this hypothesis has been revealed plausible through genetic studies, which have demonstrated that mtDNA structuring occurs between rookeries, but overlap

in foraging areas (Allard et al. 2004, Bass et al. 2006, Bowen & Karl 2007). Based on the assumption that such structuring exists, a Bayesian approach known as Mixed Stock Analysis (MSA) has been increasingly applied for determining contributions of genetically structured rookeries (stocks) to mixed sea turtle foraging aggregations (mixed stocks), employing differences in relative frequency of genetic characters (especially mtDNA) between rookeries to link feeding populations to their sources (Pella & Masuda 2001). This analysis frequently presents high standard deviations and is based on the assumption that all sources have been adequately sampled. This is often not the case, and many areas still present insufficient or even lack genetic characterization (Avise 2007). Despite these potential biases, MSA can be useful for inferences on green turtle origins in foraging habitats, as long as not over interpreted (Bowen & Karl 2007).

The relevance of identifying natal origins of mixed stocks for conservation lies in the fact that rookeries, despite being generally independent reproductively, are linked at the non-nesting phases of the female green turtle life cycle (Avise 2007). Therefore, impacts at foraging grounds and migratory routes may affect many breeding stocks at different levels. Understanding these origins, as well as determining possible migratory routes, is crucial for the elaboration of management and conservation plans (Moritz 1994, Avise 2007, Bowen & Karl 2007). Nevertheless, caution is needed when using MSA estimates to understand stock contributions to feeding grounds, and when possible, should be compared and associated with other data. The Bayesian approach to MSA allows the incorporation of relevant information available prior to the study at hand along with the sample data, and ecological data such as rookery population size and distance from source to mixture are commonly employed based on the assumption that foraging ground composition may be related to these factors. Hatchlings are considered by most authors as

“pelagic”, dispersing almost passively with ocean currents until reaching a certain size (Bolten 2003). Therefore, oceanographic data (in this case, surface drifter trajectories) can be viewed as an indicative of early life stage dispersal routes, and have potential to better improve informative MSA when compared to data such as distances between natal and feeding sites.,

Examples of green turtle MSAs employing mtDNA data from foraging areas in the Atlantic and Pacific Oceans can be seen in Bass et al. (2006), Bolker et al. (2007), Bjorndal & Bolten (2008), and Dutton et al. (2008). There are four green turtle rookeries in the central and western South Atlantic, listed in decreasing number of nesting females: Ascension Island, Trindade Island, Rocas Atoll and Fernando de Noronha. Origins of Brazilian juvenile green turtles have been described though mtDNA data using MSA for Rocas Atoll, Fernando de Noronha, Ubatuba and Almofala (Bjorndal et al. 2006, Naro-Maciel et al. 2007), with the consistent observation of prevailing contributions from Ascension Island, followed by smaller (yet significant) contributions from Trindade and almost null contribution from Rocas Atoll and Fernando de Noronha. Naro-Maciel et al. (2007) conclude that this pattern of contributions is shaped by the prevailing ocean currents flowing near rookeries.

The role of ocean currents in sea turtle dispersal and migration has been thoroughly discussed (see Luschi et al. 2003a). As cited previously, hatchlings are thought to rely on oceanic currents for dispersal until recruiting to their coastal foraging zone, and data obtained through satellite telemetry indicate that sea turtle movements and migrations are frequently shaped by ocean currents (Luschi et al. 1998, 2003a, 2003b; Craig et al. 2004). Parallels between MSA and ocean currents have been made for North Atlantic mixed stocks by Luke et al. (2004) and Bass et al. (2006), in which it is assumed that the compositions of these foraging aggregations depend on local major and minor current systems. For Brazil, it has been suggested that Ascension Island

hatchlings drift with major Equatorial currents towards South America, while hatchlings from other rookeries may drift away with prevailing currents (Naro-Maciel et al. 2007). The large-scale upper-layer (< 100 m) general circulation pattern which could influence sea turtle dispersal in the South Atlantic, affecting the composition of Brazilian foraging areas, is characterized by a dominating anticyclonic subtropical gyre. The westerly-bound Southern Equatorial Current bifurcates at the South American continental shelf at approximately 10° S originating the northern-bound North Brazil Current and the southern-bound Brazil Current (BC). The BC travels southward alongside the coast until reaching the Subtropical Convergence Zone (approximately 33-38° S), where it encounters the Falkland Current and separates from the coast forming the eastern-bound South Atlantic Current (SAC). When it approaches the African continent, part of the SAC flows to the Indian Ocean and part forms the northern-bound Benguela Current, which in turn will form the South Equatorial Current (SEC) and complete the gyre (Stramma & England 1999).

Considering that genetic studies potentially elucidate many aspects of green sea turtle biology and ecology, including foraging ground composition, hatchling dispersal and migrations, this study aimed at: a) determining genetic differences amongst the southern Brazil foraging areas Arvoredo Island (AI) and Cassino Beach (CB) and other studied mixed aggregations in the Atlantic; b) estimating contributions of different rookeries to the AI and CB mixed stocks; c) developing novel informative priors for Bayesian Mixed Stock Analysis and assessing their effect on the analysis; and d) indicating potential dispersal patterns of hatchlings from rookeries to the studied foraging areas.

Materials and Methods

Tissue sampling

Samples were collected at Arvoredo Island ($27^{\circ}51'S$ $48^{\circ}26'W$), in Santa Catarina state ($n = 66$), and Cassino Beach (from $31^{\circ}21'S$ $51^{\circ}02'W$ to $33^{\circ}44'S$ $53^{\circ}22'W$), Rio Grande do Sul state ($n = 101$). Arvoredo Island lies within the Arvoredo Marine Biological Reserve and presents rocky shores with diverse benthic organisms and frequent occurrences of green turtles, of which at least some present fidelity to the area (Reisser et al. 2008). Cassino Beach is an extensive and continuous sandy beach composed of predominantly unconsolidated substrate and few substantial hard substrates. Green turtles are frequently observed stranded at this beach (Bugoni et al. 2001), but its exact role in the life cycles of these animals is unknown. At Arvoredo Island, skin samples were collected using 5 mm disposable biopsy punches from the flippers of live individuals hand-captured through free and SCUBA dives in expeditions carried out from July 2005 to April 2008. At Cassino Beach, samples were collected using disposable scalpels from stranded live animals or carcasses found washed ashore during beach surveys conducted from January 2005 to May 2007. All samples were conserved in absolute ethanol and maintained at -20 °C until DNA extraction. Sea turtle sizes ranged from 33.5-83 cm (mean 49.2 cm) and 29-71.5 cm (mean 40.1 cm) curved carapace length (CCC), respectively for Arvoredo Island and Cassino Beach.

Laboratorial procedures

Tissue samples were macerated employing conical-shaped plastic grinders in a Tris-HCl lysis buffer containing Proteinase K, and submitted to digestion in an oven at 37 °C until complete digestion (from five to 24 hours). DNA was extracted through DNAExtraction Kits (Tissue – Bioamerica Inc.) or standard phenol:chlorophorm method with precipitation in absolute ethanol (adapted from Hillis et al. 1996). Approximately 500 bp-fragments of the mitochondrial DNA control region were amplified via polymerase chain reaction (PCR), using primers LTCM1

and HDCM1 (Allard et al. 1994) or LTCM2 and HDCM2 (longer versions of the prior primers, designed by Lahanas et al. 1994). PCR conditions for the first primers were as follows: initial denaturation of 1' at 94 °C; 35 cycles of 30'' at 94 °C, 1' at 50 °C and 1' at 72 °C; and a final 5' extension at 72°C. For the latter primers, applied conditions were: initial denaturation of 1' at 94 °C; 35 cycles of 45'' at 94 °C, 30'' at 55 °C and 45'' at 72 °C; and a final 3' extension at 72°C. Illustra GFX purification kits (GE Healthcare, U.S.A.) were employed for purification, and samples were sequenced in both directions using DYEnamic ET dye terminator kit in a MegaBACE 500 DNA sequencer (GE Healthcare, U.S.A.).

Data analysis

mtDNA sequences

Sequences were aligned using software Clustal X 1.83 (Thompson et al. 1997), and haplotypes (491 bp, according to previously-described haplotypes for *Chelonia mydas*) classified according to the Archie Carr Center for Sea Turtle Research online genetic bank (Florida University). Additional sequences for Arvoredo Island ($n = 49$, Proietti et al. 2009) were included in the analyses, totalizing 115 samples. Relationships among haplotypes were demonstrated through a statistical parsimony network, constructed using TCS 1.3 software (Clement et al. 2000). Exact tests of differentiation were conducted with Arlequin 3.11 (Excoffier et al. 2005) in order to verify differences between the study areas and other previously-described Atlantic foraging grounds, employing a Markov Chain Monte Carlo (MCMC) of 10000 steps with 1000 dememorizations ("burn-in"). This software was also used to calculate pairwise Φ -statistics for an Analysis of Molecular Variance (AMOVA), with 10000 permutations and using the Tamura-Nei model of nucleotide substitution. The Brazilian foraging grounds included in these analyses for comparative purposes were Ubatuba (SP), Almofala (CE) (Naro-Maciel et al. 2007), Rocas Atoll

(RN) and Fernando de Noronha (PE) (Bjorndal et al. 2006). The last two were grouped into one unit for all analyses due to geographic proximity (c.a. 150 km) and small sample sizes, as performed in Bjorndal et al. (2006), and will hereafter be referred to as Rocas/Noronha. Nicaragua (Bass et al. 1998), Barbados (Luke et al. 2004), Bahamas (Lahanas et al. 1998), Florida (Bass and Witzell 2000) and North Carolina (Bass et al. 2006), in the Caribbean and North Atlantic, were also considered for comparison. Genetic structure results were taken into consideration when defining certain aspects of analyses of surface drifter data and Mixed Stock Analyses (see details below).

Surface drifter trajectories and natal origins

In order to compare MSA results with surface current data and develop two novel informative priors for MSA analysis, surface drifter data available for the Atlantic and Mediterranean (5842 drifters, from February 1979 to January 2009), was downloaded from NOAA's Global Drifter Program (<http://www.aoml.noaa.gov/envids/gld>). We then evaluated the number of drifters that passed through the nesting areas considered in the natal origin analyses and reached a target area consisting in the eastern Brazilian coast, from the southernmost limit to the northeastern corner. The northern portion of the country was not included due to evidence of genetic structuring between this area and the East Brazil coast. Based on these data, the probability of a drifter which reached the target area being from a determined rookery was calculated in a Bayesian framework (see Annex 1).

The nesting areas considered as possible sources correspond to all rookeries in the Atlantic and Mediterranean with mtDNA description, as reported by Encalada et al. (1996), Kaska (2000), Bjorndal et al. (2005, 2006), Formia et al. (2006, 2007): (1) Trindade Island, (2) Rocas/Noronha (Brazil), (3) Ascension Island (United Kingdom), (4) Poilão (Guiné Bissau), (5)

Bioko Island (Equatorial Guinea), São Tomé and Príncipe (Democratic Republic of São Tomé and Príncipe), (6) Aves Island (Venezuela), (7) Matapica (Surinam), (8) Quintana Roo (Mexico), (9) Tortuguero (Costa Rica), (10) Florida (United States) and (11) Lara Bay (Cyprus). Bioko, São Tomé and Príncipe were grouped into one area due to proximity and lack of genetic differentiation, and hereafter will be referred to as Gulf of Guinea, following Bolker et al. (2007). $4^{\circ} \times 4^{\circ}$ (latitude and longitude) areas were delineated around all these considered rookeries, and in the case of non-insular rookeries, the area was designed in order to incorporate the largest possible oceanic area.

Probable natal origins were determined employing mtDNA data from the study areas and all rookeries with mtDNA description (see above), through Bayesian Mixed Stock Analysis (MSA) implemented with software Bayes (Pella & Masuda 2001). Arvoredo Island and Cassino Beach were grouped into one area due to geographic proximity and genetic similarity, and four MSAs were performed considering uninformative priors (MSA_1) and priors weighed according to: number of females/year of each source (MSA_2); probabilities calculated from surface drifter data (MSA_3); and a combination of the two previous informative priors (MSA_4). For a detailed description of priors, refer to Annex 1. Source populations considered as possible contributors to the study areas correspond to the same area used in surface drifter analysis. One MCMC was implemented for each rookery (totalizing 11 chains) in each analysis, with chain lengths varying from 10000 – 25000, according to the Gelman-Rubin convergence factor (which was maintained under 1.2, and in most cases presented values of approximately 1.0, indicating convergence) and one-half chain length discarded as “burn-in” steps (as described by Pella & Masuda 2001).

Results

Haplotype frequencies and genetic diversity

Each study area presented 12 previously-described haplotypes, of which ten were shared (CM-A5, CM-A6, CM-A8, CM-A9, CM-A10, CM-A23, CM-A24, CM-A32, CM-A42 and CM-A45) and four were not shared (CM-A3 and CM-A39, present only at AI, and CM-A25 and CM-A36, present only at CB) (Table 1). Both areas were characterized by a high predominance of haplotypes CM-A8 (61% for both areas) and CM-A5 (22% and 20% for AI and CB, respectively). All remaining haplotypes were present in frequencies lower than 5%. Rare haplotypes were observed, such as CM-A10, CM-A23 and CM-A24, encountered only at Ascension and Trindade islands; CM-A25 and CM-A32 only at Rocas Atoll and Ascension Island; CM-A39 and CM-A45 at Ascension Island; and CM-A42 in only two individuals at the Almofala foraging ground in northeast Brazil, with no observations in rookeries. The number of polymorphic sites defining these haplotypes was 19 for AI and CB, with a maximum of 12 variations distinguishing them (Figure 1).

Haplotype (h) and nucleotide (π) diversities of AI ($h = 0.5831 \pm 0.0451$; $\pi = 0.00246 \pm 0.00176$) and CB ($h = 0.5857 \pm 0.0501$ and $\pi = 0.00251 \pm 0.00178$), and the averaged diversities of all compared foraging aggregations ($h = 0.5410$; $\pi = 0.0045$), were similar, as shown in Table 2.

Genetic differentiation

Exact test of differentiation and AMOVA revealed an overall structuring among foraging areas ($p < 0.001$ for both analyses); however, genetic structuring was non-significant between AI and CB, with a slightly negative Φ_{ST} value ($\Phi_{ST} = -0.0066$, $p > 0.05$). Both analyses revealed that AI and CB are genetically different from most areas (Almofala, Nicaragua, Barbados, North

Carolina, Florida and Bahamas, $p < 0.05$), but showed no difference in relation to Ubatuba and Rocas/Noronha ($p > 0.05$), both located in the Southwestern Atlantic.

Surface drifter trajectories and natal origins

Drifters coming from Ascension and Trindade Islands were dominant at the target area, as clearly shown in Figure 2, which illustrates the trajectories of all surface drifters which passed through the eleven $4^\circ \times 4^\circ$ areas (all rookeries considered in the MSAs). Table 3 lists the total number of drifters which passed through each area (N), the number of these that reached the target area (Y), and the probability that drifters at the Brazilian coast are from each rookery (P). This table shows that only Ascension, Trindade, Rocas/Noronha and Gulf of Guinea supplied drifters to the target area. The first two areas presented posterior probabilities of around 40% of reaching the target, while Rocas/Noronha and Gulf of Guinea presented near 2% and slightly over 5%, respectively. Although Costa Rica and Guinea Bissau exhibited posterior probabilities of over 2%, they are not considered relevant due to the fact that this estimate is simply a result of the small number of drifters passing through the areas. The remaining rookeries presented probabilities lower than 1%.

All MSAs for Arvoredo Island and Cassino Beach indicated that the main contributors to the southern Brazil foraging areas were Ascension, Aves and Trindade Islands (Figure 3). Ascension Island consistently presented the largest contributions, ranging from 53.3 to 66.5% in the four performed MSAs, while Aves and Trindade Islands exhibited contributions that ranged from 21.6 to 22% and 7.6 to 17.7%, respectively. Remaining stocks presented low contributions in all MSAs (less than 1% in a general manner), with the exception of the Gulf of Guinea, with estimated contributions from 2.1 to 7.3%.

MSA₃ (which used surface drifter data as ecological information) slightly increased Ascension Island contribution estimates when compared to the uninformative MSA₁, while MSA₂ (prior weighing rookery population size) and MSA₄ (combination of both ecological priors) increased estimates in slightly over 12% for the former and 6% for the latter analysis. Contributions from Trindade Island increased to 17.7% in MSA₃, while MSA₂ decreased this contribution to slightly less than 8%. Gulf of Guinea's contributions in MSA₁ was relatively high (around 7%), but decreased to 5.2% in MSA₃, and when inserting the ecological variable rookery size (MSA₂) and the combination rookery size/surface drifters (MSA₄), contribution from this stock dropped to 2.1%. Of the largest contributors, Aves Island was the least variable throughout MSAs, varying less than 1%.

Discussion

Haplotype frequencies of Arvoredo Island and Cassino Beach were similar to other Atlantic rookeries and foraging aggregations: high CM-A8 frequency, consistent with the suggestion that this haplotype is the closest relative to an ancestral haplotype in the Atlantic basin, followed by a high occurrence of CM-A5, an extremely common haplotype encountered in Caribbean rookeries (Bjorndal et al. 2005, 2006; Formia et al. 2006, 2007; Naro-Maciel et al. 2007), and low frequencies of rarer haplotypes. Increasing sample size did not significantly change the proportion of haplotypes CM-A8 and CM-A5 or the diversity indexes found by Proietti et al. (2009) at Arvoredo Island. These authors analyzed 49 green turtle samples and encountered 64% haplotype CM-A8, 22% CM-A5, and h and π of 0.5570 ± 0.0697 and 0.0021 ± 0.0016 , respectively. The detection of rarer haplotypes, however, increased. High haplotype and low nucleotide diversity indexes for both study areas followed the general pattern found at other green turtle foraging grounds, due to the mixed characteristic of these areas and small variations

between haplotypes, respectively (Bass et al. 2006; Bjorndal et al. 2006; Naro-Maciel et al. 2007).

Arvoredo Island and Cassino Beach were extremely similar in terms of haplotype diversity and frequency, and were not significantly different from other foraging grounds in the southwestern Atlantic (Ubatuba and Rocas/Noronha). A significant structuring, however, seems to occur in relation to Almofala, a more distant feeding area located in northeast Brazil. This area presents larger frequencies of Caribbean haplotypes, explaining such differentiation, as the Caribbean region presents elevated mtDNA structuring within the Atlantic basin (Bass et al. 2006). Juvenile sea turtles may perform coastal migrations, sometimes seasonal, transiting between foraging areas according to different factors (such as variations in current intensity, water temperature, and food availability (Musick & Limpus 1996, Bass et al. 2006). Avens & Lohmann (2004) studied seasonal movements of green turtles in North Carolina, and reported that animals swam in opposite directions according to the season: northwards in the summer and southwards in the autumn. Green turtles tagged in Uruguay have been recaptured in Brazil and vice-versa, suggesting that some juveniles may perform seasonal movements, going to lower latitudes during colder periods and to higher latitudes during warmer seasons (Lopéz-Mendilaharsu et al. 2006). The possibility of inter-annual movements, or even longer intervals, cannot however be discarded. Souza & Robinson (2004) demonstrated through Langrangian measurements and analysis of Sea Surface Temperature images that the intrusion of cold waters transported by a coastal current is apparently a regular winter phenomenon occurring on the Brazilian shelf, at latitudes up to around 25° S. This intrusion was so consistent that these authors named it the “Brazilian Coastal Current”, and could favor coastal movements from Uruguay to Brazil during cold periods. The Brazil Current, on the other hand, could facilitate opposite

displacement patterns. Musick & Limpus (1996) speculated that juveniles at temperate zones perform these seasonal movements in order to seek warmer waters and avoid cold stunning. A four-year study performed at Arvoredo Island revealed moderate site fidelity of immature green turtles; however, one turtle tagged at the island was found six months later stranded on a beach at São Paulo state, over 600 km away (Reisser et al. 2008). For Cassino Beach, evidence of residency is not available, and it is possible that some animals are in fact from distant areas and simply pass by or perish, drift and strand on the over 350 km stretch of sandy beach. This indicates that the area may be not only a foraging ground, but also a migratory corridor for this species. Observations of juvenile sea turtle coastal movements have demonstrated that the genetic similarity between proximal coastal feeding areas is in accordance with the movements performed by animals at this life stage (Marcovaldi et al. 2000).

Surface drifter trajectories presented in Figure 2 clearly reveal that Ascension and Trindade Islands, and at a lesser extent Gulf of Guinea and Rocas/Noronha, present favorable conditions for conducting drifters to the eastern Brazilian coast. As highlighted before, ocean currents are considered by most authors to influence sea turtle dispersal and migration, but direct evaluations have been performed only for post-pelagic animals. Craig et al. (2004) compared the post-nesting migration routes of female green turtles satellite-tagged while nesting at Rose Atoll (Pacific Ocean) with surface drifter data. These authors found that, even though their means of navigation were not investigated, the migration routes undergone by the females closely paralleled surface ocean currents. Luschi et al. (1998) verified relationship between post-nesting movements of female green turtles satellite-tagged at Ascension Island and prevailing ocean currents, by employing a general circulation model (global isopycnic model). They noted that the turtles initially followed directions highly coincident with the prevailing current at the given

period. For satellite-tagged female leatherback turtles, post-nesting movements were monitored and paralleled to surface drifter data (surface current patterns) (Luschi et al. 2003b). Large portions of the females' routes were strikingly similar to those of surface drifters tracked in the same region, and the authors concluded that long-lasting oceanic movements of marine turtles may be shaped by oceanic circulation patterns. Although such studies are difficult to be performed with hatchling sea turtles due to their small size and evidence of a passive pelagic stage is mostly indirect, it is reasonable to suspect that sea currents play an important role in the movements of hatchlings and early juveniles of all sea turtle species (Luschi et al. 2003b).

As observed in the Brazilian sea turtle foraging grounds described by Naro-Maciel et al. (2007) the main stock contributing to the studied areas was Ascension Island (Figure 3). Large contributions from Ascension Island were also estimated by Bjorndal et al. (2006) when studying the mtDNA of a small sample ($n = 31$) from the Rocas/Noronha mixed stock. For the Gulf of Guinea foraging aggregation, Formia (2002) also found that the highest contributions were from Ascension Island, but followed by almost equal contributions from rookeries located at the Guinea area. Differently, MSA estimates of a foraging aggregation in North Carolina (Bass et al. 2006) did not reveal contribution from Ascension, being composed mainly of rookeries located in the United States, Mexico and Costa Rica, which is in accordance with the marked mtDNA structuring of the Caribbean region within the Atlantic Ocean. Aves Island was the second most important contributor in MSAs for AI and CB, followed by Trindade Island. Such high contribution from Aves Island (approximately 20% in most MSAs) was not observed for other feeding areas in Brazil, which presented a maximum contribution of 18% for Almofala, reasonable when considering that this area is located close to the Aves rookery. The low contributions from African and North-American rookeries are in accordance with Naro-Maciel et

al.'s (2007) findings for Ubatuba and Almofala. These authors detected a relatively high contribution (around 10%) only from Guinea Bissau to Ubatuba, but this contribution was considered possibly flawed due to the fixed characteristic of this rookery for haplotype CM-A8. Gulf of Guinea's contribution to the study areas, however, was noticeable in MSA₁ and MSA₃ (7.2 and 5.3%, respectively), and relatively high in the remaining MSAs (approximately 2%) when compared to other rookeries. Contributions from Cyprus were null or almost so in all MSAs, in accordance with all other studies quoted above and with the hypothesis that Mediterranean green turtles were recently separated from their relatives in the Atlantic Ocean (Kaska 2000).

The link between Brazil and Ascension Island has long been disclosed by tagging and telemetry studies of female green turtles (Meylan 1995; Luschi et al. 1998; Hays et al. 2002). There is no evidence of movements between Aves Island (second largest contribution to southern Brazil as estimated by MSA) and Brazilian foraging grounds, but other Caribbean rookeries have been shown to be linked to North Brazil (Lima et al. 2008). Marcovaldi et al. (2000) reported frequent recaptures along the Brazilian coast (from latitudes 03°45'S to 20°08'S) of female green turtles tagged at Trindade Island, demonstrating that movements between this island and coastal foraging grounds are common. Although green turtles tagged at Trindade have also been recaptured in Western Africa (Marcovaldi et al. 2000), transatlantic movements between Brazil and the west coast of Africa have not yet been confirmed by tagging programs or telemetry. Tagging efforts however have demonstrated such movements for hawksbills (Bellini et al. 2000, Grossman et al. 2007) and leatherbacks (Billes et al. 2006). Despite the scarcity of drifter data for the Gulf of Guinea area, a certain tendency of buoy to drift towards the Western Atlantic can be noted (Figure 2). Two possible explanations for the lack of evidence of a green turtle Brazil-

Africa link can be suggested: 1) green turtles born in the Atlantic coast of Africa rarely migrate to Brazil for foraging, and vice-versa; and 2) the limited amount of studies at the African continent have not yet revealed such migrations. In any one of these scenarios, further genetic and demographic studies at the western coast of Africa are necessary for enlightenment of green turtle dispersal in the Atlantic.

Despite the fact that the Ascension contribution is apparently disproportionately large when compared to other rookeries, it could in fact be reasonable. As demonstrated by drifter trajectories, surface currents favor dispersal from Ascension towards the Brazilian coast, which is also observed for Trindade Island and Rocas/Noronha. Various authors have demonstrated that a West-Southwest flow of the South Atlantic Equatorial Current is a common feature at the Ascension area (Luschi et al. 1998, Hays et al. 2002). Thus, it is reasonable to suggest that hatchlings arrive at the South American coast by means of this favorable current. Also, considering that contribution is theoretically proportional to the population size of nesting females, it would be natural to expect a larger contribution from Ascension Island, as this is the most populated of the South Atlantic rookeries and the second largest in the Atlantic Ocean (approximately 3800 females nesting annually, Broderick et al. 2006).

Few drifters left the Rocas/Noronha area and moved southwards along the Brazilian coast (Figure 2, Table 3); conditions are apparently favorable for drifting northward along the Brazilian coast, probably due to the strong North Brazil Current. These rookeries together present a small population of nesting females, approximately 100-150/year (Seminoff 2002), which could also be a possible explanation for the low estimated contribution. Aves Island was not shown by drifter data to be linked to Brazilian waters, but presented large contribution as estimated by MSA. The high frequency (90%) of CM-A5 at this rookery and frequent occurrence of this haplotype at AI

and CB is a probable cause for such estimation, which should be interpreted with caution. Despite presenting a relatively large nesting population (300-500 females nesting annually; Seminoff 2002), the number of analyzed samples is fairly low ($n = 30$), and contribution could be overestimated. Haplotype CM-A5 is present at some other rookeries in low frequencies (Mexico, Costa Rica and Gulf of Guinea), and composed 86% of the observed haplotypes in a small sample ($n = 15$) from Surinam. Contributions from these areas however were almost zero, and the reason why Aves presented such high contribution needs further investigation. Trindade Island is the second largest rookery in the South Atlantic, with between 600-800 females/year over the last nesting seasons (Soares L, pers comm). It is also close to the Brazilian coast, and clearly presents favorable ocean currents for arrival at the target area (see Figure 2). Some factors may lead to a negative influence on the number of green turtles reaching the coast. It is possible that at Trindade female fecundity and egg eclosion success is lower, and hatchling and small juvenile mortality due to predation higher, than at other rookeries; also, currents could transport animals from the island to low temperature areas, lowering the likelihood of arrival. When compared to other South Atlantic rookeries for example, Trindade is located at a relatively high latitude, and in Figure 2 it can be noted that some buoys passing through Trindade drift to higher latitudes, and consequently, lower-temperature areas. Another possibility raised by Figure 2 is that green turtles born at Trindade may arrive at the Brazilian coast at a size too small for recruitment and drift with the South Atlantic Current to the African coast, or even be carried directly from Trindade to West Africa, where they may recruit. Such factors require further investigation in order to verify their relevance. In any case, we believe that Trindade's contribution could be underestimated, and suggest additional genetic analysis of this rookery and verification of mixed stock composition in southwest Africa, as well as the analysis of additional molecular markers and elaboration of

different statistical approaches to MSA. An example of a novel approach to the analysis of mixed stock compositions is the ‘many-to-many’ MSA (Bolker et al. 2007), which has demonstrated that incorporating multiple mixed stocks in the analysis might modify contribution estimates. For northeastern Brazil, these authors reported that this analysis greatly altered results, increasing estimates from Trindade Island and decreasing contributions from Ascension and Aves Islands.

As stated above, MSA may present wide confidence limits and some limitations. The available mtDNA markers cannot precisely distinguish all rookeries, and interpretation of results must take this into account by assuming regional origins as opposed to exact nesting beaches (Avise et al. 2007). Also, MSA assumes that all nesting areas have been sampled, when in fact many lack genetic studies and many others present small sample sizes (see Table 1). The presence of “orphan” haplotypes, that is, haplotypes which have been encountered in foraging grounds but not in nesting ground samples (Bolker et al. 2007), confirms this statement. The most commonly employed software for MSA estimates, BAYES, requires removal of such “orphans”, which leads to the exclusion of rare haplotypes, such as haplotype CM-42, encountered until today only at Almofala and at Arvoredo Island and Cassino Beach.

One of the advantages of analyzing data in a Bayesian framework is the possibility of incorporating previous knowledge as informative priors. Reproductive data can be extremely useful ecological priors in MSA, altering estimates by providing the proportion of hatchlings that each area contributes to sea (i.e., the larger the nesting population size, the higher the potential contribution). Employing data such as number of hatchlings that effectively reach the ocean (hatching survival) could be useful, but unfortunately reproductive data collected at nesting areas are not standardized. Due to the fact that the most commonly available data is the number of nesting females per year, we acquiesced to adopt this information for representing the

reproductive population size. This information, however, is often biased. For example, Bjorndal et al. (2006), Bolker et al. (2007) and Naro-Maciel et al. (2007) considered as 3000 the number of females nesting annually at Trindade Island (according to Seminoff 2002), which is a highly overestimated value. Therefore, it is important to standardize the collection of such data and provide updated, reliable estimates of rookery size, in order to develop more realistic ecological priors. Another frequently used ecological prior is distance from source to mixture, in the form of great circle distances. These distances are simply a measure of the shortest geodesic route from one point to the next, and do not represent actual travelled distances, especially when considering ocean currents, with their meanders, eddies and circulation patterns. Naro-Maciel et al. (2007) noted that this type of distance was not significantly related to contribution, and we suggest that the surface drifter data used in this work are probably more informative for MSA when considering the life history traits of sea turtles.

Some caveats exist in considering surface drifter data as ecological information for green turtles: surface buoys present 15 m drogues to measure mixed layer currents (Lumpkin & Pazos 2007) and therefore do not consider surface wind drag which could influence hatchling movement; data were not limited to the hatching seasons of turtles, due to the large reduction in number of available drifter data; and the life-span of a drifter (approximately 400 days, see Lumpkin & Pazos 2007) is generally shorter than the pelagic phase of hatchlings. However, the use of such data has the potential to provide a more thorough understanding of sea turtle dispersal and the role of ocean currents in that dispersal, and greatly improve traditional distances inserted as priors in MSA. We suggest that future MSA analyses further advance prior development, modeling the afterlife of drifters and considering the effect of wind drag on surface currents, and consequently, hatchling dispersal.

The insertion of ecological priors did not greatly alter MSA estimates (Figure 3). When using rookery size (number of females/year) as an ecological prior for MSA of Rocas/Noronha, Bjorndal et al. (2006) found a decrease in contributions from Ascension and Aves Islands and an increase in contribution from Trindade. For Ubatuba and Almofala, insertion of the same prior increased contribution estimates from Ascension and Trindade Islands, while the Aves Island contribution decreased (Naro Maciel et al. 2007). However, as mentioned above, these authors overestimated the number of females nesting annually at Trindade, and therefore such alterations in stock estimates are unreliable. Bass et al. (2006) reported noticeable alterations when incorporating population size estimated in MSA of the North Carolina foraging area, and concluded that such estimates are biologically more truthful than estimates obtained with uninformative priors. In the present work, we believe that MSA₄, which incorporated the combination of different types of ecological information, is the ideal scenario for obtaining realistic stock contribution estimates. Nonetheless, the combination of all priors performed in MSA₄ resulted in estimates quite similar to the uninformative MSA.

Arvoredo Island and Cassino Beach were very similar in terms of green turtle mtDNA, and were grouped for Mixed Stock Analysis. Despite being genetically indistinct units, we suggest that different management strategies be adopted at the due areas due to some differences in terms of green turtle occurrence and habitat use. Mean curved carapace size of sampled animals revealed that green turtles at AI and CB are at different stages of their life cycles, with CB hosting smaller animals than AI. This is in accordance with sizes registered by Bugoni et al. (2003) and Reisser et al. (2008), of respectively 37.7 cm and 50.1 cm for CB and AI. These different stages present different vulnerabilities and their survival influences population growth rates in different manners, with larger juveniles, due to their higher reproductive value,

contributing more than small individuals (Crouse et al. 1987; Crouse et al. 1999). Also, these habitats may represent different roles in the life cycles of juvenile green turtles. While at least short-term fidelity has been observed at Arvoredo Island, the role of Cassino Beach is still unclear due to lack of mark-recapture or telemetry studies and it is possibly more important as a migratory corridor than a feeding area when compared to Arvoredo Island, perhaps due to factors such as lower preferred food availability and temperatures. Marine turtle bycatch in fisheries is today one of the major obstacles for the recovery of populations reduced by overexploitation and habitat degradation, and in southern Brazil, it has been evidenced that sea turtle mortality due to fishery interaction, as well as ingestion of human debris, is an issue of concern (Bugoni et al. 2001, Domingo et al. 2006). Such impacts on the developmental stages of green turtles prevent the fulfillment of their ecological role of reaching maturity and reproducing, and the conservation of juveniles along the coast leads to the protection of rookeries which are frequently thousands of kilometers away (Naro-Maciel et al. 2007). The highly migratory behavior of green turtles, which may occupy the waters of many countries as demonstrated by demographic and genetic studies, makes international cooperation essential for the conservation of these animals. The identification of stock contributions to mixed aggregations, through MSA, has important conservation implications, and if is to be seriously considered as a tool for the adequate elaboration of conservation and management plans, it is necessary that nesting populations be adequately described in terms of mtDNA, in order to provide complete and accurate baseline genetic data for estimates of natal origins. The use of other genetic markers is also advisable for better description of populations and possible inclusion in MSA.

Acknowledgements

We greatly acknowledge Pata da Cobra Diving and the Brazilian Navy for logistic support in expeditions, and all personnel involved in field work. We acknowledge Projeto Tamar-ICMBIO for partnership, and Núcleo de Educação e Monitoramento Ambiental (NEMA) and Centro de Recuperação de Animais Marinhos (CRAM) for samples from Cassino Beach. The authors also thank Liane Artico, Daniela Volcan, Ana Studzinsky for generous laboratorial aid, and Fabrício Santos, Sarah Vargas and the Laboratório de Biodiversidade e Evolução Molecular (UFMG) staff for partnership in sequencing. This is a contribution of the Research Group “Ecologia e Conservação da Megafauna Marinha – EcoMega”. M.C. Proietti and J.W. Reisser are graduate students of the Programa de Pós-graduação em Oceanografia Biológica (FURG) and received scholarships from the Conselho Nacional de Pesquisa (CNPq – Brazil). E.R.Secchi was sponsored by CNPq (PQ 305219/2008-1). Research was conducted thanks to financial support from the Rufford Small Grants (RSG – UK) and People’s Trust for Endangered Species (PTES – UK).

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Figures

Figure 1. Parsimony network of haplotypes encountered at Arvoredo Island and Cassino Beach.

Black bars represent 1 basepair substitutions between haplotypes.

Figure 2. Surface drifter trajectories in the Atlantic Ocean, with study areas (black circles), rookeries ($4^{\circ} \times 4^{\circ}$ squares), and target area. Letters stand for Rocas/Noronha (R/N), Ascension Island (AS), Trindade Island (TR), Guinea Bissau (GB), Gulf of Guinea (GG), Aves Island (AV), Mexico (MX), Costa Rica (CR), Suriname (SU), Florida (FL), Cyprus (CY).

Figure 3. Mixed Stock Analyses estimates for the southern Brazil foraging aggregations, with C.I.s and weights of employed priors. MSA_1 – uninformative prior; MSA_2 – prior reflecting surface drifter data; MSA_3 – prior reflecting number of females nesting per year at each rookery; MSA_4 – prior constructed to weigh both previous priors. Prior weights are represented in black, and MSA estimates in gray. Letters stand for Rocas/Noronha (R/N), Ascension Island (AS), Trindade Island (TR), Guinea Bissau (GB), Gulf of Guinea (GG), Aves Island (AV), Mexico (MX), Costa Rica (CR), Suriname (SU), Florida (FL), Cyprus (CY).

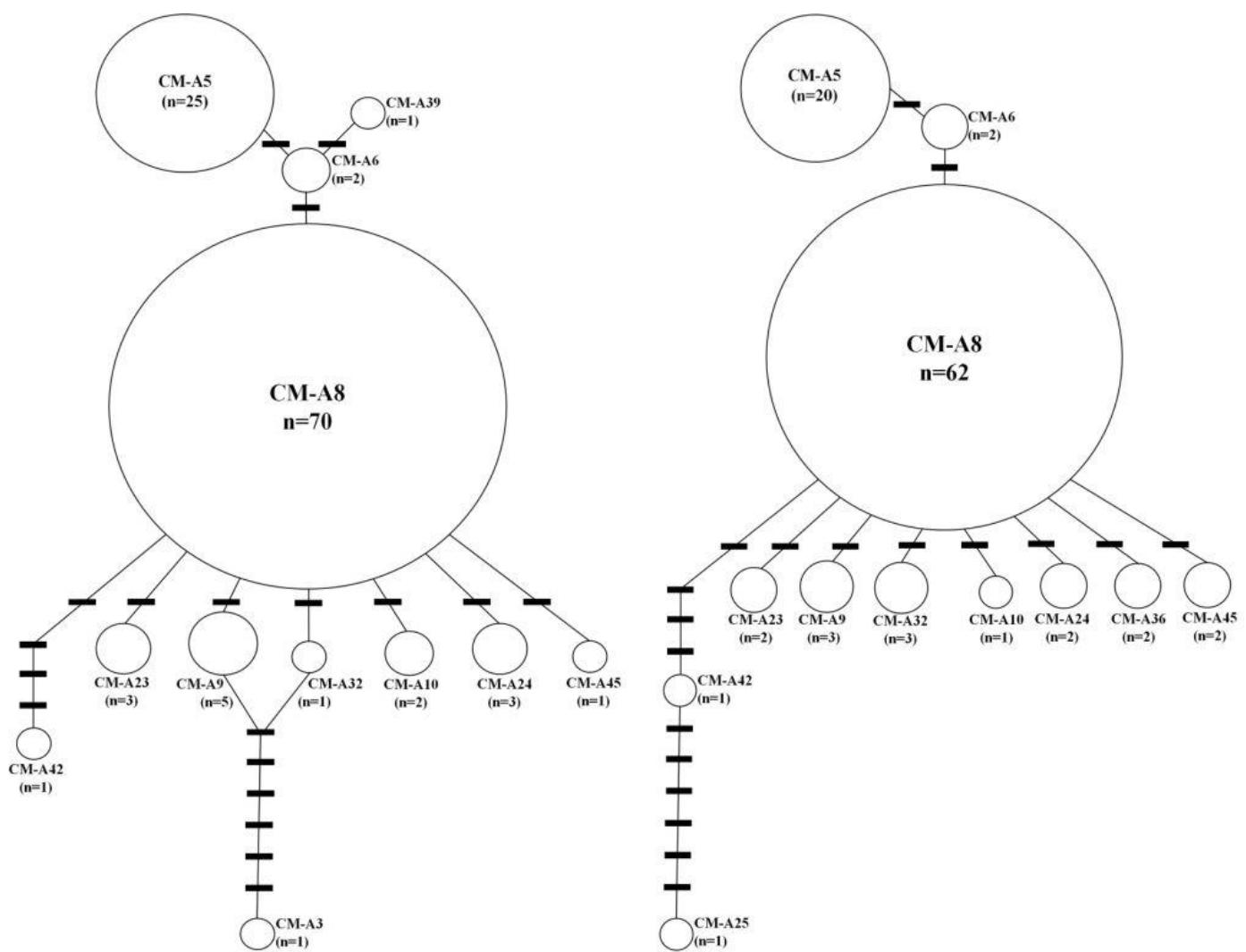


Figure 1

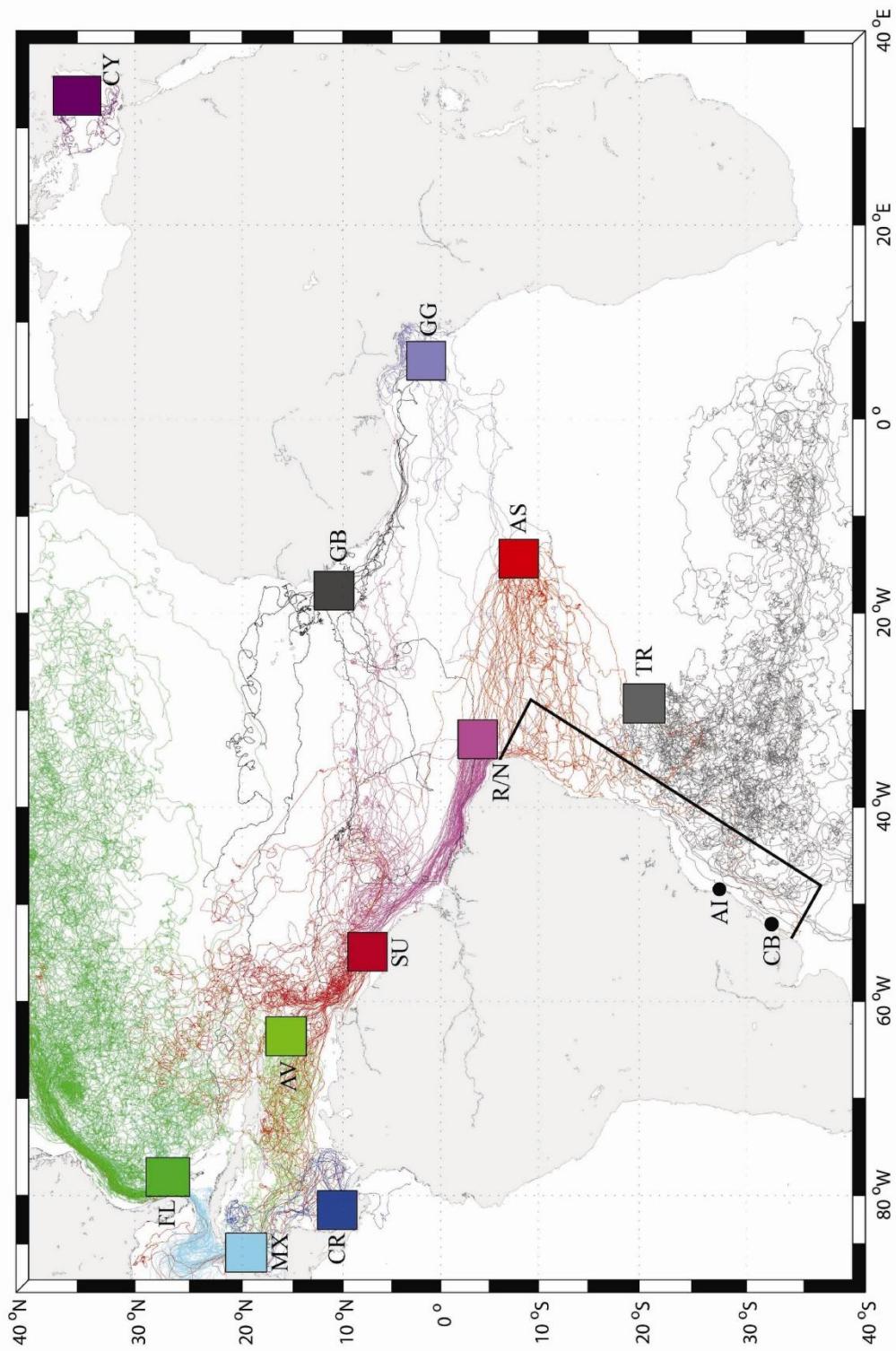


Figure 2

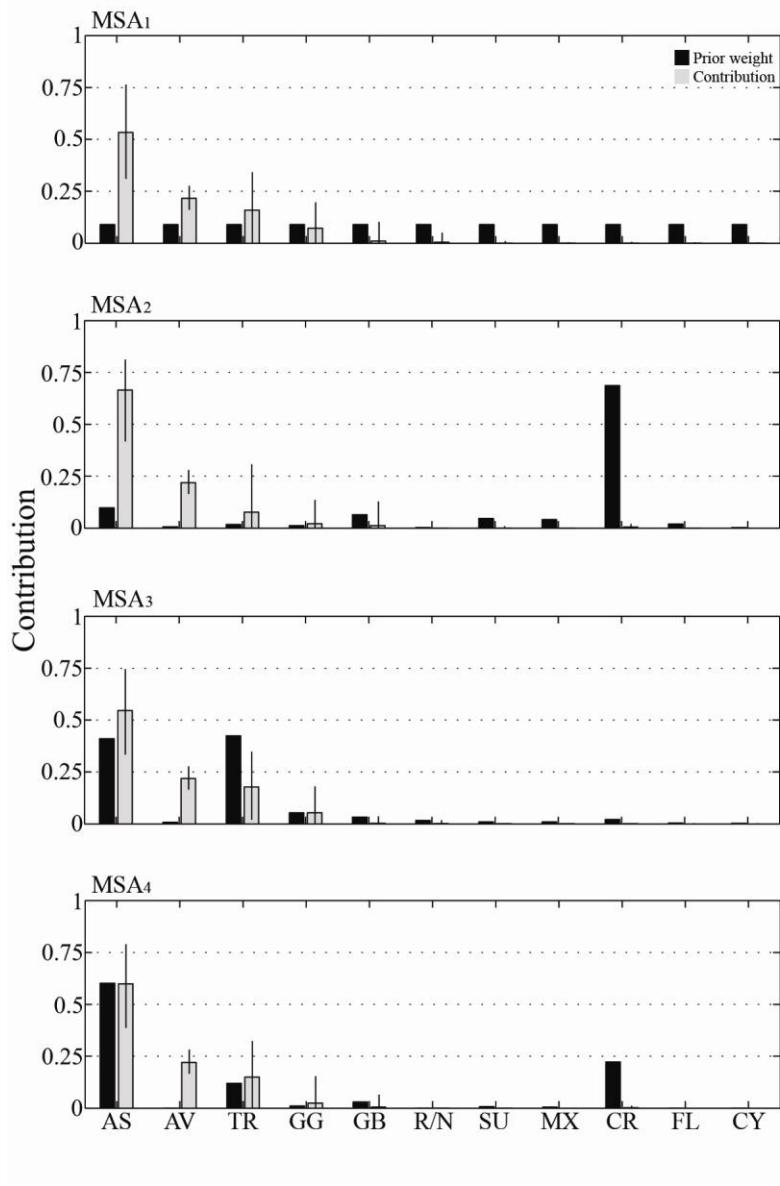


Figure 3

Tables

Table 1. Haplotype frequencies for all cited foraging and nesting areas in the Atlantic, with total number of haplotypes and samples per area. Letters stand for: Arvoredo Island (AI), Cassino Beach (CB), Ubatuba (UB), Almofala (AF), Rocas/Noronha (R/N), North Carolina (NC), Nicaragua (NI), Bahamas (BH), Florida (FL), Barbados (BB), Ascension Island (AS), Trindade Island (TR), Guinea Bissau (GB), Gulf of Guinea (GG), Aves Island (AV), Mexico (MX), Costa Rica (CR), Suriname (SU), Cyprus (CY).

Haplotype	Foraging grounds										Rookeries										
	AI	CB	UB ^a	AF ^a	R/N ^b	NC ^c	NI ^d	BH ^e	FL ^f	BB ^g	AS ^{h,i,j}	R/N ^b	TR ^b	FL ^h	GB ⁱ	GG ⁱ	AV ^h	MX ^h	CR ^k	SU ^h	CY ^{h,l}
CM-A1	-	-	-	-	-	34	-	2	12	7	-	-	-	11	-	-	-	7	-	-	-
CM-A2	-	-	-	-	-	2	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-
CM-A3	1	-	2	18	-	43	54	62	43	21	-	-	-	12	-	-	3	5	395	-	-
CM-A4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
CM-A5	25	20	14	28	5	5	6	10	3	13	-	-	-	-	-	1	27	1	32	13	-
CM-A6	2	2	-	3	2	-	-	-	-	-	11	-	-	-	-	6	-	-	-	1	-
CM-A7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
CM-A8	70	62	83	53	20	7	-	1	-	14	204	50	67	-	70	62	-	-	-	-	-
CM-A9	5	3	4	3	3	-	-	-	-	1	9	7	19	-	-	-	-	-	-	-	-
CM-A10	2	1	3	4	1	-	-	-	-	2	5	2	-	-	-	-	-	-	-	-	-
CM-A11	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
CM-A12	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-
CM-A13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25
CM-A14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
CM-A15	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
CM-A16	-	-	-	1	-	2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
CM-A17	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	2	-	-	-
CM-A18	-	-	-	-	-	3	-	-	2	-	-	-	-	-	-	-	-	3	-	-	-
CM-A20	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	2	-	-
CM-A21	-	-	-	1	-	-	-	3	-	-	-	-	-	-	-	-	-	-	3	-	-
CM-A22	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-
CM-A23	3	2	-	-	-	-	-	-	-	-	1	-	6	-	-	-	-	-	-	-	-
CM-A24	3	2	2	1	-	-	-	-	-	-	7	-	1	-	-	-	-	-	-	-	-
CM-A25	-	1	-	-	-	-	-	-	-	-	1	3	-	-	-	-	-	-	-	-	-
CM-A32	1	3	2	1	-	-	-	-	-	-	1	1	4	-	-	-	-	-	-	-	-
CM-A33	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
CM-A35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
CM-A36	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-
CM-A37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
CM-A38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-
CM-A39	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
CM-A42	1	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CM-A44	-	-	1	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
CM-A45	1	2	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
CM-A46	-	-	1	-	1	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
CM-A50	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
CM-A55	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

N. hap.	12	12	10	13	5	8	2	6	6	8	13	7	7	3	1	7	2	7	5	3	2
N. samples	115	101	113	117	31	97	60	79	62	60	245	69	99	24	70	76	30	20	433	15	26

^aNaro-Maciel et al. 2007, ^bBjorndal et al. 2006, ^cBass et al. 2006, ^dBass et al. 1998, ^eLahanas et al. 1998, ^fBass & Witzell 2000, ^gLuke et al. 1994, ^hEncalada et al. 1996, ⁱFormia et al. 2006, ^jFormia et al. 2007, ^kBjorndal et al. 2005, ^lKaska 2000.

Table 2. Haplotype (h) and nucleotide (π) diversity estimates \pm standard deviations for all compared foraging aggregations. For references see Table 1.

Foraging ground	h	π
Arvoredo Island	0.5831 ± 0.0451	0.0024 ± 0.0017
Cassino Beach	0.5857 ± 0.0501	0.0020 ± 0.0015
Ubatuba	0.4460 ± 0.0556	0.0021 ± 0.0016
Rocas/Noronha	0.5887 ± 0.0911	0.0019 ± 0.0015
Almofala	0.7168 ± 0.0306	0.0067 ± 0.0039
Barbados	0.7734 ± 0.0276	0.0105 ± 0.0057
Bahamas	0.3703 ± 0.0650	0.0066 ± 0.0038
Nicaragua	0.1831 ± 0.0621	0.0039 ± 0.0025
Florida	0.4855 ± 0.0668	0.0032 ± 0.0021
North Carolina	0.6778 ± 0.0310	0.0052 ± 0.0031
<i>Average</i>	0.5410	0.0045

Table 3. Global drifter data from the Atlantic Ocean and ecological priors for MSA. N = total number of drifters per $4^\circ \times 4^\circ$ area; Y = number of drifters reaching the target area of the Brazilian coast; P = posterior probability that a drifter that arrived at the target area is from the rookery.

Stock	N	Y	P
Ascension Island	56	30	0.410
Trindade Island	58	30	0.424
Rocas/Noronha	140	2	0.017
Gulf of Guinea	45	2	0.053
Guinea Bissau	23	0	0.033
Cyprus	29	0	0.003
Costa Rica	36	0	0.021
Surinam	84	0	0.009
Mexico	93	0	0.009
Aves Island	106	0	0.008
Florida	195	0	0.004

Annex 1

Four MSAs were performed considering uninformative priors (MSA_1) and priors weighed according to: number of females/year of each source (MSA_2); posterior probabilities calculated from surface drifter data (MSA_3); and a combination of the two previous informative priors (MSA_4).

For MSA_1 , prior probabilities were uninformative, meaning that all possible sources (rookeries – R_j , $j = 1, \dots, 11$) have equal prior probabilities of reaching the coast, that is: $P(R_j) = \frac{1}{11}$.

In MSA_2 , we consider that the probability is proportional to the number of females nesting per year at a certain rookery, that is, the $P(R_j) \propto N_j$. Since $\sum P(R_j) = 1$, $P(R_j) = \frac{N_j}{\sum_{i=1}^{11} N_i}$

Information provided by drifters was summarized as the number of drifters which passed through the possible sources (n_j , $j = 1, \dots, 11$) and reached a target area (y_j) consisting in the eastern Brazilian coast, from the southernmost limit to the northeastern corner.

Considering that θ_j is the probability that a drifter passing through a possible source rookery (R_j , $j = 1, \dots, 11$) will reach the target area at the Brazilian coast, for each R_j we have: $y = [Y_1, Y_2, \dots, Y_j]$, with:

$Y = 0$ for drifters that did not reach the coast (failure);

$Y = 1$ for drifters that reach the coast (success); the vector Y will have length n (number of drifters leaving the source).

In order to estimate the probability (θ_j) that a drifter passing through an R_j will reach the target area, a uniform prior between zero and one was used for θ_j (uninformative prior) in a binomial sampling model:

$$p(\mathbf{y}_j | \theta_j) = \text{Bin}(\mathbf{y}_j | n_j, \theta_j) = \binom{n_j}{y_j} \theta_j^{y_j} (1 - \theta_j)^{n_j - y_j} \quad (1)$$

Applying Bayes' Theorem: $p(\theta_j | \mathbf{y}_j) \propto p(\mathbf{y}_j | \theta_j) p(\theta_j)$ to equation 1, we obtain a posterior density following a Beta distribution:

$$\theta_j | \mathbf{y}_j \sim \text{Beta}(y_j + 1, n_j - y_j + 1) \quad (2)$$

By estimating posterior values of the Beta probability distribution parameters for each source area, the posterior distributions of θ_j can be calculated. Since we are interested in the probability a_j that a new success (that is, a drifter reaching the Brazilian coast) is from an R_j , the posterior distribution of the variable must be considered, corresponding to:

$$a_j = P(R_j | \text{success}) = \frac{P(\text{success} | R_j) P(R_j)}{P(\text{success})} ;$$

since $P(\text{success} | R_j) = \theta_j$ and $P(\text{success}) = \sum_{i=1}^{11} \theta_i P(R_i)$, the equation is modified to:

$$a_j = \frac{\theta_j \frac{1}{11}}{\frac{1}{11} \sum_{i=1}^{11} \theta_i} \quad (3)$$

Bayes estimates were chosen for point estimates of a_j . When considering that $\hat{\theta}_j$ is estimated by $\frac{y_j + 1}{n_j + 2}$, equation 3 is modified to:

$$a_j = \frac{\hat{\theta}_j}{\sum \hat{\theta}_i} \quad (4)$$

These average values were directly used as an informative prior in MSA₃. Additionally, another prior was developed and used in MSA₄, by combining the average values cited above and the number of females of each rookery (N_j), through the equation:

$$a_j = \frac{N_j \hat{\theta}_j}{\sum N_i \hat{\theta}_i} \quad (5)$$