NOT JUST ANOTHER MIXED STOCK ANALYSIS: GREEN TURTLES OF ESPIRITO SANTO, BRAZIL

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ABSTRACT. **In the Southwestern Atlantic Ocean, sea turtles are exposed to myriad threats including disease, fisheries bycatch, and industrial or coastal development, but protected by effective conservation organizations. In Espírito Santo, Brazil, green turtles (*Chelonia mydas*) with relatively high incidence of fibropapillomatosis tumors routinely strand in the vicinity of the state capital, Vitória, a highly urbanized area that encompasses the effluent discharge channel of a local steel plant. This is also a particularly interesting population because of its relative proximity to the regionally important Trindade Island rookery, whose feeding grounds have not been convincingly identified to date. To investigate the population distribution of the at-risk turtles, we sequenced a segment of the mitochondrial control region (862 bp; n = 132). Eight mtDNA haplotypes were revealed, of which the most common were CMA-08 and CMA-05. Haplotypes CMA-06 and CMA-09 were each found in six individuals, and rare haplotypes CMA-03, CMA-10, CMA-23, and CMA-32 were also detected. Two kinds of "many-to-many" mixed stock analyses were carried out, taking into account or alternately disregarding source nesting population size. The same approach was taken with traditional MSAs ("one-to-many"), and the main differences between the "one-to-many" and "many-to-many" results are reported. The analyses that included population size and all available data were most consistent with expectations. We recommend caution when employing different mixed stock analysis methods, and emphasize the importance of exploring alternate ways of investigating the origins of mixed stocks, including modeling approaches. These data will provide insight into population isolation and conservation priorities necessary to establish whether areas should be managed as independent units or as regional populations, and will clarify questions of scale in conservation and management, providing a scientific basis for conservation prioritization.**

INTRODUCTION

Despite the endangered status of green sea turtles worldwide (IUCN 2009) and considerable scientific interest, aspects of their biology important for research and conservation remain unknown. For example, conclusive identification of linkages among groups of highly migratory green turtles is important for comprehensive protection and understanding population biology. This can be especially relevant when considering at-risk groups, such as the green turtles found with high incidence of fibropapillomatosis tumors (about 34%) in the effluent discharge channel of a steel plant in Espírito Santo, Brasil (Torezani et al. 2009; Figures 1, 2). Genetic analysis is a powerful tool for determining such relationships, and methods for investigating the natal origins of mixed stocks have been developed and refined, including:

- "One-to-many" mixed stock analyses (MSAs; Pella and Masuda 2001, 2005), in which the natal origins of a single feeding ground are elucidated with respect to nesting areas using Bayesian analysis; and
- "Many-to-many" MSAs (Bolker et al. 2007), an extension of the "one-to-many" in which data from multiple feeding grounds and nesting areas are simultaneously analyzed.

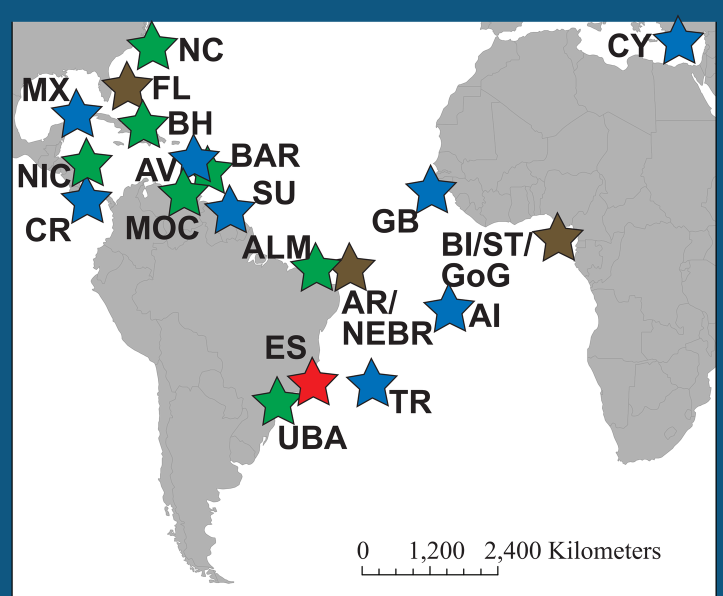

Figure 1. Map of the study site (red star), with respect to regional nesting (blue stars), feeding (green stars), and nesting/feeding (brown stars) areas. Abbreviations are as follows: Almofala (ALM); Ascension Island (AI); Atol das Rocas (AR); Northeast Brazil (NEBR, feeding grounds at Atol das Rocas and Fernando de Noronha); Aves Island (AV); Barbados (BAR); Bahamas (BH); Bioko (BI); Costa Rica (CR); Cyprus (CY); Espirito Santo (ES); Florida (FL); Guinea Bissau (GB); Gulf of Guinea (GoG); Mexico (MX); Mochima (MOC); Nicaragua (NIC); North Carolina (NC); Sao Tome (ST); Surinam (SU), Trindade (TR), and Ubatuba (UBA).

OBJECTIVES

We aim to apply genetic methods to advance the research and conservation of juvenile green sea turtles found in the effluent discharge channel of a steel plant in Brasil (Figure 1, 2). Specifically, our objectives are to: 1) Determine their genetic composition at the mitochondrial control region; 2) Assess genetic differentiation between this group and other Atlantic populations, as well as among years and seasons at the study site; 3) Elucidate the natal origins of these turtles; and 4) Consider effects of population size, geographic distance, natal homing, disease (fibropapillomatosis), mortality, and ocean currents on genetic composition. For this presentation we focus on objective 3.

Figure 2. A) Study site in the effluent discharge panel of a steel plant in Espírito Santo, Brazil. B) A high incidence of turtles with fibropapillomatosis disease has been reported at this site (Torezani et al. 2009). Photo credits: TAMAR Image Bank.

MATERIALS AND METHODS

Juvenile green sea turtles (n = 132) were captured in the effluent discharge channel of a steel plant in Espírito Santo, Brazil (Figure 1, 2) from 2004-2005. These turtles were visually examined, measured, and sampled for genetic analysis. Laboratory work followed previously described methods (Naro-Maciel et al. 2007) for sequencing about 800 bp of the mitochondrial control region, using primers designed by Abreu et al. (2006). Measures of genetic diversity and differentiation were calculated as described by Naro-Maciel et al. (2007).

To investigate the natal origins of this mixed stock, as well as contributions of other regional feeding grounds to nesting areas, four MSAs were carried out:

- MSA 1. One-to-many taking into account source population size;
- MSA 2. One-to-many not taking into account source population size;
- MSA 3. Many-to-many with source population size;
- MSA 4. Many-to-many without source population size.

In addition, to investigate the distribution of turtles feeding at Espírito Santo among regional nesting areas, two "rookery-centric" many-to-many analyses (one that considered, and one that disregarded rookery population size) were carried out.

RESULTS AND DISCUSSION

"Rookery-centric" analyses. Turtles of Espírito Santo contributed the most to the Surinam, Trindade, and Ascension nesting populations. However these turtles did not contribute substantially to five of the twelve rookeries included in the study (Florida, Costa Rica, Cyprus, Mexico, and Sao Tome; Figure 3).

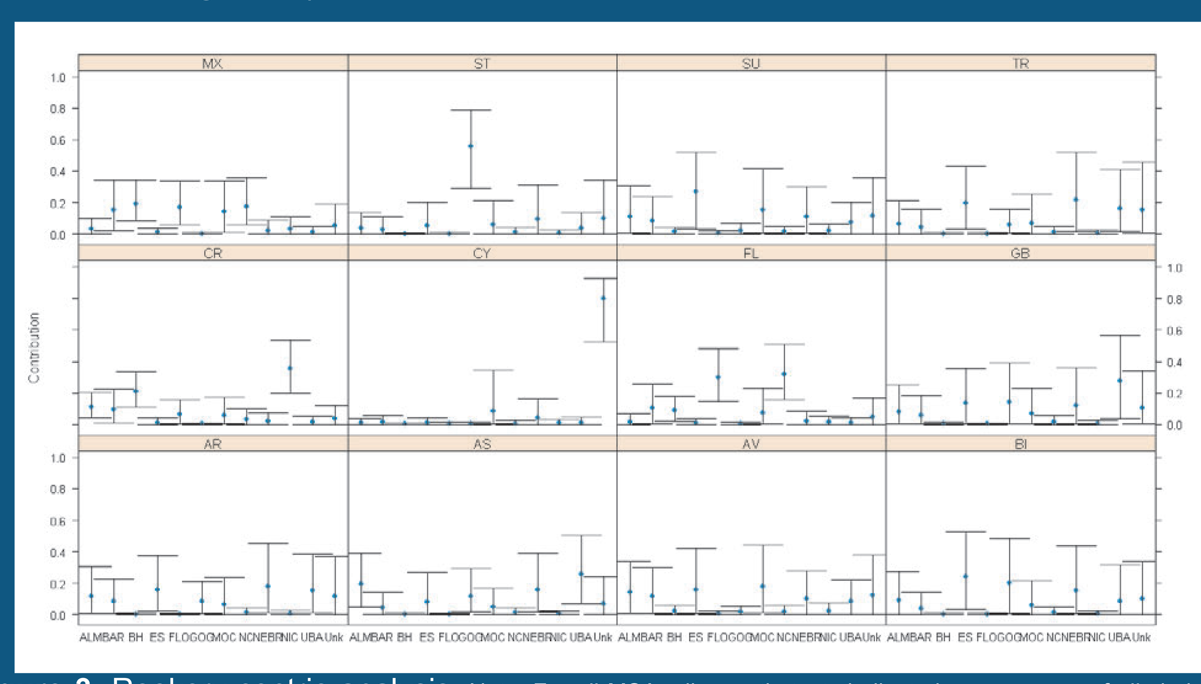

Figure 3. Rookery-centric analysis. Note: For all MSAs diagnostic tests indicated convergence of all chains. All available data on rookeries and feeding grounds (Bass et al. 1998, Bass and Witzell 2000, Bass et al. 2006, Bjorndal et al. 2005, Bjorndal et al. 2006, Bjorndal and Bolten 2008, Bolker et al. 2007, Encalada et al. 1996, Formia et al. 2002, Formia et al. 2006, Kaska 2000, Lahanas et al. 1998, Luke et al. 2004, Naro-Maciel et al. 2007) were included, with the exception of nesting areas with fewer than 10 samples or 20 nesting females. For the analyses incorporating source population size, the following numbers of nesting females were used; Ascension Island (AI): 3709; Atol das Rocas (AR): 115; Aves (AV): 267; Bioko (BI): 407; Costa Rica (CR): 24,000; Cyprus (CY): 100; Florida (FL): 779; Guinea Bissau (GB): 1500; Mexico (MX): 1587; Sao Tome (ST): 90; Surinam (SU): 1814 (references in Bolker et al. 2007 and Naro-Maciel et al. 2007) and Trindade (TR): 900 (Almeida et al. In preparation).

Mixed Stock Analyses: Espírito Santo. Five of the twelve nesting areas did not contribute over 5% to Espírito Santo in any analyses (Florida, Costa Rica, Cyprus, Mexico, and Sao Tome). Two of the nesting areas, Surinam and Ascension Island, had over 5% mean contribution in all MSAs. In all but one analysis, Trindade accounted for over 5% of turtles at Espírito Santo, while Guinea Bissau was only an important source in the many-to-many analyses (and not in the one-to-many analyses). The small colonies of Aves Island and Atol das Rocas were only identified as main sources when population size was not factored. In contrast, Bioko could only be considered an important source when population size was not included in the analysis. The estimates incorporating population size differed from those not including population size by >5% in nine of the twenty-four comparisons (Table 1, highlighted in yellow). The confidence intervals were generally smaller for analyses that included population size (Table 1).

STOCK	One-to-many	MEAN	2.5%	97.5%	Many-to-many	MEAN	2.5%	97.5%
Florida	$MSA_{1\ pop.\ size}$	0.000	0.000	0.002	$MSA_{3\ pop.\ size}$	0.007	0.000	0.027
	$MSA_{2\ no\ pop.\ size}$	0.001	0.000	0.010	$MSA_{4\ no\ pop.\ size}$	0.007	0.000	0.026
Mexico	$MSA_{1\ pop.\ size}$	0.000	0.000	0.005	$MSA_{3\ pop.\ size}$	0.008	0.000	0.027
	$MSA_{2\ no\ pop.\ size}$	0.001	0.000	0.009	$MSA_{4\ no\ pop.\ size}$	0.008	0.000	0.026
Aves	$MSA_{1\ pop.\ size}$	0.030	0.000	0.341	$MSA_{3\ pop.\ size}$	0.021	0.001	0.075
	$MSA_{2\ no\ pop.\ size}$	0.124	0.000	0.381	$MSA_{4\ no\ pop.\ size}$	0.119	0.005	0.294
Costa Rica	$MSA_{1\ pop.\ size}$	0.010	0.000	0.035	$MSA_{3\ pop.\ size}$	0.012	0.000	0.042
	$MSA_{2\ no\ pop.\ size}$	0.001	0.000	0.013	$MSA_{4\ no\ pop.\ size}$	0.008	0.000	0.029
Surinam	$MSA_{1\ pop.\ size}$	0.305	0.000	0.421	$MSA_{3\ pop.\ size}$	0.294	0.204	0.381
	$MSA_{2\ no\ pop.\ size}$	0.213	0.000	0.414	$MSA_{4\ no\ pop.\ size}$	0.200	0.027	0.345
Atol das Rocas	$MSA_{1\ pop.\ size}$	0.000	0.000	0.000	$MSA_{3\ pop.\ size}$	0.009	0.000	0.033
	$MSA_{2\ no\ pop.\ size}$	0.029	0.000	0.257	$MSA_{4\ no\ pop.\ size}$	0.121	0.018	0.257
Trindade	$MSA_{1\ pop.\ size}$	0.032	0.000	0.224	$MSA_{3\ pop.\ size}$	0.122	0.020	0.263
	$MSA_{2\ no\ pop.\ size}$	0.071	0.000	0.269	$MSA_{4\ no\ pop.\ size}$	0.146	0.031	0.285
Ascension	$MSA_{1\ pop.\ size}$	0.605	0.329	0.728	$MSA_{3\ pop.\ size}$	0.341	0.070	0.561
	$MSA_{2\ no\ pop.\ size}$	0.465	0.000	0.706	$MSA_{4\ no\ pop.\ size}$	0.061	0.002	0.200
Sao Tome	$MSA_{1\ pop.\ size}$	0.000	0.000	0.000	$MSA_{3\ pop.\ size}$	0.004	0.000	0.013
	$MSA_{2\ no\ pop.\ size}$	0.004	0.000	0.037	$MSA_{4\ no\ pop.\ size}$	0.042	0.001	0.140
Bioko	$MSA_{1\ pop.\ size}$	0.006	0.000	0.082	$MSA_{3\ pop.\ size}$	0.044	0.001	0.146
	$MSA_{2\ no\ pop.\ size}$	0.066	0.000	0.427	$MSA_{4\ no\ pop.\ size}$	0.182	0.026	0.352
Guinea Bissau	$MSA_{1\ pop.\ size}$	0.011	0.000	0.137	$MSA_{3\ pop.\ size}$	0.134	0.005	0.329
	$MSA_{2\ no\ pop.\ size}$	0.025	0.000	0.249	$MSA_{4\ no\ pop.\ size}$	0.100	0.005	0.248
Cyprus	$MSA_{1\ pop.\ size}$	0.000	0.000	0.000	$MSA_{3\ pop.\ size}$	0.004	0.000	0.016
	$MSA_{2\ no\ pop.\ size}$	0.001	0.000	0.007	$MSA_{4\ no\ pop.\ size}$	0.008	0.000	0.028

Table 1. Mixed stock analyses of green sea turtles from Espírito Santo. MSA1: One-to-many with priors weighted to reflect population size; MSA2: one-to-many with equal priors; MSA3: many-to-many with priors weighted to reflect population size; MSA4: many-to-many with equal priors. The mean estimates are given as well as the 95% confidence interval. Boxes outlined in red represent the mean estimates that include the most information, and that we feel are the most reliable.

Mixed Stock Analyses: All feeding grounds. The study also revealed the relationships among other feeding grounds and nesting areas in the Atlantic that had not previously been subjected to many-to-many analysis including all currently available published data (Figure 4).

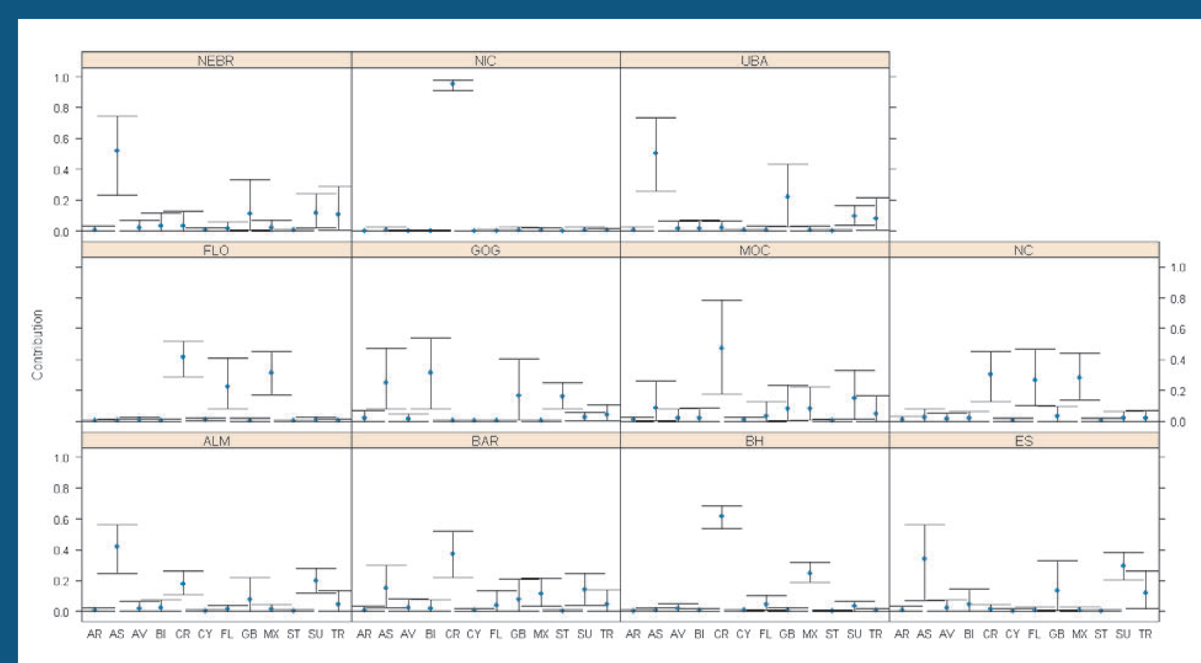

Figure 4. Results of the many-to-many MSA incorporating nesting population size of all published green sea turtle feeding area and rookery data available to date.

CONCLUSIONS

Although broadly consistent, the different MSAs did have varying mean estimates and confidence intervals particularly with regard to contributions from the Ascension Island, Trindade Island, African, and small rookeries. We conclude that the estimates which include the most information - the many-to-many analyses with source population size (MSA3) - are the most reliable and consistent with tag and satellite data. These analyses do indicate a greater contribution to the Espírito Santo feeding grounds from distant African rookeries consistent with previous reports (Bolker et al. 2007). However, the confidence intervals remain high. We therefore recommend examining other sources of information to corroborate these findings, particularly modeling of hatchlings in oceanographic currents (Blumenthal et al. 2009).

REFERENCES available upon request

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