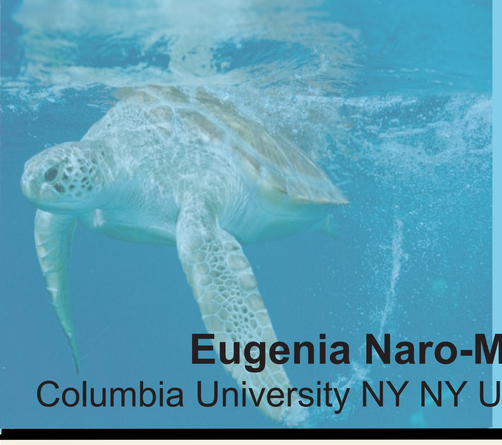

# Connectivity and Intra-Population Structure of Western South Atlantic Green Sea Turtle (*Chelonia mydas*) Foraging Populations

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## Introduction

Population genetic methods are being employed to test hypotheses about connectivity and intra-population structure of major Western South Atlantic green sea turtle foraging groups. The project's significance stems from ensuing insights into *Chelonia mydas* population structure in a key yet insufficiently characterized area of the range, with applications to other species worldwide.

The first research objective is to understand relationships between the study groups and other Atlantic green turtle populations. This will provide insight into dispersal, migration, genetic variation, disease, and mortality. Conservation applications include informing management priorities, assessing distinctiveness of protected populations, and establishing a baseline for forensic work. This study further aims to examine rarely addressed aspects of intra-population genetic structure. These include temporal and spatial variation, as well as relationships between genetic composition and demographic factors affecting survivorship and dispersal.

## Hypotheses

Green turtle foraging population structure in the Western South Atlantic region will be characterized by testing hypotheses for the two major feeding groups of Ubatuba and Almofala, Brazil (Figure 1). Hypotheses progress logically from expectations under simple models to more complex scenarios incorporating elements of populations sub-structure, and encompass 3 general areas:

- **Relationship between the study groups and regional nesting populations**
- **Relationship between the focal areas and regional foraging groups**
- **Intra-population structure at the study sites**


**Figure 1:** Research sites (*italics*) and selected Atlantic foraging groups (*italics*) and rookeries.

## Materials and Methods

Study sites were chosen based on biological importance and conservation concern (Marcovaldi and Marcovaldi 1999). Sampling spanned two-year periods for temporal comprehensiveness. Tissue collection employed standard safe procedures. DNA extraction, sequencing, screening for microsatellite polymorphism, and genotyping follow protocols commonly used at the AMNH, and use published primers (including FitzSimmons 1998; Lahanas 1998). Study populations will be compared to other Atlantic groups with adequate sample sizes submitted to genetic analysis. Research to date assessing origins of foraging sea turtles (H 1-4) is based on Mixed Stock Analysis of mtDNA data. This study will optimize MSA performance by using appropriate sampling and updated methods (Pella and Masuda 2001; Bolker et al. 2003), and explore alternate procedures. For accuracy, the MSA will not be performed until the assumption that all sources are included is reasonably met. Instead, each foraging group is tested for homogeneity against available single Atlantic rookeries, and a preliminary data examination based on common and private alleles is reported. Population genetic structure was assessed through Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992) and pairwise population comparisons using conventional F-statistics based on haplotype frequencies only (Weir and Cockerham 1984), as well as exact tests of population differentiation (10000 steps in Markov chain; Raymond and Rousset 1995) implemented by the Arlequin program (v. 2.000; Schneider et al. 2001). Significance values were obtained from 1000 permutations. In addition, indices of molecular variation (Nei 1987) and minimum spanning networks were calculated.

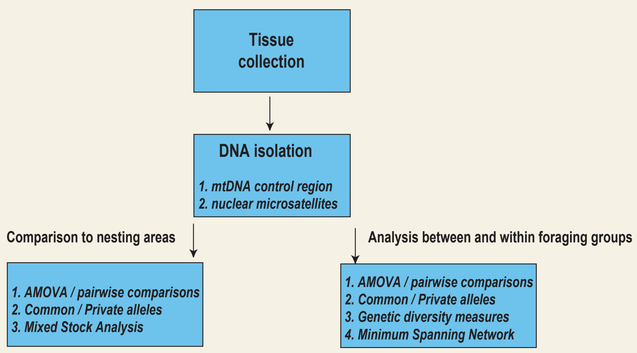


**Figure 2:** Summary of Methods

## Results

At Almofala 19 polymorphic sites defined 13 mitochondrial control region haplotypes, all of which had been previously identified (n=121). The Ubatuba population consists of 11 haplotypes defined by 13 polymorphic sites (n=118). Of these, nine had been previously named, 1 was a confirmed heteroplasmy at site 164, and 1 matched no published sequences (temporarily designated "new"). This haplotype differed by 1 bp from haplotype cm05. Eight of the 15 Cheloniidae microsatellite loci screened for variation in Ubatuba were polymorphic. The intra-population results discussed here were obtained from approximately 40 samples genotyped for 3 variable loci (cm3, cm58 and cm72).

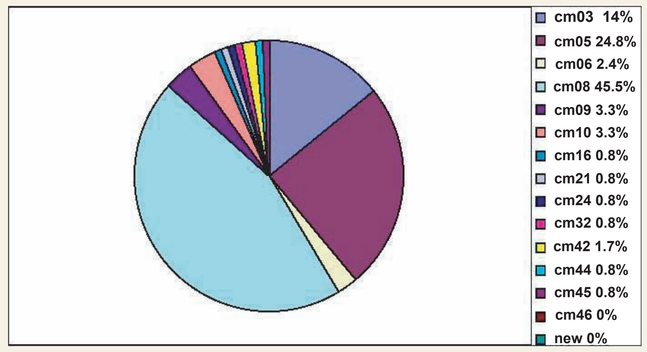


**Figure 3:** Almofala control region haplotypes

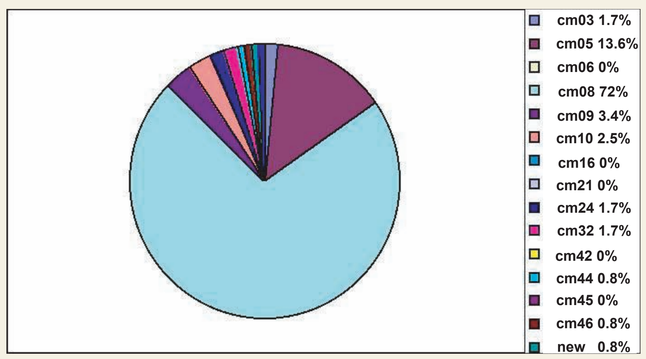


**Figure 4:** Ubatuba control region haplotypes

### Relationship between study groups and regional nesting areas

**The hypothesis of homogeneity between each feeding area and single Atlantic rookeries was rejected (exact P < 0.05; Fst P < 0.05).**
The rookeries tested were Florida, Mexico, Costa Rica, Aves VZ, Suriname, Atol BR, Ascension UK, Guinea Bissau, Bioko, Sao Tome, and Cyprus (Encalada et al. 1996; Formia et al. 2003). The hypothesis was not rejected by both tests in one instance, the pairwise comparison between Ubatuba and Sao Tome (Fst = 0.00735, P = 0.24805 +- 0133).

**The most frequent haplotypes at Almofala are cm08, cm05 and cm03. Cm08 and cm05 are also characteristic of Ubatuba (Figures 2 and 3).**
Cm08 is common (>50%) in equatorial oceanic island rookeries such as Ascension Island, Atol das Rocas BR, and Sao Tome and Principe, as well as in West Africa (Encalada et al. 1996; Formia et al. 2003). Cm05 occurs in high frequencies at Aves Island and Suriname, while cm03 is almost fixed at Tortuguero, being common also in Florida (Encalada et al. 1996). Alleles found only at the study feeding areas and one other rookery were all rare (< 5%) and primarily from Ascension Island, although cm16 was found in Almofala and Mexico (Encalada et al. 1996; Formia et al. 2003).

### Relationship between study groups and regional foraging areas

**Overall homogeneity between Ubatuba and Almofala was rejected (exact P = 0.0000; Fst = 0.07090, P = 0.0000).**

**Homogeneity between each study group and other Atlantic feeding areas was rejected for all pairwise comparisons (P = 0.0000) excluding Uruguay**
(Ubatuba/Uruguay: exact P = 0.3438 +_ 0.0280; Fst = 0.01871, P = 0.15315 +_ 0.0273; Almofala/Uruguay exact P = 0.5471 +_ 0.0236; Fst = -0.00624, P = 0.48649 +_ 0.0309). The Atlantic foraging groups tested were East Central Florida (Bass and Witzell 2000), Bahamas (Lahanas et al.1998), Nicaragua (Bass et al. 1998), Gulf of Guinea (Formia et al. 2003) and Uruguay (Caraccio et al. In press).

**Comparison of genetic diversity measures between both Brazilian sites and other Atlantic groups revealed above average allele number.**
At Almofala gene (0.7146+-0.0303) and nucleotide (0.006395 +- 0.003693) diversities were high, and the latter measure was similar to the Bahamas. At Ubatuba gene diversity was average (0.4637+- 0.0536). Nucleotide diversity was at the low end of the range (0.002037+- 0.001533) and similar to Uruguay and the Gulf of Guinea. High gene diversity and allele number are likely due to large sample sizes and detection of rare alleles. Low nucleotide diversity at Ubatuba reflects the few differences between haplotypes there, most of which belong to one mitochondrial lineage.

### Relationships within study sites

**Overall homogeneity between years at Ubatuba was rejected by the preliminary microsatellite data set (Fst P = 0.00901), although not by the mitochondrial results.**

**Homogeneity between six month periods at Ubatuba was rejected by the control region data set (P = 0.003).**

**Seasonal and yearly homogeneity were not rejected at Almofala based on mtDNA results ( Fst P > 0.05).**

**Homogeneity among size classes at Ubatuba was rejected.**
Small and medium turtle groups were significantly different (exact P = 0.00035 +_ 0.0001; Fst = 0.05725, P = 0.0000), and variation among other classes was indicated pending in depth microsatellite work.

## Conclusions

**The rejection of homogeneity between the study groups and single Atlantic rookeries was an expected result.**
Atlantic green sea turtle foraging populations researched to date are mixed stocks. Natal origins have been traced to diverse rookeries, with contributions influenced by geographic proximity and/or source population size (References in Results).

**Common alleles at Almofala link the population to both northern and southern Atlantic rookeries.**
Extensive mark-recapture studies (Figure 4; Marcovaldi et al. 2000; Lima and Troeng 2001) and satellite work documenting juvenile dispersal from Almofala in both directions (Godley et al. In press) substantiate these results. Although this population is composed mainly of juveniles (Marcovaldi et al. 2000b), the well established connectivity between *C. mydas* adults in Northern Brazil and the Ascension Island and Suriname rookeries (Papi et al. 2000; Luschi et al. 1998; Pritchard 1976; Schulz 1975; Carr 1975), as well as links to Tortuguero (Meylan 1995), further support this conclusion.

**A strong equatorial/South Atlantic natal link to Ubatuba is indicated by the frequency of the cm08 allele, the private alleles, and evidence from tag returns, all of which have occurred below 17 S latitude (Figure 4).**
The role of Suriname and Aves, which lack cm08 but contain cm05 at high frequencies, will be clarified when the presence of the latter allele in other major Atlantic nesting areas is determined. While the hypothesis that this group originates entirely from the Sao Tome rookery was not rejected in all tests, the proximity and large size of the nesting population at Trindade Island strongly point to this area as an important source.

**The lack of homogeneity between Ubatuba, Almofala, and most other Atlantic foraging groups is consistent with previous research.**
All feeding populations surveyed to date are significantly different from each other, with the exception of Nicaragua and the Bahamas (Bass and Witzell 2000; Lahanas et al. 1998; ENM data not shown). The lack of subdivision between Ubatuba and Uruguay is expected due to close geographic proximity and mark-recapture results (Figure 4). Insignificant differences between Almofala and Uruguay were unexpected. Allele frequencies in the southern population (Caraccio et al. In press) are intermediate between the two Brazilian groups. It is possible this result would change once the pilot study (n=20) is extended to include a larger sample number over a comprehensive time-span, especially considering the absence of characteristic northern haplotypes such as cm03 in Uruguay (Caraccio et al. In press) . Connectivity between the Almofala and Nicaraguan foraging groups revealed by tag returns (Lima et al. 1999; Lima et al. 2003) may be insufficient to completely homogenize the two areas genetically.

**Temporal and size-related heterogeneity in allele frequencies at Ubatuba are consistent with variation in demographic parameters at the site (Gallo et al. In review), clearly demonstrating the importance of comprehensive sampling for genetic studies.**


**Figure 5:** Projeto TAMAR tag returns (Marcovaldi et al. 2000) at Almofala and Ubatuba

**References:** Available from the author upon request.

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