GENETIC STOCK COMPOSITION OF NESTING HAWKSBILL TURTLES, IN MARIE GALANTE (GUADELOPE/FRENCH WEST INDIES)

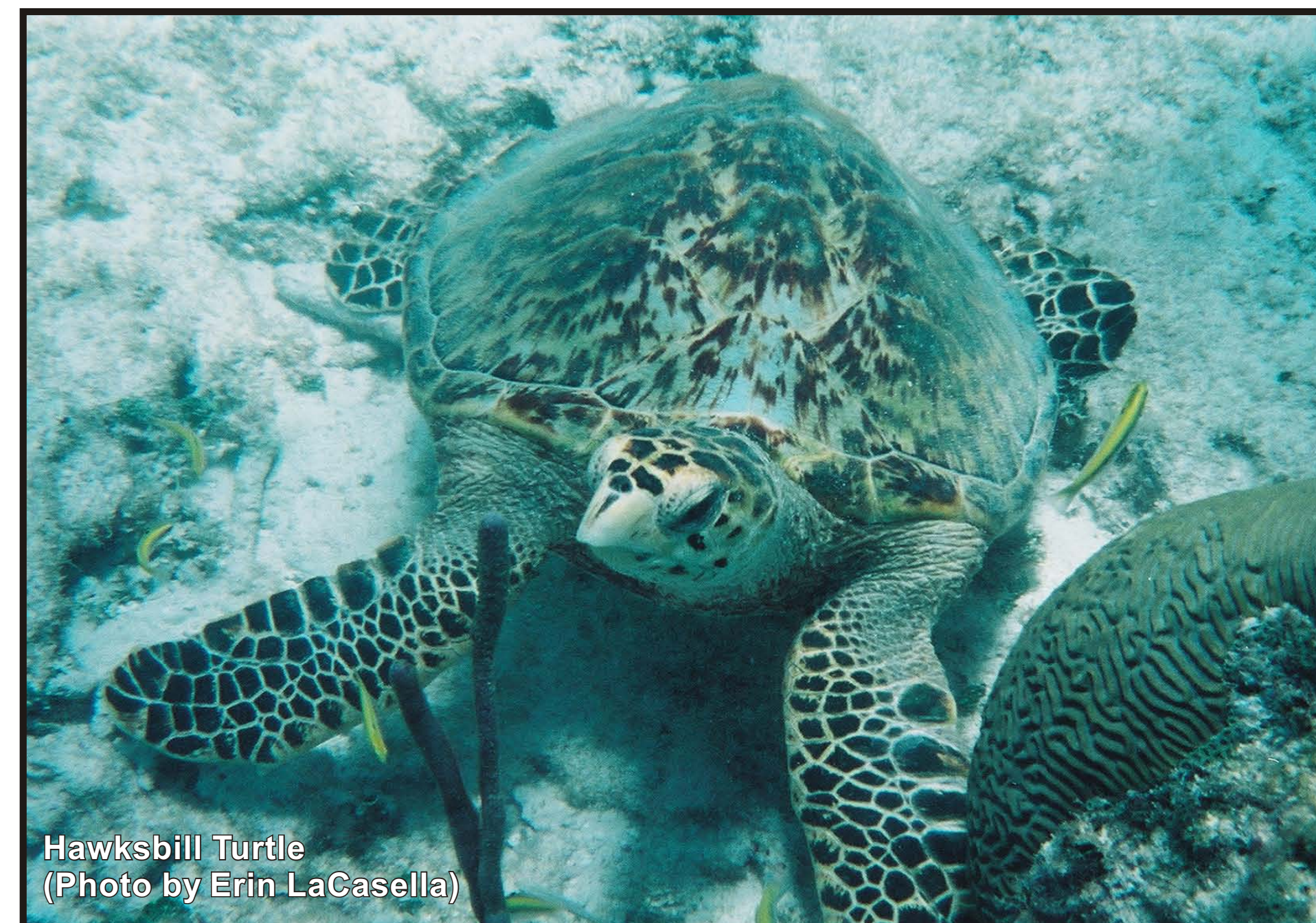## Robin A. LeRoux[1], Johan Chevalier[2,3], and Peter H. Dutton[1]

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INTRODUCTION

Hawksbill turtles (*Eretmochelys imbricata*) are widely distributed throughout the Caribbean. Recent studies present a foundation for understanding the mitochondrial (mtDNA) genetic make-up of several major nesting beaches in this region (Bass et al. 1999, Diaz-Fernandez et al. 1999, and Bowen et al. 1996). This study attempts to fill in a portion of the genetic map by reporting haplotype frequencies obtained from hawksbill females nesting at Trois Islets beach on the island of Marie Galante in Guadeloupe (Chevalier et al. 2002; Figure 1). We compare our results with published data (Bass et al. 1999, Diaz-Fernandez et al. 1999) to examine regional population structure. An important goal of these nesting stock surveys is to ultimately provide baseline data for assessing stock composition at foraging grounds, so that impacts of harvest or other activities on specific stocks can be evaluated. However, previous mixed stock analyses (MSA) have been limited by lack of data from many of the potential source nesting stocks. We re-ran the MSA using previously published foraging ground data to see how inclusion of Guadeloupe might change the conclusions of these studies.

Figure 1

Map of the Caribbean. Nesting sites addressed are highlighted in yellow.

METHODS

The 480 base pair (bp) mtDNA control region of 55 hawksbill females that nested at Trois Islets beach in 2002 were sequenced using standard laboratory techniques as outlined in Dutton et al. 1999. The results obtained are presented in table 1.

Table 1

MtDNA haplotype frequencies for nesting populations in the Caribbean based on Bass et al. (1999) and Diaz-Fernandez (1999). with data from Trois Islet, Guadeloupe 2002. *Denotes Haplotype F based on Bass mtDNA sequence of 384 base pairs. Forty-nine of the 51 Guadeloupe samples collected were EiA009/F/c when analyzed with 480 base pairs. **Data for the F haplotype in the Puerto Rico nesting site was sequenced as EiA011/PR1 using 480 base pairs in Diaz-Fernandez et al. (1999).

Location	EIA001 (A)	EIA003 (B)	EIA005 (C)	EIA006 (D)	EIA008 (E)	EIA009 (F)	EIA011 (PR1)(G)	EIA012 (H)	EIA013 (I)	EIA014 (J)	EIA016 (K)	EIA017 (L)	EIA018 (M)	EIA019 (N)	EIA020 (O)	EIA021 (P)	EIA022 (Q)	EIA023	EIA030	n
Belize	-	-	-	-	-	11	-	-	1	-	1	-	1	-	-	-	-	-	-	14
Mexico	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	64	-	-	67
Puerto Rico	1	-	-	-	-	1*	12*	-	-	-	2	1	2	2	12	2	-	-	-	22
US Virgin Is.	3	-	-	-	-	28	-	-	-	-	-	-	-	-	-	-	-	-	-	31
Antiqua	9	4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15
Barbados	11	-	-	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15
Cuba	62	-	-	-	-	-	-	-	5	-	-	-	-	-	-	1	1	70
Guadeloupe	2	-	-	-	-	51*	-	-	2	-	-	-	-	-	-	-	-	-	-	55

ANALYSES AND DISCUSSION

Part I: Isolation of Rookeries

Table 2

Fst values (above diagonal) and level of significance (below diagonal) for pairwise comparisons among nesting sites based on 3024 permutations. Levels of significance are denoted as "*" for p-values < 0.05, "**" for p-values < 0.01 and "***" for p-values < 0.0001.

Location	Guadeloupe	Belize	Mexico	Puerto Rico	Virgin Islands	Antigua	Barbados	Cuba
Guadeloupe		P=0.109	P=0.000	P=0.000	P=0.545	P=0.000	P=0.000	P=0.000
Belize	-		P=0.000	P=0.003	P=0.174	P=0.000	P=0.000	P=0.000
Mexico	***	***		P=0.000	P=0.000	P=0.000	P=0.000	P=0.000
Puerto Rico	***	***	***		P=0.000	P=0.000	P=0.000	P=0.000
Virgin Islands	-	-	***	***		P=0.000	P=0.000	P=0.000
Antigua	***	***	***	***	***		P=0.101	P=0.000
Barbados	***	***	***	***	***	-		P=0.001
Cuba	***	***	***	***	***	***	*	P=0.027

Pairwise comparisons suggest:

- Homogeneity among nesting populations at Guadeloupe, Belize and USVI, suggesting shared recent common ancestry
- Homogeneity between Antigua and Barbados (Bass et al. 1999) populations confirmed
- Remaining nesting sites represent distinct stocks
- Significant differences between Guadeloupe and Antigua (approx. 130 km) indicate nest site fidelity and regionalized sub-structuring
- Sequencing of 480 base pairs allows for more complete genetic distinction among rookeries

Part 2: Assignment of Rookeries to Foraging Sites

Bayesian methods using Markov Chain Monte Carlo (MCMC) estimations were used to re-analyze published data in order to determine if stock contributions from the Guadeloupe nesting colony could be detected in three main foraging areas; Mexico, Puerto Rico, and Cuba. Analysis was first conducted using rookery data presented in both Bass et al. (1999) and Diaz-Fernandez et al. (1999). Since the Diaz-Fernandez et al. control region sequences were longer than the sequences presented by Bass et al. and revealed several additional haplotypes, the data presented in the Diaz-Fernandez paper were initially combined to use the haplotype nomenclature presented in Bass. New nomenclature for published Hawksbill sequences in this region proposed by Alberto Abreu (unpublished) is represented in Table 1 as EiA001, EiA002 etc. The results presented should be interpreted with caution due to errors associated in the estimates.

MSA using 384 bp sequence fragments:

- Re-analysis indicates Guadeloupe is a potential source rookery for the Cuba and Puerto Rico foraging populations
- The addition of the Guadeloupe nesting colony decreased several of the stock contribution assignments to the Cuba and Puerto Rico foraging colonies
- Contributions to the Mexico foraging area remained relatively consistent with numbers previously reported

Table 3

Various estimates of nesting stock contributions to the Puerto Rico foraging area based on haplotype frequencies from 384 and 480 bp mtDNA fragments as published in Bass et al. 1999 and Diaz-Fernandez et al. 1999. Results using Bayesian MSA are also included.

Location	Contribution Rates using GIRLSEM Bass et. al 1999	Bass et. al 1999 standard deviation	Contribution Rates using ratios *Diaz-Fernandez et al. 1999	Bayes analysis using 384 bp region	Standard deviation (384 bp)	Bayes analysis using 480 bp region	Standard deviation (480 bp)
Belize	0.0344	0.0294	Not compared	0.0452	0.1075	Not compared	Not compared
Mexico	0.1353	0.0296	0.1000	0.1134	0.0387	0.1016	0.0431
Puerto Rico	0.1376	0.0382	0.4100	0.1306	0.0546	0.5015	0.0730
US Virgin Is.	0.4150	0.0625	Not compared	0.1959	0.2126	Not compared	Not compared
Antigua	0.0414	0.0270	Not compared	0.0054	0.0190	0.0644	0.0947
Cuba	0.2361	0.0461	0.2900	0.2266	0.1233	0.2106	0.1218
Barbados	0.0000	0.0000	0.0000	0.0614	0.1107	0.0370	0.0834
Guadeloupe	Not reported	Not reported	0.2000	0.2215	0.1958	0.0849	0.0319

Table 4

Various estimates of nesting stock contributions to the Cuban foraging area based on haplotype frequencies from 384 and 480 bp mtDNA fragments as published in Bass et al. 1999 and Diaz-Fernandez et al. 1999. Results using Bayesian MSA are also included.

Location	Contribution Rates using GIRLSEM Bass et. al 1999	Bass et. al 1999 standard deviation	Contribution Rates using ratios *Diaz-Fernandez et al. 1999	Bayes analysis using 384 bp region	Standard deviation (384 bp)	Bayes analysis using 480 bp region	Standard deviation (480 bp)
Belize	0.0821	0.0700	Not compared	0.0127	0.0253	Not compared	
Mexico	0.0545	0.0306	0.1100	0.1069	0.0232	0.0476	0.0188
Puerto Rico	0.0604	0.0308	0.2700	0.1483	0.0193	0.3193	0.0556
US Virgin Is.	0.0870	0.0929	Not compared	0.1168	0.0913	Not compared	Not compared
Antigua	0.0483	0.0464	Not compared	0.0109	0.0182	0.0090	0.0136
Cuba	0.6673	0.0850	0.5000	0.5209	0.0464	0.5618	0.0578
Barbados	0.0000	0.0000	0.0000	0.0027	0.0083	0.0028	0.0075
Unknown	Not reported	-	0.1200	Not compared	-	-	-
Guadeloupe	Not compared	-	Not compared	0.0807	0.0809	0.0595	0.0180

Estimates of stock composition at the Puerto Rico, and Cuba foraging areas were also re-analyzed using the longer sequence versions reported in Diaz-Fernandez et al. (1999). However, Belize and the USVI had to be dropped from the analysis because these data were based on Bass's shorter sequences (384 bp).

MSA using 480 bp sequence fragment indicate:

- Assignment of Cuba and Puerto Rico foraging animals among remaining nesting stocks were redistributed due to the longer sequence data, most notably:
  - Guadeloupe continues to contribute to Cuban and Puerto Rican foraging areas, however contributions to Puerto Rico were reduced (Tables 3 & 4)
  - Cuba foragers had a reduced assignment to Mexico (Table 4)
  - Assignment to Puerto Rico increased for both Cuba and Puerto Rico foraging sites (Tables 3 & 4)
  - Cuba nesting site contributing over 50% to Cuba foraging area which is consistent with previous published results (Table 4)
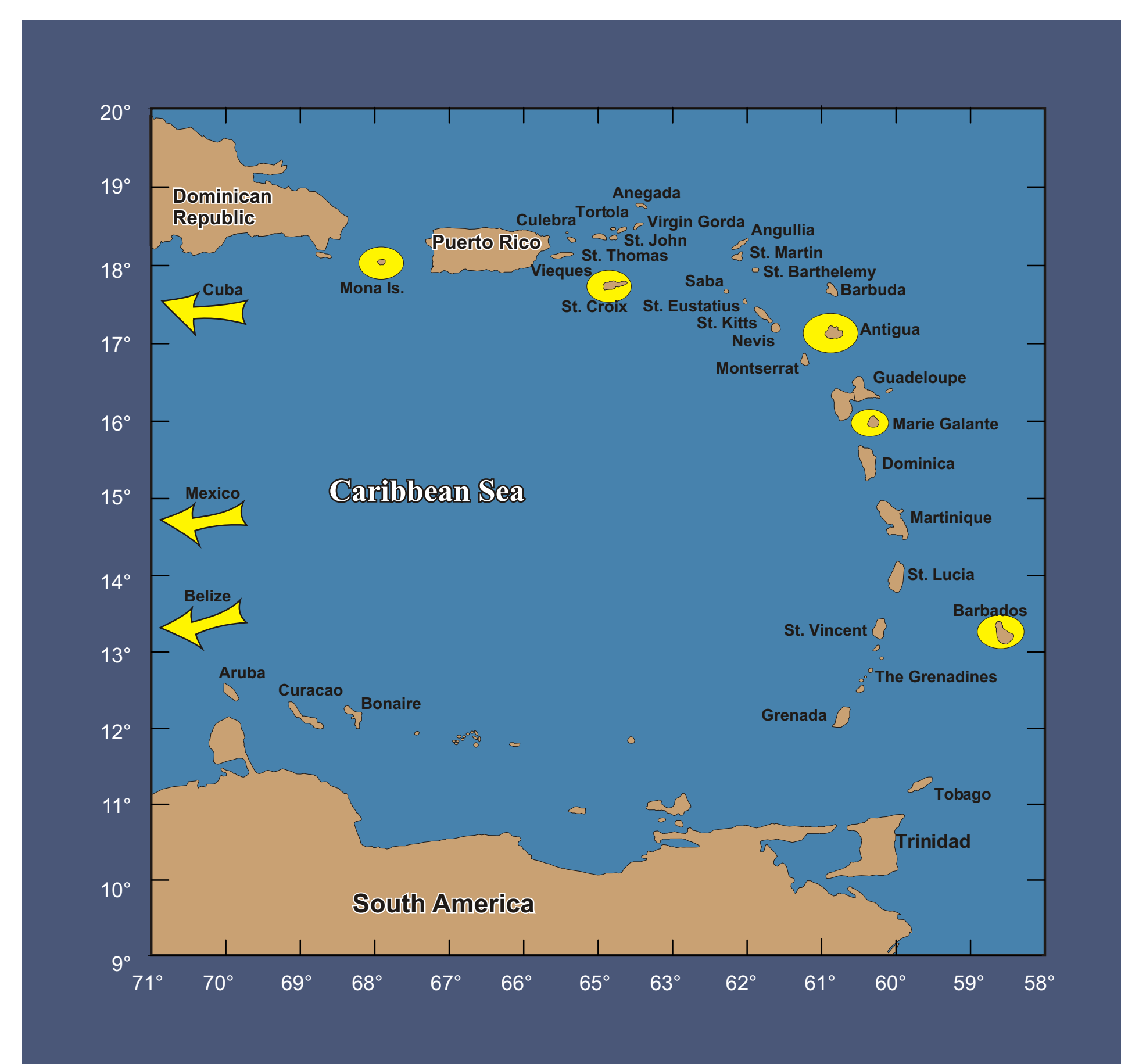 - Contributions from Puerto Rico to Puerto Rico foraging area increased.

IMPLICATIONS FOR CONSERVATION

While our results further confirm earlier studies that show several of the hawksbill nesting populations within the Caribbean are genetically distinct suggesting strong nesting site fidelity, they also show the Guadeloupe rookery is genetically indistinguishable from Belize and USVI based on current mtDNA data. However, this does not necessarily imply contemporary demographic homogeneity, since these results may indicate recent common ancestry, rather than ongoing gene flow. Until genetic characterizations of all potential rookeries are complete, a precautionary approach should be taken by treating rookeries as unique management units. Recovery plans should also address the possibility that previously undetected rookeries contribute to foraging populations in Cuba and Puerto Rico. Our results do not necessarily show that Guadeloupe is the real source of some of these animals, but that inclusion of new rookery data can influence the outcome of MSA. Large scale efforts to collect samples for genetic analysis are needed before these regional stock boundaries can be accurately characterized.

CONCLUSION

The results illustrate the potential use of mtDNA as a tool for conservation of hawksbills in the Caribbean. However, further genetic studies which include samples from all potential source stocks are needed. Increasing the sample size from existing source stocks is also critical for the analysis to become more clearly defined. Current projects to analyze the control region of previous samples collected with longer sequence data are also underway. This fine scale resolution of haplotypes combined with data from multiple nuclear markers will enable us to better address the questions of hawksbill nesting and foraging ecology using molecular genetic techniques.

LITERATURE CITED

Bass, A.L. (1999) Genetic Analysis to elucidate the natural history and behavior of hawksbill turtles (Eretmochelys imbricata) in the Wider Caribbean: a review and re-analysis. Chelonian Conservation and Biology 3(2):195-199.

Bowen B.W., A.L. Bass, A. Garcia-Rodriguez, C.E. Diez, R. van Dam, A. Bolten, K.A. Bjorndal, M.M. Miyamoto and R.J. Ferl (1996) Origin of hawksbill turtles in a Caribbean feeding area as indicated by genetic markers. Ecological Applications 6(2) 566-572.

Chevalier J., D. Guyader, E. Boitard, F. Creantor, E. Delcroix, M. Deries, T. & X. Deville, S. Guilloux, L. Nelson, C. Pavis, M. Roulet, J. Seman, and B. Thuaire (2002) Discovery of an important hawksbill turtle (Eretmochelys imbricata) nesting site in the Lesser Antilles: Trois Ilets beach in Marie Galante (Guadeloupe Archipelago - French West Indies). Proceedings of the 22nd Annual Sea Turtle Symposium. U.S. Dept of Commerce. In Press.

Diaz-Fernandez, R., T. Okayama, T. Uchiyama, E. Carrillo, G. Espinosa, R. Marquez, C. Diez, and H. Koike (1999) Genetic sourcing for the hawksbill turtle, Eretmocheyls imbricata, in the northern Caribbean region. 3(2) 296-300.

Dutton, P.H., B.W. Bowen, D.W. Owens, A.R. Barragan, and S. Davis. (1999). Global phylogeography of the leatherback turtle (Dermochelys coriacea). J. Zool. 248, 397-409.

Masuda, M. (2002) BAYES: Bayesian Stock-Mixture Analysis Program. National Marine Fisheries Service, Alaska Fisheries Science Center, Auke Bay Laboratory, Juneau, AK

Schneider, S., D. Roessli, and L. Excoffier (2000) Arlequin ver. 2.000: A software for population genetic data analysis. Genetics and Biometry Laboratory, University or Geneva, Switzerland.