GENETIC ASSESSMENT OF SEA TURTLES FORAGING AGGREGATIONS OF BONAIRE, NETHERLANDS ANTILLES

Final Report submitted to Sea Turtle Conservation Bonaire

(Pictures by Robert P. van Dam)

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Abstract

As a result of dispersal and migratory behavior, sea turtles establish a complex interconnection between their original natal rookeries and their developmental and foraging grounds. Elucidating this interconnection is of vital importance as it provides a regional perspective of conservation priorities and initiatives to recover and maintain their populations. In addition it provides important clues to gain a better understanding of the migratory behavior of sea turtles and the factors influencing their spatial and temporal distribution. In this study, we assessed the genetic variation of a section of the mtDNA control region to investigate the genetic diversity and origin of the green sea turtle and hawksbill sea turtle foraging aggregations (FAs) of Bonaire, Netherlands Antilles. Furthermore, we conducted a mixed stock analysis with a hierarchical Bayesian approach to estimate the most likely future contribution of the Bonaire FAs to adult recruitment of Caribbean and Atlantic nesting rookeries. After analyzing 169 individuals (hawksbills=75, greens=94) a relatively high genetic diversity characterizing both aggregations was observed, with the green aggregation exhibiting the highest genetic diversity so far observed for a foraging aggregation. Evidence was obtained that confirms the cosmopolite character of the FAs with individuals exclusively from Caribbean rookeries recruiting to the hawkbill foraging grounds. Interestingly, a contribution of rookeries from South Atlantic and probable from West Africa was suggested for the green turtle foraging ground. This confirms the migratory capabilities of the green sea turtles and provides the framework to further test hypotheses about the mechanism influencing such long-distance dispersal. Results from this study not only confirm the regional connectivity established between the Island of Bonaire and the rest of the Caribbean nesting rookeries but also reveals that recruitment from rookeries far as Africa exists and highlights the importance of a regional collaboration to conserve these areas as they represent the future of reproductive recruitment.

Introduction

As a result of complex behavior sea turtles establish multiple connections between their natal rookeries, reproductive areas and foraging grounds during their life cycle (Velez-Zuazo et al. 2008a). Right after hatching, sea turtles engage in a pelagic journey drifting with oceanic currents while associated to "living rafts" most likely composed of *Sargassum sp* (Carr and Meylan 1980). For some species like green sea turtles (*Chelonia mydas*) and hawksbill sea turtles (*Eretmochelys imbricata*) this pelagic journey ends after approximately two years when recruiting to benthic habitats for foraging and development. In these benthic areas they will establish permanently until reaching adulthood when starting regular migrations back and forward from the feeding grounds to their original natal grounds to breed and nest. In this way, sea turtles move from foraging to breeding to nesting grounds at a constant basis and during these complex dynamics of dispersal and migration the occupy both marine and terrestrial habitats and determine levels of connectivity among these grounds.

The use of molecular tools have made possible to elucidate the connections established by sea turtles during their life history and have provided relevant insights into their migratory behavior. Investigations of nucleotide variation in the mitochondrial genome supported the hypothesis that nesting grounds have a distinctive genetic signature as a result of females' philopatric behavior (Meylan et al. 1990, Bass et al. 1996). In addition, genetic analyses have provided evidence that the foraging grounds are composed by individuals from diverse origin (Lahanas et al. 1998, see review for hawksbill sea turtles in Bowen et al. 2007) and that adult males exhibit natal phylopatry when recruiting to their breeding grounds (Fitzsimmons et al. 1997, Velez-Zuazo et al. 2008). Therefore, the use of genetic analysis allows making inferences about important aspects of the biology and ecology of organisms and in this specific case about the migratory behavior of sea turtles.

While nesting females and adult breeding males exhibit natal phylopatry, the migratory behavior of young sea turtles is less understood and there is no conclusive evidence to

explain if recruitment from pelagic habitats to benthic habitats is the result of life-history traits alone, in combination with extrinsic factors (i.e. environmental parameters), or the result of alternative mechanisms. In this light, genetic studies on foraging grounds can help to shed light about the most likely factors influencing sea turtle recruitment.

There are numerous identified foraging grounds for the green and hawksbill sea turtle in the Caribbean, however, given the abundance of sea grass and coral reef ecosystems in the Caribbean it very likely that more critical areas for sea turtle foraging and development exist. Since 2003, the Sea turtle Conservation Bonaire (STCB) has been conducting in-water surveys to identify sea turtles foraging grounds around the island of Bonaire (see Figure 1). Multiple observations of green and hawksbills sea turtle have been recorded. Their sampling efforts, however, have been focused mainly in the area of Lac Bay due to the abundance of sea turtles in that area. Lac Bay, located in the southeast of Bonaire, is composed of an inner area characterized by the presence of sea grass beds with scarce coral reef formations and an outside area composed of a barrier of coral reef. In these two areas high abundance of green and hawksbill sea turtles have been recorded. Last year only around 129 green turtles and 70 hawksbill turtles were captured in Lac Bay and this represents a slightly increment compared to previous years (STCB 2007). Given the abundance observed in this area and compared with other foraging grounds in the Caribbean, Lac Bay represents an important foraging ground for these two species of sea turtles. Furthermore, the island of Bonaire and the foraging grounds identified around it are situated in the southern most end of the Caribbean and represent a unique opportunity to investigate the recruitment patterns of pelagic sea turtles to benthic areas and discuss the importance of these foraging grounds for future adult recruitment (Velez-Zuazo et al. 2007).

In this study, I used the sequence variation of the mitochondrial DNA to investigate the genetic diversity and structure of the foraging aggregations of green and hawksbill sea turtles from Bonaire. In addition, I combined the genetic information obtained in this study with genetic diversity and structure data from nesting rookeries and foraging grounds in the Caribbean and South Atlantic to elucidate the most likely origin of the

green and hawksbill turtle's aggregations and to estimate the future contribution of these aggregations for future adult recruitment. The genetic information obtained in this study will help to further hypothesize about the most likely mechanisms influencing sea turtle recruitment into benthic habitats. This will provide us a better understanding of the connectivity established between rookeries and foraging grounds within the Caribbean.

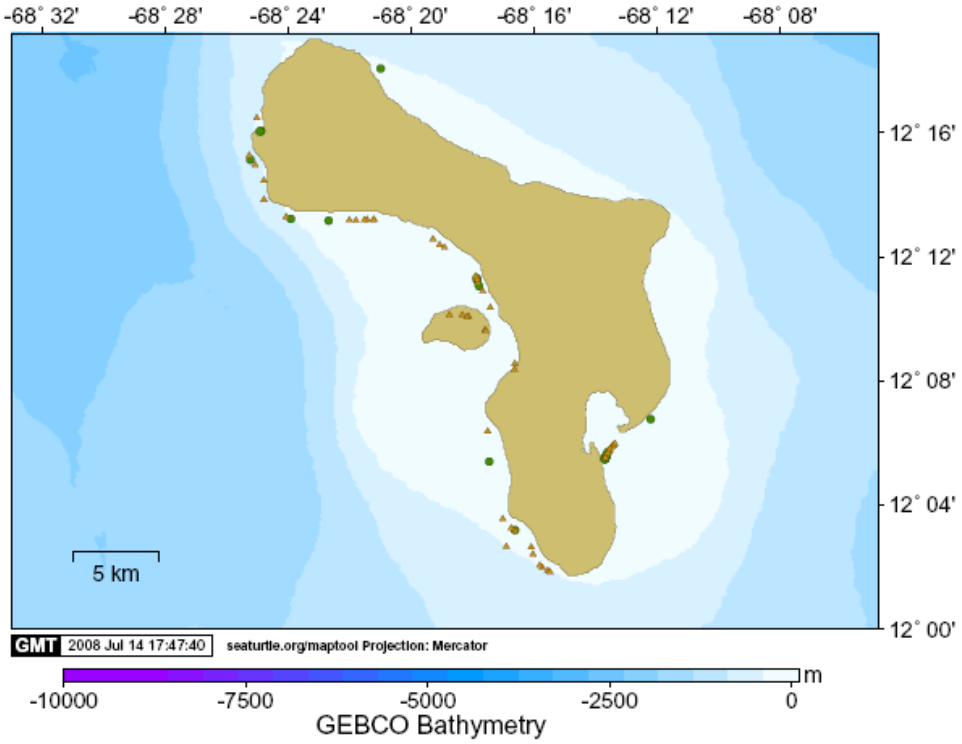

Figure 1. Map of the Island of Bonaire, Netherland Antilles showing the geographic positions were foraging green sea turtles (green circles) and hawksbill sea turtles (orange triangles) have been observed and samples during in-water surveys conducted by STCB since 2003.

Sea turtle foraging grounds are not only aggregations of individuals from different natal origin but also at different developmental stages (i.e. recruit, juveniles, and adults). For these reasons, foraging grounds are priority areas for conservation since they represent the source of future adult recruitment for many reproductive aggregations and because as potential mating areas they can affect gene flow dynamics among populations and therefore influence the structure of their populations. Given this, it is priority to characterize genetically the foraging grounds to have a better understanding of the level

of connectivity between these areas and the natal grounds to design realistic conservation programs both locally and regionally.

Aims of this study

- To elucidate the rookery of origin of the mixed aggregation of juvenile hawksbill and green sea turtles using a molecular approach
- To estimate the relative contribution of the rookeries of origin to the juvenile aggregations
- To investigate the future contribution of the juvenile aggregations to the adult aggregations in the Caribbean
- To increase knowledge of the most likely migratory pathways influencing the recruitment to the feeding habitats of Bonaire
- To increase the knowledge about the sea turtles of Bonaire to improve conservation practices both locally and regionally

Methods

Study site and sample collection

The island of Bonaire, along with the Island of Curacao, Saba, Sint Eustatius and Sint Marteen comprises the Netherlands Antilles. In all these islands nesting or foraging aggregations of sea turtles are largely observed; however, the Island of Bonaire is the area with the highest density of sea turtles reported. Here, the Sea Turtle Conservation Bonaire (STCB) established a conservation program that aims to protect the sea turtles through the monitoring of their nesting and foraging. Foraging aggregations are monitored by conducting in-water surveys and collecting relevant biological information of each encountered individual (i.e. size, weight, and health condition) as well as environmental parameters. Detailed information about the in-water survey methodology and study area can be found in the progress reports submitted by STCB (2006, 2007).

During 2006 and 2007, tissue samples were collected from each sea turtle captured by hand or by entanglement. A 4mm tissue plug was obtained from the right shoulder area of each individual with a disposable biopsy punch (Acuderm®) or obtained from the front flipper during application of plastic Roto-tags. In all cases, the samples was preserved either in 95% ethanol or in salt-saturated 20%DMSO-20% EDTA and stored at room temperature.

mtDNA genotyping

Genomic DNA was isolated using a QIAGEN® DNeasy kit for blood and tissue samples following the manufacturer instructions and final elutions were made in 50µl of AE buffer. To confirm successful isolation, 1µl of the elution was run in a 0.8% agarose gel along with 2µl of gel star dye and 1µl of loading buffer 5X and the spectrophotometer NanoDrop (Thermo Fisher Scientific) was used to quantify DNA yield concentration (ng/µl). Amplification by the polymerase chain reaction (PCR) was conducted using primers LTEi9 and H950 (A Abreu-Grobois et al. 2006). This primer combination targets approximately 800bp of the mtDNA control region improving sequences resolution as it covers the largest fragment so far sequenced for sea turtle genetic studies. For a 10ul PCR reaction we combined 1 µl of 10ng/µl genomic DNA, 4µl of QIAGEN Taq Master mix, 0.5µM of each primer, and ultrapure water and place the mix on a BIO-RAD thermocycler under the following cycling conditions: an initial denaturing step of 5min at 94ºC, followed by 30 cycles of 30sec at 94ºC, 30sec at 52ºC, and 1min at 72ºC. Finally, an extension step of 5min at 72ºC was added. To confirm successful amplification of target fragment, 1ul of PCR product was run by electrophoresis in 1% agarose gel. Final concentration of PCR product was estimated by running in the same gel 2ul of Low DNA Mass Ladder (Invitrogen). Amplified fragments were sequenced in both directions using the PCR primers with ABI Big-dye® terminator chemistry on an automated station ABI 3130XL sequencer (Applied Biosystems) and run on the automated sequencer station ABI 3130xl (Applied Biosystems) and the forward and reverse sequences were assembled and aligned by eye using SEQUENCHER 4.5 (Gene Codes) and later exported to MEGA version 4 to conduct molecular evolutionary analyses (Tamura, Dudley, Nei, and Kumar 2007).

Diversity estimates and mixed stock analyses

To identify unique haplotypes and estimate absolute haplotype frequencies we used DNAsp (Rozas et al. 2003). Unique haplotypes of each turtle species were compared with previously identified haplotypes in other nesting and foraging aggregations using the Basic Local Alignment Search tool (BLAST) from the National Center for Biotechnology information (http://www.ncbi.nlm.nih.gov/), the Green turtle genetic database maintained by the Archie Carr Center for Sea Turtle Research (http://accstr.ufl.edu/) and the Hawksbill sea turtle genetic database maintained by Dr. Alberto Abreu (UNAM, Mexico) and named accordingly.

To conduct molecular evolutionary analyses first we estimated the most likely model of molecular evolution that better explains the pattern of sequence variation in our samples using Modeltest implemented in PAUP (Posada and Crandall 1998). To estimate the diversity of haplotypes, genetic (h) and nucleotide diversity (π) we used the program Arlequin version 3.1 (Excoffier et al. 2005). Arlequin was also used to identify the number and type of nucleotide substitutions on the mtDNA sequences.
In addition, I was interested in testing if there were significant differences in the genetic structure and diversity of the foraging aggregations of Bonaire compared to other foraging aggregations in the Caribbean. For this purpose Arlequin was used to conduct a pairwise analysis using F-statistics to measure the genetic structure of the foraging aggregations (Wright 1969).

To estimate the most likely origin of the turtles from each foraging aggregation a mixed stock analysis (MSA) was conducted using Mixstock (Bolker et al. 2007). Mixstock is a library written in R-language and executable using the program R (www.r-project.org) that uses a hierarchical Bayesian approach to estimate, from a pool of potential origins, the most probable rookeries of origin of a given mixed aggregation (i.e. foraging centric) and to estimate their contribution. In addition, Mixstock offers the opportunity to conduct a reverse analysis ("many to many" analisis) to estimate the most likely total contribution of a given rookery to a mixed aggregation that can also be interpreted as the contribution of that mixed aggregation to the future recruitment of a given rookery (i.e.

rookery centric). To conduct the analyses, all the available genetic information from nesting rookeries and foraging aggregations of the green (Encalada et al. 1996, Lahanas et al. 1998, Luke et al. 2004, Bass et al. 2006, Formia et al. 2006, Naro-Maciel et al. 2007, Velez-Zuazo 2007) and hawksbill (Bass et al. 1996, Bowen et al. 1996, Diaz-Fernandez et al. 1999, Bowen et al. 2007, Velez-Zuazo et al. 2008) sea turtle was compiled and used as baseline information (see Table 1). Rookery size, in the form of number of yearly nesting females (Seminoff 2004, Mortimer and Donnelly 2007), was used to establish weighted priors and five chains were set to start the estimations with 12000 iterations for each chain. Convergence of the chains was confirmed if a value less of 1.2 was obtained after running a Gelman-Rubin diagnostic; otherwise, an increment of 5000 iterations was made every time was necessary until convergence of the chains was obtained.

Table 1. Genetic diversity and structure of nesting rookeries and foraging grounds of the hawksbill (A) and green sea turtle (B) in the Caribbean. Haplotype information is provided for mtDNA fragments of 380bp length for the two species data set. This information was used as baseline and input data to conduct mixed stock analysis of the foraging aggregations of Bonaire Island. Population size is represented as the average number of nests deposited yearly in every rookery

(A) Nesting rookeries: CUB (Cuba), MON (Mona Island, PR), USVI (Buck Island, US Virgin Islands), ATG (Antigua), BRB (Barbados), CR (Costa Rica), BLZ (Belize), MX (Mexico). Foraging grounds: BNR (Bonaire), Mon (Mona Island), US (Texas), BHM (Bahamas), CUBa (Cuba Southeast), CUBb (Southwest), CUBd (Northeast), DR (Dominican Republic), USVI (US Virgin Islands), MX (Mexico).

	Nesting rookery								Foraging aggregation									
HP (380bp)	**CUB**	**MON**	**USVI**	**ATG**	**BRB**	**CR**	**BLZ**	**MX**	BNR	Mon	US	BHM	CUBa	CUBb	CUBd	DR	USVI	MX
A	62	1	5	9	20				51	38		28	28	46	18	42	28	
B			1	4						2		1			1		2	1
C				2														
D					1													
E					3													
F	1	71	43			33	11		15	62	3	20	6	34	13	30	17	2
G						6	1		1						1	2		
H							1											
I							1											
L		1				4			1	3				1	2	1		
N		32								8		2		10	3		6	
O		6																
P								3				1						2
Q		2						64	4	18	38	21	3	11	8	6	9	13
BI1			1					1										
alpha						10			1	5		1	2	1	2	6	3	
gamma	5												1	5	5		1	
CU3	1					4						3	2	2	2			
CU4	1																	
Mx1a								1			1							
n	**70**	**113**	**50**	**15**	**24**	**57**	**14**	**69**	73	136	42	77	42	110	55	87	66	18
Rookery size	1400	697	196	438	1691	35	28	1869										

(B) Nesting rookeries: MX (Mexico), FL (Florida), CR (Tortuguero, Costa Rica), AV (Aves, Venezuela), GNC (Guanacabibes, Cuba), SF (San Felipe, Cuba), SUR (Surinam), TRI (Trindade, Brazil), RC (Rocas, Brazil), ASC (Ascensión Islands), GUI (Guinea Bissau), SAOT (Sao Thome). Foraging grounds: BNR (Bonaire), EcFL (East Central Florida), NC (North Carolina, US), NIC (Nicaragua), BRB (Barbados), CULB (Culebra, PR), UTAT (Ubatuba, Brazil), ALM (Almofala, Brazil), RCS (Rocas, Brazil).

	Nesting Rookeries													Foraging Aggregations									
HP	MX	FL	CR	AV	GNC	SF	SUR	TRI	RC	ASC	GUI	BIOK	SAOT	BNR	BAH	EcFL	NC	NIC	BRB	CULB	UTAT	ALM	RCS
CM1	7	11			3									2	2	12	34	54	7	14			
CM2		1												1		1	2			1			
CM3	5	12	395	3	8	8								40	62	43	43		21	53	2	18	
CM4			1																				
CM5	1		32	27			13						1	39	1	3	5	6	13	20	14	28	5
CM6							1			3		5	1	1								3	2
CM7							1																
CM8								67	36	43	19	45	13	4	1		7		14	6	83	53	13
CM9								19	7										1		4	3	2
CM10									2										2		3	4	
CM11								1	1														
CM12									5														
CM15	1																1						
CM16	1																2			2		1	
CM17	2													2					1	1			
CM18	3															2	3			3			
CM20			2											1	1								
CM21			3											1	3							1	
CM23								6															
CM24								1		1											2	1	
CM26									1								2			1			
CM27					1												2			2			
CM28					1												3						
CM32								4	1												2	1	
CM33								1															
CM35													1										
CM36													1										
CM37													1										
CM38													2										
CM39										1													
CM45										1												1	
CM46										1											1		1
CM48					2	3																	
CM56					1																		
CM57					1																		
n	**20**	**24**	**433**	**30**	**17**	**11**	**15**	**99**	**53**	**50**	**19**	**50**	**20**	91	70	61	104	60	59	103	111	114	23
Rookery size	1600	787	27511	267	150	50	1816	3000	125	3709	2530	454	100										

Results

Hawksbill foraging aggregation

Since 2003, 83 tissue samples were collected from hawksbill turtles around Bonaire (2003=5, 2004=11, 2005=3, 2006=28, and 2007=36). More than half of the samples were collected from different sites around the whole island (n=49) while the rest were obtained in Lac Bay (n=25) and Klein Bonaire (n=9, see Figure 1). From the total of samples collected, 75 (90%) individuals were successfully genetically analyzed and a mtDNA fragment of 740bp was sequenced from each individual. The rest of the samples failed for different reasons that included: absence of tissue on vial, tissues were collected from hatchlings, failure to isolate DNA, failure to amplify mtDNA fragment and failure to sequence mtDNA fragment.

Along the 740bp fragments, 17 polymorphic sites were observed; 16 transitions and 1 transversion, determining 11 different haplotypes. Ei-A01 was the most common

haplotype and it was observed in 64% (n=48) of the samples, followed by haplotype Ei-A11 (10%, n=9) and Ei-A09 (6%, n=5). For comparison purposes, however, we truncated the fragments to 380bp length; the fragment historically explored and for which more genetic information is currently available (see Table 2). As a result, the total number of haplotypes collapsed to seven haplotypes and used to conduct comparative molecular diversity estimates.

Table 2. Mitochondrial DNA haplotype composition at 380bp and 740 bp length fragments for the hawksbill sea turtle foraging aggregation of Bonaire. Detailed information if presented for the two main sites: Klein Bonaire (KB) and Lac Bay (Lac) and well as for the rest of the Island. Total sample sizes represent five (5) years of in-water surveys.

Haplotype			SITE		
380bp	740bp	n	KB	Lac	Rest of BNR
A	EiA01	48	3	17	28
A	EiA51	3	1		2
alpha	EiA02	2			2
F	EiA09	5	2		3
F	EiA11	9		5	4
F	EiA63	1			1
G	EiA12	1			1
b	EiA28	1		1	
Q	EiA42	3		1	2
Q	EiA43	1			1
L	EiA47	1		1	
n		75	6	25	44

Overall, the foraging aggregation of hawksbill turtles in Bonaire had a relatively low haplotype (h) and nucleotide (π) diversity estimate when compared with other foraging aggregations (Table 3). The nucleotide polymorphisms observed in the mtDNA fragments were analyzed to obtain the best model of molecular evolution that best explain the nucleotide substitution pattern suggesting the Tamura-Nei model (Trn, Tamura and Nei 1993). This model was used to conduct the analysis of molecular variance (AMOVA) among feeding grounds.

Table 3. Molecular diversity estimates for all the foraging aggregations of hawksbill sea turtles genetically surveyed in the Caribbean. Information included sample size (n), number of haplotypes (HP), haplotype diversity (h) and nucleotide diversity (π) as well as their respective standard deviation values (SD)

Foraging Ground	Country	n	HP	h	±SD	π	±SD
Texas	US	42	3	0.1800	0.0768	0.0005	0.0007
Bahamas	Bahamas	78	9	0.7396	0.0238	0.0091	0.0052
Southeast	Cuba	43	7	0.5592	0.0834	0.0080	0.0047
Southwest	Cuba	111	9	0.7204	0.0273	0.0100	0.0056
Northeast	Cuba	56	11	0.8214	0.0311	0.0101	0.0057
Jaragua	Dominican Republic	90	9	0.6687	0.0337	0.0099	0.0056
Mona Island	Puerto Rico	138	9	0.7050	0.0267	0.0090	0.0051
Buck Island	US Virgin Island	69	10	0.7575	0.0354	0.0120	0.0066
Bonaire	Netherland Antilles	73	6	0.4726	0.0597	0.0076	0.0045
Rio Lagartos	Mexico	21	6	0.6095	0.1114	0.0021	0.0018

The analysis of molecular variance indicated a low but significant (*P-value*<0.001) genetic structure among foraging grounds, with 13% of the variation distributed among aggregations while the most of the genetic diversity (87%) was estimated to be distributed within aggregations. This can be explained with the results of F_{st} analysis and the exact test of differentiation where there seems to be groups of aggregations with similar genetic diversity and composition. For example, the hawksbill aggregation from Bahamas was statistically indistinguishable from the aggregations of Cuba (Northeast) and Buck Island (USVI). A possible explanation for these genetic similarities observed among foraging aggregations has been proposed in early studies and is related to the effect that oceanic currents can have on dispersal patterns during the early pelagic years experienced by young turtles before recruitment (Luke et al. 2004).

The results from the mixed stock analysis confirmed that the hawksbill foraging aggregation of Bonaire is a mix aggregation composed of individuals from diverse rookeries of origin. The rookery-centric MSA suggested that the Bonaire hawksbill aggregation is composed by approximately 60% individuals recruiting from the Barbados nesting rookery and in less but still important extent by individuals recruiting from the Cuban rookery (20%). There was an estimated contribution of other rookeries; however, in negligible extent that made up the left 20% of the composition (see Figure 2).

The foraging-centric MSA also suggested a strong genetic connectivity between the Bonaire hawksbill foraging aggregation and the Barbados rookery estimating a contribution of the Bonaire rookery to the 20% of total adult recruitment (see Figure 2). In lesser extent, but still important, the Bonaire rookery is suggested to contribute similarly to the adult recruitment to rookeries as close as Antigua, USVI and Costa Rica and as far as Belize and Cuba. These results highlight the importance of the hawksbill foraging aggregation from Bonaire for the adult recruitment of close as well as more distant nesting rookeries throughout the Caribbean.

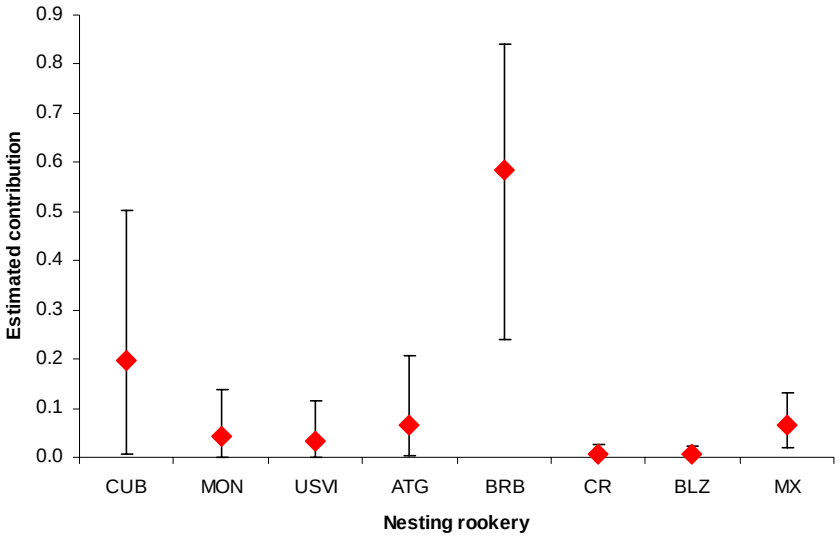

Figure 2. Most likely contribution of the Caribbean hawksbill nesting rookeries to the foraging aggregation of Bonaire Island as estimated with mixed stock analysis. The MSA was conducted using rookery size as *a-priori* information to improve estimations. Point estimations are in orange polygons and bars indicate the 2.5% (upper) and 97.5% (lower) bounds of the 95% confidence intervals.

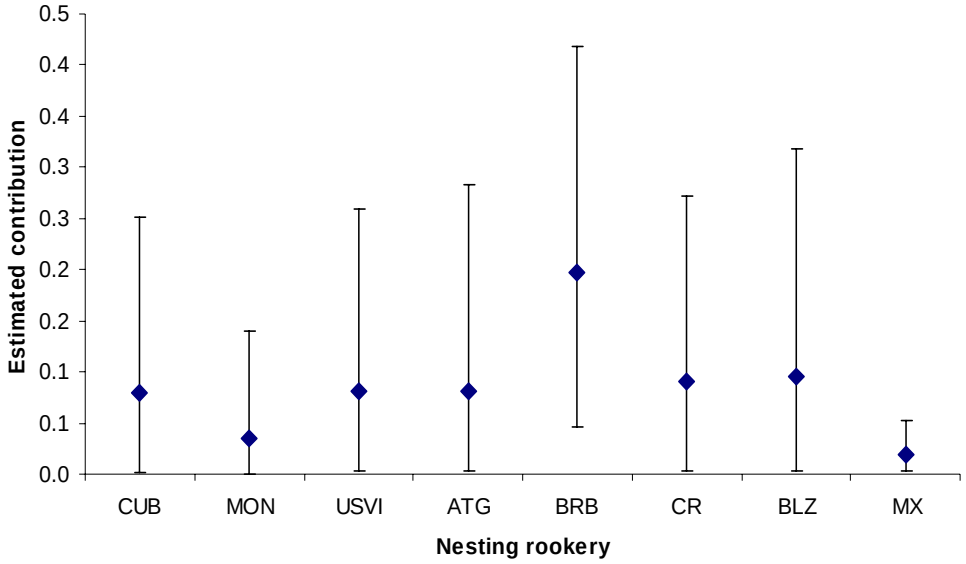

Figure 3. Most likely contribution of the Bonaire hawksbill foraging aggregation to adult recruitment of Caribbean nesting rookeries as estimated by the foraging-centric mixed stock analysis. Point estimations are in blue polygons and bars indicate the 2.5% (upper) and 97.5% (lower) bounds of the 95% confidence intervals.

Green turtle foraging aggregation

As a result of in-water surveys conducted around the Island of Bonaire, 97 foraging green sea turtles were sampled for genetic analyses (*2004*=1, *2005*=4, *2006*=51, *2007*=40, and *ND*=1). Approximately 85% (n=82) of the total were surveyed in Lac Bay while the rest of green turtles were sampled in other areas (n=14) and only one individual was sampled in Klein Bonaire. Genetic analysis, however, were completed successfully for 94 individuals due to similar troubleshooting experienced with the hawksbill sample set.

MtDNA fragments of 751bp were sequenced and aligned by eye allowing the identification of 21 polymorphic sites (20 transversions and one transition) determining 12 unique haplotypes. All haplotypes have been previously observed and reported in nesting rookeries and foraging grounds in the Caribbean and Atlantic basin (See Table 1). Similarly to the hawksbill data set, all mtDNA fragments were truncated to the historically 380bp fragments to allow comparisons of diversity and composition among rookeries and foraging grounds. However, the number of haplotypes remained the same,

contrasting with the hawksbill haplotype data set, where it was a significant reduction of haplotype diversity.

The green turtle foraging aggregation genetic composition of Bonaire was mostly characterized by two haplotypes: CM-A3 (n=40) and CM-A5 (n=39) in almost equal frequency that made the 84% of the total genetic diversity. Nevertheless, the overall haplotypic diversity was relative high (h=0.6502) and over the average when compared to the mean haplotypic diversity of Caribbean green turtle foraging grounds (h= 0.5679). This was due to the presence of additional haplotypes in very low frequency (See Table 1). In addition, the diversity of haplotypes and the number of polymorphic sites resulted in the highest nucleotide diversity reported so far for a green sea turtle foraging aggregation in the Caribbean (π= 0.0113, min=0.0021, max= 0.0113, see Table 4). Haplotypes CM-A3 and CM-A5 are common haplotypes characterizing the nesting rookeries of the Caribbean basin and West Atlantic. CM-A3 is the dominant haplotype in Eastern Caribbean rookeries while CM-A5 dominates the genetic composition of rookeries at the west end of the Caribbean (Aves Island) and part of West Atlantic (i.e. Suriname).

Table 4. Estimates of molecular diversity for the foraging aggregations of the Caribbean green sea turtle. In this table is presented information of sample size (n), diversity of haplotypes (HP), haplotypic diversity (h) and nucleotide diversity (π) with the corresponding values of standard deviation (±SD).

Foraging Ground	Country	n	HP	*h*	±SD	π	±SD
Bahamas	Bahamas	79	6	0.3703	0.0650	0.0066	0.0038
East Central Florida	USA	62	6	0.4855	0.0668	0.0032	0.0021
Nicaragua	Nicaragua	60	2	0.1831	0.0621	0.0035	0.0023
Barbados	Barbados	60	8	0.7734	0.0276	0.0105	0.0057
North Carolina	USA	106	12	0.7294	0.0301	0.0054	0.0032
Utatuba	Brazil	113	10	0.4460	0.0556	0.0021	0.0016
Almofala	Brazil	117	13	0.7168	0.0306	0.0067	0.0038
Rocas	Brazil	23	5	0.6443	0.0917	0.0022	0.0017
Bonaire	Netherland Antilles	94	12	0.6502	0.0308	0.0113	0.0061
Culebra	Puerto Rico	103	10	0.6804	0.0397	0.0086	0.0048

Similar to the hawksbill sequences data set, the model that best fitted the pattern of nucleotide substitution was the Tamura-Nei model (TrN, Tamura and Nei 1993). AMOVA estimates indicated that 39.7% of the total genetic diversity is distributed among foraging grounds while the rest (i.e. 60.3%) is distributed within foraging grounds. Still, the analysis of population structure indicated a medium (Fst=0.39732) but highly significant structure among Caribbean foraging grounds ($P<0.001$). In contrast to the structure observed among the foraging grounds of the hawksbill turtle where various aggregations were genetically indistinguishable, the green turtle aggregations presented a distinguished genetic signature with the exception of the Brazilian aggregations (i.e. Utatuba, Almofala and Rocas) that behave as a single foraging aggregation, genetically speaking.

The rookery-centric mixed stock analysis conducted to the green sea turtle foraging aggregation of Bonaire confirmed that it is indeed an aggregation composed of individuals from diverse origins in the Caribbean. As observed for other Caribbean foraging grounds, the higher contribution came from Tortuguero beach (49.9%), the rookery with the highest nesting density of green turtles in the Caribbean. The rookery of Surinam, another important rookery for the green sea turtle also had a relatively high estimated contribution to the Bonaire foraging aggregation (30%). Interestingly, the MSA estimated a contribution of rookeries from South Atlantic (i.e. Brazil and West Africa) that altogether add up to the 10% of the foraging composition. This contrasts with the MSA results from the hawksbill aggregation where no contribution of rookeries in the south Atlantic was detected.

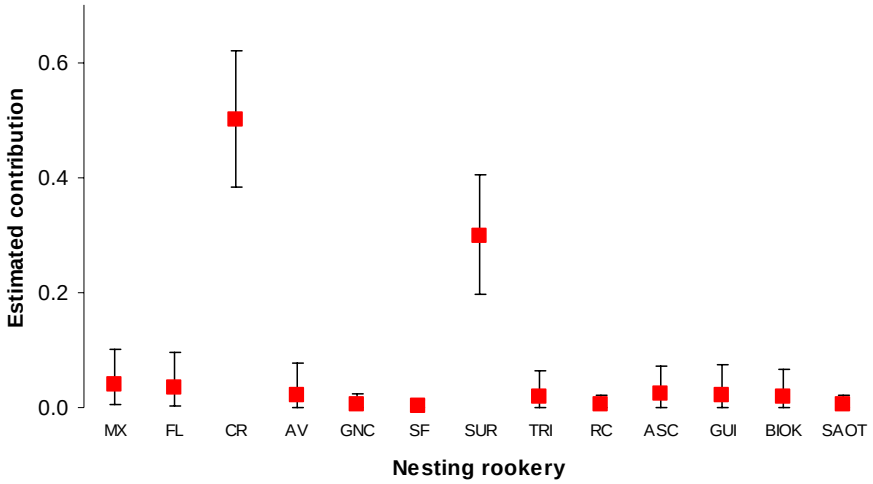

Figure 4. Origin and contribution of green turtle nesting rookeries to the composition of the foraging aggregation of Bonaire. Orange squares indicate MSA point estimates and bars are the upper and lower bounds of the 95% confidence interval.

While the rookery-centric MSA analysis revealed the high density of foraging green sea turtle recruiting from the Tortuguero rookery in Costa Rica, the foraging-centric analysis suggested the rookery of Surinam as the principal rookery where the Bonaire foraging green turtles will recruit as adults. The Bonaire FG also contributes, although in less extent, to the near rookery of Aves (Venezuela) and to rookeries as far as West Africa (i.e. Bioko and Sao Thome).

The best explanation for the low contribution of the Bonaire FG to the adult recruitment of the Tortuguero rookery is the high population density of the latter which receives contribution of numerous foraging grounds but mostly from the Bahamas FG which holds one of the biggest foraging grounds in Caribbean and with a direct interchange of individuals with the Tortuguero rookery as has been previously reported. In general, the population size of the Tortuguero rookery is reflected in its contribution to the Caribbean FGs.

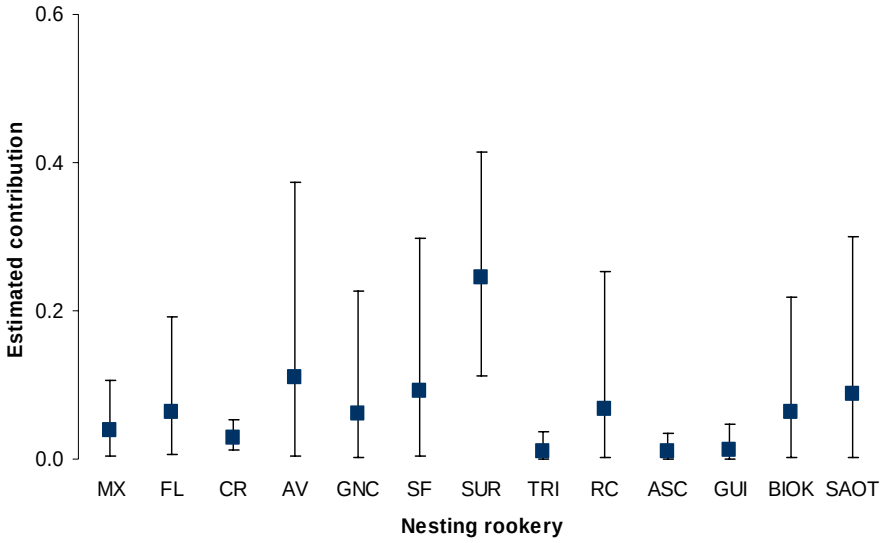

Figure 5. Contribution of the Bonaire foraging ground to the nesting rookeries of the Caribbean green sea turtle as estimated by the foraging-centric mixed stock analysis. Point estimations are showed in blue squares and bars denotes the lower and upper bounds of the confidence interval.

Conclusions

- The Island of Bonaire holds two important foraging aggregations: the green sea turtle and the hawksbills sea turtle foraging aggregations
- Results of genetic analyses indicated high levels of gene diversity for both aggregations. The green sea turtle aggregation, however, exhibited the highest diversity compared to the hawksbill aggregation.
- In addition, the green turtle aggregation exhibited the highest gene diversity ever reported for a green foraging aggregation in the Caribbean.
- The hawksbill aggregation was composed of 11 haplotypes at 740bp and seven haplotypes at 380bp. Two haplotypes (Ei-A01/A and Ei-A11/F) were the most common haplotypes representing almost the 90% of the individuals assessed.
- These two haplotypes are the two most common haplotypes in the Caribbean and dominate the genetic composition of the Caribbean nesting rookeries
- Similarly, the green turtle aggregation was dominated by two haplotypes; CM-A3 and CM-A5 which also characterize most of the green sea turtle nesting rookeries. However, CM-A3 is a dominant haplotype for rookeries located in the Eastern

Caribbean while CM-A5 dominates the genetic composition of rookeries in west Caribbean and West Atlantic.

- At the Caribbean level, there was genetic structure among foraging grounds. Both, the hawksbill and the green sea turtle aggregations were significantly different compared to other conspecific foraging grounds.
- As a result of their genetic composition and structure, the rookery-centric mixed stock analysis indicated that the foraging aggregations of the green sea turtle and hawksbill sea turtle from the Island of Bonaire are composed of individuals from diverse origins in the Caribbean and South Atlantic.
- While the contribution to the hawksbill aggregation comes exclusively from rookeries in the Caribbean basin, the green aggregation receives recruitment from rookeries as far as West Africa as revealed by the mixed stock analysis
- Both foraging aggregations exhibited a similar recruitment pattern that was characterized by the dominant contribution of nesting rookeries harboring high density of nesting females and situated in the close vicinity of the foraging ground.
- The foraging-centric analysis, in the other hand, estimated the importance of the hawksbill foraging aggregation for the future recruitment of turtles to the rookeries of Barbados and Cuba. The same analysis conducted to the green aggregation revealed the influence of this aggregation to the adult recruitment of the rookeries of Suriname and Aves Island.
- In general, the results from this study highlight the complex migratory behavior of sea turtles while providing insights into the patterns of sea turtle recruitment to feeding areas.
- This study elucidated the origin of the sea turtle aggregated in the foraging areas of Bonaire and the genetic connectivity established between Bonaire and the Caribbean and South Atlantic and reinforces the concept of a regional-focused effort to conserve sea turtles and their habitats.

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References

Abreu-Grobois A, Horrock J, Formia A, Dutton P, LeRoux R, Velez-Zuazo X, Soares L and P Meylan. 2006. New mtDNA Dloop primers which work for a variety of marine turtles species may increase the resolution of mixed stock analyses.

Bass AL, Good DA, Bjorndal KA*, et al.* (1996) Testing models of female reproductive migratory behaviour and population structure in the Caribbean hawksbill turtle, Eretmochelys imbricata, with mtDNA sequences. Molecular Ecology 5, 321-328.

Bass AL, Epperly SP, Braun-McNeill J. 2006. Green turtle (Chelonia mydas) foraging and nesting aggregations in the Caribbean and Atlantic: impact of currents and behavior on dispersal. Journal of Heredity. 97:346–354.

Bolker M. Bolker, Okuyama T, Bjorndal KA, and AB Bolten. Incorporating multiple mixed stocks in mixed stock analysis: 'many-to-many' analyses. Molecular Ecology, 16:685–695, 2007

Bowen BW, Bass AL, GarciaRodriguez A*, et al.* (1996) Origin of hawksbill turtles in a Caribbean feeding area as indicated by genetic markers. Ecological Applications 6: 566-572.

Bowen BW, Grant WS, Hillis-Star Z, Shaver DJ, Bjorndal KA, Bolten AB, Bass AL. 2007. Mixed-stock analysis reveals the migrations of juvenile hawksbill turtles (*Eretmochelys imbricata*) in the Caribbean Sea. Molecular Ecology 16: 49-60.

Carr A, Meylan AB. Evidence of Passive Migration of Green Turtle Hatchlings in Sargassum. *Copeia* 2: 366-368

Encalada SE, Lahanas PN, Bjorndal KA, Bolten AB, Miyamoto MM, Bowen BW. 1996. Phylogeography and population structure of the Atlantic and Mediterranean green turtle Chelonia mydas: a mitochondrial DNA control region sequence assessment. Mol Ecol. 5:473–483.

Excoffier, L. G. Laval, and S. Schneider (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50

FitzSimmons NN, Limpus CJ, Norman JA, Goldizen AR, Miller JD, Moritz C. 1997. Philopatry of male marine turtles inferred from mitochondrial DNA markers. Proceedings of the National Academy of Sciences 94:8912-8917.

Formia A, Godley BJ, Dontaine J-F, Bruford MW. 2006. Mitochondrial DNA diversity and phylogeography of endangered green turtle (*Chelonia mydas*) populations in Africa. Conserv Genet. 7:353–369.

Lahanas PN, Bjorndal KA, Bolten AB, Encalada SE, Miyamoto MM, Valverde RA, Bowen BW. 1998. Genetic composition of a green turtle (*Chelonia mydas*) feeding ground population: evidence for multiple origins. Mar Biol. 130:345–352.

Luke K, Horrocks JA, Le Roux RA, Dutton PH. 2004. Origins of green turtle (*Chelonia mydas*) feeding aggregations around Barbados, West Indies. Mar Biol. 144:799–805.

Meylan AB, Bowen BW, Avise JC. 1990. A genetic test of the natal homing versus social facilitation models for green turtle migration. *Science* **248**: 724

Mortimer J, Donelly M. 2007. Marine Turtle Specialist Group 2007 IUCN Red list Status Assessment. Hawksbill sea turtle (Eretmochelys imbricata). Final Assessment. 121 pages.

Naro-Maciel, E, JH Becker, EHSM. Lima, MA Marcovaldi, and R DeSalle. 2007. Testing dispersal hypotheses in foraging green sea turtles (*Chelonia mydas*) of Brazil. *Journal of Heredity* 98(1): 29-39

Posada D and Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics Vol 14: 817-818

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics **19:** 2496-2497.

Sea Turtle Conservation Bonaire. 2007. Progress Report for year 2007. 31 pages.

Seminoff JA. 2004. Global status assessment green turtle (Chelonia mydas). Gland (Switzerland): Marine Turtle Specialist Group, The World Conservation Union IUCN.

Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol. Biol. Evol. 24:1596-1599. 2007.

Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526.

Velez-Zuazo X, Ramos WD, van Dam RP, Diez CE, Abreu-Grobois A and WO McMillan. 2008. Dispersal, recruitment and migratory behavior of a hawksbill sea turtle population. Molecular Ecology 17:839-853

Velez-Zuazo X. 2007. Origin and contribution of a green turtle feeding ground affected by fibropapillomatosis. In: Diez CE and RP van Dam. In-water surveys for marine turtles at foraging grounds of Culebra Archipelago, Puerto Rico. Progress Report FY 2006-2007.

Wright S. 1969. Evolution and the Genetics of Populations, vol. 2, The Theory of Gene Frequencies. University of Chicago Press, Chicago, IL.

Appendix I. Haplotype composition by individual for the hawksbill sea turtle foraging aggregation of Bonaire.

Lab ID	Tag (1)	Tag (2)	Spp (DNA)	HP 380bp	HP740bp	Date capture	Location
BNR001	BX1257	WH1244	Ei	A/CU1	EiA01	9-Mar-07	Playa Franz
BNR002	134734490A		Ei	F/c	EiA09	7-Mar-07	Reserve
BNR003	BX1222		Ei	Q	EiA42	28-Nov-06	Out of Lac
BNR004	BX1218		Ei	A/CU1	EiA01	28-Nov-06	Out of Lac
BNR005	BX1256	WH1243	Ei	F/PR1	EiA11	9-Mar-07	Playa Franz
BNR006	BX1271	WH1259	Ei	A/CU1	EiA01	20-Mar-07	Lac
BNR007	BX1268	WH1256	Ei	F/PR1	EiA11	20-Mar-07	Lac
BNR008	BX1219		Ei	A/CU1	EiA01	28-Nov-06	Out of Lac
BNR009	BX1277	WH1265	Ei	A/CU1	EiA01	22-Mar-07	Lac
BNR010	BX1205		Ei	A/CU1	EiA01	21-Nov-06	Lac
BNR011	BX1220		Ei	A/CU1	EiA01	28-Nov-06	Out of Lac
BNR012	BX1204		Ei	A/CU1	EiA01	21-Nov-06	Lac
BNR013	WH1053	WH1023	Ei	alpha/g	EiA02	12-Feb-07	Karpata/reserve
BNR014	BX1223		Ei	A/CU1	EiA01	28-Nov-06	Out of Lac
BNR015	BX1170		Ei	F/PR1	EiA11	28-Mar-06	Lac
BNR016	BX1221		Ei	A/CU1	EiA01	28-Nov-06	Out of Lac
BNR017	MUERTA	23-11-06	Ei	A/CU1	EiA01		
BNR018	BX1262	WH1249	Ei	A/CU1	EiA01	19-Mar-07	Lac
BNR019	BX1299	WH1289	Ei	b	EiA28	27-Mar-07	Out of Lac
BNR020	BX1288	WH1278	Ei	A/CU1	EiA01	26-Mar-07	Out of Lac
BNR022	134966290A		Ei	G	EiA12	16-Feb-07	Nukove
BNR023	133728690A		Ei	A/CU1	EiA01	21-Feb-07	Playa Frans
BNR024	WE4209	WE4208	Ei	A/CU1	EiA01	23-Jul-04	Chogogo
BNR025	WH1209	WH1210	Ei	A/CU1	EiA01	26-Feb-07	Sweet Dreams
BNR027	BX1241	WH1222	Ei	A/CU1	EiA01	9-Feb-07	1.000 steps
BNR028	133926726A		Ei	Q	EiA42	5-Mar-07	Witches Hut
BNR029	BX1247	WH1206	Ei	A/CU1	EiA01	16-Feb-07	Nukove
BNR030	BX1253	WH1228	Ei	F/PR1	EiA11	26-Feb-07	Margae Bay
BNR031	133632565A		Ei	A/CU1	EiA01	7-Mar-07	Reserve
BNR032	BX1248	WH1202	Ei	F	EiA63	16-Feb-07	BOPEC
BNR033	WH1018	WH1017	Ei	F/c	EiA09	31-Jan-07	White Slave
BNR034	WH1233	WH1232	Ei	A/CU1	EiA01	2-Mar-07	Petries Piller
BNR035	WE4058	WH4059	Ei	F/PR1	EiA11	7-Feb-03	Andrea
BNR036	WH1138	WH1139	Ei	A/CU1	EiA01	2-Mar-07	Andrea I
BNR037	BX1391	WH1042	Ei	L/PR3	EiA47	24-Mar-06	Out of Lac
BNR038	WH1230	WH1231	Ei	A/CU1	EiA01	28-Feb-07	Punt Vierkant
BNR039	WH1023	WH1053	Ei	A/CU1	EiA01	10-Feb-06	Reserve1
BNR040	BX1158	WH1083	Ei	A/CU1	EiA01	26-Mar-06	Out of Lac
BNR041	BX1356NESTI	WH1208TRAN	Ei	A/CU1	EiA01	26-Oct-05	No name beach
BNR042	WH1220	WH1219	Ei	Q/Mx2	EiA43	5-Feb-07	Divi Flamingo Beach
BNR043	BX1239	WH1189	Ei	A/CU1	EiA01	14-dec-06	Flamingo Beach
BNR044	BX1250	WH1201	Ei	A/CU1	EiA01	22-Jan-07	Sweet dreams
BNR045	BX1247	WH1194	Ei	A/CU1	EiA01	12-Feb-07	Karpata/reserve
BNR046	WH1223	WH1224	Ei	A/CU1	EiA01	9-Feb-07	1.000 steps
BNR047	WH1238	WH1239	Ei	A/CU1	EiA01	7-Mar-07	Reserve
BNR048	BX1254	WH1229	Ei	alpha/g	EiA02	28-Feb-07	Punt Vierkant
BNR049	BX1390	WH1041	Ei	A/CU1	EiA01	28-Mar-07	Lac
BNR050	BX1370	WH1063	Ei	A/CU1	EiA01	22-Feb-06	S. Fisherman Huts
BNR051	BX1164	WH1089	Ei	A/CU1	EiA01	27-Mar-06	Lac
BNR052	BX1193	WH1142	Ei	Q	EiA42	7-Nov-06	Ebo's Reef
BNR053	BX1191	WH1132	Ei	A/CU1	EiA01	18-Aug-06	Plaza hotel Marina
BNR054	BX1392	WH1093	Ei	A/CU1	EiA01	24-Mar-06	Out of Lac
BNR055	WH1192	WH1193	Ei	A/CU1	EiA01	24-Jan-07	
BNR056	133729750A		Ei	A/CU1	EiA01	29-Jan-07	Sweet Dreams
BNR057	WH1190	WH1191	Ei	F/PR1	EiA11	22-Jan-07	Atlantis
BNR058	BX1362	WH1021	Ei	F/c	EiA09	3-Feb-06	Andrea II
BNR059	BX1190	WH1124	Ei	A/CU1	EiA01	31-May-06	No Name
BNR060	BX1181	WH1107	Ei	F/PR1	EiA11	4-Apr-06	Lac
BNR061	BX1373	WH1069	Ei	A/CU1	EiA01	8-Mar-06	kas di gezaghebber
BNR062	BX1359	WH1016	Ei	A/CU1	EiA01	18-Jan-06	North of Fisherman huts
BNR063	BX1398	WH1049	Ei	F/PR1	EiA11	24-Mar-06	Lac
BNR065	BX1380	WH1031	Ei	F/PR1	EiA11	22-Mar-06	Lac
BNR066	BX1360	WH1019	Ei	A/CU1	EiA01	1-Feb-06	Cliff
BNR067	03-068	18-Jun-03	Ei	A/CU1	EiA01	18-Jun-03	KB
BNR069	BX1120	11-Jun-06	Ei	A/CU1	EiA51	11-Jun-04	KB
BNR070	BX1060		Ei	A/CU1	EiA01	1-Nov-03	KB
BNR071	BX1123	23-Jun-04	Ei	F/c	EiA09	18-Jun-04	KB north
BNR074	SAT08364	WE4211/BX11	Ei	A/CU1	EiA01	22-Nov-04	No Name beach
BNR080	NIDO75	KB 27-DEC-	Ei	A/CU1	EiA01		
BNR084	BX1125	27-Jun-04	Ei	A/CU1	EiA51	27-Jun-04	
BNR085	BX1124	20-Jun-04	Ei	F/c	EiA09	20-Jun-04	KB
BNR098	BX1055	WE4121	Ei	A/CU1	EiA01	24-Oct-03	KB
BNR099	BX1130 04-	13-Jul-04	Ei	A/CU1	EiA51	1-Sep-05	No name
BNR102	BX1293	WH1283	Ei	A/CU1	EiA01	27-Mar-07	Out of Lac
BNR120	WH1315	WH1316	Ei	A/CU1	EiA01	30-Mar-07	Lac

Appendix II. Haplotype composition by individual for the green sea turtle foraging aggregation of Bonaire.

Lab ID	Tag (1)	Tag (2)	Spp (DNA)	HP740bp	Date capture	Location
BNR081	BX1292	WH1282	Cm	CM-A3	27-Mar-07	Out of Lac
BNR090	NIDO PLAYA	CHIKITU03-NOV-04	Cm	CM-A5		
BNR103	BX1298	WH1288	Cm	CM-A5	27-Mar-07	Out of Lac
BNR104	BX1295	WH1285	Cm	CM-A5	27-Mar-07	Out of Lac
BNR105	BX1290	WH1280	Cm	CM-A5	27-Mar-07	Out of Lac
BNR106	BX1121	WH1291	Cm	CM-A5	28-Mar-07	Lac
BNR108	WH1305	WH1306	Cm	CM-A3	30-Mar-07	Out of Lac
BNR110	BX1283	WH1272	Cm	CM-A5	23-Mar-07	Lac
BNR111	BX1282	WH1271	Cm	CM-A3	23-Mar-07	Lac
BNR112	BX1273	WH1261	Cm	CM-A3	21-Mar-07	Lac
BNR113	BX1235		Cm	CM-A5		
BNR114	BX1280	WH1268	Cm	CM-A3	22-Mar-07	Out of Lac
BNR116	BX1281	WH1270	Cm	CM-A5	22-Mar-07	Out of Lac
BNR117	BX1300	WH1290	Cm	CM-A5	28-Mar-07	Lac
BNR119	WH1309	WH1310	Cm	CM-A29	30-Mar-07	Lac
BNR121	WH1298	WH1299	Cm	CM-A8	29-Mar-07	Lac
BNR123	BX1279	WH1267	Cm	CM-A5	22-Mar-07	Lac
BNR124	BX1242		Cm	CM-A3	12-Feb-07	Karpata
BNR125	WH1022	BX1365	Cm	CM-A5	3-Feb-06	Andrea II
BNR126	BX1274	WH1262	Cm	CM-A5	21-Mar-07	Lac
BNR127	BX1284	WH1273	Cm	CM-A3	23-Mar-07	Lac
BNR128	WE4256	BX1329	Cm	CM-A5	30-Mar-05	Lac Bay
BNR129	BX1286	WH1275	Cm	CM-A5	23-Mar-07	Lac
BNR130	BX1276		Cm	CM-A3	22-Mar-07	Lac
BNR131	BX1155	WH1078FP	Cm	CM-A3	25-Mar-06	Lac
BNR132	BX1278		Cm	CM-A3	22-Mar-07	Lac
BNR133	BX1289	WH1277	Cm	CM-A3	24-Mar-07	Lac
BNR134	BX1285	WH1274	Cm	CM-A3	23-Mar-07	Lac
BNR135	BX1275	WH1264	Cm	CM-A5	21-Mar-07	Lac
BNR136	WH1245	BX1258	Cm	CM-A21	9-Mar-07	Slaagbai
BNR137	BX1287	WH1276	Cm	CM-A1	24-Mar-07	Lac
BNR138	BX1203	WH1154	Cm	CM-A17	22-Nov-06	Lac
BNR139	BX1229	WH1180	Cm	CM-A3	29-Nov-06	Lac
BNR140	BX1265	WH1252	Cm	CM-A5	19-Mar-07	Lac
BNR141	BX1395	WH1046	Cm	CM-A8	30-Nov-06	Lac
BNR142	BX1236	WH1237	Cm	CM-A3	01-dec-07	Lac
BNR143	BX1269	WH1257	Cm	CM-A3	20-Mar-07	Lac
BNR144	BX1227	WH1178	Cm	CM-A5	29-Nov-06	Lac
BNR145	BX1225	WH1176	Cm	CM-A5	29-Nov-06	Lac
BNR146	BX1264	WH1251	Cm	CM-A3	19-Mar-07	Lac
BNR147	BX1210	WH1160	Cm	CM-A3	22-Nov-06	Lac
BNR148	BX1198	WH1150	Cm	CM-A20	21-Nov-06	Lac
BNR149	BX1267	WH1255	Cm	CM-A3	20-Mar-07	Lac
BNR150	BX1232	WH1183	Cm	CM-A3	30-Nov-06	Lac
BNR151	BX1200	WH1152	Cm	CM-A3	21-Nov-06	Lac
BNR152	BX1272	WH1260	Cm	CM-A5	21-Mar-07	Lac
BNR153	BX1231	WH1182	Cm	CM-A22	30-Nov-06	Lac
BNR154	BX1212	WH1162	Cm	CM-A3	23-Nov-06	Lac
BNR155	BX1228	WH1175	Cm	CM-A3	29-Nov-06	Lac
BNR156	BX1207	WE4043	Cm	CM-A5	22-Nov-06	Lac
BNR157	BX1259	WH1250	Cm	CM-A5	19-Mar-07	Lac
BNR158	BX1238	WH1118	Cm	CM-A3	01-dec-07	Lac
BNR159	BX1263	WH1248	Cm	CM-A3	19-Mar-07	Lac
BNR160	BX1216	WH1166	Cm	CM-A3	23-Nov-06	Lac
BNR161	BX1266	WH1254	Cm	CM-A5	20-Mar-07	Lac
BNR162	BX1202	WH1153	Cm	CM-A5	21-Nov-06	Lac
BNR163	BX1311	WE4237	Cm	CM-A3	26-Mar-05	Lac Bay
BNR164	BX1255	WH1242	Cm	CM-A3	9-Mar-07	Playa Franz
BNR165	BX1230	WH1181	Cm	CM-A8	29-Nov-06	Lac
BNR166	BX1224	WH1175	Cm	CM-A5	29-Nov-06	Lac
BNR167	BX1234	WH1185	Cm	CM-A5	30-Nov-06	Lac
BNR168	BX1235	WH1186	Cm	CM-A5	01-dec-06	Lac
BNR169	BX1226	WH1177	Cm	CM-A3	29-Nov-06	Lac
BNR170	BX1214	WH1164	Cm	CM-A5	23-Nov-06	Lac
BNR171	BX1215	WH1165	Cm	CM-A5	23-Nov-06	Lac
BNR172	BX1270	WH1258	Cm	CM-A5	20-Mar-07	Lac
BNR173	BX1261	WH1246	Cm	CM-A5	19-Mar-07	Lac
BNR174	BX1233	WH1184	Cm	CM-A3	30-Nov-06	Lac
BNR175	BX1199	WH1151	Cm	CM-A3	21-Nov-06	Lac
BNR176	BX1213	WH1163	Cm	CM-A3	23-Nov-06	Lac
BNR177	BX1211	WH1161	Cm	CM-A5	22-Nov-06	Lac
BNR178	BX1378	WH10228	Cm	CM-A3	22-Mar-06	Lac
BNR179	BX1197	WH1149	Cm	CM-A5	20-Nov-06	Lac
BNR180	BX1217FP	WH1167	Cm	CM-A3	23-Nov-06	Lac
BNR181	BX1381	WH1032	Cm	CM-A5	22-Mar-06	Lac
BNR182	BX1208	WH1158	Cm	CM-A47	22-Nov-06	Lac
BNR183	BX1377	WH1027	Cm	CM-A6	22-Mar-06	Lac
BNR184	BX1209	WH1184	Cm	CM-A8	22-Nov-06	Lac
BNR185	WH1074	WH1075	Cm	CM-A3	24-Mar-06	Lac
BNR186	BX1369	WH1062	Cm	CM-A5	17-Feb-06	Wayaka
BNR187	BX1260	WH1247	Cm	CM-A5	19-Mar-07	Lac
BNR188	BX1194	WH1148	Cm	CM-A3	13-Nov-06	Pakuna
BNR189	BX1366	WH1056	Cm	CM-A3	10-Feb-06	Bopec
BNR190	BX1361	WH1020	Cm	CM-A5	3-Feb-06	Petres Pillars
BNR191	BX1376	WH1026	Cm	CM-A17	22-Mar-06	Lac
BNR192	BX1379	WH1029	Cm	CM-A3	22-Mar-06	Lac
BNR193	BX1367	WH1061	Cm	CM-A5	13-Feb-06	Salina di Tamp
BNR194	BX1201	WH1144	Cm	CM-A3	20-Nov-06	Lac
BNR195	BX1195	WH1145	Cm	CM-A1	14-Nov-06	Blue Hole
BNR196	BX1372	WH1068	Cm	CM-A5	6-Mar-06	Punt Vierkant
BNR197	BX1371	WH1065	Cm	CM-A3	1-Mar-06	N.Fisherman huts
BNR198	BX1374	WH1071	Cm	CM-A5	13-Mar-06	Andrea I
BNR199	BX1375	WH1030	Cm	CM-A3	22-Mar-06	Lac
BNR200	BX1196	WH1147	Cm	CM-A2	20-Nov-06	Lac