Molecular Ecology (2008) **17**, 839–853 doi: 10.1111/j.1365-294X.2007.03635.x

Dispersal, recruitment and migratory behaviour in a hawksbill sea turtle aggregation

XIMENA VELEZ-ZUAZO,* WILLY D. RAMOS,* ROBERT P. VAN DAM,† CARLOS E. DIEZ,†‡ ALBERTO ABREU-GROBOIS§ and W. OWEN MCMILLAN*¶

**Department of Biology, University of Puerto Rico, Rio Piedras campus, PO Box 23360, San Juan, PR 00931, †Chelonia Inc., PO Box 9020708, San Juan, PR 00902, ‡Departamento de Recursos Naturales y Ambientales, PO Box 366147, San Juan, PR 00936, §Apartado Postal 811, Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, UNAM, Mazatlán, Sinaloa, México 82000, ¶Department of Genetics, North Carolina State University, PO Box 7614 Raleigh, NC 27695, USA*

Abstract

We investigated the dispersal, recruitment and migratory behaviour of the hawksbill sea turtle (*Eretmochelys imbricata*), among different life-history stages and demographic segments of the large hawksbill turtle aggregation at Mona Island, Puerto Rico. There were significant differences in both mitochondrial DNA (mtDNA) haplotype diversity and haplotype frequencies among the adult males, females and juveniles examined, but little evidence for temporal heterogeneity within these same groups sampled across years. Consistent with previous studies and the hypothesis of strong natal homing, there were striking mtDNA haplotype differences between nesting females on Mona Island and nesting females in other major Caribbean rookeries. Breeding males also showed strong, albeit weaker, genetic evidence of natal homing. Overall, Bayesian mixed-stock analysis suggests that Mona Island was the natal rookery for 79% (65–94%) of males in the aggregation. In contrast, the Mona Island rookery accounted for only a small subset of the new juvenile recruits to the foraging grounds or in the population of older juvenile hawksbills turtles on Mona. Instead, both new recruits and the older juvenile hawksbill turtles on Mona more likely recruited from other Caribbean rookeries, suggesting that a mechanism besides natal homing must be influencing recruitment to feeding habitats. The difference in the apparent degree of natal homing behaviour among the different life-history stages of hawksbill turtles at Mona Island underscores the complexity of the species' life-history dynamics and highlights the need for both local and regional conservation efforts.

Keywords: conservation genetics, *Eretmochelys imbricata*, mitochondrial DNA, mixed stock analysis, Mona Island, natal homing

Received 26 August 2007; revision received 31 October 2007; accepted 9 November 2007

Introduction

The population genetic structure of a species is the result of the historical interplay between intrinsic and extrinsic forces (Greenwood 1980; Slatkin 1987; Johnson & Gaines 1990). In some species, adult behaviour leaves a profound imprint on the genetic structure of natural populations. For example, the foraging behaviour of killer whales and the natal philopatry of humpback whales are important forces in the distribution of genetic variation in these populations (Baker *et al*. 1998; Hoelzel *et al*. 2007). In other species, particularly marine organisms with sedentary adults and highly dispersive larval stages, physical environmental features can have a strong effect on patterns of genetic variation (e.g. butterflyfishes, McMillan & Palumbi 1995; shrimps, Aubert & Lightner 2000; stomatopods, Barber *et al*. 2006; corals, Baums *et al*. 2006).

In many organisms, however, the relative importance of different intrinsic and extrinsic forces depends on the age or sex of the organism and this combination of forces will influence the architecture of genetic variation in complex ways. For example, the hawksbill turtle (*Eretmochelys imbricata*), similar to most marine turtles, has a life history

Correspondence: Ximena Velez-Zuazo, Fax: +1 (787) 764–3875; E-mail: xvelezuazo@gmail.com

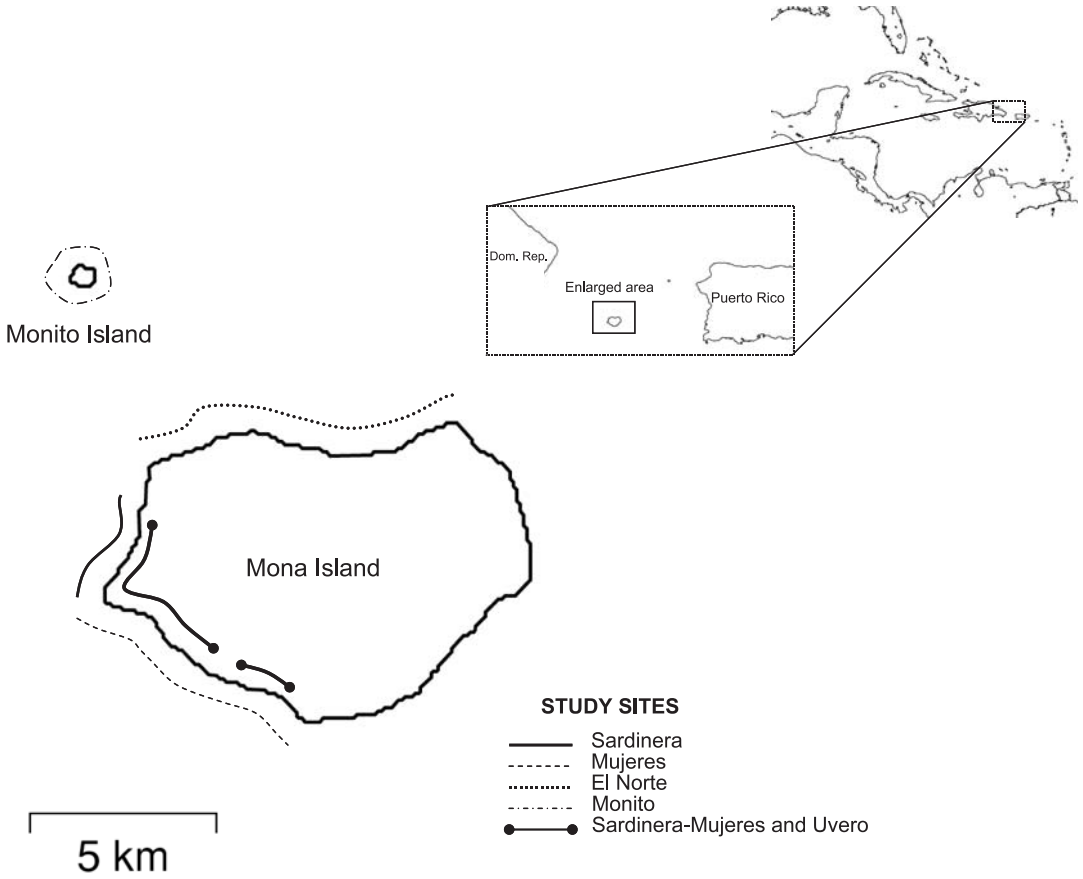

Fig. 1 Map of the Caribbean showing the location of Mona Island and Monito Island and the location of in-water and nesting beach transects conducted during our study.

characterized by a highly dispersive juvenile stage, marked habitat shifts through development and long-distance migrations by adults (Limpus 1992; León & Diez 1999; Meylan & Donelly 1999; van Dam *et al*. 2008). For this species, and other species with similarly complex life histories, a full appreciation of how dispersal and migratory behaviour shape population boundaries and influence the demographic composition of local aggregations requires genetic analysis of all life-history stages.

In this study, we examined mitochondrial DNA (mtDNA) haplotype diversity between sexes, among different life-history stages (adults vs. juveniles; new recruits vs. older juveniles) and across different breeding seasons in a single *focal* hawksbill turtle aggregation. Genetic studies of hawksbill turtles have extensively used mtDNA as a marker to trace matriarchal lineages. Most have taken a regional perspective. Such studies provide exceptional data about genetic differences among regional populations and provide the backdrop for research designed to identify the likely origins of migratory adults and juveniles (Bowen *et al*. 1996, 2007; Bass 1999). However, these studies do not necessarily capture the underlying complexity of local populations, which are composed of mixtures of recruits, juveniles, and adults and where temporal shifts among these different components of the population are possible.

We focused our research efforts on the Mona Island hawksbill turtle aggregation. The beaches and adjacent waters of Mona Island and its satellite Monito Island harbour one of the largest hawksbill turtle colonies in the Caribbean (Fig. 1). The Mona Island rookery comprises approximately 600 adult females (unpublished data), which nest on Mona Island's beaches. In addition, the surrounding reefs and cliff walls host a large aggregation of foraging turtles composed of all life-history stages. Importantly, Mona Island's near-shore benthic habitat, which is characterized by a narrow shelf region of clear water surrounded by open ocean, allows for the sampling of both juveniles and breeding adults. The unusual accessibility of juvenile and male turtles, combined with a large group of breeding females, presents an unparalleled opportunity to conduct studies on the reproductive and population biology of hawksbill turtles. Our genetic results are combined with data from 14 years of mark–recapture efforts at Mona Island to address the following three questions:

1 Is there temporal variation in the genetic composition of nesting females on Mona Island? Previous molecular studies of female hawksbill turtles have collected samples from one nesting season and assumed that the genetic profile provided by that temporal snapshot is constant between years. This assumption has never been explicitly tested, but appears well justified for long-lived organisms, such as marine turtles. Nonetheless, studies of nesting females on Mona Island examined in consecutive years showed highly significant different mtDNA haplotype profiles (Bass *et al*. 1996; Diaz-Fernandez *et al*. 1999). This suggests that temporal genetic heterogeneity may be observed in sea turtles as has been observed in other natural populations (e.g. salmon, Moffett & Crozifr 1996; Brykov *et al*. 1999; Garant *et al*. 2000; cicadas, Lloyd & Dybas 1966; Yang 2004). A genetic profile that best reflects the genetic structure of the nesting female aggregation over time is of importance as a reference for other works, such as those, to determine the origin of turtles on feeding grounds.

2 Do males show evidence for strong natal homing behaviour? Female hawksbill turtles are philopatric, and there is a strong genetic signature of natal homing among different hawksbill turtle rookeries across the Caribbean (Bass *et al*. 1996; Bowen *et al*. 2007). However, nothing is yet known about the genetic composition of breeding males at any hawksbill turtle rookery. It has been assumed that male hawksbill turtles do not show strong fidelity to their natal nesting area and that male dispersal is important for maintaining genetic exchange among distinct rookeries (Karl *et al*. 1992). However, this conclusion has never been directly tested in hawksbill turtles and has been challenged on genetic grounds in the green turtle (FitzSimmons *et al*. 1997a).

3 Are there differences in the mtDNA profile of newly recruiting juvenile hawksbill turtles relative to larger juvenile residents at Mona Island? Starting at approximately 20 cm straight carapace length (SCL), hawksbill turtles recruit into neritic areas around Mona Island where they feed on sponges and other coral reef-associated invertebrates (van Dam & Diez 1996; Diez & van Dam 2002). Previous mtDNA studies, including those concerning juvenile hawksbill turtles sampled at Mona Island, have shown that hawksbill turtle foraging aggregations are composed of individuals originating from different natal beaches (Bowen *et al*. 1996, 2007; Lahanas *et al*. 1998; Diaz-Fernandez *et al*. 1999; Bass & Witzell 2000). It is thought that immature sea turtles recruit to feeding areas as a result of a combination of nonstochastic (e.g. natal homing, Bowen *et al*. 2004) and stochastic (e.g. oceanic currents, Luke *et al*. 2004) factors. Nevertheless, feeding aggregations are not homogenously distributed across the Caribbean Sea (Bowen *et al*. 2007), and it is unclear if, or how, natal homing influences the architecture of mtDNA variation in these groups. Recruitment into feeding aggregations may be a multistep process, with initial recruitment being influenced largely by stochastic forces followed by the movement of larger juvenile-size classes towards their natal habitats. Every year, approximately 40 small and untagged turtles recruit to the shallow benthic habitats around Mona and Monito Island (R. P. van Dam and C. E. Diez, unpublished data), allowing us to determine differences in the mtDNA haplotype profiles between the new recruits and the larger resident juveniles that make up the bulk of this feeding ground aggregation.

Our study is the first description of the complex spatial patterns of variation resulting from the interaction of different life-history components of a population. This type of information is critical for the design of scientifically-based conservation and management policies for hawksbill turtles. The global population size of hawksbill turtles is estimated to have plummeted by nearly 80% over the last 105 years (Meylan & Donelly 1999). Within the Caribbean, recent estimates place the number of nesting females per year at less than 5000 (~22% of historical annual nesting levels, McClenachan *et al*. 2006), and many nesting areas have been decimated (Bjorndal & Jackson 2003). The species is currently listed as critically endangered by the International Union for the Conservation of Nature and Natural Resources (Baillie & Groombridge 1996). Nonetheless, hunting pressure remains intensive in some areas, and even in the areas where conservation measures may be effective, habitat degradation and habitat loss threatens existing populations (Pandolfi *et al*. 2003).

Materials and methods

Study site and sample collection

Mona Island (18°05′N, 67°54′W) and its smaller satellite island Monito Island are located in the middle of the Mona Passage between the islands of Hispaniola and Puerto Rico (Fig. 1). Both Mona Island and Monito Island are uninhabited natural reserves managed by the Department of Natural and Environmental Resources of Puerto Rico. Sea turtles have been legally protected within the reserve since the introduction of the Endangered Species Act of 1973.

During the hawksbill turtle breeding and early in the nesting season (August to September), we conducted daily in-water surveys of breeding males (2003 and 2004) and juvenile turtles (2004 and 2006). These surveys covered four aquatic transects: two along the cliff walls of Mona Island ('El Norte') and Monito Island ('Monito') and two coral reef areas near Mona Island ('Sardinera' and 'Carabinero–

Mujeres'; Fig. 1). We captured hawksbill turtles by hand following the protocol of van Dam & Diez (1998). All turtles were measured [straight carapace length (SCL) and body mass] and marked with a combination of plastic and metal tags in the front flippers. Tissue samples were taken from the right shoulder area with a 6-mm disposable biopsy punch (Acuderm) and preserved in a salt-saturated 20% DMSO 20% EDTA solution. We collected blood samples from each individual, which we preserved in a lysis buffer [100 mM Tris-HCl, pH 8; 100 mM EDTA, pH 8, 10 mM NaCl; 1.0% (w/v) SDS, ratio 1:10 blood-buffer]. Our study also included tissue from breeding males collected during the 2001 breeding season and blood samples from juvenile hawksbill turtles collected in 2000, 2001, 2003 and 2006. During our field research, we surveyed nesting females across the peak of the breeding season (August to December 2003, 2004 and 2005) along the main nesting beaches at Sardinera, Mujeres and Uvero (Fig. 1). As with males and juveniles, we collected morphometric data (curved carapace length — CCL) and tissue samples from the nesting females which were also tagged to avoid sampling duplication. Sampling occurred during oviposition, a specific stage of the nesting activity that minimizes disturbance to the nesting turtle.

Life-history stage identification

All nesting females in this study were examined during their nesting activity. Males and juvenile turtles were caught by hand during in-water surveys. Males were considered reproductively active through direct observation of male–female mounting or if they showed physical signs of recent breeding activity, such as injuries on the tail, flippers and neck (Limpus 1992) and plastron softness (Wibbels *et al*. 1991). Juveniles were distinguished from adults based primarily on size. Adult male hawksbill turtles feature an elongated tail and are generally smaller than females. The smallest breeding male recorded (66.9 cm) was used to set the upper size limit of juveniles.

In addition, we divided juveniles into two classes, new recruits and residences based on both size and field observations. In-water saturation tagging has been conducted on Mona Island for the past 14 years (R.P. van Dam and C.E. Diez, unpublished data). Juveniles were classified as new recruits if they were smaller than 35 cm SCL and were untagged, indicating that they had never been captured previously within the study area. Most of these individuals also possessed several pelagic commensals such as goose barnacles (*Lepas anatifera*) and pelagic crabs (*Nautilograpsus* sp.), indicating recent settlement to the neritic feeding grounds (Carr 1987). Conversely, individuals were classified as resident juveniles if they were larger than 35 cm and had been captured or observed on Mona Island over at least two field seasons.

mtDNA haplotype characterization

We isolated genomic DNA following two protocols: a standard phenol-chloroform extraction protocol followed by ethanol precipitation for tissue samples and using a QIAGEN DNeasy kit for the blood samples. We suspended DNA in an elution buffer (Tris HCl 10 mM, EDTA 0.1 mM, pH 8.0), quantified DNA concentrations using a spectrophotometer (NanoDrop ND-3300) and stored the sample at –20 °C. We amplified a 740-bp fragment of the mtDNA control region (CR) using primers LTEi9 (GGGAA TAATCAAAAGAGAAGG-3') and H950 (GTCTCGGATTTA GGGGTTT-3') (A. Abreu-Grobois, www.iucn-mtsg.org/genetics/meth/primers/abreu_grobois_etal_new_dloop_primers.pdf), which included the region historically surveyed. Our 10 μL polymerase chain reaction (PCR) included the following: 10 ng of genomic DNA, 1× PCR Buffer, 0.2 mM dNTP, 0.5 μM of each primer and 0.05 U/μL of *Taq* polymerase. After an initial 5-min denaturing step (94 °C), our PCR protocol consisted of 30 cycles of the following temperature regime: 30 s at 94 °C (denaturing), 30 s at 52 °C (annealing) and 90 s at 72 °C (extension). In addition, we included a final extension step of 5 min at 72 °C. Along with the samples, we included both positive and negative (no DNA) controls. Following PCR, we removed single-stranded DNA by digesting 5 μL of PCR product with 3 μL of a combined Exonuclease I and Shrimp Alkaline Phosphatase solution. This solution consisted of 6000 U of Exonuclease I and 0.46 U of Shrimp Alkaline Phosphatase. The reaction was incubated for 20 min at 37 °C, followed by 10 min incubation at 85 °C to inactivate the two enzymes.

We sequenced both forward and reverse strands using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences) run on an automated DNA sequencer (MegaBACE 500 Amersham Biosciences or ABI PRISM 3130) at the Sequencing Facilities of the University of Puerto Rico. For the sequencing reaction, we used 1 μL of our PCR product at a concentration of 50 ng/μL under the following conditions: 60 cycles consisting of an initial denaturing of 3 s at 94 °C, 5 s at 50 °C (annealing) and 90 s at 60 °C (extension). We used SEQUENCHER 4.2 (Gene Code Corporation) to generate a single consensus strand from the aligned forward and reverse sequences. All trimmed sequences were aligned by eye and unique haplotypes were identified by collapsing sequences using COLLAPSE 1.2 (available from http://darwin.uvigo.es). Each haplotype was compared to previously assigned haplotypes and named using the standardized nomenclature conventions recently established for the Caribbean hawksbill turtles. All new haplotypes were named in order of appearance.

To assess the genetic diversity of each group (i.e. females, males and foraging aggregation), we estimated gene (haplotype) diversity (h) and nucleotide diversity (π, Nei

Table 1 Haplotype frequencies of the turtle populations that were used as the baseline for the mixed stock analyses. Haplotype nomenclature is based on 380 bp mtDNA fragment. The relative rookery size was estimated based on the average number of nests deposited per year (nest/year) over the five available consecutive years (Antigua, Cuba, Barbados, Mexico, Mona Island and US Virgin Islands) for most of the rookeries. For the Belize rookery, the average number of nest/year was calculated as the product of the average number of females nesting in a year multiplied by four nest/female. For the Costa Rica rookery, average number of hawksbill turtle nest/year was obtained from long-term records (Meylan 1999; Table 1). Rookery size data was compiled from the following sources: ANT, Jumby Bay, Antigua (P. Mason, personal communication); BRB, Barbados (J. Horrocks, personal communication); BLZ, Belize (Meylan 1999); CUB, Doce Leguas Cays, Cuba (F. Moncada, personal communication); MX, Yucatán Peninsula, Mexico (E. Cuevas, personal communication); USVI, Buck Island, Saint Croix, US Virgin Islands (Z. Hillis, personal communication); CR, Tortuguero, Costa Rica (S. Tröeng, personal communication); MN, Mona Island, Puerto Rico (Proyecto Carey, unpublished data)

Haplotype	ANT	BRB	BLZ	CUB	MX	USVI	CR	MN	MN*
A	9	20	—	62	—	5	—	1	1
Alpha	—	—	—	—	—	—	10	—	—
B	4	—	—	—	—	1	—	—	—
C	2	—	—	—	—	—	—	—	—
D	—	1	—	—	—	—	—	—	—
E	—	3	—	—	—	—	—	—	—
F	—	—	11	1	—	43	33	71	1
G	—	—	1	—	—	—	6	—	—
Gamma	—	—	—	5	—	—	—	—	—
H	—	—	1	—	—	—	—	—	—
I	—	—	1	—	—	—	—	—	—
J	—	—	—	—	—	—	—	—	2
K	—	—	—	—	—	—	—	—	1
L	—	—	—	—	—	—	4	1	1
M									2
N	—	—	—	—	—	—	—	32	6
O	—	—	—	—	—	—	—	6	1
P	—	—	—	—	1	—	—	—	—
Q	—	—	—	—	52	—	—	2	—
CU3	—	—	—	1	—	—	4	—	—
CU4	—	—	—	1	—	—	0	—	—
BI1	—	—	—	—	—	1	—	—	—
Total	15	24	14	70	53	50	57	113	15
Rookery size	203	1504	13	130	3800	158	25	740	

*from Bass *et al*. (1996).

1987) using ARLEQUIN version 3.0 (Excoffier *et al*. 2005). In addition, we used a randomized chi-square test (CHIRXC, Zaykin & Pudovkin 1993) to determine if there were significant shifts in haplotype frequencies between nesting beaches, years, and among life-history stages. Finally, using ARLEQUIN version 3.0 we conducted an AMOVA using Tamura–Nei model of nucleotide substitution with gamma correction to measure how genetic variation is partitioned in the hawksbill turtle population of Mona Island and a pairwise analysis of genetic structure among the groups (Φ_{ST}, Excoffier *et al*. 1992).

Mixed stock analysis of individuals

We assembled baseline information for the mixed stock analysis (MSA) using the available mtDNA haplotype frequencies of eight nesting rookeries: Antigua (Jumby Bay), Belize (Gales Point, Bass *et al*. 1996), US Virgin Islands (Buck Island), Barbados (Bass *et al*. 1996; Bowen *et al*. 2007), Cuba (Doce Leguas), Mexico (Yucatan, Diaz-Fernandez *et al*. 1999), Costa Rica (Tortuguero, Tröeng *et al*. 2005; Bowen *et al*. 2007) and Puerto Rico (Mona Island, Diaz-Fernandez *et al*. 1999; this study) (Table 1). The data set reported here and the Diaz-Fernandez *et al*. (1999) data set were combined and truncated for these analyses to include only the 380-bp region of the control region for comparison with the earliest genetic studies of hawksbill turtles.

We used a conditional maximum likelihood (ML; SPAM 3.7, ADGF 1999) and a Bayesian approach (BAYES, Pella & Masuda 2001) to estimate the most likely origin of each individual and the contribution of the different Caribbean nesting rookeries to both the breeding male and juvenile hawksbill turtles of Mona Island. The main difference between these two approaches is the incorporation of a prior distribution parameter in the latter, which allows researchers to alter their expectations based on biologically

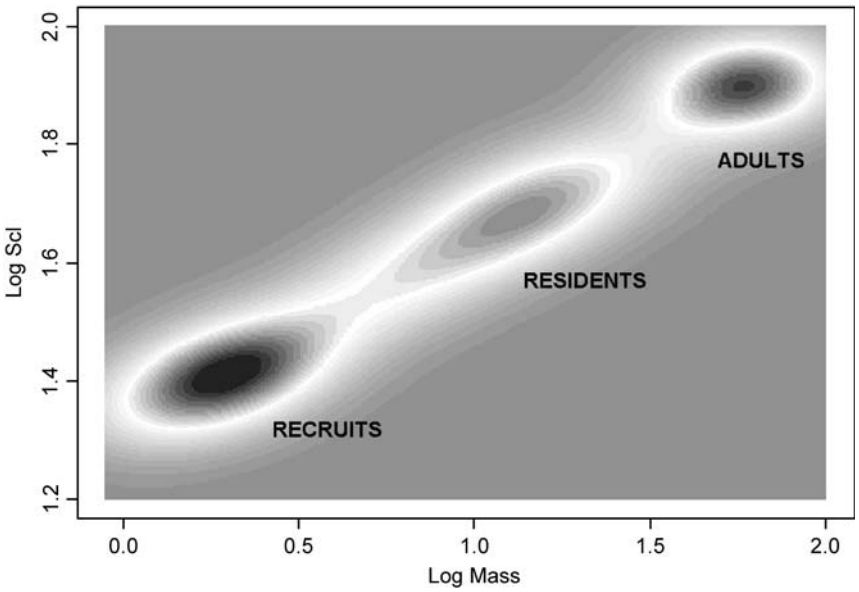

Fig. 2 Two-dimension comparison using smoothing curves of the logarithm of the straight carapace length (SCL-nt) and body mass of 14-year mark–recapture records of immature hawksbill sea turtles. The *x* axis represents the logarithm of the mass while the *y* axis represents the logarithm of the carapace length. The plot was drawn using a nonparametric smoothing technique in two dimensions developed by Bowman & Azzalini (1997) and implemented in S-PLUS version 6.2.

relevant information (Okuyama & Bolker 2005). Our Bayesian analysis was conducted using both uniform and weighted priors. In our analysis with uniform priors, each of the eight rookeries was equally likely to contribute individuals to the Mona Island aggregations. For our weighted analysis, similar to Bass *et al*. (2004), we weighted the potential contribution of different rookeries to the Mona Island aggregation relative to the size of the rookery. Thus, large rookeries were assumed to have contributed more individuals to the Mona Island aggregation. In addition, we examined the importance of distance between major rookeries. Under this prior model, hawksbill turtle rookeries near to Mona had a greater probability of contributing individuals to the Mona Island aggregation than more distantly located rookeries.

For the Bayesian MSA analysis, we conducted 50 000 Markov chain Monte Carlo (MCMC) runs for every chain where a chain represents a potential rookery of origin and a burn-in of 25 000 runs to calculate the posterior distribution of every chain and for the total of chains combined. The estimates were considered for further analysis in our study after computing a diagnostic to test the convergence of the chains (i.e. Gelman and Rubin shrink factor). This diagnostic compares the variation of a single chain to the total variation among chains, and convergence is verified if the shrink factor is less than 1.2 for each chain (Pella & Masuda 2001).

In both our ML and Bayesian MSA, individuals with haplotypes not observed in any of the nesting rookeries were removed from the analysis. We adopted this strategy because their absence in the baseline sample biases the origin estimations and contributions (Pella & Masuda 2001). After we obtained our results from the MSA, we added these individuals back into the analysis and calculated the contribution of the 'unknown' rookeries to the stock-mixture.

Results

Size classes

Over a 14-year period of in-water surveys, a total of 275 individual adults and 668 juvenile hawksbill turtles were tagged and measured. Three distinctive constellations of individuals were distinguished by the two-dimensional plot of the log-transformed SCL vs. mass (Fig. 2). All individuals classed as new recruits based on both size and observational data fell into the first cluster, whereas most of the resident juvenile population fell into the second cluster. All adults sampled fell into the third cluster. The presence of these distinctive clusters suggests differences in underlying growth parameters or migratory behaviour that seem to correlate with major life history transformations (Charnov 1993).

Diversity of haplotypes of the hawksbill turtle population from Mona Island

For 270 individuals, we sequenced a 740-bp fragment that spans most of the mitochondrial control region. This region included the 380-bp and 480-bp segment previously sampled in regional population genetic analysis of hawksbill turtles. Our genetic sample consisted of temporal samples of 93 nesting females [2003 (n = 48), 2004 (n = 20) and 2005 (n = 25)], 59 breeding males [2001 (n = 10), 2003 (n = 38) and 2004 (n = 11)], 118 juveniles including 62 recruits [2000 (n = 8), 2001 (n = 16), 2003 (n = 9), 2004 (n = 18) and 2006 (n = 11)] and 56 residents (2004 n = 56) (Table 2).

Table 2 Haplotype composition of the nesting females (2003–2005), breeding males (2001, 2003 and 2004), recruits (2000, 2001, 2003, 2004 and 2006) and juveniles (2004) from the Mona Island hawksbill turtle population

								Foraging aggregation					
Haplotype		Females			Males			Recruits					Residents
380–480 bp	740 bp	2003	2004	2005	2001	2003	2004	2000	2001	2003	2004	2006	2004
F/PR1	Ei-A11	28	16	15	6	18	5	2	6	2	7	2	17
N/PR2	Ei-A20	16	2	7		7	2		1				2
O/PR4	Ei-A21	2	2	1	1								
Q/MX2	Ei-A43	1		1	1	1							1
A/CU1	Ei-A01			1	1	3	1	3	6	6	8	7	15
F/c	Ei-A09	1						1		1	1	1	6
CU3	Ei-A29												2
Q/MX1	Ei-A41												2
F/PR1	Ei-A45												2
Q/MX1	Ei-A23					1			1				1
alpha/g	Ei-A02							1				1	1
P/MX3	Ei-A22												1
B/e	Ei-A03										1		1
n	Ei-A36												1
A/CU1	Ei-A51												1
	Ei-A59												1
	Ei-A60												1
A	Ei-A68												1
	Ei-A28							1					
L/PR3	Ei-A47					1			1		1		
Q/MX2	Ei-A24					1			1				
L/PR3	Ei-A18					4	2						
Q/MX2	Ei-A42				1	1							
a	Ei-A27					1							
	Ei-A58						1						
Total		48	20	25	10	38	11	8	16	9	18	11	56
h (SD)		0.5273 (0.0450)			0.7259 (0.0540)			0.6753 (0.0448)					0.8312 (0.0338)
π (SD)		0.0025 (0.0016)			0.0048 (0.0027)			0.0077 (0.0041)					0.0079 (0.0042)

The longer control region fragment identified four new polymorphic sites at the 5′ end of the control region and increases the genetic resolution of our study relative to previous studies based on the smaller 380-bp region (Table 3). For example, with the longer section we were able to distinguish 25 mtDNA haplotypes among the 270 individuals sampled rather than 16 haplotypes had we restricted our sampling to the smaller 380-bp region (Table 3). Importantly, several haplotypes that are reported in other rookeries around the Caribbean could potentially be subdivided further (see Tables 1 and 3).

Temporal analysis of nesting females, breeding males and recruits

The nesting beaches of Mona Island (i.e. Sardinera–Mujeres and Uvero) had similar genetic composition ($P > 0.05$) and the data was pooled for the temporal analysis.

We found little evidence for temporal heterogeneity among the different segments of the hawksbill turtle population (Table 2). Consistent with previous studies (Bass *et al*. 1996; Diaz-Fernandez *et al*. 1999), the nesting rookery on Mona Island had a characteristic mtDNA haplotype profile that was significantly different ($P < 0.01$) from the profiles of other major rookeries across the Caribbean. Importantly, over our 3-year sampling period, we found no evidence for temporal structure in the female mtDNA haplotype frequencies (2003 vs. 2004 $P = 0.29$, 2003 vs. 2005 $P = 0.57$, 2004 vs. 2005 $P = 0.51$). Our combined 3-year nesting aggregation was not significantly different from the female haplotype frequencies observed by Diaz-Fernandez *et al*. (1999) in 1994 ($P = 0.5$), but was significantly different from the mtDNA haplotype frequencies reported for females collected in 1993 (Bass *et al*. 1996). This later conclusion was true whether or not nesting seasons were compared individually (1993 vs. 1994 $P < 0.01$, 1993 vs. 2003 $P < 0.01$, 1993 vs. 2004 $P < 0.01$, 1993 vs. 2005 $P < 0.01$),

Table 3 Polymorphic sites within the 740 base-pair fragment of the mtDNA control region sampled in these studies. Shaded areas represent polymorphic sites defining new haplotypes. Haplotype names are a combination of Bass *et al*. (1996), Diaz-Fernandez *et al*. (1999) and this study

			Polymorphic sites																											
Bass_380 bp						13	20	21	62	78	96	119	158	164	192	210	244	255	257	309	310	317	341	372						
D-F_480 bp			38	100	124	138	145	146	185	201	219	242	281	288	315	333	367	378	380	432	433	440	464	495					GenBank	
D-loop_740 bp			23	85	109	123	130	131	172	188	206	229	268	274	302	320	354	265	367	419	420	427	451	482	502	578	651	664	Accession no.	
Bass	D-F	HP																												
A	CU1	Ei-A01	T	C	A	A	C	T	T	A	A	A	C	A	A	T	G	T	A	T	G	T	G	A	G	T	G	C	EF210779	
A	CU1	Ei-A51	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	EF210780	
A		Ei-A68	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	EF210801	
alfa	g	Ei-A02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	EF210781	
B	e	Ei-A03	.	T	G	G	.	.	.	.	G	.	T	.	.	C	.	.	.	C	.	.	A	.	C	C	.	.	EF210782	
F	c	Ei-A09	.	T	.	G	.	.	C	.	G	.	T	.	.	C	.	.	.	C	.	.	A	.	C	C	.	.	EF210783	
F	PR1	Ei-A11	.	T	G	G	.	.	C	.	G	.	T	.	.	C	.	.	.	C	.	.	A	.	C	C	.	.	EF210784	
F	PR1	Ei-A45	C	T	G	G	.	.	C	.	G	.	T	.	.	C	.	.	.	C	.	.	A	.	C	C	.	.	EF210785	
L	PR3	Ei-A18	.	T	G	G	.	.	C	.	.	.	T	.	.	C	.	.	.	C	A	.	A	.	.	.	.	.	EF210786	
L	PR3	Ei-A47	.	T	G	G	.	.	C	.	.	.	T	.	.	C	.	.	.	C	A	.	A	.	C	C	.	.	EF210787	
N	PR2	Ei-A20	.	T	G	G	.	.	C	.	G	.	T	.	.	C	.	.	.	C	A	.	A	.	C	C	.	.	EF210788	
O	PR4	Ei-A21	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	EF210789	
P	MX3	Ei-A22	.	T	.	G	.	.	C	G	.	.	T	.	.	C	.	.	.	C	.	.	A	.	.	.	A	.	EF210790	
Q	MX1	Ei-A23	.	T	.	G	.	.	C	.	.	.	T	.	.	C	.	.	.	C	.	.	A	.	.	.	A	.	EF210791	
Q	MX1	Ei-A41	.	T	.	G	.	.	C	.	.	.	T	.	.	C	.	.	.	C	.	.	A	.	.	.	.	.	EF210793	
Q	MX2	Ei-A24	.	T	G	G	.	.	C	.	.	.	T	.	.	C	.	.	.	C	.	.	A	.	.	.	A	.	EF210792	
Q	MX2	Ei-A42	.	T	G	G	.	.	C	.	.	.	T	.	.	C	.	.	.	C	.	.	A	.	.	.	.	T	EU113048	
Q	MX2	Ei-A43	.	T	G	G	.	.	C	.	.	.	T	.	.	C	.	.	.	C	.	.	A	.	C	C	.	.	EF210794	
	a	Ei-A27	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	EF210795	
	CU3	Ei-A29	.	T	G	G	.	.	.	.	.	.	T	.	.	C	.	.	.	C	.	.	A	.	.	.	.	T	EF210796	
	b	Ei-A28	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	EU113049	
	n	Ei-A36	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	EF210797	
		Ei-A58	.	T	G	G	.	.	C	.	.	.	T	.	.	C	A	.	.	C	A	.	A	.	C	C	.	.	EF210798	
		Ei-A59	.	T	G	G	.	.	C	.	.	.	T	.	G	C	.	.	.	C	.	.	A	.	C	C	.	.	EF210799	
		Ei-A60	.	T	G	G	T	.	C	.	G	G	T	.	.	C	.	.	.	C	.	C	A	.	C	C	.	.	EF210800	

or if years 2003–2005 were grouped into a single unit ($\chi^2 = 48.39$, $P < 0.01$).

Similarly, we found no evidence for temporal heterogeneity between males sampled in different years [2001–2003 ($\chi^2 = 8.08$, $P = 0.24$), 2001–2004 ($\chi^2 = 8.06$, $P = 0.31$) or 2003–2004 ($\chi^2 = 2.20$, $P = 0.88$)], nor in our sample of recruits arriving during 5 years (2000, 2001, 2003, 2004 and 2006; $\chi^2 = 29.39$, $P = 0.9$). This lack of temporal heterogeneity was consistent in all pairwise comparisons among recruitment years ($\chi^2 = 0.9$–8.25, $P = 0.4$–1.0). As a result, we grouped individuals together for subsequent analysis.

mtDNA haplotypes and diversities along groups

Overall, there were significant differences in mtDNA diversity among the nesting females, breeding males and juveniles ($\Phi_{ST} = 0.19$, $P < 0.001$). Pairwise comparisons indicated strong genetic structure between males vs. juveniles ($\Phi_{ST} = 0.17$, $P < 0.001$) and females vs. juveniles ($\Phi_{ST} = 0.31$, $P < 0.0001$). In contrast, differentiation between females and males were much less pronounced, yet still significant ($\Phi_{ST} = 0.06$, $P < 0.05$). We observed similarly weak genetic differences (and in this case not significant) between the newest recruits and the older juveniles in the Mona Island aggregation ($\Phi_{ST} = 0.04$, $P = 0.06$).

Examination of the haplotype distributions provided more detailed insight into this pattern. Females showed the lowest haplotype ($h = 0.53 \pm 0.04$) and nucleotide ($\pi = 0.0025 \pm 0.0016$) diversity and 13 polymorphic sites identified five haplotypes. Haplotype Ei-A11 was the most common and, with Ei-A20, accounted for approximately 90% of the nesting females. Nucleotide and haplotype diversity was considerably higher in males ($h = 0.73 \pm 0.04$, $\pi = 0.0048 \pm 0.0027$); however, similar to females, haplotypes Ei-A11 and Ei-A20 were found in the highest frequency and occurred in 65% of the males sampled. The major difference in mtDNA diversity between males and females was the presence of a large proportion of individuals (nearly 34%) that possessed unique mtDNA haplotypes, including one haplotype, Ei-A18, which was present in about 10% of the males sampled, but absent from both our juvenile and female sample.

Haplotype and nucleotide diversities among juveniles were similar but slightly higher than these in males. However, unlike in the adult population, haplotypes Ei-A11 and Ei-A20 were less common in both the resident and recruiting juveniles, with two haplotypes (A/CU1 and F/c) that were rare in adult populations but present in almost 50% of the juveniles sampled. In addition, there was a large number of rare haplotypes that were absent in the adults. This pattern was mainly driven by a general buildup in haplotype diversity in the standing juvenile population vs. the new recruits most likely as a result of the accumulation of common and rare haplotypes over time (Table 2).

Mixed stock analysis

Consistent with the strongly overlapping mtDNA haplotype profiles between males and nesting females, the mixed stock analysis suggested Mona Island as the most likely origin of the majority of the breeding males (Fig. 3a). Indeed, the weighted Bayesian MSA suggested that 79% (0.79, CI 0.65–0.94) of the males that we sampled on Mona Island were likely born there. Contribution estimates were similarly high, albeit slightly lower (0.58, CI 0.38–0.78), using ML (see Fig. 3a). Confidence intervals in both the Bayesian and ML MSA were large, reflecting the presence of a number of haplotypes in the Mona Island population that are broadly distributed in the other Caribbean hawksbill turtle populations.

In contrast, the MSA results suggested that Mona Island provides a relatively small contribution of individuals to the foraging aggregation (weighted Bayesian = 0.08, CI 0.00–0.1; Fig. 3b). This result was consistent whether or not we conducted the MSA using the ML approach or the Bayesian approach with uniform priors or using priors based on the estimated size rookery or proximity to Mona Island. Instead, as has been previously reported (Bowen *et al*. 1996, 2007), the MSA supported a model where the juvenile aggregation was composed of individuals from rookeries around the Caribbean and Gulf of Mexico. There was no strong evidence that either rookery size or rookery location had a significant effect on the composition of mtDNA haplotypes in the juvenile aggregation on Mona Island (size $P = 0.8$; distance from Mona $P = 0.8$). Nonetheless, the nearby US Virgin Island and Cuban rookeries had the highest estimated contribution; however, similar to the pattern in males, confidence intervals around point estimates were quite large (Fig. 3b).

It is important to point out that our MSA was conducted with the data on nesting female haplotype profiles collected during this study and combined with Diaz-Fernandez *et al*. (1999). When we used the data reported by Bass *et al*. (1996) to estimate the contribution of the rookeries to the males, we observed fundamentally different patterns. In particular, the relative importance of the Mona Island rookery to the juvenile population on Mona increased substantially, with Mona Island estimated to have been the source of over one-third of the juvenile aggregation (0.37, CI 0.18–0.62).

Discussion

Our study is the largest focal genetic study of any hawksbill turtle aggregation and the first to examine mtDNA haplotype differences among different life-history stages and demographic segments, and across consecutive breeding seasons. The genetic data obtained permit us to address several specific hypotheses about the relative importance of natal homing in structuring different demographic

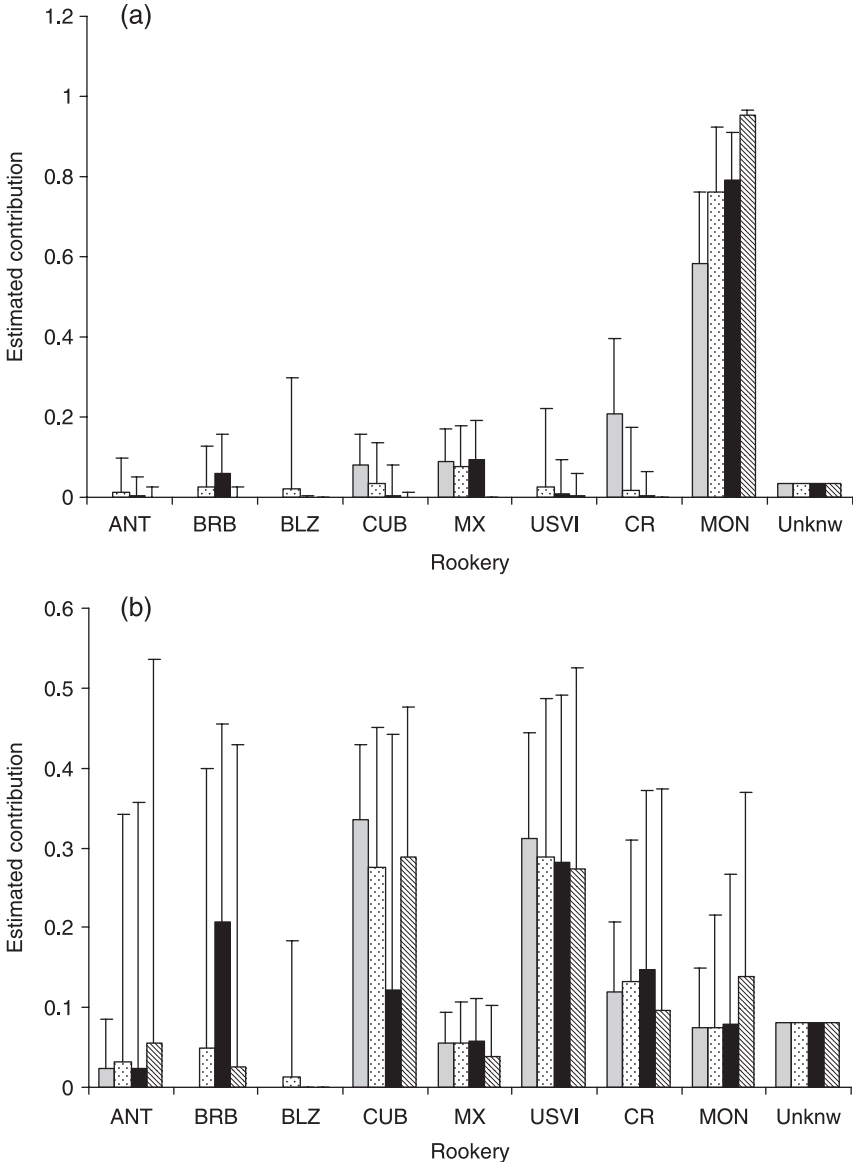

Fig. 3 The contribution of different Caribbean rookeries to the (a) breeding males and (b) foraging aggregation of the Mona Island hawksbill sea turtle population. Estimates were generated using maximum likelihood (grey), Bayesian with uniform priors (white), Bayesian with weighting prior probabilities relative to rookery size (black) and relative to geographical distance (diagonal black and white bars; see Materials and methods). Posterior probability estimates and their 95% credible intervals (similar to confidence intervals) are given for the eight major hawksbill rookeries in the Caribbean. In addition, estimates for unknown haplotypes were calculated as described in the text. ANT, Jumby Bay, Antigua; BRB, Barbados; BLZ, Belize; CUB, Doce Leguas, Cuba; MX, Yucatán, Mexico; USVI, Sandy Point, US Virgin Islands; CR, Tortuguero, Costa Rica; MN, Mona Island, Puerto Rico.

segments of a population and to suggest additional explanations for the dispersal, recruitment and migratory behaviour of sea turtles.

Temporal stability among nesting female cohorts

We found no evidence for temporal differences in mtDNA haplotype distributions among nesting hawksbill turtle females across three consecutive breeding seasons. Furthermore, mtDNA haplotype profiles were similar to the haplotype profile of females sampled a decade earlier by Diaz-Fernandez *et al*. (1999). Together, these data do not support the idea that there are temporally discrete nesting aggregations of hawksbill turtles (Diaz-Fernandez *et al*. 1999), a hypothesis originally put forward based on significant differences in haplotype diversity and frequency between females collected on Mona Island in 1993 and 1994 (Bass *et al*. 1996; Diaz-Fernandez *et al*. 1999).

Temporal genetic stability has been observed in other species with strong migratory behaviour, including birds (Degnan 1993), fishes (e.g. herrings, King *et al*. 1987; shads, Brown *et al*. 1996) and marine mammals (e.g. beluga whales, Gladden *et al*. 1997), and has been mostly explained by natal philopatry, although extrinsic factors (e.g. oceanographic conditions) may also play an important role (Rocha-Olivares & Vetter 1998). In the specific case of the hawksbill turtles, we suggest that the staggered remigration to rookeries has a mixing effect on the seasonal nesting aggregations and blurs any temporal signal that might arise either by stochastic (variance in reproductive success) or deterministic (immigration) forces. Although female hawksbill turtles tend to nest every 2 years, there is variation

in the remigration interval (Richardson *et al*. 1999; R.P. van Dam and C.E. Diez, unpublished data). On Mona Island, recapture data of 35 nesting females from 2000 to 2005 show that six females exhibited a 2- to 3-year mix remigration interval, while the rest exhibited a 2-year interval. The ability to nest is probably influenced by the physical state of the female and may be correlated to environmental factors that affect food quality and availability on foraging grounds (Carr & Carr 1970; Broderick *et al*. 2001; Wallace *et al*. 2006; Saba *et al*. 2007). Females nesting on Mona Island migrate there from different feeding grounds (van Dam *et al*. 2008) that likely differ in food quality and abundance. Thus, it is easy to imagine how any genetic distinctions between temporal cohorts would quickly erode over time.

In this light, it is difficult to explain the differences in female mtDNA diversity the shift in the frequency of major haplotypes observed by Bass *et al*. (1996) in 1993 relative to the values observed in 1994 and across our multiyear study. This discrepancy is not the result of differences in sampling site and/or sampling time, as all samples were collected during the same time of the year and from the same beaches. However, we cannot discard the possibility that this discrepancy is a remnant of Caribbean hawksbill sea turtle fisheries of past decades. Haplotypes originally observed in low frequency in the 1993 nesting aggregation (i.e. J, K and M) may have gone extinct in the Mona nesting rookery. This would suggest a rather sharp population decline and, although hawksbill turtle populations have declined sharply since the 1950s, there has been no obvious single year drop and the number of nesting females on Mona has actually been increasing since the mid-1990s (Proyecto Carey, unpublished data). In addition, it remains difficult to explain the shift in the dominant haplotype from N in 1993 to F in 1994, 2003, 2004 and 2005.

Whatever the underlying cause, the consistency of our temporal samples and the similarity to the 1994 nesting females profile argue that these data provide a better estimate of the underlying diversity of mtDNA haplotypes in the female hawksbill nesting population on Mona Island and should be used in any future analysis. This revised female haplotype profile has several practical applications; foremost, it provides a stronger genetic signature of Puerto Rican stock in mixed stock analyses. There are two distinctive Mona Island haplotypes (N and O) not observed in any other nesting stock. Furthermore, we identified additional polymorphic sites in several of the broadly distributed haplotypes (e.g. A, F, and Q) that might also prove to be locally endemic upon higher resolution analysis of other key rookeries across the Caribbean. In addition, the temporal stability of the mtDNA haplotype profiles argues that descriptions of genetic diversity based on one sample provide a reasonable approximation of the underlying population genetic structure. However, our sample sizes are too small to detect subtle temporal fluctuations in mtDNA haplotype profiles. Furthermore, rarefaction analysis using ANALYTICAL RAREFACTION 1.3 (http://www.uga.edu/~strata/software/Software.html) of the pooled female data (93 individuals) suggested that temporal sample sizes of approximately 100 individuals are needed to identify rare haplotypes in the population — haplotypes that are better indicators of real-time female migration among different hawksbill rookeries.

Strong natal homing in males

We also demonstrated that adult male hawksbill turtles, similar to females, exhibit the genetic signature of strong natal homing behaviour. Male natal homing behaviour has previously been observed in green turtles (*Chelonia mydas*) in Australia (Fitzsimmons *et al*. 1997a) where males and females from three nesting rookeries showed nearly identical mtDNA haplotype profiles to each other. In contrast, male natal homing in hawksbill turtles appears to be more relaxed. Although male and female mtDNA haplotype profiles were strongly overlapping, haplotype diversity was considerably higher in males than females, and a number of males had mtDNA haplotypes that suggested that they originated from other rookeries. For example, haplotype Ei-A18, which was observed in 10% of the male aggregation but was absent in our female sample, has been reported only in the Costa Rican rookery (Tröeng *et al*. 2005).

Somewhat surprisingly, the mtDNA data suggest that the Mona Island foraging ground was not necessarily the source of recruitment of 'non-natal' males into the breeding aggregation. Nearly 17% ($n = 10$) of the sampled males exhibited mtDNA haplotypes unique to males and not found in the juvenile foraging aggregation (Table 3). This discrepancy suggests a more complex mode of male recruitment into the breeding population than previously envisioned. At least three possible scenarios might explain how 'non-natal' males recruit into the Mona Island breeding population: (i) social facilitation — neophyte adult males in the same foraging ground but originating from other rookeries, might follow experienced adult males from the Mona Island rookery to their breeding grounds at Mona Island; (ii) social status — adult males excluded from breeding at their natal beaches might migrate to seek mating opportunities at other breeding grounds; (iii) rookery lost — adult males that belong to a rookery where breeding activity is not sustainable as a result of past fishing activities or habitat loss might disperse to other breeding areas. Of course, none of these scenarios are mutually exclusive, but all suggest that males utilize alternate migratory behaviours that do not rely on strict natal homing or stochastic processes to locate breeding aggregations. This flexibility has ramifications for male-mediated gene flow and demographic connectivity among hawksbill turtle rookeries. Male-mediated gene flow is important in

maintaining genetic similarity among distant rookeries in green sea turtles (Fitzsimmons *et al*. 1997b; Roberts *et al*. 2004) and, given that nearly 21% of the males we sampled probably originated from other rookeries, our results suggest that hawksbill turtles will show a similar pattern. This assumes that 'non-natal' males have similar reproductive success as 'natal' males, a hypothesis that we are currently testing using microsatellite markers.

No evidence for natal homing in the juvenile aggregation

Bowen *et al*. (2004) concluded that post-pelagic Atlantic loggerhead sea turtles exhibit natal homing behaviour based on the observed strong similarities in the genetic structure of the nesting grounds and their adjacent feeding grounds. From that study, we expected a similar behaviour for juvenile hawksbill sea turtles. Instead, the juvenile population on Mona Island appears to be largely composed of individuals originating from other rookeries across the Caribbean. A large number of the recruits had mtDNA haplotypes that were either absent or occurred in very low frequency in the nesting female aggregation, including haplotype Ei-A01/A, the dominant haplotype in rookeries in Cuba, Barbados and Antigua. This pattern extended to the resident juveniles, where there was a notable buildup in haplotype diversity rather than a shift towards female mtDNA haplotypes that would indicate post-recruitment natal homing. The build-up in haplotype diversity likely reflects the long resident time of post-recruit juveniles. Site fidelity of juveniles is quite strong and the same juveniles spend years at the feeding areas of Mona (van Dam & Diez 1998 and unpublished data).

Our data reinforce previous studies on feeding aggregations of hawksbill turtles, which suggested that recruitment into feeding areas is largely influenced by oceanic mixing during the extended (2–3 years) pelagic stage (Luke *et al*. 2004; Bass *et al*. 2006). Nonetheless, there was a clear regional influence on juvenile recruitment. mtDNA diversity in Caribbean and Western Atlantic hawksbill turtle rookeries is characterized by a number of regionally distributed haplotypes. For example, haplotype A is found in Cuba, most rookeries of the Lesser Antilles (Barbados, Antigua), Venezuela (Bowen *et al*. 2007) and Brazil (Lara-Ruiz *et al*. 2006), whereas haplotype F is found in rookeries in Mona Island, USVI, and Central America (Tortuguero, Costa Rica; Gales Point, Belize), and the closely related haplotype Q is almost totally restricted to rookeries in the Yucatán Peninsula (Table 1 and references). Our sample was composed of large and approximately equal numbers of F-type (Ei-A01, Ei-A09, Ei-A45) and A-type (Ei-A01, Ei-A51, Ei-A68) haplotypes, suggesting that the juvenile aggregation on Mona is composed of recruits from across the wider Caribbean region. In contrast, only five juveniles possessed a haplotype (Q) characteristic of the large Yucatán Peninsula hawksbill turtle rookery, one of which (EiA43) was found in nesting females on Mona, and thus, may represent another example of local recruitment. A similar pattern was observed in the larger regional study of juvenile feeding aggregations (Bowen *et al*. 2007). In that study, most juvenile populations sampled in the Caribbean showed a high and similar proportion of F and A mitochondrial haplotypes, with a much smaller proportion of individuals with a Q mitochondrial haplotype while the Bahamas feeding area, in the Western Atlantic, in contrast, was composed of approximately equal numbers of all three major haplotypes.

This larger regional context aside, it is impossible to determine with precision, the natal origins of juveniles in the Mona Island feeding aggregation. The presence of common and broadly distributed haplotypes makes point-source estimation difficult. Our MSA estimations showed wide confidence intervals and varied depending on the underlying model and analytical approach (Fig. 3b). Nevertheless, our mtDNA data do underscore how relatively unimportant self-recruitment was to juvenile feeding aggregation on Mona Island. Indeed, only three out of 118 juveniles sampled possessed mtDNA haplotypes unique to the Mona Island rookery (and therefore likely originating on Mona Island), and the MSA suggested that both Mona Island and the small rookery in Costa Rica contributed an equal (and low) number of individuals to the standing juvenile population on Mona Island.

Fine-scale genetic structure of the hawksbill sea turtle aggregation of Mona Island

We showed that both stochastic and deterministic forces influence the population genetic architecture of the Mona Island hawksbill turtle aggregation and that the relative importance of these forces depends on gender and life-history stage. Together, these data suggest a generalized model for the Mona aggregation (Fig. 4). Under this model, juvenile recruitment into Mona is driven primarily by oceanic mixing. Recruits are mainly drawing from rookeries across the wider Caribbean region and the long residence time of juveniles in the aggregation results in a general build-up in mtDNA haplotype diversity in this segment of the population. In contrast, natal imprinting returns both adult females and males to the Mona aggregation during the breeding season. Imprinting and natal fidelity is quite strong in females and is responsible for observed mtDNA haplotype distinction of the Mona rookery. Males show less natal site fidelity and approximately 20% of the breeding males we sampled were not born on Mona. These males are not obviously recruiting from the juvenile aggregation, but instead use other cues, possibly social, to locate Mona Island. Presently, it is unclear if this class of males consistently returns to Mona to breed as what appears to be the case in some fishes where social facilitation plays an important

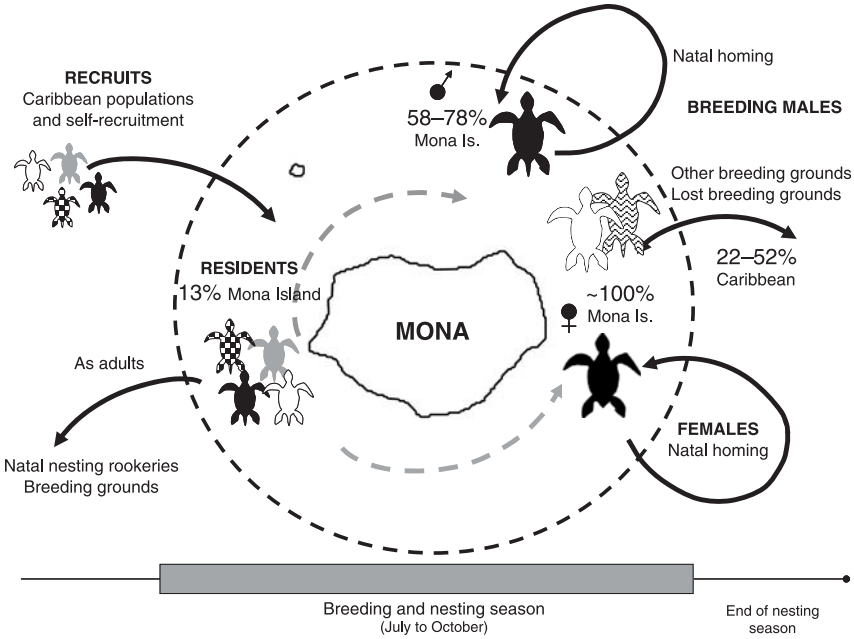

Fig. 4 Generalized model of the population structure of the Mona Island hawksbill turtles as a result of self-recruitment and input from other Caribbean rookeries during their life history.

role in spawning site fidelity (McQuinn 1997). Alternatively, these males may move between breeding sites. In either case, they are potentially important sources of gene exchange among the distinctive hawksbill turtle rookeries in the Western Atlantic and Caribbean.

The comprehensive view of the Mona Island population also highlights both the local and regional nature of hawksbill turtle conservation. At the local level, strong female fidelity to natal nesting beaches results in a high degree of mtDNA endemism. Nevertheless, turtles hatched at particular rookeries are dispersed broadly across the region. For example, individuals possessing the unique Mona Island mtDNA haplotype N (Ei-A20) were found in seven of the 10 feeding areas surveyed by Bowen *et al*. (2007). An identical, yet even more pronounced, pattern was evident in turtles likely originating from the large hawksbill turtle rookery on the Yucatán Peninsula. These individuals were distributed across the Caribbean, Gulf of Mexico and Western Atlantic, and this single rookery was the likely origin for over 20% of 626 juveniles sampled in that survey (Bowen *et al*. 2007). Feeding habitat fidelity of juveniles is strong and the juvenile aggregations are genetically diverse assemblages of individuals from many rookeries. Given these complex dynamics, it is easy to see how the effects of local harvesting of either adults or juveniles will resonate across the broader regional population.

Acknowledgements

We would like to acknowledge the help of the personnel of Mona Island for their invaluable collaboration to make this study possible, especially to Gaspar Pons and Tony Nieves for logistical support and to park rangers for help during the nightly beach patrols. Geoff Gearheart, Mónica Bustamante, Alberto Alvarez, Michelle Schärer, Gustavo Olivieri, Mabel Nava, 'Funchi' Egbreghts, Hana Lopez and volunteers during the nesting seasons [2003 (Marina Rujas, Camelia Manrique and Martha Rincon), 2004 (Jessica Castro and Maria Jose Andrade) and 2005 (M.J. Andrade and Diana Perez)] provided invaluable field assistance and helped with sample collection. Special thanks to Peri Mason, Julia Horrocks, Felix Moncada, E. Cuevas, Zandy Hillis-Star and Sebastian Tröeng for kindly providing rookery size data and to the students and staff from W.O.M.'s Ecological and Evolutionary Genetics Laboratory at the University of Puerto Rico for their assistance (Xaymara Serrano and Aida T. Miro) and technical advice (Manuel Ramirez, Jenny Acevedo, Silvia Planas, Ivania Ceron and Elsie Rivera) during laboratory analyses. Thanks to Mitch Aide for his insightful comments and early reviews of the manuscript and to Valerie McMillan for editing in the final phases of manuscript preparation. Michelle Masuda provided valuable advice with BAYES program. Carla Restrepo kindly provided guidance on the morphometric analysis. All field activities were conducted under DNER and USFWS permits. This project was funded by the National Science Foundation through the CREST-CATEC Program at UPR-RP (HRD 0206200), the DNER-PR and Japan Bekko Association. Sequences were generated at the University of Puerto Rico Sequencing and Genotyping Facility which is supported by grants from NIH-S.C.O.R.E. (S06 G08102), NIH-I.N.B.R.E. (P20 RR16470) and NSF CREST (HRD 0206200).

References

ADGF (Alaska Department of Fish and Game) (1999) *SPAM, Version 3.7: Statistics Program for Analyzing Mixtures*. Alaska Department of Fish and Game, Commercial Fisheries Division, Anchorage, Alaska.

Aubert H, Lightner VV (2000) Identification of genetic populations of the Pacific blue shrimp *Penaeus stylirostris* of the Gulf of California, Mexico. *Marine Biology*, **137**, 875–885.

Baillie J, Groombridge B (1996) *IUCN Red List of Threatened Animals*. International Union for the Conservation of Nature and Natural Resources, Gland, Switzerland.

Baker CS, Medrano-Gonzalez L, Calambokidis J *et al*. (1998) Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific. *Molecular Ecology*, **7**, 695–707.

Barber PH, Erdmann MV, Palumbi SR (2006) Comparative phylogeography of three codistributed stomatopods: origin and timing of regional lineage diversification in the coral triangle. *Evolution*, **60**, 1825–1839.

Bass AL (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**, 195–199.

Bass AL, Witzell WN (2000) Demographic composition of immature green turtles (*Chelonia mydas*) from the east central Florida coast: Evidence from mtDNA markers. *Herpetologica*, **56**, 357–367.

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 (2004) Multi-year analysis of stock composition of a loggerhead turtle (*Caretta caretta*) foraging habitat using maximum likelihood and Bayesian methods. *Conservation Genetics*, **5**, 783–796.

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.

Baums IB, Paris CB, Chérubin LM (2006) A bio-oceanographic filter to larval dispersal in a reef-building coral. *Limnology and Oceanography*, **51**, 1969–1981.

Bjorndal K, Jackson JBC (2003) Roles of sea turtles in marine ecosystems: reconstructing the past. In: *The Biology of Sea Turtles* (eds Lutz PL, Musick JA, Wyneken J), Vol. 2. CRC Press, Boca Raton, FL.

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, Bass AL, Chow S-M *et al*. (2004) Natal homing in juvenile loggerhead turtles (*Caretta caretta*). *Molecular Ecology*, **13**, 3797–3808.

Bowen BW, Grant WS, Hillis-Star Z *et al*. (2007) Mixed-stock analysis reveals the migration of juveniles hawksbill turtle (*Eretmochelys imbricata*) in the Caribbean Sea. *Molecular Ecology*, **16**, 49–60.

Bowman AW, Azzalini A (1997) *Applied Smoothing Techniques for Data Analysis*. Oxford University Press, Oxford, UK.

Broderick AC, Godley BJ, Hays GC (2001) Trophic status drives interannual variability in nesting numbers of marine turtles. *Proceedings of the Royal Society B: Biological Sciences*, **268**, 1481–1487.

Brown BL, Epifanio JM, Smouse PE, Kobak CJ (1996) Temporal stability of mtDNA haplotype frequencies in American shad stocks: to pool or not to pool across years? *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 2274–2283.

Brykov VA, Polyakova N, Skurikhina LA *et al*. (1999) Analysis of mtDNA indicates weak temporal genetic heterogeneity in pink salmon spawning runs in two rivers on Sakhalin Island. *Journal of Fish Biology*, **55**, 617–635.

Carr A (1987) New perspectives on the pelagic stage of sea turtle development. *Conservation Biology*, **1**, 103–121.

Carr A, Carr MH (1970) Modulated reproductive periodicity in *Chelonia*. *Ecology*, **51**, 335–337.

Charnov EL (1993) Life history invariants: some explorations of symmetry in evolutionary ecology. *Oxford Series in Ecology and Evolution*. Oxford University Press, Oxford, UK.

van Dam RP, Diez CE (1996) Diving behavior of immature hawksbills (*Eretmochelys imbricata*) in a Caribbean cliff-wall habitat. *Marine Biology*, **127**, 171–178.

van Dam RP, Diez CE (1998) Home range of immature hawksbill turtles (*Eretmochelys imbricata* Linnaeus) at two Caribbean islands. *Journal of Experimental Marine Biology and Ecology*, **220**, 15–24.

van Dam RP, Diez CE, Balazs GH, Colón LAC, McMillan WO, Schroeder B (2008) Sex-specific migration patterns of hawksbill turtles from Mona Island, Puerto Rico. *Endangered Species Research*, in press.

Degnan SM (1993) Genetic variability and population differentiation inferred from DNA fingerprinting in silvereyes (Aves: Zosteropidae). *Evolution*, **47**, 1105–1117.

Diaz-Fernandez R, Okayama T, Uchiyama T *et al*. (1999) Genetic sourcing for the hawksbill turtle, *Eretmochelys imbricata*, in the northern Caribbean region. *Chelonian Conservation and Biology*, **3**, 296–300.

Diez CE, van Dam RP (2002) Habitat effect on hawksbill turtle growth rates on feeding grounds at Mona and Monito Islands, Puerto Rico. *Marine Ecology Progress Series*, **234**, 301–309.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Excoffier L, Laval G, Schneider S (2005) ARLEQUIN version 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

FitzSimmons NN, Limpus CJ, Norman JA *et al*. (1997a) Philopatry of male marine turtles inferred from mitochondrial DNA markers. *Proceedings of the National Academy of Sciences, USA*, **94**, 8912–8917.

Fitzsimmons N, Moritz C, Limpus CJ *et al*. (1997b) Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and male biased gene flow. *Genetics*, **147**, 1843–1854.

Garant D, Dodson JJ, Bernatchez L (2000) Ecological determinants and temporal stability of the within-river population structure in Atlantic salmon (*Salmo salar* L.). *Molecular Ecology*, **9**, 615.

Gladden JGB, Ferguson MM, Clayton JW (1997) Matriarchal genetic population structure of North American beluga whales *Delphinapterus leucas* (Cetacea: Monodontidae). *Molecular Ecology*, **6**, 1033–1046.

Greenwood PJ (1980) Mating systems, phylopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140–1162.

Hoelzel AR, Hey J, Dahlheim ME *et al*. (2007) Evolution of population structure in a highly social top predator, the killer whale. *Molecular Biology and Evolution*, **24**, 1407–1415.

Johnson ML, Gaines MS (1990) Evolution of dispersal: theoretical models and empirical tests using birds and mammals. *Annual Review of Ecology and Systematics*, **21**, 449–480.

Karl SA, Bowen BW, Avise JC (1992) Global population genetic structure and male-mediated gene flow in the green turtle (*Chelonia mydas*): RFLP analyses of anonymous nuclear loci. *Genetics*, **131**, 163–173.

King DP, Ferguson A, Moffett IJ (1987) Aspects of the population genetics of herring *Clupea harengus* around the British Isles UK and in the Baltic Sea. *Fisheries Research*, **6**, 35–52.

Lahanas PN, Bjorndal KA, Bolten AB *et al*. (1998) Genetic composition of a green turtle (*Chelonia mydas*) feeding ground population: evidence for multiple origins. *Marine Biology*, **130**, 345–352.

Lara-Ruiz P, Lopez GG, Santos FR, Soares LS (2006) Extensive hybridization in hawksbill turtles (*Eretmochelys imbricata*) nesting in Brazil revealed by mtDNA analyses. *Conservation Genetics*, **7**, 773–781.

León Y, Diez CE (1999) Population structure of hawksbill turtles on a foraging ground in the Dominican Republic. *Chelonian Conservation and Biology*, **3**, 230–236.

Limpus CJ (1992) The hawksbill turtle, *Eretmochelys imbricata*, in Queensland: population structure within a southern Great Barrier Reef feeding ground. *Wildlife Research*, **19**, 489–506.

Lloyd M, Dybas HS (1966) The periodical cicada problem. I. Population ecology. *Evolution*, **20**, 133–149.

Luke K, Horrocks JA, LeRoux RA *et al*. (2004) Origins of green turtle (*Chelonia mydas*) feeding aggregations around Barbados, West Indies. *Marine Biology*, **144**, 799–805.

McClenachan L, Jackson JBC, Newman MJH (2006) Conservation implications of historic sea turtle nesting beach. *Frontiers in Ecology and the Environment*, **4**, 290–296.

McMillan WO, Palumbi SR (1995) Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. *Proceedings of the Royal Society of London, Series B*, **260**, 229–236.

McQuinn IH (1997) Metapopulations and the Atlantic herring. *Reviews in Fish Biology and Fisheries*, **7**, 297–329.

Meylan AB, Donelly M (1999) Status justification for listing the hawksbill hurtle (*Eretmochelys imbricata*) as critically endangered on the 1996 IUCN Red List of Threatened Animals. *Chelonian Conservation and Biology*, **3**, 200–224.

Moffett IJJ, Crozifr WW (1996) A study of temporal genetic variation in a natural population of Atlantic salmon in the River Bush, Northern Ireland. *Molecular Ecology*, **48**, 302.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York, NY.

Okuyama T, Bolker BM (2005) Combining genetic and ecological data to estimate sea turtle origins. *Ecological Applications*, **15**, 315–325.

Pandolfi JM, Bradbury RH, Sala E *et al*. (2003) Global trajectories of the long-term decline of coral reef ecosystems. *Science*, **301**, 955–958.

Pella JJ, Masuda M (2001) Bayesian methods for analysis of stock mixtures from genetic characters. *Fisheries Bulletin*, **99**, 151–167.

Richardson JI, Bell R, Richardson TH (1999) Population ecology and demographic implications drawn from an 11-year study of nesting hawksbill turtles, *Eretmochelys imbricata*, at Jumby Bay, Long Island, Antigua, West Indies. *Chelonian Conservation and Biology*, **3**, 244–250.

Roberts MA, Schwartz TS, Karl SA (2004) Global population genetic structure and male-mediated gene flow in the green sea turtle (*Chelonia mydas*): analysis of microsatellite loci. *Genetics*, **166**, 1857–1870.

Rocha-Olivares A, Vetter RD (1998) Effects of oceanographic circulation on the gene flow, genetic structure, and phylogeography of the rosethorn rockfish (*Sebastes helvomaculatus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 803–813.

Saba VS, Santidrian-Tomillo P, Reina RD *et al*. (2007) The effect of the El Nino Southern Oscillation on the reproductive frequency of Eastern Pacific leatherback turtles. *Journal of Applied Ecology*, **44**, 395–404.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science*, **236**, 787–792.

Tröeng S, Dutton PH, Evans D (2005) Migration of hawksbill turtles *Eretmochelys imbricata* from Tortuguero, Costa Rica. *Ecography*, **28**, 394–402.

Wallace BP, Kilham SS, Paladino SS *et al*. (2006) Energy budget calculations indicate resource limitation in Eastern Pacific leatherback turtles. *Marine Ecology Progress Series*, **318**, 263–270.

Wibbels T, Owens DW, Rostal D (1991) Soft plastra of adult male sea turtles: an apparent secondary sexual characteristic. *Herpetological Review*, **22**, 47–49.

Yang LH (2004) Periodical cicadas as resource pulses in North American forests. *Science*, **306**, 1565–1567.

Zaykin DV, Pudovkin AI (1993) Two programs to estimate the significance of χ^2 values using pseudoprobability tests. *Journal of Heredity*, **84**, 152.