

# Origin of juvenile loggerhead turtles (*Caretta caretta*) in a tropical developmental habitat in Caribbean Panamá

Tag N. Engstrom<sup>1,3</sup>, Peter A. Meylan<sup>1</sup> and Anne B. Meylan<sup>2</sup>

<sup>1</sup> Natural Sciences Collegium, Eckerd College, 4200 54th Ave S., St. Petersburg, FL 33711, USA

<sup>2</sup> Florida Fish and Wildlife Conservation Commission, Florida Marine Research Institute, 100 8th Avenue SE, St. Petersburg, FL 33701, USA

<sup>3</sup> Present address: Section of Evolution and Ecology and Center for Population Biology, University of California, One Shields Avenue, Davis, CA 95616, USA

(Received 16 April 2001; accepted 26 November 2001)

## Abstract

Maximum likelihood mixed stock analysis was used to identify the natal origin of immature loggerhead turtles (*Caretta caretta*) in a tropical developmental habitat in Caribbean Panamá. Approximately 65–70% of the loggerhead turtles in Chiriquí Lagoon originate from South Florida nesting beaches, and the other 30–35% originate from Mexico. Haplotype frequencies of the Chiriquí Lagoon loggerhead population are significantly different from those observed in the pelagic environment in the eastern Atlantic, and estimated nesting beach contributions to Chiriquí Lagoon are significantly different from values expected if recruitment were based solely on the size of nesting populations. These observations suggest that dispersal of loggerheads into benthic developmental habitats from the pelagic environment is not random. The occurrence of US and Mexican loggerheads in tropical developmental habitats has not been previously recognized. Exploitation and other mortality factors operating in the Caribbean area must be taken into account in demographic models and management plans for these two populations. This exploitation could be particularly important for the small, demographically vulnerable Mexican population and for other small populations for which no genetic data are currently available.

## INTRODUCTION

The migratory lifestyle and temporally protracted life history of marine turtles present difficulties for the effective conservation of these species (Carr, Carr & Meylan, 1978; Bowen, 1995). Hatchlings leave the nesting beach immediately after emerging from the nest, swim directly offshore, and enter a pelagic life-history stage for 10 or more years prior to recruiting as juveniles to near-shore developmental habitats (Carr, 1986, 1987; Bolten & Balazs, 1995). Turtles either may remain in the same developmental habitat through maturation (Limpus, Couper & Read, 1994*a,b*), or they may migrate among different developmental habitats and then migrate to a separate adult feeding ground where maturation takes place (Carr *et al.*, 1978; Ehrhart, 1989; A. Meylan & P. Meylan, unpublished data). Adult turtles make periodic reproductive migrations from feeding grounds to nesting beaches that may be separated by tens, hundreds or

thousands of kilometers (Meylan, 1982; Limpus *et al.*, 1992; Bowen *et al.*, 1995). Despite subdivision of populations on the nesting beaches, extensive migration of post-hatchling, juvenile and adult turtles provides opportunity for these reproductively isolated stocks to mix in foraging habitats, thus making it difficult to assess how exploitation of turtles on feeding grounds will affect reproductive populations (Carr *et al.*, 1978; Bowen & Karl, 1997).

All sea turtle nesting populations examined to date show a pattern of strong population subdivision in mitochondrial DNA (mtDNA). This pattern is consistent with the natal homing hypothesis (Meylan, Bowen & Avise, 1990) and also suggests that genetically distinct nesting aggregations are demographically independent and must be treated as separate management units (Bowen & Karl, 1997). Differences in mtDNA haplotype frequencies among nesting populations provide genetic markers that are powerful tools for the identification of the natal origin of turtles captured away from nesting beaches (Bowen & Karl, 1997). Maximum likelihood based techniques of mixed stock analysis (Pella & Milner, 1987) are now widely applied to estimate nesting beach

All correspondence to: Tag N. Engstrom.  
Section of Evolution and Ecology, University of California,  
One Shields Ave, Davis, California 95616. Tel.: 530-752-1112;  
Fax: 530-752-1449; E-mail: tngstrom@ucdavis.edu.

contributions to marine turtle feeding grounds (Bowen *et al.*, 1996; Bass, Lageux & Bowen, 1998; Bolten *et al.*, 1998; Laurent *et al.*, 1998; Bass & Witzell, 2000; Rankin-Baransky *et al.*, in press). This information has provided valuable insights into previously intractable questions regarding the ecological geography of marine turtles and has been particularly useful in tracking migration, dispersal and recruitment patterns of juvenile turtles. Information about the origin of turtles on foraging grounds has direct applications to conservation because incidental and direct take of turtles on foraging grounds has the potential to affect reproductive populations on a wide geographic scale (Bowen & Karl, 1997).

Since 1987, the Bocas Turtle Project has studied the ecology and migrations of the immature green (*Chelonia mydas*) loggerhead (*Caretta caretta*) and hawksbill turtles (*Eretmochelys imbricata*) that use the eastern end of the Chiriquí Lagoon as developmental habitat (A. Meylan & P. Meylan, unpublished data). Chiriquí Lagoon is an approximately 2000 km<sup>2</sup> bay located on the Caribbean coast of Panamá in Bocas del Toro Province, at 09° 00' N, 081° 50' W (Fig. 1). No major nesting beaches for loggerhead turtles currently exist in the tropics of the western Atlantic, so the occurrence of immature loggerheads in this part of the tropical Atlantic seems anomalous, and the origin of the turtles in Chiriquí Lagoon has been completely unknown. In this component of the larger long-term study, we use sequence data from the mtDNA control region to estimate the contribution of Atlantic Ocean stocks to the Chiriquí Lagoon foraging ground. We use our results to test predictions regarding patterns of recruitment of juvenile marine turtles to developmental habitats, and we evaluate the potential that ongoing exploitation in Chiriquí Lagoon, although small-scale, may adversely affect small, vulnerable nesting populations that are geographically far removed.

## MATERIALS AND METHODS

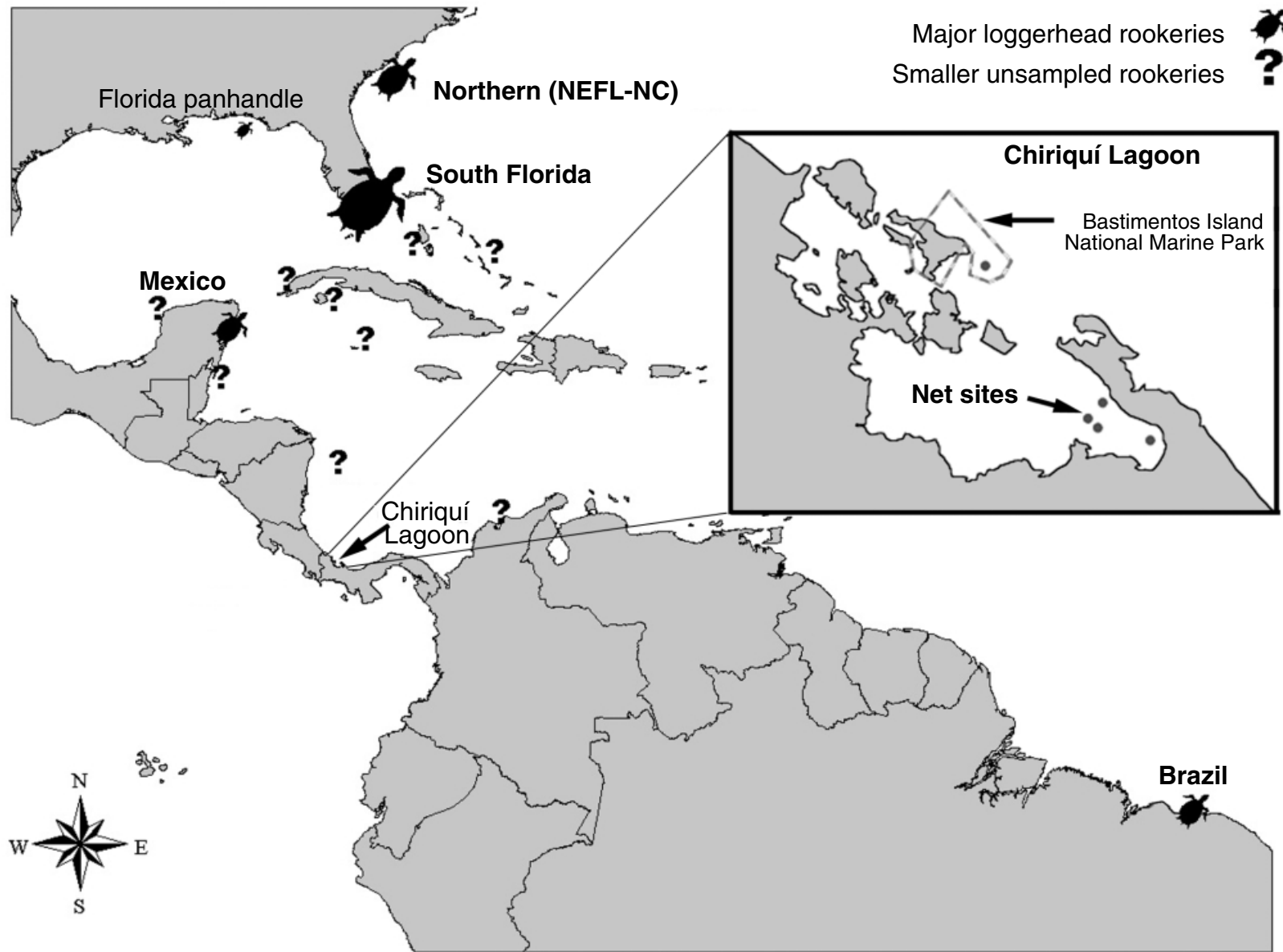
Turtles for this study were captured in the eastern end of Chiriquí Lagoon using a netting technique that is common in the turtle fisheries throughout the Caribbean region. Large-mesh (25–40 cm) tangle nets 10–80 m long are held in place at each end by an anchor and a balsa wood buoy. In lieu of a lead line, the net is held vertical in the water by tying a series of small rocks to the bottom mesh, thus allowing an entangled turtle to breathe by bringing the net to the surface. Entangled turtles were removed from nets at least twice daily and brought back to a base camp on shore for measurement and sample collection.

The straight carapace length (notch-to-notch, standard and maximum) and width, curved carapace length, plastron length and length of tail to the cloaca and to the tip were measured using standard techniques as described by Bolten (1999). Laparoscopic examination of the gonads was used to determine the sex and maturity of 16 loggerhead turtles captured during 1989–1991 and 1997 following the criteria of Limpus & Reed (1985).

In subsequent years maturity was assessed by size. Blood samples for genetic analysis were collected from the 45 immature loggerhead turtles captured in Chiriquí Lagoon during May–June field seasons of 1993, 1994 and 1997. Blood was drawn from the cervical sinus as described by Owens & Ruiz (1980) and preserved in a lysis buffer consisting of 10 mM EDTA, 100mM Tris-HCl and 1.0% SDS at pH 8.0 at a blood:buffer ratio of 1:10. Blood samples were stored at ambient temperature in the field until transport to the laboratory where they were stored at 4°C. Genomic DNA was isolated from blood samples using standard phenol:chloroform extraction methods (Palumbi, 1996) and stored at –20° C.

A ~390 base pair (bp) segment of mtDNA control region was amplified by the polymerase chain reaction (PCR) (Saiki *et al.*, 1986) using the primer pair TCR5 (5'-TTGTACATCTACTTATTTACCAC-3') TCR6 (5'-GTACGTACAAGTAAAACCTACCGTATGCC-3') (Norman, Moritz & Limpus, 1994). Standard precautions including the use of negative controls were taken to avoid contamination. PCR products were purified using either streptavidin-coated beads (Megabeads, Promega Corp., Madison, WI), or 30,000 ng DNA filters (Ultrafree-MC Millipore Corp., Bedford, MA) and sequenced at the DNA Sequencing Core Lab at the University of Florida or at the Division of Biological Sciences DNA Sequencing Facility at the University of California, Davis. Sequences were aligned by eye with previously published loggerhead control region sequences (Bolten *et al.*, 1998; Encalada *et al.*, 1998) and compared with haplotypes in the online haplotype registry maintained by the Archie Carr Center for Sea Turtle Research (ACCSTR <http://accstr.ufl.edu>). Throughout this paper we use standardized haplotype nomenclature established by ACCSTR but also include the letter code of the previous convention in parentheses where appropriate. Sequences that showed ambiguity or did not match a previously published sequence were sequenced in both the forward and reverse direction. We tested for significant differences in haplotype frequencies among samples from Chiriquí Lagoon, pelagic habitats (Bolten *et al.*, 1998) and nesting beaches (Encalada *et al.*, 1998; Laurent *et al.*, 1998) by using Monte Carlo simulation as implemented in the program Monte Carlo RxC (W. Engles, University of Wisconsin).

Encalada *et al.* (1998) identified six genetically distinct stocks among loggerhead turtle nesting colonies in the Atlantic and Mediterranean. Laurent *et al.* (1998) identified an additional Mediterranean stock in Turkey and examined 60 more turtles from throughout the southeastern Mediterranean. Differences in haplotype frequencies between Encalada's and Laurent's samples from Greece and the southeastern Mediterranean were not significant ( $P > 0.25$ ) so all are combined as a single Greece–southeastern Mediterranean stock in our analyses. In this paper we sometimes refer to this Greece–SE Med population and the genetically distinct Turkey nesting population collectively as the Mediterranean populations. We use the names established by the Turtle Expert Working Group (TEWG,



**Fig. 1.** Map of the western Atlantic showing the location of loggerhead turtle nesting beaches in relation to Chiriquí Lagoon, Panamá. Locations of major nesting populations are indicated with the figure of a turtle, which is roughly proportional to the size of the nesting population (see Table 3). Locations of smaller populations for which no mtDNA information is available are indicated with question marks. Sites of turtle capture are indicated by dots on the inset map of Chiriquí Lagoon. The current boundary of the Bastimentos Island National Marine Park is outlined with a broken line

2000) for nesting populations in the Atlantic and Gulf of Mexico.

We assumed equal contributions as a starting point for iterations of two different maximum likelihood mixed stock analyses: (1) including only Atlantic nesting beaches as possible source populations; (2) including both Atlantic and Mediterranean nesting populations as source populations. In each of these two analyses, relative contributions of nesting populations in the Atlantic–Mediterranean basin to the Chiriquí Lagoon foraging ground were estimated by using three different mixed stock analysis programs: CONSQRT, UCON (Masuda, Nelson & Pella, 1991) and SHADRACQ (Xu, Kobak & Smouse, 1994). Population estimates for each nesting population in the Atlantic–Mediterranean basin (Laurent *et al.*, 1998; TEWG, 2000) were used to calculate nesting beach contributions that would be expected under a model of random recruitment. These expected contributions were compared with our estimated contributions by using adjusted  $X^2$  goodness-of-fit test (Sokal & Rohlf, 1995).

## RESULTS

Since 1987, a total of 81 loggerheads have been captured in Chiriquí Lagoon. The only mature *Caretta* captured at this site was a 98-cm adult male, which had recently lost its entire right front flipper. The remaining 80 turtles ranged in size from 45.5 to 76.5 cm straight carapace length notch-to-notch (SCLN), with a mean length of 59.8 cm ( $N = 80$ ,  $SD = 6.91$ ), and were judged to be immature on the basis of laparoscopy ( $N = 16$ ), small size, and the lack of development of secondary sex characteristics. The 45 turtles examined in this genetic study ranged from 48.1 to 73.3 cm SCLN with a mean length of 60.1 cm ( $N = 45$ ,  $SD = 7.07$ ).

Aligned nucleotide sequences for 380 bp of the mtDNA control region from these turtles yielded a total

of 25 polymorphic sites consisting of 22 transitions, no transversions and three insertion/deletions (indels). These polymorphic sites defined eight haplotypes among the 45 Chiriquí Lagoon turtles. Six of the haplotypes observed in Chiriquí Lagoon match haplotypes reported from nesting beaches (Encalada *et al.*, 1998; Laurent *et al.*, 1998). Two turtles from Chiriquí Lagoon showed haplotypes that have not yet been observed on any nesting beach. Haplotype CC-A12 (L) was previously found in a single pelagic-stage loggerhead (Bolten *et al.*, 1998); CC-A19 was reported from one stranded loggerhead from Georgia (A. L. Bass and B. W. Bowen, unpublished data). Haplotype CC-A12 differs from the endemic Mexican haplotype, CC-A10 (J), by a single transition at position 63. Haplotype CC-A19, differs from the common CC-A2 (B) haplotype by a single transition at position 259. Haplotype frequencies in Chiriquí Lagoon did not differ across years ( $P = 0.56$ ) and thus were pooled for all analyses (Table 1).

In pair-wise comparisons, haplotype frequencies in Chiriquí Lagoon differ significantly from those of each individual nesting population ( $P < 0.05$  in all cases) except for South Florida ( $P = 0.19$ ). However, the presence of two endemic Mexican haplotypes, CC-A9 (I) and CC-A10 (J), in our sample indicates that turtles from the Mexican population are also present in Chiriquí Lagoon. Haplotype frequencies in Chiriquí Lagoon also differ significantly from those observed in the pelagic habitat in the eastern North Atlantic ( $P = 0.043$ ) (Bolten *et al.*, 1998).

Because the mixed stock analysis programmes used here required that all individuals in the mixed population be assigned to types occurring in the included source populations, our analysis was limited to the 43 turtles with haplotypes already identified from nesting beaches (Encalada *et al.*, 1998; Laurent *et al.*, 1998). In analyses including only Atlantic nesting beaches as possible contributing sources (left columns of Table 2), contri-

**Table 1.** Distribution of mtDNA haplotypes of loggerhead turtles (*Caretta caretta*) in Chiriquí Lagoon, Panamá (developmental habitat), the eastern Atlantic (pelagic habitat), and seven Atlantic and Mediterranean nesting populations. Haplotypes occurring in Chiriquí Lagoon are indicated with an asterisk

Haplotype letter code	Chiriquí Lagoon	Eastern Atlantic	Florida panhandle	South Florida	Northern NEFL-NC	Mexico	Brazil	Greece–SE Med	Turkey
CC-A1*	10	60	34	22	104				
CC-A2*	26	50	4	24	1	11		78	19
CC-A3*	3	7	2	2		2			13
CC-A4							11		
CC-A5				1					
CC-A6								2	
CC-A7*	1		2	1					
CC-A8		1				1			
CC-A9*	1					1			
CC-A10*	2	3				5		1	
CC-A11		1							
CC-A12*	1	1							
CC-A13		2							
CC-A14		3							
CC-A15		1							
CC-A16		1							
CC-A17		1							
CC-A19*	1								
Totals	45	131	42	50	105	20	11	81	32

butions to the Chiriquí Lagoon foraging ground were divided between the South Florida (0.658 to 0.712) and Mexico (0.281 to 0.342) populations, while no other nesting beaches make significant contributions. If the Mediterranean stocks are included among possible sources (right columns of Table 2), the estimated contribution of these stocks is uniformly high (0.367–0.436 for Greece–SE Med.; 0.095–0.103 for Turkey), the contribution of Mexico remains similar (0.171 to 0.208), and the contribution from the Florida panhandle population, which consists of only a few hundred nesting females, is also very high (0.233–0.292). In these analyses the estimated contribution from the largest nesting population in the Atlantic, South Florida, is minimal (0.0–0.116). Contributions of Atlantic nesting beach populations estimated by each of the three mixed stock analysis programmes differ significantly from expected nesting beach contributions based on numbers of nests recorded at Atlantic and Mediterranean nesting beaches (Table 3).

## DISCUSSION

### Mixed stock analysis

On general principles one would hope that the best mixed stock analysis result would proceed from the inclusion of all available genetic data from all possible sources. In this case, with the genetic markers and analytical tools we have available, we believe that this simple approach is not the most effective and that it is

reasonable to use additional non-genetic data to rule out unrealistic maximum likelihood solutions. Although it is possible that some of the turtles in Chiriquí Lagoon are from Mediterranean beaches, the inclusion of these beaches as possible sources leads to the conclusion that over half of the turtles in Chiriquí Lagoon are from the Mediterranean and another third are from the tiny Florida panhandle population. If this were true it would represent an extraordinary biological phenomenon. The only record of recruitment of juvenile turtles from Mediterranean stocks to developmental or pelagic habitats in the Atlantic involves three animals out of a sample of 300 from developmental habitats in east Florida (D. Bagley, unpublished data). No other study has reported any contribution of Mediterranean stocks (Sears *et al.*, 1995; Bass *et al.*, 1998; Bolten *et al.*, 1998; Lahanas *et al.*, 1998; Bass & Witzell, 2000; Rankin-Baransky *et al.*, in press). It would also be difficult to propose a reasonable biological mechanism to explain the 20–30% contribution of the Florida panhandle population, whereas the neighbouring South Florida population, which is several orders of magnitude larger, contributes nothing.

Although the biological improbability of these results does not preclude their veracity, such an extraordinary result does encourage a closer examination of the methodology for possible sources of bias. The maximum likelihood algorithms employed here are known to produce spurious results under certain conditions: (1) if the ratio of observed haplotypes to populations examined is less than 1.5 (Xu *et al.*, 1994); (2) if haplotype frequencies

**Table 2.** Estimated nesting beach contributions to the Chiriquí Lagoon, Panamá, developmental habitat based on three maximum likelihood mixed stock analysis programmes. Results for analyses including only Atlantic nesting beaches as possible contributing sources are shown in the left-hand columns. Results for analyses including both Atlantic and Mediterranean nesting beaches are shown on the right.

Population	CONSQR contribution (SD)		UCON contribution		SHADRACQ contribution	
	Atlantic only	Atl. & Med.	Atlantic only	Atl. & Med	Atlantic Only	Atl. & Med.
South Florida	0.6814 (0.1326)	0.0172 (0.0199)	0.7119	0.1162	0.6580	0.0000
Mexico	0.3181 (0.1326)	0.2079 (0.1162)	0.2811	0.1775	0.3420	0.1712
Northern (NEFL–NC)	0.0000 (0.0000)	0.0000 (0.0000)	0.0002	0.0028	0.0000	0.0000
Florida panhandle	0.0006 (0.0006)	0.2896 (0.0784)	0.0068	0.2331	0.0000	0.2921
Brazil	0.0000 (0.0000)	0.0000 (0.0000)	0.0000	0.0000	0.0000	0.0000
Greece/SE Med	NA	0.3905 (0.1314)	NA	0.3669	NA	0.4357
Turkey	NA	0.0948 (0.1087)	NA	0.1035	NA	0.1009

**Table 3.** Comparison between maximum likelihood estimates of stock contributions and expected contributions of nesting beaches based on proportions of nesting females. Population size estimates for US beaches are based on data compiled by the Turtle Expert Working Group (TEWG, 2000) and represent mean number of females nesting annually between 1989 and 1995. Population estimates for Mexico are from Zurita, Herrera & Prezas (1993) and for Brazil are from Marcovaldi & Laurent (1996). Contribution proportions from Table 2 are converted into number of turtles expected in our sample of 43.

Nesting population	Population estimate	Expected contribution		Maximum likelihood estimates					
		Proportion	# Turtles	CONSQR		UCON		SHADRACQ	
				%	#	%	#	%	#
South Florida	15,595	85.75%	36.87	68.14	29.30	71.19	30.61	65.80	28.29
Mexico	561	3.08%	1.33	31.81	13.68	28.11	12.09	34.20	14.71
Northern (NEFL–NC)	1519	8.35%	3.59	0.000	0	0.02	0.01	0.00	0
Florida Panhandle	111	0.61%	0.26	0.06	0.02	0.68	0.29	0.00	0
Brazil	400	2.20%	0.95	0.00	0	0.00	0	0.00	0
				$\chi^2 \geq 110.06$ $P < 0.001$	$\chi^2 \geq 83.75$ $P < 0.001$		$\chi^2 \geq 129.60$ $P < 0.001$		

differ by less than 30% among populations (Broderick *et al.*, 1994); (3) if genetic diversity of source populations differs greatly and the haplotypes in the less diverse populations are a subset of those found in the more diverse populations (Davies, Villablanca & Roderick, 1999). All three of these conditions exist in the data set that includes the Mediterranean beaches. Furthermore, haplotype CC-A6 (F), which is the only endemic Mediterranean haplotype, was not detected in our sample, so all contributions ascribed to the Mediterranean could also be attributed to Atlantic populations.

We suggest that the exclusion of some source populations from mixed stock analyses is justified if it is biologically the most reasonable alternative and if the inclusion of those populations produces analytical conditions that are known to bias results. We feel that both biological and analytical lines of evidence strongly indicate that analyses that exclude the Mediterranean beaches are more accurate. Thus we have chosen to focus on results of analyses that exclude these populations. The development of molecular markers that unambiguously distinguish the Mediterranean from the Atlantic nesting populations will be an important avenue for future studies.

#### Patterns of juvenile loggerhead recruitment

Lahanas *et al.* (1998) observed that dispersal during the pelagic stage provides the potential thoroughly to mix turtles from separate nesting populations and that the content of this pelagic melting pot may be reflected in subsequent recruitment to benthic developmental habitats. Under this turtle soup model, the composition of the pool of post-hatchling turtles in the pelagic stage would be proportional to the sizes of contributing stocks. If subsequent recruitment of juvenile turtles to benthic developmental habitats represented an unbiased spoonful from this pool of potential recruits, then the proportions of juvenile turtles in benthic developmental habitats would also be expected to reflect the sizes of contributing stocks. Studies of pelagic-stage loggerhead turtles (Bolten *et al.*, 1998) in the eastern Atlantic and green turtles (*Chelonia mydas*) in developmental habitat in the Bahamas (Lahanas *et al.*, 1998) are consistent with this turtle soup model. Alternatively, any number of oceanographic and behavioural factors, including proximity to nesting beaches (Lahanas *et al.*, 1998), local current anomalies (Carr & Meylan, 1980) and selective settlement or directed migration, could lead to disproportionate nesting beach contributions to developmental habitats even if contributions to the pelagic habitats are proportional to rookery size.

Evidence both from direct comparisons of haplotype frequencies in Chiriquí Lagoon with haplotype frequencies in the pelagic habitat and from mixed stock analysis indicates that recruitment of juvenile loggerheads to Chiriquí Lagoon is not consistent with the simple, turtle soup model. Bolten *et al.* (1998) found that nesting beach contributions to pelagic loggerhead habitat in the eastern Atlantic were proportional to the sizes of

contributing rookeries. Haplotype frequencies in the Chiriquí Lagoon developmental habitat differ significantly from the haplotype frequencies in the eastern Atlantic pelagic sample, indicating that loggerhead turtles in Chiriquí Lagoon do not represent a random selection from the pool of potential recruits in the eastern Atlantic pelagic habitat. Similarly, mixed stock analysis estimates of the Chiriquí sample indicate that approximately 70% of the turtles in Chiriquí Lagoon originate from South Florida and 30% from Mexico. If recruitment to Chiriquí Lagoon were proportional to the sizes of nesting beaches in the Atlantic, approximately 86% of the loggerhead turtles captured in Chiriquí Lagoon would originate from South Florida, 8% from the Northern population (northeast Florida–North Carolina), 3% from Mexico and 2% from Brazil. The overrepresentation of Mexican turtles in our sample indicates that recruitment to this developmental habitat is not random and is likely driven by oceanographic or behavioural factors other than simply the size of contributing populations. Evidence is slowly accumulating for the predominance of this non-random recruitment in marine turtles (Sears *et al.*, 1995; Laurent *et al.*, 1998; Bass & Witzell, 2000; Rankin-Baransky *et al.*, in press; D. Bagley, unpublished data). As a caveat, it is important to realize that although we have used both nesting beach population estimates and maximum likelihood estimates in quantitative analyses, there are inherent limitations of these data for such purposes. The calculations of expected nesting beach contributions are based on population estimates with unknown error. The mixed stock analysis is hampered by the small sample size of 43 individuals available for this study and by incomplete nesting beach sampling. Given these limitations, it is important that even the quantitative results presented here be interpreted in a qualitative manner.

#### Conservation implications

Chiriquí Lagoon supports a significant population of immature loggerheads. More than one-third (81 of 216) of the turtles captured in Chiriquí Lagoon by the Bocas Turtle Project have been loggerheads. The presence of significant numbers of Mexican and US turtles in Chiriquí Lagoon shows that this area, and perhaps other, as-yet-undescribed feeding grounds in the Caribbean, are important developmental habitats for these nesting populations. Mortality sources in Caribbean developmental habitats are not currently included in demographic models for Florida or Mexican loggerhead populations (TEWG, 2000). Mortality sources in Caribbean developmental habitats could be particularly detrimental to the persistence of the Mexican loggerhead population because it is small (~500 females) and therefore demographically vulnerable. Demographic models for marine turtles indicate that population growth is sensitive to mortality of the subadult size class that is present in Chiriquí Lagoon (Crouse, Crowder & Caswell, 1987) and that harvest of just a few hundred subadult individuals from a small population can lead to

population decline (Heppell *et al.*, 1996). Tag return data indicate that at least seven of the 81 (8.6%) loggerhead turtles tagged in Chiriquí Lagoon since 1987 have been subsequently captured in subsistence fisheries in Chiriquí Lagoon and elsewhere in the Caribbean. Thus, the persistence of the Mexican loggerhead population may be threatened because subadult turtles from this population are concentrated in Chiriquí Lagoon, and perhaps in other developmental habitats in the Caribbean where they are subject to harvest in small-scale fisheries. Although the Mexican nesting population is small, it is the most genetically diverse loggerhead population in the Atlantic or Mediterranean (Encalada, Zurita & Bowen, 1999). The loss of this nesting population would result in the loss of 30% of the mtDNA diversity in the Atlantic basin (Encalada *et al.*, 1999).

The presence of two new haplotypes in a sample of just 45 individuals from Chiriquí Lagoon indicates that genetic diversity on loggerhead nesting beaches is not completely known. The novel haplotypes observed in Chiriquí Lagoon may be present at a low frequency in the populations surveyed (Encalada *et al.*, 1998) and may have simply escaped detection because of sampling error. Alternatively, they may represent markers present in other nesting populations that have not yet been surveyed. In addition to the major nesting beaches in the US, Mexico and Brazil, loggerhead nesting occurs in several areas throughout the Atlantic (Fig. 1), including Colombia, the Caribbean coast of Central America, Cuba, Jamaica, the Cayman Islands, the Dominican Republic, Haiti, Puerto Rico and the Bahamas (Kaufman, 1975; Carr *et al.*, 1982; Ehrhart, 1989; Addison & Morford, 1996; Amorocho, Córdoba & Milkin, 1999; Aiken *et al.*, 2001). It is possible that these other small nesting populations are also contributing to Caribbean feeding grounds and would be similarly adversely affected by fisheries in the region. It is particularly interesting to consider the possibility that a Caribbean pool of advanced juveniles may persist from the once substantial Colombian rookery, which has collapsed in the last 20 years because of overharvesting (Amorocho *et al.*, 1999). Expanding the current nesting beach sampling to include Colombia and other small Caribbean beaches will enable us to identify the origin of turtles bearing these unknown haplotypes and to determine what, if any, contribution the small, nesting populations make to Chiriquí Lagoon and other Caribbean feeding grounds.

Based on the results of this study, we are able to make several recommendations regarding future research and conservation efforts specific to Chiriquí Lagoon and for marine turtle conservation in general.

Consideration should be given to extending the existing Bastimentos Island National Marine Park (Fig. 1) to include the eastern part of the Chiriquí Lagoon in which important marine turtle developmental habitat is found. By doing so, the government of Panamá would be granting protection to loggerhead turtles from Florida, from the vulnerable Mexican nesting population, and perhaps from other small, Caribbean nesting populations.

The documentation of incidental and direct take and the collection of genetic samples for mixed stock analysis should be a standard part of all studies of marine turtles on feeding grounds. These two types of data are necessary to access accurately the possible effects of harvest and incidental take in an area. In this study, we have shown that a disproportionately large number of Mexican loggerhead turtles inhabit Chiriquí Lagoon and have suggested that the local artisanal fishery may compromise the Mexican population. Although some data have been collected on this fishery (R. Landrum & P. Meylan, unpublished data), no attempt has been made to describe quantitatively the level of harvest. This is an important area of future research in Chiriquí Lagoon. It is essential that these data on mortality in Caribbean habitats be included in demographic models and management plans of both Mexican and Florida populations.

An effort should be made to increase sampling at surveyed beaches and to get samples from smaller, unsurveyed beaches, particularly in Colombia, in order to detect rare haplotypes. A concomitant effort should be made to identify and genetically characterize other tropical feeding grounds. The combination of these data would allow us to describe more accurately the geographic distribution of feeding grounds used by each nesting population and thereby predict more accurately the effects of mortality on those feeding grounds.

Finally, our results reinforce once again that marine turtles, when viewed as a resource, must be considered to be an internationally shared resource. Management of the western Atlantic marine turtle populations requires cooperation from countries spanning large political, environmental and geographic spectra, because these animals respect neither political nor climatological boundaries. Turtles from temperate nesting beaches in the US and from the more southerly populations from Mexico mix in tropical developmental habitat in Panamá. It is clear that marine turtle conservation efforts must focus not only on protection of nesting habitat but also on identification and protection of turtles in pelagic, developmental and adult foraging habitats.

### Acknowledgements

This research was carried out with permission from the Panamanian Protected Areas and Wildlife, National Authority for the Environment (ANAM). Blood samples were imported to the US under CITES permit US758093. Major funding was provided by a grant to P. & A. Meylan from the Wildlife Conservation Society, with additional funding or support from Eckerd College, the Ford Foundation, Florida Fish and Wildlife Conservation Commission, and an NSF grant to H. B. Shaffer. Invaluable logistical and technical support was provided by the Smithsonian Tropical Research Institute. The Chiriquí Land Company provided transportation on numerous occasions and contributed sterilized drums for our water supply. Allen Foley, Jim Quinn, Blair Whitherington and an anonymous reviewer provided helpful comments on the manuscript. We thank

D. Bagley, Anna Bass and Brian Bowen for use of unpublished information and the ACCSTR for information on haplotype designation. R. S. Duncan, R. Landrum, L. Castillo, G. Castillo and Poppa D. provided assistance in the field. Chenco Castillo was an indispensable source of turtle knowledge, history, humour and myths.

## REFERENCES

- Addison, D. S. & Morford, B. (1996). Sea turtle nesting activity on the Cay Sal Bank, Bahamas. *Bahamas J. Sci.* **3**: 31–36.
- Aiken, J. J., Godley, B. J., Broderick, A., Austin, T., Ebanks-Petrie, G. & Hays, G. C. (2001). Two hundred years after a commercial marine turtle fishery: the current status of marine turtles nesting in the Cayman Islands. *Oryx* **35**(2):145–151.
- Amorcho, D., Córdoba, B. J. A. & Milkin, H. S. (1999). Current status of nesting sea turtles in northern Colombian Caribbean. *Mar. Turt. Newslet* **85**: 6–7.
- Bass, A. L., Lagueux, C. J. & Bowen, B. W. (1998). Origin of green turtles, *Chelonia mydas*, at 'sleeping rocks' off the north-east coast of Nicaragua. *Copeia* **1998**: 1064–1069.
- Bass, A. L. & Witzell, W. N. (2000). Demographic composition of immature green turtles (*Chelonia mydas*) from the east central Florida coast: evidence from mtDNA markers. *Herpetologica* **56**: 357–367.
- Bolten, A. B. (1999). Techniques for measuring sea turtles. In *Research and management techniques for the conservation of sea turtles*: 110–114. Ekert, K. L., Bjorndal, K. A., Abreu-Grobois, F. A. & Donnelly, M. (Eds). IUCN/SSC Marine Turtle Specialist Group Publication No. 4.
- Bolten, A. B. & Balazs, G. H. (1995). Biology of the early pelagic stage: the 'lost year'. In *Biology and conservation of sea turtles*: 579–581. Bjorndal, K. A. (Ed.). Washington, DC: Smithsonian Institution Press.
- Bolten, A. B., Bjorndal, K. A., Martins, H. R., Dellinger, T., Biscoito, M. J., Encalada, S. E. & Bowen, B. W. (1998). Transatlantic developmental migrations of loggerhead turtles demonstrated by mtDNA sequence analysis. *Ecol. Applic.* **8**: 1–7.
- Bowen, B. W. (1995). Molecular genetic studies of marine turtles. In *Biology and conservation of sea turtles*: 585–587. Bjorndal, K. A. (Ed.). Washington, DC: Smithsonian Institution Press.
- Bowen, B. W., Abreu-Grobois, F. A., Balazs, G. H., Kamezaki, N., Limpus, C. J. & Ferl, R. J. (1995). Trans-Pacific migrations of the loggerhead turtle (*Caretta caretta*) demonstrated with mitochondrial DNA markers. *Proc. Natl. Acad. Sci. USA*. **92**: 3731–3734.
- Bowen, B. W., Bass, A. L., Garcia-Rodriguez, A., Diez, C. E., van Dam, R., Bolton, A., Bjorndal, K. A., Miyamoto, M. M. & Ferl, R. J. (1996). Origin of hawksbill turtles in a Caribbean feeding area as indicated by genetic markers. *Ecol. Applic.* **6**: 566–572.
- Bowen, B. W. & Karl, S. A. (1997). Population genetics, phylogeography, and molecular evolution. In *The biology of sea turtles*: 29–50. Lutz, P. & Musick, J. (Eds). Boca Raton, FL: CRC Press, Inc.
- Broderick, D., Moritz, C., Miller, J. D., Guinae, M., Prince, R. I. T. & Limpus, C. J. (1994). Genetic studies of the hawksbill turtle *Eretmochelys imbricata*: evidence for multiple stocks in Australian waters. *Pacific Conserv. Biol.* **1**: 123–132.
- Carr, A. F. (1986). Rips, FADS and little loggerheads. *BioScience* **36**: 92–100.
- Carr, A. F. (1987). New perspectives on the pelagic stage of sea turtle development. *Conserv. Biol.* **1**: 103–121.
- Carr, A. F., Carr, M. H. & Meylan, A. B. (1978). Ecology and migrations of sea turtles, 7: The west Caribbean green turtle colony. *Bull. Am. Mus. Nat. Hist.* **162**: 1–46.
- Carr, A. F. & Meylan, A. B. (1980). Evidence of passive migration of green turtle hatchlings in Sargassum. *Copeia* **1980**: 366–368.
- Carr, A. F., Meylan, A. B., Mortimer, J. A., Bjorndal, K. A. & Carr, T. (1982). *Surveys of sea turtle populations and habitats in the Western Atlantic*. US Department of Commerce, Panama City, FL.
- Crouse, D. A., Crowder, L. B. & Caswell, H. (1987). A stage-based population model for loggerhead turtles and implications for conservation. *Ecology* **68**: 1412–1423.
- Davies, N., Villablanca, F. X. & Roderick, G. K. (1999). Determining the source of individuals: multilocus genotyping in nonequilibrium population genetics. *TREE* **14**: 17–21.
- Ehrhart, L. (1989). Status report of the loggerhead turtle. In *Proceedings of the Second Western Atlantic Turtle Symposium*: 122–139. Ogren, L. et al. (Eds). NOAA Technical Memorandum NMFS-SEFC-226.
- Encalada, S. E., Bjorndal, K. A., Bolten, A. B., Zurita, J. C., Schroeder, B., Possardt, E., Sears, C. J. & Bowen, B. W. (1998). Population structure of loggerhead turtle (*Caretta caretta*) nesting colonies in the Atlantic and Mediterranean as inferred from mitochondrial DNA control region sequences. *Mar. Biol.* **130**: 567–575.
- Encalada, S. E., Zurita, J. C. & Bowen, B. W. (1999). Genetic consequences of coastal development: the sea turtle rookeries at X'acel, Mexico. *Mar. Turt. Newslet*, **83**: 8–10.
- Heppell, S. S., Limpus, C. J., Crouse, D. T., Frazer, N. B. & Crowder, L. B. (1996). Population model analysis for the loggerhead sea turtle, *Caretta caretta*, in Queensland. *Wildl. Res.* **23**: 143–159.
- Kaufman, R. (1975). Studies of the loggerhead sea turtle, *Caretta caretta* (Linné) in Colombia, South America. *Herpetologica*. **31**: 323–326.
- Lahanas, P. N., Bjorndal, K. A., Bolten, A. B., Encalada, S. E., Miyamoto, M. M., Valverde, R. A. & Bowen, B. W. (1998). Genetic composition of a green turtle (*Chelonia mydas*) feeding ground population: evidence for multiple origins. *Mar. Biol.* **130**: 345–352.
- Laurent, L., Casale, P., Bradai, M. N., Godley, B. J., Gerosa, G., Broderick, A. C., Schroth, W., Schierwater, B., Levy, A. M., Freggi, D., Abd El-Mawla, E. M., Hadoud, D. A., Gomati, H. E., Domingo, M., Hadjichristophorou, M., Kornaraky, L., Demirayak, L. F. & Gautier, C. (1998). Molecular resolution of marine turtle stock composition in fishery bycatch: a case study in the Mediterranean. *Mol. Ecol.* **7**: 1529–1542.
- Limpus, C. J., Couper, P. J. & Read, M. A. (1994a). The green turtle, *Chelonia mydas*, in Queensland: population structure in a warm temperate feeding area. *Mem. Queensland Mus.* **35**: 139–154.
- Limpus, C. J., Couper, P. J. & Read, M. A. (1994b). The loggerhead turtle, *Caretta caretta*, in Queensland: population structure in a warm temperate feeding area. *Mem. Queensland Mus.* **37**: 195–204.
- Limpus, C. J., Miller, J. D., Parmenter, C. J., Reimer, D., McLachlan, N. & Webb, R. (1992). Migration of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles to and from eastern Australian rookeries. *Wildl. Res.* **19**: 347–358.
- Limpus, C. J. & Reed, P. C. (1985). The loggerhead turtle, *Caretta caretta*, in Queensland [Australia]: observations on interesting behavior. *Austral. Wildl. Res.* **12**: 535–540.
- Marcovaldi, M. A. & Laurent, A. (1996). A six season study of marine turtle nesting at Praia do Forte, Bahia, Brazil, with implications for conservation and management. *Chelonian Conserv. Biol.* **2**: 55–59.
- Masuda, M., Nelson, S. & Pella, J. (1991). *User manual for GIRSEM, GIRLSYM and CONSORT. USA-DOC-NOAA-NMFS*. US-Canada Salmon Program, Juneau, Alaska.
- Meylan, A. B. (1982). Sea turtle migration: evidence from tag returns. In *Biology and conservation of sea turtles*: 91–100.



- Bjorndal, K. A. (Ed.). Washington, DC: Smithsonian Institution Press.
- Meylan, A. B., Bowen, B. W. & Avise, J. C. (1990). A genetic test of the natal homing versus social facilitation models for green turtle migration. *Science* **248**: 724–727.
- Norman, J. A., Moritz, C. & Limpus, C. J. (1994). Mitochondrial DNA control region polymorphisms: genetic markers for ecological studies of marine turtles. *Mol. Ecol.* **3**: 363–373.
- Owens, D. W. & Riuz, G. J. (1980). New methods of obtaining blood and cerebrospinal fluids from marine turtles. *Herpetologica* **36**: 17–20.
- Palumbi, S. R. (1996). Nucleic acids II: the polymerase chain reaction. In *Molecular systematics, second edition*: 205–247. Hillis, D. M., Maritz, C. & Mable, B. K. (Eds). Sunderland, MA: Sinauer Associates.
- Pella, J. J. & Milner, G. B. (1987). Use of genetic marks in stock composition analysis. In *Population genetics and fishery management*: 247–276. Ryman, N. & Utter, F. (Eds). Seattle: University of Washington Press.
- Rankin-Baransky, K., Williams, C. J., Bass, A. L., Bowen, B. W. & Spotila, J. R. (In press). Origin of loggerhead turtles stranded in the northeast United States as determined by mtDNA analysis. *J. Herpetol.*
- Saiki, R. K., Bugawan, T. L., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1986). Analysis of enzymatically amplified beta globin and Hla-Dq-alpha DNA with allele-specific oligonucleotide probes. *Nature* **324**: 163–166.
- Sears, C. J., Bowen, B. W., Chapman, R. W., Galloway, S. B., Hopkins-Murphy, S. R. & Woodley, C. M. (1995). Demographic composition of the feeding population of juvenile loggerhead turtles (*Caretta caretta*) off Charleston, South Carolina: evidence from mitochondrial DNA markers. *Mar. Biol.* **123**: 869–874.
- Sokal, R. R. & Rohlf, F. J. (1995). *Biometry: the principles and practice of statistics in biological research*. New York: W. H. Freeman.
- TEWG (Turtle Expert Working Group) (2000). *Assessment update for the Kemp's Ridley and loggerhead sea turtle populations in the western North Atlantic*. US Department of Commerce, NOAA Technical Memorandum NMFS-SEFCS-444.
- Xu, S., Kobak, C. J. & Smouse, P. E. (1994). Constrained least squares estimation of mixed population stock composition from mtDNA haplotype frequency data. *Can. J. Fish. Aq. Sci.* **51**: 417–425.
- Zurita, J. C., Herrera, R. & Prezas, B. (1993). Biología y conservación de las tortugas marinas en el litoral de Quintana Roo, Temporada (1990). In *IV Taller regional sobre programas de conservación de tortugas marinas en la península de Yucatán*. Frazier, J. (Ed.). Mexico: Universidad Autónoma de Yucatán, Mérida, Yucatán, México.

