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Annual variation in source contributions to a mixed stock: implications for quantifying connectivity

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Abstract

Connectivity among populations of highly migratory species is an area of active research and is often quantified with genetic markers. We determined mitochondrial DNA (mtDNA) sequences in 350 green turtles, *Chelonia mydas*, in 10 annual samples over a 12-year period from an aggregation of immature green turtles in the southern Bahamas. We found significant temporal structuring in haplotype frequencies among years for all turtles and for recruits. These significant differences were reflected in substantial variation in the relative contributions from different rookeries among years estimated by a Bayesian hierarchical model. Because this foraging aggregation has been the subject of a demographic study for over 30 years, we were able to determine that, among the three potential causes of temporal structuring–differential recruitment, mortality and emigration–recruitment accounts for most of this variation. We found that estimates of connectivity and genetic diversity in sea turtle populations are affected by the level of temporal variation reported here. More studies on the extent of temporal variation in composition of mixed stocks of other migratory species are needed to determine how this affects measures of connectivity.

Keywords: connectivity, marine turtles, migratory species, population structure, recruitment, temporal variation

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Introduction

Connectivity among populations has been the focus of a rapidly increasing number of studies over the past 15 years (Crooks & Sanjayan 2006a), driven both by theoretical interests in how populations function and by the need to maintain population connections to conserve species (Webster *et al*. 2002; Crooks & Sanjayan 2006b; Waples & Gaggiotti 2006). Quantification of connectivity is essential for conservation strategies, particularly to protect a migratory species throughout its range (Martin *et al*. 2007). Here, we refer to connectivity in the ecological sense as a concept used to describe the movement or exchange of organisms between geographically distinct habitats.

The earliest studies focused largely on terrestrial systems, often on issues of habitat fragmentation, but marine species and habitats have received more attention in recent years. Studies of connectivity in marine systems have focused on wide ranging species–from invertebrates with pelagic larvae to whales–in which different life stages inhabit widely separated habitats (DiBacco *et al*. 2006; Harrison & Bjorndal 2006). For example, green turtles (*Chelonia mydas*), like most sea turtle species, exhibit a complex life-history pattern (Bolten 2003) which may extend across ocean basins. An early dispersal of hatchlings from nesting beaches into oceanic waters is followed, after several years, by immature turtles recruiting to neritic foraging grounds (Reich *et al*. 2007). After recruitment, green turtles may undertake extensive developmental migrations among neritic foraging grounds. These foraging aggregations are mixed stocks from widespread sources (populations of nesting females at different beaches, hereafter called rookeries). After several decades, sexual maturity is attained, and green turtles then make periodic reproductive migrations to their natal nesting beaches that may be thousands of kilometers from their foraging areas. This distribution and mixing pattern characterize green turtles as having relatively weak migratory connectivity (*sensu* Webster *et al*. 2002). That is, individuals do not move between a single foraging ground and single breeding area (which would be strong connectivity); but rather, individuals at a foraging ground return to different

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breeding areas and individuals at a breeding area travel to different foraging grounds.

As efforts to evaluate connectivity in migratory species based on genetic 'tags' increase, a greater understanding of the effects of temporal variation is essential. Many marine species are characterized by populations that overlap extensively on foraging grounds, either in oceanic or neritic habitats. There is great interest in determining connectivity in these mixed stocks, or the relative contributions from source breeding stocks, particularly in species that are commercially important or endangered–such as Atlantic coast striped bass (Wirgin *et al*. 1993), great white sharks (Pardini *et al*. 2001), Pacific and Atlantic salmon (Shaklee *et al*. 1999; Crozier *et al*. 2004) and Atlantic bluefin tuna (Carlsson *et al*. 2007). Effective management of these migratory species requires knowledge of temporal as well as spatial patterns of movements and distribution.

Connectivity in sea turtle populations has been evaluated in a number of studies that have determined contributions from various rookeries to mixed stocks on foraging grounds (e.g. Luke *et al*. 2004; Bass *et al*. 2006; Carreras *et al*. 2006; Bolker *et al*. 2007; Bowen & Karl 2007; Bowen *et al*. 2007; and references cited therein) based on genetic sequences. All but three studies have evaluated a single sample from each mixed stock–either from one sampling time or a composite collected over several years–and thus have not evaluated temporal variation in spatial structuring. The three exceptions evaluated temporal variation in foraging aggregations of immature turtles (i) in a three-year study of loggerheads, *Caretta caretta* (Bass *et al*. 2004); (ii) in a two-year study of green turtles (Naro-Maciel *et al*. 2007); and (iii) in a five-year study of hawksbills, *Eretmochelys imbricata* (Velez-Zuazo *et al*. 2008). None of these studies found significant changes in haplotype frequency over time. However, as the respective authors noted, lack of significant variation may have resulted from the short time intervals of the first two studies, and from relatively small sample sizes in the third study (8–18 hawksbills per year).

Temporal variation in genetic structure in foraging aggregations could have significant effects on estimates of differentiation among foraging aggregations and would violate the assumption of stability in mixed-stock analyses (Naro-Maciel *et al*. 2007). We use mitochondrial DNA (mtDNA) sequence data collected over a 12-year period to assess temporal variation in the genetic composition of an aggregation of immature green turtles in the southern Bahamas and in the pattern of connectivity between this foraging aggregation and rookeries throughout the Atlantic. This study represents the longest study of genetic structuring in a sea turtle aggregation to date, and this aggregation has been the focus of a 30-year demographic study, which provides demographic parameters to evaluate possible mechanisms of temporal variation. We compare our results with those from this study site reported in an earlier paper (Lahanas *et al*. 1998), based on the first year of data reported here, to emphasize the importance of incorporating annual variation. We explore implications of temporal variation for the evaluation of connectivity in sea turtle populations and genetic diversity in sea turtle foraging aggregations.

Methods

Study site and data collection

Union Creek Reserve (UCR), on the north coast of Great Inagua in the southern Bahamas (21.12°N, 73.56°W), covers an area of approximately 20 km^2. In the Bahamas, the term 'creek' is applied to saltwater bays. UCR is within the Bahamas National Park system, and green turtles in UCR are protected from exploitation. Immature green turtles enter UCR, remain for varying lengths of time and then emigrate to other habitats throughout the Greater Caribbean prior to sexual maturity (Bjorndal *et al*. 2003).

Our study of green turtles at UCR began in 1975; data presented here were collected each year from 1992 through 2003, except 1995 and 1999. Each year, we captured as many turtles as possible (see Table 1 for sample sizes) during a 7–10 day interval and applied four flipper tags with identification numbers to each turtle. Turtles captured without tags are defined as recruits. Although some recruits may represent turtles that had been missed in the previous year's survey, such misidentification was minimal because our capture probabilities were high and tag loss was very low (Bjorndal *et al*. 2003). Over the course of our study, only 1.4% of all tags were lost, but all turtles were identified by other tags still attached. Blood or skin samples were collected from each turtle and stored at room temperature. Blood samples were preserved in lysis buffer (100 mM Tris-HCl, 100 mM EDTA, 10 mM NaCl, 1% SDS; pH 8.0), and skin samples in either saturated NaCl aqueous solution (250 mM EDTA, pH 7.0; 20% DMSO) or 70% ethanol.

mtDNA sequence analysis and data analyses

DNA isolations were conducted at the Genetics Analysis Laboratory at the University of Florida using a standard methodology. A 481 base-pair fragment at the 5′ end of the control region of the mitochondrial genome was amplified via polymerase chain reaction (PCR) methodology using primers LTCM2 and HDCM2 (Allard *et al*. 1994). Cycle sequencing reactions with fluorescently labelled dideoxynucleotides were performed and sequencing products were analysed with an automated DNA sequencer (Applied Biosystems model 373A) at the DNA Sequencing Core at the University of Florida. All samples were sequenced in the forward direction; novel haplotypes were also sequenced in the reverse direction to assure the accuracy of DNA-sequence designations. Sequences were aligned using

Table 1 Diversity values: haplotypes is number of haplotypes, *h* is haplotype diversity, and π is nucleotide diversity; *n* is sample size. Results from 1-sample Kolmogorov–Smirnov tests for uniform distribution (*ks*) and *P*-values; significant values are in bold

	n	Haplotypes	*h*	π
All turtles				
1992	80	7	0.39 ± 0.07	0.0066 ± 0.0038
1993	57	6	0.41 ± 0.07	0.0074 ± 0.0042
1994	57	9	0.56 ± 0.07	0.0067 ± 0.0039
1996	61	8	0.61 ± 0.06	0.0059 ± 0.0035
1997	62	11	0.62 ± 0.06	0.0060 ± 0.0035
1998	69	12	0.63 ± 0.06	0.0055 ± 0.0033
2000	64	8	0.63 ± 0.05	0.0048 ± 0.0030
2001	100	11	0.68 ± 0.04	0.0050 ± 0.0030
2002	95	10	0.59 ± 0.05	0.0051 ± 0.0031
2003	74	11	0.74 ± 0.04	0.0066 ± 0.0038
Composite	350	19	0.59 ± 0.03	0.0058 ± 0.0034
ks & *P* without composite sample		0.233, *P* = 0.57	0.296, *P* = 0.285	0.189, *P* = 0.802
ks & *P* with composite sample		**0.448, *P* = 0.016**	0.320, *P* = 0.169	0.181, *P* = 0.805
Recruits only				
1992	32	6	0.50 ± 0.09	0.0082 ± 0.0047
1993	22	4	0.45 ± 0.12	0.0072 ± 0.0042
1994	17	4	0.63 ± 0.08	0.0023 ± 0.0018
1996	20	5	0.75 ± 0.06	0.0074 ± 0.0044
1997	26	6	0.47 ± 0.12	0.0066 ± 0.0039
1998	25	8	0.63 ± 0.10	0.0046 ± 0.0029
2000	33	6	0.59 ± 0.07	0.0037 ± 0.0024
2001	60	11	0.68 ± 0.06	0.0050 ± 0.0031
2002	35	6	0.60 ± 0.08	0.0056 ± 0.0034
2003	26	8	0.81 ± 0.06	0.0082 ± 0.0047
ks & *P* without composite sample		**0.414, *P* = 0.044**	0.201, *P* = 0.744	0.219, *P* = 0.647

the program CLUSTAL X version 1.81 (Thompson *et al*. 1997). Haplotype designations were assigned according to the Marine Turtle DNA sequences website maintained by the Archie Carr Center for Sea Turtle Research at the University of Florida (http://accstr.ufl.edu/genetics.html).

The mtDNA haplotype was identified for every green turtle captured between 1992 and 2003. Analyses were conducted on annual samples and a composite sample. The composite sample was created by counting each individual turtle captured in UCR once; it is not the pooled annual samples. All analyses of haplotype diversity (*h*), nucleotide diversity (π), analysis of molecular variance (AMOVA), and exact tests of population differentiation (Raymond & Rousset 1995) were conducted using the software ARLEQUIN (version 2.0; Schneider *et al*. 2000). Estimates of sequence divergence for π used the Tamura–Nei model of nucleotide substitutions with no gamma correction (Tamura & Nei 1993). To assess significance of temporal structuring among annual samples from UCR, we used both exact tests of population differentiation and AMOVA. F_{ST} values were computed using conventional *F* statistics from haplotype frequencies, and significance was assessed by comparison to values generated from at least 20 000 random permutations of haplotypes among the UCR samples. To avoid excessive multiple pairwise comparisons of F_{ST} values, we tested the following groups: haplotype distribution for all turtles in 1992 [the sample previously published (Lahanas *et al*. 1998)] was compared with those of each of the annual samples, and the composite sample was compared with each of the annual samples. For recruits, we selected a pair of adjacent years (1996 and 1997) with very different haplotype frequencies to illustrate the level of difference that has been exhibited in the aggregation. Rookery contributions to UCR green turtle aggregations were assessed with a Bayesian hierarchical model (Okuyama & Bolker 2005). For rookery data, see Bolker *et al*. (2007); data available upon request. To determine whether there is annual variation in the extent to which mixed stock composition is affected by the size of the rookery populations and/or the distance between the rookery and the mixed stock, we used multiple regression [following Lahanas *et al*. (1998)] and rookery size and distance data from Lahanas *et al*. (1998) and Bolker *et al*. (2007). Distance estimates are minimum in-water distances (i.e. distances were not measured across land) and may well underestimate the distances travelled by the turtles. Statistical analyses were conducted with SPLUS software (version 7.03); alpha was 0.05.

Results

Nineteen haplotypes were found in the UCR samples including four new haplotypes: haplotype CM-A26 (GenBank

Accession No. AF366255) in five turtles, and CM-A28 (AF366257), CM-A34 (AF366263) and CM-A47 (EF406117) in one turtle each. Complete sequences are presented at the website http://accstr.ufl.edu/cmmtdna.html. Note that in the 1992 sample, the haplotype CM-A19 reported by Lahanas *et al*. (1998) in error, is now correctly assigned to CM-A18.

We determined haplotype frequencies for each of 10 years over a 12-year period (Table S1, Supplementary material) and compared three indices of genetic diversity (Table 1) among the samples. Number of haplotypes in the annual samples ranged from six to 12 and was 19 in the composite sample (the sample in which each individual turtle captured in UCR is counted once) of 350 individual green turtles (Table 1). This basic measure of diversity did not vary significantly among the annual samples (1-sample Kolmogorov–Smirnov tests for uniform distribution), but when the composite sample was included, variation among these samples was significant. Number of haplotypes ranged from four to 11 in annual samples of recruits, and annual variation was significant.

Haplotype diversity (h) varied by a factor of 1.9 and nucleotide diversity (π) by a factor of 1.5 for the annual samples and composite sample (Table 1). Neither of these measures varied significantly among samples with or without the composite sample. Haplotype diversity varied by a factor of 1.8 and nucleotide diversity varied by a factor of 3.6 for annual samples of recruits. Variation was not significant in either measure for recruits.

Haplotype frequencies (Table S1, Supplementary material; Fig. 1) varied significantly among annual samples (composite sample not included) for all turtles (AMOVA, $F_{ST} = 0.019$, $P = 0.0004$) and for recruits (AMOVA, $F_{ST} = 0.031$, $P = 0.004$). These F_{ST} values, although highly significant, were low, with only 2–3% of the variance attributed to among-sample variation. However, to put these values in perspective, five widely separated green turtle foraging aggregations in the Greater Caribbean (Bahamas, Florida, Nicaragua, Barbados and Venezuela) also had a highly significant ($P < 0.0001$) F_{ST} value, but only 6.4% of the variation was attributed to differences among aggregations (Bolker *et al*. 2007). Exact tests of population differentiation yielded similar results. The global test of differentiation among samples was significant ($P = 0$) and, of the 45 pairwise comparisons among years, 15 were significant (P-values from 0.031 to 0).

Comparisons of the haplotype frequency from 1992 [the one-year sample from UCR upon which Lahanas *et al*. (1998) was based] with annual and composite samples revealed that all samples were significantly different from 1992 except 1993 and 1994 (Table 2). Comparisons of the composite sample with annual samples revealed significant differences with 1992, 1993 and 2003; the difference between the composite and 2001 approached significance ($P = 0.088$). The pairwise comparison selected to illustrate the extent of difference between a pair of adjacent years for recruits (1996 and 1997) revealed a highly significant difference (pairwise $F_{ST} = 0.214$, $P = 0.0005$).

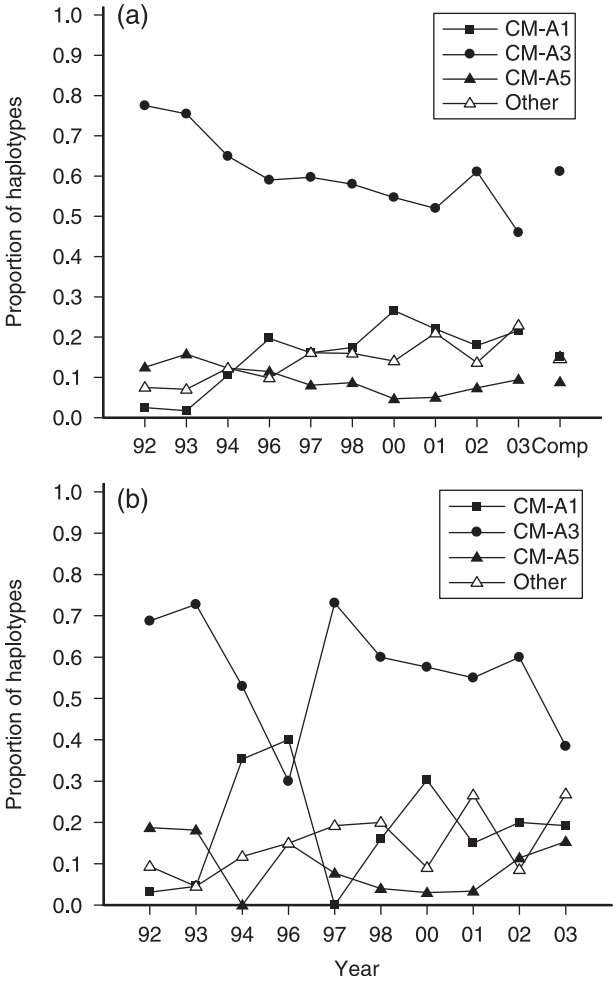

Fig. 1 Proportions of green turtle haplotypes for 10 years in Union Creek Reserve (UCR), Bahamas, for (a) all turtles and (b) recruits. Sample sizes are in Table 1. Haplotypes with fewer than five individuals ($n = 16$) in all of the annual samples were combined in 'Other.' 'Other' value for the composite sample in (a) overlapped with that for CM-A1. For a table of all haplotypes, see Table S1, Supplementary materials.

Mixed-stock analysis requires significant structuring among source populations; this requirement was satisfied by extensive structuring among rookeries (AMOVA, $F_{ST} = 0.669$, $P < 0.0001$). Many of the point estimates from the mixed stock analyses have broad 95% confidence intervals (Figs S1 and S2, Supplementary material), due primarily to the sharing of haplotypes among rookeries and unsampled or poorly sampled rookeries. Thus, the point estimates should be interpreted with caution.

Three rookeries (Tortuguero, Costa Rica; Yucatán, Mexico; and Florida, USA) were the major sources of turtles in UCR (Figs 2a and S1, Supplementary material). Relative proportions provided by these rookeries varied widely across the

Table 2 Pairwise comparisons of haplotype frequencies between 1992 and each other year and between the composite sample and each individual year (F_{ST} and P values). The composite is the sample in which each individual is included once, not the pooled annual samples. Significant values are in bold

	1992		Composite	
	F_{ST}	P	F_{ST}	P
1992	—		**0.033**	**0.002**
1993	–0.012	0.944	**0.030**	**0.009**
1994	0.011	0.14	–0.005	0.673
1996	**0.053**	**0.005**	–0.005	0.752
1997	**0.042**	**0.01**	–0.007	0.971
1998	**0.050**	**0.004**	0.007	0.977
2000	**0.098**	**0.0003**	0.009	0.101
2001	**0.086**	**< 0.0001**	0.007	0.088
2002	**0.044**	**0.004**	–0.005	0.985
2003	**0.105**	**< 0.0001**	**0.015**	**0.031**
Composite	**0.033**	**0.002**	—	

samples. For example, estimated proportions from Costa Rica ranged from 0.35 to 0.88 in the annual samples (composite sample = 0.58). Recruit samples had an even greater range of proportions from Costa Rica from 0.13 to 0.76 (Figs 2b and S2, Supplementary material). All rookeries combined, other than Costa Rica, Mexico and USA, provided approximately 0.06 to 0.10 to the annual samples, 0.06 to the composite sample, and 0.07 to 0.18 to the annual recruit samples, respectively. Even with cautious interpretation of the point estimates, visual inspection of Figs S1 and S2 indicates that the pattern of rookery contributions in 1992 and 1993, which was dominated by Costa Rica, differed from that of later years, such as 2003, when contributions from Florida and Mexico approached, or perhaps exceeded, that from Costa Rica. The extent to which mixed stock composition was related to the size of the rookery populations and/or the distance between the rookery and the mixed stock varied among years (Table 3).

Discussion

Mechanisms of temporal variation

We found significant temporal structuring in haplotype frequencies among years for all turtles and for recruits in UCR. These significant differences were reflected in substantial variation in the relative contributions from different rookeries among years. Three potential causes of temporal structuring are differential recruitment, mortality and emigration of green turtles in UCR from different rookeries. The long-term demographic studies on this population, initiated in 1975, allow us to distinguish among these factors (Bjorndal *et al*. 2000, 2003, 2005a). Body-size distributions of green turtles in UCR have been consistent over years, indicating that immature green turtles entered and left our study area at relatively consistent sizes (Bjorndal *et al*. 2000). Annual probabilities of emigration and of mortality were generated using combined live–recaptures and dead-recoveries models (Burnham models; Burnham 1993), based on both recaptures within UCR and tag returns from turtles killed outside of UCR. Probabilities of emigration and mortality have been constant over the years and were low (0.12 and 0.11, respectively) for the first three years after turtles recruit to UCR. After three years, emigration and mortality probabilities increase as larger turtles leave the protected area and are exposed to human-induced mortality (Bjorndal *et al*. 2003).

From these characteristics, we conclude that the variation in rookery contributions among years was not a result of differential emigration probabilities (which would result in different residence times) or differential mortality among turtles in UCR from different rookeries. Differential annual recruitment to UCR from the source rookeries was apparently the major cause of variation in annual composition. Number of recruits varied among years sufficiently to result in a five-fold variation in population abundance (Bjorndal *et al*. 2005a). The greater variation in annual recruit classes than

Table 3 Relationship of rookery contributions to two parameters: rookery size and distance between rookery and mixed stock. R^2 and P-values are presented for the model and t and P values for each parameter from multiple regressions. Significant values are in bold

	Multiple Regression		Rookery Size		Distance	
Sample	R^2	P	t	P	t	P
1992	0.973	**< 0.0001**	15.72	**< 0.0001**	–0.87	0.406
1993	0.968	**< 0.0001**	14.49	**< 0.0001**	–0.60	0.565
1994	0.947	**< 0.0001**	10.66	**< 0.0001**	–1.86	0.100
1996	0.819	**0.0011**	4.41	**0.002**	–2.54	**0.035**
1997	0.819	**0.0011**	4.41	**0.002**	–2.54	**0.035**
1998	0.791	**0.0019**	3.87	**0.005**	–2.54	**0.035**
2000	0.723	**0.0059**	3.02	**0.017**	–2.33	**0.048**
2001	0.526	0.0505	1.97	0.084	–1.52	0.167
2002	0.845	**0.0006**	5.30	**0.001**	–2.13	0.066
2003	0.528	0.0496	1.66	0.136	–1.86	0.100
Composite	0.892	**0.0001**	6.68	**0.0002**	–2.33	**0.048**

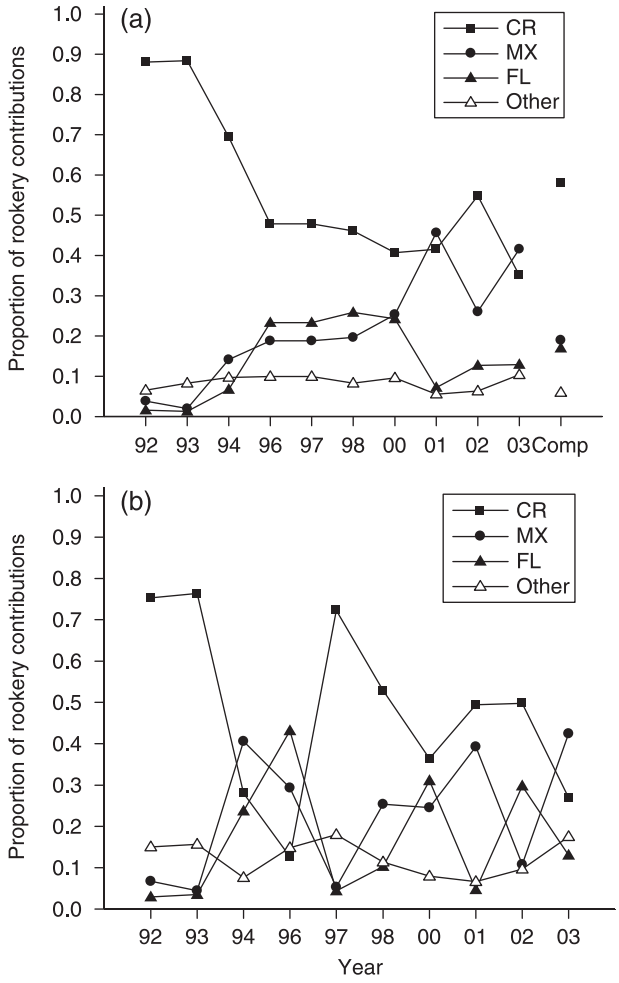

Fig. 2 Estimates of rookery contributions to green turtles for 10 years in Union Creek Reserve (UCR), Bahamas, based on Bayesian hierarchical model (Okuyama & Bolker 2005) for (a) all turtles and (b) recruits. CR is Tortuguero, Costa Rica; MX is Yucatán, Mexico; FL is Florida, USA; 'Other' is combination of remaining eight rookeries. For estimates for each rookery, and 95% confidence intervals around each estimate, see Figs S1 and S2, Supplementary materials.

in annual samples of all turtles is clear from the significantly greater variation in number of haplotypes and in haplotype frequencies in recruits and from a comparison of Fig. 2(a, b). The contribution from Costa Rica to each year's recruit class had sharp increases and decreases in adjacent years, for example, whereas the contributions from Costa Rica to the annual samples of all turtles exhibited smaller and almost unidirectional changes.

What are the mechanisms of annual variation in recruitment from source rookeries? Three mechanisms are most probable and are not mutually exclusive: (i) annual variation in haplotype frequencies at the rookeries; (ii) variation in annual productivity of hatchlings from the different rookeries (including differential survival during the oceanic stage); and (iii) changes in ocean current patterns. The first mechanism, significant annual variation in haplotype frequencies at the rookeries, is unlikely for two reasons. First, individual green turtles often shift among reproductive intervals of two, three or more years, resulting in extensive mixing among annual nesting aggregations (Carr *et al*. 1978; Solow *et al*. 2002) and probably maintaining genetic homogeneity among years. Second, no significant annual variation has been found at rookeries of three species of sea turtles: loggerheads from four genetically distinct rookeries in Japan, each sampled in two distinct years (Hatase *et al*. 2002); green turtles in Costa Rica in 2001 and 2002 (Bjorndal *et al*. 2005b); and hawksbills in Puerto Rico in 1994, 2003, 2004 and 2005 (Velez-Zuazo *et al*. 2008). In the last study, a significantly different distribution from 1993 was discounted because of differences in methods of sample collection and analyses (Velez-Zuazo *et al*. 2008; X. Velez-Zuazo, personal communication). However, most of these studies were only of two or three years' durations. Long-term studies are needed to assess the level of variation among years.

The second mechanism is more likely. Hatchling production at a rookery can vary substantially among years because of dramatic fluctuations in the numbers of sea turtles that nest each year, particularly for green turtles (Broderick *et al*. 2001; Solow *et al*. 2002), and because of long-term increasing or decreasing trends in abundance (Marine Turtle Specialist Group 2004; Chaloupka *et al*. 2008). Hatchling production at a rookery is also highly stochastic, with high success in some years and little or no production in other years as a result of storms, variation in predator populations, and variable offshore currents. These may, in some years, transport large proportions of hatchlings to habitats, such as waters off the British Isles, that cannot sustain them (Carr 1986; Hays & Marsh 1997). The third mechanism, changes in ocean current patterns, can cause differential recruitment by affecting sea turtle distribution during the early, oceanic life-stage and where they emerge from the oceanic habitat when they recruit to neritic foraging grounds.

To evaluate the importance of rookery production, we determined if a signal from rookery annual nesting numbers was apparent in the recruitment pattern at UCR. We used numbers of nests deposited annually at Tortuguero, Costa Rica (Solow *et al*. 2002); Yucatán, Mexico (A. Abreu, personal communication); and Florida, USA (http://research.myfwc.com) as a proxy for hatchling production from each of the three rookeries. Beginning in 1988, Costa Rica had ≥ 80% of the nests deposited at the three rookeries each year. Thus, no change in nest numbers could account for the variation from 13% (1996) to 76% (1993) in the contribution of Costa Rica to UCR recruits. This lack of signal could mean that rookery contributions to the recruit class each year are a result of recruitment pulses from one or more rookeries, or portions of rookeries, that escaped the stochastic calamities outlined above (storms, high predator

abundance, changes in current patterns) and produced progeny that reached recruitment size. Hedgecock (1994) developed the concept of 'sweepstakes' recruitment, in which a small portion of individuals can produce the great majority of recruits for a population because only a few individuals–through chance–reproduce at a time and place that provide the combination of biological and physical conditions required for successful larval survival to recruitment. Green turtles may exhibit 'sweepstakes' recruitment in which the large variance in the annual success of rookeries in producing hatchlings that survive the early oceanic stage means that only portions of a few rookeries win the 'sweepstakes' each year. For example, in a year in which storms destroy the vast majority of nests in Costa Rica, an off-shore current carries most of the hatchlings from Florida to Greenland where they freeze and Mexican rookeries enjoy high productivity of hatchlings, Mexico would win the sweepstakes for that year and produce the majority of recruits. Evidence for sweepstakes recruitment has been found for a number of marine species (Ruzzante *et al*. 1996; Grant & Bowen 1998).

However, lack of concordance between rookery nest numbers and recruitment levels at URC could also result from incomplete mixing in the oceanic stage and changes in currents that cause progeny from different rookeries to emerge from the oceanic habitat either near or far from UCR. Such changes in 'larval delivery' by currents can cause genetic heterogeneity in recruits in marine organisms (Selkoe *et al*. 2006). A region-wide survey in the Greater Caribbean or the entire Atlantic basin of recruits to many foraging grounds over several years could provide evidence for whether variation in hatchling production or ocean currents is more important for annual variation in recruitment.

Implications of temporal variation

For many species, source contributions are determined for mixed stocks to evaluate biological phenomena, such as patterns of connectivity and metapopulation boundaries, as well as to address important issues in conservation. If temporal structuring in mixed stocks is ignored, estimated source contributions may not be representative and misinterpretations may result. We present two examples here.

First, temporal variation can affect quantification of connectivity. Many studies of sea turtle mixed stocks have evaluated connectivity and have asked whether mixed stock composition is affected by the size of the rookery populations and/or the distance between the rookery and the mixed stock (reviewed in Bowen *et al*. 2007). Conclusions have varied among studies, and authors have invoked various reasons for these discrepancies. However, temporal variation may help explain the conflicting results. Using the 1992 UCR sample, Lahanas *et al*. (1998) evaluated the relationship between rookery contribution and rookery size or distance to rookery and found that size had a significant relationship but distance did not. We repeated their analysis with additional rookeries (see Bolker *et al*. 2007) and found the same results for 1992: size had a significant relationship and distance did not (Table 3). However, results were not consistent among years. In two of the 11 samples (annual and composite), rookery size did not have a significant relationship with rookery contribution. In five of the 11 samples, distance between rookery and UCR had a significant relationship with rookery contribution. These different conclusions underscore the importance of incorporating temporal variation into our evaluations of connectivity.

Second, temporal variation can affect priority rankings for conservation. Conservation of wide-ranging endangered species requires setting priorities for areas which should receive greater protection, and therefore greater investment of limited funds and effort. When ranking foraging grounds, priority may be given to those with higher genetic diversity or to those with greater contributions from more vulnerable rookeries. Our study demonstrates how temporal variation can influence these metrics and thus priority rankings. Naro-Maciel *et al*. (2007) ranked nine Atlantic green turtle foraging aggregations by three diversity indices: haplotype number (range 2–13), haplotype diversity (0.1831–0.7734) and nucleotide diversity (0.0022–0.0103). In their presentation, which was not intended to establish conservation priorities, UCR, based on the 1992 year class values from Lahanas *et al*. (1998), tied for fifth in haplotype number, ranked eighth in haplotype diversity, and ranked third in nucleotide diversity. However, if other annual samples from Table 1 were used in the comparison, UCR's rank would vary from the most diverse to nearly the least diverse in the Atlantic.

In regard to vulnerable rookeries, green turtle rookeries in the Greater Caribbean vary substantially in size, population trends and conservation status. The Costa Rican green turtle population is more robust than the smaller and more vulnerable rookeries in Aves Island, Florida, Mexico and Suriname. If priority rankings were assigned by the degree to which protection would help conserve vulnerable rookeries, the priority status of UCR would vary considerably among the annual samples, because the contribution of the four more vulnerable rookeries ranges from 9 to 61% in the extreme years of 1993 and 2003, respectively.

For migratory species, how common is significant temporal variation in contributions of source stocks to mixed stocks? Temporal variation should relate inversely to the strength of connectivity. Species that exhibit weak connectivity among aggregations–that is, the connection between a single foraging ground and single breeding area is weak (Webster *et al*. 2002)–should be more likely to have significant temporal variation. In addition, within and among species, aggregations most likely to exhibit significant temporal variation are those in which individuals recruit at relatively

young ages and remain resident for relatively short periods of time. In green turtles, young age at recruitment ensures that any variation in annual production of hatchlings from rookeries or in currents that distribute turtles will have the greatest probability of being exhibited. Short residence time maximizes the effect of annual variation. The average four-year residence time of green turtles in UCR results in the rookery contributions for any given year for the entire aggregation being a moving average of recruit classes of that and the previous three years. If turtles remained longer, more recruitment (year) classes would be mixed and would dampen variation in the aggregation among years. A similar pattern has been reported for the long-lived blue rockfish (*Sebastes mystinus*), in which genetic heterogeneity of recruit year-classes is transformed to genetic homogeneity as many year-classes of adults accumulate (Burford & Larson 2007). Thus, differential recruitment from source stocks among years can be masked in aggregations with long residence times unless recruits are evaluated separately.

The importance of incorporating temporal variation in analyses will depend on the question. As is clear from our study, one-year samples can yield very different conclusions depending upon which year is selected. Temporal variation should be considered in mixed-stock analyses and studies of population structure. Additional long-term studies of genetic structuring in breeding and foraging aggregations, particularly in migratory species with weak connectivity, are needed to evaluate the extent of temporal variation in natural populations. Studies with other markers, such as nuclear microsatellites, would also help determine the level of temporal variation.

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Karen Bjorndal's research focus is on the roles of sea turtles in marine ecosystems. Alan Bolten studies variation in sea turtle life history patterns. Both enjoy the privilege of studying sea turtles in The Bahamas.

Supplementary material

The following supplementary material is available for this article:

Table S1 Haplotype frequencies for annual samples of all green turtles at Union Creek Reserve. Comp is the composite sample of all individual turtles

Fig. S1 Estimates of rookery contributions to all green turtles for 10 years in Union Creek Reserve (UCR), Bahamas, based on Bayesian hierarchical model (Okuyama & Bolker 2005). Diamonds are point estimates; bars are 95% confidence intervals. Rookeries are Yucatán, Mexico (MX); Tortuguero, Costa Rica (CR); Florida, USA (FL); Aves Island, Venezuela (AV); Matapica, Suriname (SU); Atol das Rocas and Fernando de Noronha, Brazil (BR); Trindade Island, Brazil (TR); Ascension Island, UK (AS); Pailoa, Guinea Bissau (GB); Bioko and São Tome and Principe, Gulf of Guinea (GG); and Lara Bay, Cyprus (CY). Composite sample is composed of the individual green turtles.

Fig. S2 Estimates of rookery contributions to green turtle recruits for 10 years in Union Creek Reserve, Bahamas, based on Bayesian hierarchical model (Okuyama & Bolker 2005). Diamonds are point estimates; bars are 95% confidence intervals. Rookery abbreviations are given in Fig. S1.

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