



Composition of Hawaiian green turtle foraging aggregations: mtDNA evidence for a distinct regional population

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ABSTRACT: To examine the stock composition of Hawaiian foraging populations and evaluate current life-history hypotheses, mtDNA control region sequences from immature and adult green turtles that forage around the Hawaiian Islands were compared to potential source nesting populations across the Pacific. We examined the stock composition of the feeding ground (FG) populations at 5 index sites across the Hawaiian Archipelago, as well as animals stranded in areas outside these index sites. Six haplotypes, based on mtDNA sequences, were observed among the 788 green turtles sampled around the Hawaiian Islands. Stock mixture analysis shows that the Hawaiian FG populations comprise one genetic stock derived from the nesting population at French Frigate Shoals (FFS), based on a mean estimate of 99.9% from FFS as opposed to other potential source stocks. We identified only 3 turtles with haplotypes not found at FFS, indicating that Hawaiian FGs might occasionally, albeit rarely, be visited by animals from rookeries outside the Hawaiian Archipelago, both in the eastern and western Pacific. These findings lead us to conclude that the numerous foraging aggregations around the Hawaiian Islands can be considered part of a distinct regional population for management. The finding that FGs scattered across a distance of over 2400 km belong to one genetic stock is unique among sea turtles, and allows Hawaiian green turtles to be assessed separately from other Pacific stocks with respect to risk. We explore the unique population ecology of Hawaiian green turtles with reference to the complex life history of this marine megaherbivore.

KEY WORDS: Population genetics · Control region · Mitochondrial DNA · mtDNA · Sea turtles

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INTRODUCTION

The green turtle *Chelonia mydas* occurs throughout the many coral reef and coastal foraging grounds (FG) within the Hawaiian Archipelago, which consists of more than 130 islands and reefs spanning approximately 2400 km (see Fig. 1). The only significant nesting assemblage occurs at French Frigate Shoals (FFS), a mainly uninhabited reef with several sand islets located in the middle of the archipelago (see Fig. 1), although it appears that some nesting occurred at the southeastern end of the archipelago prior to European settlement (Balazs 1985a), and low-level nesting occurs elsewhere

in the northwestern Hawaiian Islands. The FFS nesting population of threatened green turtles has been monitored since 1973 and is one of the few populations in the Pacific that appears to be increasing in numbers (Balazs & Chaloupka 2004a, 2006, Hays 2004, Chaloupka & Balazs 2007), along with Ogasawara (Japan), and Heron Island (Australia) (Chaloupka et al. 2008). Although the Hawaiian nesting population is genetically distinct from other Pacific stocks (Dutton et al. unpubl. data), there is no genetic information on stock composition among FGs within the Hawaiian Archipelago. This reflects a more general limitation in the way sea turtle populations are defined, assessed, and managed. Popu-

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lations are often considered to be either a rookery or a group of rookeries of various geographic scales (Dutton et al. 1999, Reece et al. 2005), since it is more challenging to delineate stock boundaries that include foraging, migratory and developmental regions in the marine environment (Chaloupka 2004, Chaloupka et al. 2004, Bowen et al. 2005). This is because sea turtles, like other highly migratory marine vertebrates, have complex life histories. Adults breed at rookeries and migrate to FGs that usually draw animals from multiple genetic stocks spanning a large area (see Bowen 1997). Usually, adults and juveniles from one rookery also disperse to several different FGs. Furthermore, juvenile sea turtles usually have an early pelagic phase that may encompass large areas of open ocean.

In the Hawaiian Archipelago, immature green turtles generally recruit to the southern FGs once they reach sizes of approximately 35 to 40 cm straight carapace length (SCL) after pelagic development in the northern Pacific Ocean (Balazs 1982, Zug et al. 2002, Balazs & Chaloupka 2004a,b), although juveniles as small as 30 cm SCL may occasionally be found around the northwestern portion of the archipelago (Balazs 1976). Based on growth rates, the duration of this oceanic developmental phase is estimated at 6 yr or more for Hawaiian green turtles (Zug et al. 2002). It is thought that the Hawaiian foraging populations are derived primarily from the FFS rookery (Balazs et al. 1987); however, it has been difficult to evaluate this hypothesis based on tagging data alone, since it has not been possible to track hatchlings from the nesting beaches to adult FGs using traditional tagging methods.

Studies of maternally inherited mtDNA in green turtles have been useful in understanding the population structure and reproductive behavior of these highly migratory marine animals (Bowen et al. 1992, FitzSimmons et al. 1997) and in demonstrating the existence of distinguishable stocks for management (Moritz 1994, Bowen & Karl 1997). The presence of fixed or nearly fixed differences in haplotype frequencies between nesting populations is now sufficiently characterized in green turtles (Lahanas et al. 1994, Norman et al. 1994, Encalada et al. 1996, Dethmers et al. 2006, Bourjea et al. 2007) that mtDNA alleles can be used as genetic markers to determine the natal origins of juvenile turtles sampled in their developmental habitats or along migratory pathways (Bass et al. 1998, 2006, Lahanas et al. 1998, Bass & Witzell 2000, Luke et al. 2004). All studies to date indicate that FG aggregations are made up of stocks of mixed origins, with relative stock mixes differing significantly from location to location. Recent mtDNA surveys of Pacific green turtle nesting populations show that the FFS rookery is distinct from others in the eastern and western Pacific (Bowen et al. 1992, Chassin-Noria et al. 2004, Dethmers et al. 2006, P. H. Dutton et al. unpubl.

data, and these studies provide a baseline to evaluate stock composition of the Hawaii FGs.

The present study investigates the genetic stock composition of foraging green turtle aggregations in the Hawaiian Archipelago to infer dispersal patterns of green turtles in the central Pacific, and to determine whether these foraging aggregations are part of the same breeding population. We use this genetic approach of mtDNA sequence analysis, combined with information on life history and demographics, to expand knowledge of the ecology of green turtles in this region. Our findings are relevant to the status and current conservation issues relating to recognition of the Hawaiian green turtle population as a distinct demographic entity or management unit (MU, see Moritz 1994, Fraser & Bernatchez 2001), and correct classification of this population segment under the US Endangered Species Act (ESA).

MATERIALS AND METHODS

Field sampling. MtDNA control region sequences were obtained from blood or skin samples collected during 1995 to 2003 from juvenile, sub-adult and adult green turtles captured in 5 Hawaiian Archipelago FGs as part of a long-term Capture-Mark-Recapture (CMR) program (Balazs 1976, 1982, 2000, Balazs et al. 1994, 2005, Balazs & Chaloupka 2004b). Blood samples were collected using protocols designed not to harm the turtles, as described in Dutton (1996). The FG samples were obtained from Midway Atoll, Kane'ohe Bay (O'ahu), Pala'au (Moloka'i), and from the east and west sides of the island of Hawai'i at Kiholo Bay and Punalu'u Bay (Fig. 1). Details of the study sites and the

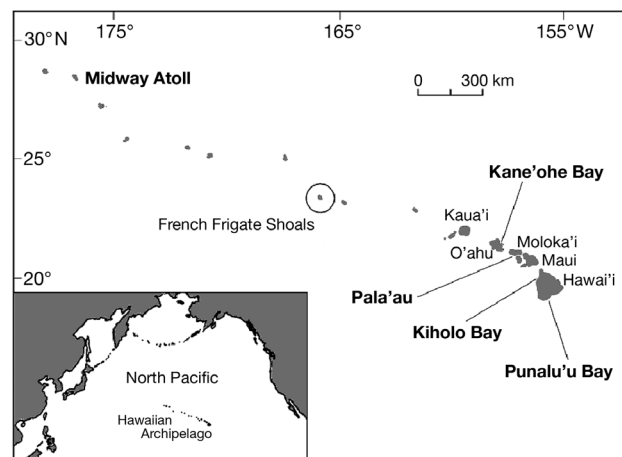


Fig. 1. Location of the 5 Hawaiian green turtle foraging-ground study sites sampled (**bold**). The major rookery is at French Frigate Shoals located in the middle of the Hawaiian Archipelago

Hawaiian Archipelago can be found in Balazs (1976, 1980, 1982) and Balazs & Chaloupka (2004b).

Turtles were captured using several methods, including tangle nets, bullpen or pound nets, scoop nets, and hand capture from small boats or by SCUBA or snorkel. Additionally, some turtles were captured while basking ashore at Kiholo, Punalu'u, and Midway. Further details of the capture and handling methods are given in Balazs (1982), Balazs et al. (1987), and Balazs & Chaloupka (2004b). Blood samples were centrifuged and the packed red cells frozen. In addition, 115 skin samples were collected from an array of green turtles stranding at locations throughout the Hawaiian Islands (see Work et al. 2004).

Laboratory analysis. Specimens included packed red blood cells that were frozen or stored in lysis buffer or small skin biopsies preserved in a 20% dimethyl sulphoxide (DMSO) solution saturated with sodium chloride (Dutton 1996). DNA was isolated from these samples using either standard phenol/chloroform extraction techniques (Sambrook et al. 1989) or the Fast Prep DNA isolation kit (Bio101®). Amplification of mtDNA was performed by PCR (Innis et al. 1990) using the primers HDCM2 and LTCM2, designed to target 488 bp at the 5' end of the control region of the mitochondrial genome (Lahanas et al. 1994). Template DNAs were amplified in 50 µl PCR reactions on a Perkin Elmer 480 thermocycler using the following profile: initial denaturation at 94°C for 2 min, followed by 36 cycles of DNA denaturing at 94°C for 50 s, primer annealing at 52°C for 2 min, and primer extension at 72°C for 1 min 30 s, followed by a final primer extension for 5 min at 72°C. The sizes of the amplified products were determined using electrophoresis in a 2% agarose gel stained with ethidium bromide. PCR products were then purified using the Qiaquick PCR Purification Kit (Qiagen® 1995) and stored at 4°C. Direct cycle-sequencing reactions of the light strand were performed on 2 µl of purified PCR product combined with 2 µl of ABI Prism® dRhodamine Terminator Cycle Sequencing Kit, 3 µl of primer LTCM2 and 5 µl of purified water. The labeled extension products were purified via ethanol precipitation and analyzed with an Applied Biosystems model 377 automated DNA Sequencer. The sequences were analyzed for uncalled and miscalled bases using either Gene Codes Sequencer 3.1.1 or ABI SeqED v. 1.0.2. Sequences were aligned against reference data from the 384 bp segment of the mtDNA control region corresponding to the region reported by Norman et al. (1994). Haplotype nomenclature follows that reported by the Southwest Fisheries Science Center (<http://swfsc.noaa.gov/prd-turtles.aspx>) and were based on the same 384bp fragment used by Norman et al. (1994), and Dethmers et al. (2006) to assign haplotypes.

Statistical analysis. The mtDNA haplotypes that were identified at all of the Hawaiian FGs were compared with published and unpublished data for all rookeries sampled in the Pacific and Indian Ocean to identify potential source stocks to use in the mixed stock analysis (MSA) (Norman et al. 1994, Chassin-Noria et al. 2004, Dethmers et al. 2006, Bourjea et al. 2007, P. H. Dutton et al. unpubl. data). Haplotype frequencies were compared among the 5 FGs using a χ^2 test (Roff & Bentzen 1989), as implemented in the program CHIRXC (Zaykin & Pudovkin 1993). The FG haplotype frequencies were then compared with those reported for 4 Pacific rookeries that contain haplotypes we found present at the Hawaiian FG. We performed MSA using Markov chain Monte Carlo (MCMC) methods as implemented in the program BAYES (Pella & Masuda 2001). Estimates of contributions by different nesting populations to the Hawaii FG were based on Bayesian analysis using MCMC estimation from 6320 re-samplings of 4 stock mixtures composed of green turtles from 4 potential source stocks (Chassin-Noria et al. 2004, P. H. Dutton et al. unpubl. data). Western Pacific and Indian Ocean nesting stocks were not included as potential sources in this analysis, since haplotypes that characterize these stocks were not found at the Hawaiian FG.

RESULTS

Six haplotypes were identified from sequences for the 788 green turtles sampled from all of the Hawaiian Archipelago FGs and strandings (Table 1). Samples were available which allowed between-year comparisons of haplotype frequencies for turtles sampled at one of the FGs (Pala'au, Molokai) and we found no significant differences ($p > 0.5$, $df = 1$) (see LeRoux et al. 2003). This indicates temporal stability among haplotype composition and that annual sampling was representative for this site within the time frame of our study. There were no significant differences in haplotype frequencies among the 5 Hawaiian Archipelago FGs ($\chi^2 = 12.19$, $df = 16$, $p > 0.5$), so the data from all FG were combined for subsequent analysis. There was also no significant difference among haplotype frequencies of the strandings, compared with the FGs. Haplotype CMP1 was the most common, found in 64% of the FG samples, with CMP3 (15%) and CMP2 (10%) making up most of the rest (Table 1). The FFS rookery is the only breeding site where CMP2 has been detected, and neither CMP 1 nor CMP3 have been detected at any of the western Pacific rookeries (Dethmers et al. 2006, D. Broderick pers. comm., P. H. Dutton unpubl. data), and only at one eastern Pacific rookery (Revillagigedos, Table 1). Three additional haplotypes (not detected in

Table 1. Green turtle haplotype composition for the Hawaiian feeding ground populations and strandings compared with nesting population data (from Chassin-Noria et al. 2004; P. H. Dutton et al. unpubl. data) used in mixed stock analysis. MICH = Michoacan rookery; REV = Revillagigedos; FFS = French Frigate Shoals

Haplo-type	Nesting population				Feeding ground population						Hawaii strandings
	Mexico MICH	Mexico REV	Galapagos	Hawaii FFS	Pala'au	Kiholo	Kane'ohe Bay	Midway	Punalu'u	Total	
CMP1		1		156	227	57	91	45	57	477	76
CMP2				34	42	3	14	14	9	82	16
CMP3		11		39	54	10	22	10	16	112	22
CMP4	82	23	95								1
CMP5	34										
CMP6		50			1					1	
CMP7	2										
CMP8	2										
CMP9		2									
CMP10		1									
CMP11		1									
CMP12	3										
CMP13		1									
CMP15			3								
CMP20					1					1	
Total	123	90	98	229	325	70	127	69	82	673	115

the FFS rookery) were found in 3 of the 788 FG and stranded turtles (Table 1). Two of these came from the Pala'au FG, and the third case was a turtle missing both front flippers that stranded alive on the northeast coast of Oahu. Haplotype CMP20 is primarily found in the western Pacific (Dethmers et al. 2006), while haplotypes CMP4 and CMP6 occur in eastern Pacific rookeries, with CMP6 only identified at the Mexican rookery in the Revillagigedos Archipelago (Table 1; P. H. Dutton unpubl. data).

Haplotype frequencies were not significantly different from those at the FFS rookery (Table 1; $\chi^2 = 3.1133$, $df = 5$, $p > 0.5$). The MSA also confirmed that the Hawaii FG comprises animals of FFS nesting stock origin (Table 2).

Table 2. Mean estimated stock mixtures of green turtles from FG and strandings in the Hawaiian Archipelago using BAYES (Pella & Masuda 2001). Analysis consisted of 10000 resamplings (20000 MCMC samples) of 4 stock mixtures composed of green turtles from 4 potential nesting stocks, including French Frigate Shoals (FFS, Hawaii), Islas Revillagigedos (Mexico-REV), Michoacan (Mexico-MICH) and Galapagos Islands (Ecuador). Median and 95% confidence limits (2.5 and 97.5% quantiles) are shown

Nesting stock	Mean	SD	Median	Lower quantile	Upper quantile
FFS	0.999	0.002	0.998	0.993	1.000
Mexico-REV	0.001	0.002	0.001	0.000	0.006
Mexico-MICH	0.000	0.001	0.000	0.000	0.003
Galapagos	0.000	0.001	0.000	0.000	0.003

DISCUSSION

Our results show that the foraging aggregations of green turtles in the Hawaiian Archipelago are from this north central Pacific nesting stock and can be considered a discreet MU separate from other Pacific stocks. Results also indicate that strandings are part of the same genetic stock, and that the individuals that strand are representative of the FG populations. The discovery of 3 individuals with haplotypes only found at rookeries other than FFS, suggests that the Hawaiian FGs are occasionally, albeit rarely, visited by turtles from rookeries outside of the Hawaiian Archipelago. It is possible that these 3 haplotypes are so rare that they were not detected at FFS, despite the relatively large sample size (Table 1; Dutton et al. in prep). However, 2 of these turtles (haplotypes CMP4 and CMP6) were visibly different from typical Hawaiian FG and FFS turtles; they both had a dark grey plastron, black carapace with distinct posterior indentations, typically associated with eastern Pacific green turtle stocks (see Karl & Bowen 1999). The stranded turtle (CMP4, Table 1), while missing both flippers, was generally healthy, and believed to be a pelagic turtle that, having lost its ability to swim, had been carried by the northeast trade winds into the coastal waters of the exposed north shore of Oahu. We believe this animal would not normally have been found at the Hawaiian FGs. Juvenile green turtles of eastern Pacific stock origin have been caught by pelagic longlines on the high seas in the Central Pacific, suggesting occasional pelagic

wandering by eastern Pacific green turtles (Dutton et al. 2000). The third non-endemic haplotype (CMP20), has been found at western Pacific rookeries in Micronesia and Melanesia (Dethmers et al. 2006). These rare wanderers might provide an avenue for historic colonization of Hawaii.

Our results show that the Hawaiian green turtle population is made up of a single primary rookery that is the source of the FG populations spanning a wide geographic range of more than 2400 km throughout the archipelago (Fig. 1). This finding contrasts with all the other FG studies of green turtles in the Atlantic (Bass et al. 1998, 2006, Lahanas et al. 1998, Bass & Witzell 2000, Luke et al. 2004, Bowen et al. 2007) that have detected FG populations of mixed stock origins over comparable geographic scales. The general model that has emerged from these studies of long-range oceanic transport of juveniles from multiple nesting stocks facilitating settlement into overlapping FGs clearly is not applicable to the central North Pacific.

Dispersal patterns

Our results suggest that, after hatching at FFS, pelagic juveniles spend their oceanic years in the north central Pacific region and settle into FGs around the Hawaiian Archipelago (see Balazs 1976). The extent to which Hawaiian green turtles disperse to foraging areas in either the eastern or western Pacific is unknown, and further research that includes sampling of multiple FGs around the Pacific should address this point. Satellite telemetry has shown that nesters from FFS also forage in the waters around Johnston Atoll, immediately south of the Hawaiian Archipelago (Balazs 1985b, 1994); however, it appears that the range of Hawaiian green turtles does not extend beyond the central Pacific region. Preliminary genetic results from foraging areas in Southern California, Baja California, and the eastern tropical Pacific do not indicate the presence of Hawaiian green turtles (P. H. Dutton, unpubl. data). Similarly, the strandings from the coast of Alaska (Hodge & Wing 2000), Oregon and northern California that have been analyzed to date have all been of eastern Pacific stock origin (Dutton 2003, unpubl. data). Likewise, studies to date have not found green turtles of Hawaiian origin in the western Pacific (Norman et al. 1994, Dethmers & Broderick 2003, P. H. Dutton unpubl. data) or at Melanesian FGs (P. H. Dutton unpubl. data, see Boyle 1998). This indicates that dispersal from the Hawaiian population may be constrained by geographic isolation and oceanographic conditions that prevent successful migration to FGs beyond the central Pacific. Further work is needed to examine stock composition of pelagic juveniles to

test the hypothesis that these early stage juveniles are also from the Hawaiian genetic stock. In addition, oceanographic studies that model current patterns would enable better understanding of the transport processes that may influence dispersal and delineate the boundaries of this population. Surface drift trajectories compiled from average current measurements suggest that hatchlings entering the ocean at FFS during the hatching season would be transported in a northwesterly direction (Balazs 1976). However, the longer range processes are poorly studied and little is known about the pelagic ecology of sea turtles in general.

Our genetic findings are consistent with ecological studies of local dispersal of green turtles in the Hawaiian Archipelago. Long-term CMR studies have indicated that green turtles take up residence at local FGs as juveniles and that there is limited movement among various FGs (Balazs 1980, 1983, see Balazs & Chaloupka 2004b). Adult green turtles are also resident in these FGs and migrate every few years to breed and nest at FFS (Balazs 1983, 1994).

Our study builds on previous demographic and ecological studies by integrating genetic data to produce a simple model describing the structure of the population of green turtles in the Hawaiian Archipelago. The population model we propose (Fig. 2) for the Hawaiian Archipelago contrasts with more complex ones typical of green turtles, and sea turtles in general (Chaloupka 2004), in which a genetically distinct stock comprises multiple rookeries and FGs that overlap with several other genetic stocks (Lahanas et al. 1998, Limpus et al. 2003, Chaloupka 2004, Bass et al. 2006). Chaloupka & Balazs (2007) have characterized the Hawaiian green turtle FG populations ecologically as a metapopulation of geographically dispersed and disjunct FGs, with dispersal among nearby populations more likely than dispersal among distant populations. Our genetic results show that these FGs are part of a single panmictic

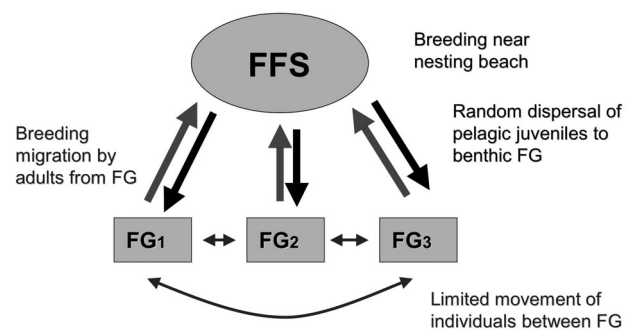


Fig. 2. Simplified schematic model of dispersal and gene flow among the Hawaiian green turtle population (FFS = French Frigate Shoals breeding sites). Feeding grounds (FG1, FG2, FG3, etc.) represent generalised feeding grounds that occur within the Hawaiian Archipelago

breeding population (Fig. 2). In terms of risk management, specific threats (such as disease, anthropogenic impacts) acting differentially at different FGs may lead to local depletions of foraging aggregations. However, these aggregations have the potential of rapid recovery as long as hatchling production in the nesting population is maintained.

This contrasts sharply with other more socially complex migratory marine vertebrates, such as humpback whales, who recruit to different FGs by learning the migratory routes as calves from their mothers. In the North Atlantic, humpback whales consist of demographically isolated FG populations which are genetically differentiated from each other in terms of matrilineal mtDNA lineages. Adults, however, migrate from these FGs to interbreed at a single wintering ground in the West Indies, thus maintaining panmixia at the nuclear level, since nuclear DNA is inherited from both parents (Larsen et al. 1996, Palsböll et al. 1997, Smith et al. 1999). If a FG population of humpback whales in Norway was decimated by a catastrophic event, it might take decades to re-establish, since the migratory routes are learned from the mothers. However, in the case of Hawaiian green turtles, a depleted FG population would re-establish itself relatively rapidly, since juveniles would recruit there randomly. This process might help maintain a buffer for the population as a whole against localized catastrophic impacts on a FG, but not if the threat was persistent (e.g. disease, habitat degradation, or over-harvest), since the risky FG would remain as a 'drain' on the population. Furthermore, unlike other sea turtle FGs containing animals from multiple nesting stocks, the Hawaiian population, supported by a single primary rookery, would be more vulnerable to impacts at the breeding sites at FFS.

One such impact of recent concern is the potential effect of sea level rise on the northern islets of FFS (Baker et al. 2006). For example, Whale-Skate was once a primary nesting site at FFS (Balazs 1976) but by 2004 the island, along with several other prominent nesting islands, had been reduced by erosion to a fraction of its size recorded in 1963 (Amerson 1971, Antonelis et al. 2006). The reasons for this loss of habitat are not clearly understood, but turtle nesting has shifted to the other islets in the FFS, such as Tern Island, where there has been periodic artificial shoreline restoration.

Conservation implications

The green turtle is listed as 'Threatened' under the US ESA throughout its Pacific range, except for the population nesting on the Pacific coast of Mexico that is listed under the ESA as 'Endangered' (NMFS and US-

FWS 1998). ESA objectives focus on recognizing the biological and ecological importance of discrete populations (DPS) and taking action when necessary to preserve them (USFWS-NOAA 1996). Inability to clearly define stock boundaries, and an incomplete understanding of population structure and dispersal patterns have made it difficult to recognize appropriate demographic units for conservation purposes. This has led to a bias toward defining sea turtle Evolutionary Significant Units (ESUs) and MUs (Moritz 1994) based solely on their rookery stock structure. Previous studies involving the FFS rookery have focused on phylogeographic-level divergence of mtDNA which reveals ancient divergence among ocean basins and identifies FFS as an ESU and demographically discrete MU (Bowen et al. 1992, Moritz 1994, Bowen & Avise 1995, P. H. Dutton unpubl. data). Our genetic findings, combined with information on phylogeography, ecology, habitat and population trends, support the conclusion that the foraging and nesting populations of green turtles throughout the Hawaiian Archipelago essentially comprise a panmictic, demographically discrete and biologically and ecologically significant population of green turtles. These findings should influence future decisions about DPS designations for this species.

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