

## Genetic stock composition of foraging green turtles off the southern coast of Molokai, Hawaii

Robin A. LeRoux<sup>1</sup>, George H. Balazs<sup>2</sup>, and Peter H. Dutton<sup>1</sup>

<sup>1</sup> National Marine Fisheries Service, Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, La Jolla, California 92037, USA

<sup>2</sup> National Marine Fisheries Service, Southwest Fisheries Science Center, Honolulu Lab, 2570 Dole St., Honolulu, Hawaii 96822, USA

### INTRODUCTION

The Hawaiian Archipelago contains some of the principal nesting and foraging sites for the green turtle (*Chelonia mydas*) in the central Pacific. This population of threatened green turtles has been monitored for many years and is one of the few populations within the Pacific that is increasing in numbers (Balazs 1998, Wetherall et al. 1997). Our study focuses on one of the largest resident foraging populations in the Hawaiian Archipelago, Palaau. The Palaau foraging site is located off the south-central coast of Molokai at approximately 21°06'N, 157°07'W (Balazs et al. 1987). We have used molecular genetics to examine the stock composition of the Palaau forage population by analyzing mtDNA sequences obtained from the large number of samples provided by ongoing tag-recapture studies. mtDNA haplotype frequencies were compared with those found at the nesting beaches at French Frigate Shoals (FFS) and other key nesting beaches throughout the Pacific basin in order to determine whether this is a mixed or genetically isolated stock. Previous studies have indicated that green turtles from genetically distinct nesting populations share common foraging grounds (Fitzsimmons et al. 1997, Lahanas et al. 1998). However, it is thought that the Hawaiian foraging population is derived from a single nesting stock located at FFS (Balazs et al. 1987).

### MATERIALS AND METHODS

Samples for genetic analysis were routinely collected throughout the Hawaiian Archipelago. mtDNA control region sequences were obtained from blood or skin samples collected at FFS and Palaau during 1995, 1996 and 1997. Juvenile, sub-adult, and adult green turtles foraging off the coast of Southern Molokai were caught using the bullpen capture method as described in Balazs et al. (1998). Skin samples from females nesting at FFS were collected during the nesting season which takes place May through August (Balazs et al. 1987). Standard laboratory techniques were used in order to isolate and analyze DNA samples (Sambrook et al. 1989). Statistical analyses of haplotype frequencies found in Palaau and FFS were carried out using a permutation Chi-Square test (Roff and Bentzen 1989).

### RESULTS AND DISCUSSION

Three main haplotypes that differ by four variable sites were found within the 428 sequences analyzed. These haplotypes distinguish the FFS nesting stock, which is considered a distinct management unit (P. Dutton unpubl. data). Haplotype B was found to be the most common haplotype showing up in 69% of the samples from FFS (n=229) and 72% of the samples from Palaau (n=205). Although less common, haplotypes C and D appeared in both sample sets with an average frequency of 15% each (Table 1). Of the 205 samples collected at Palaau, only 2 exhibited haplotypes that were different than the three main haplotypes. Haplotype FijA is primarily found in the western Pacific, while haplotype G is commonly found in eastern Pacific stocks, indicating that animals from other nesting stocks rarely recruit into the Hawaiian forage grounds.

Between-year comparisons of haplotype frequencies for turtles sampled at Palaau and FFS during 1995, 1996, and 1997 found no significant differences indicating each site is in genetic equilibrium. Samples were then pooled across years and frequencies at the nesting grounds and foraging area were compared (Table 2). Analysis revealed no significant difference between the haplotype frequencies found at Palaau versus those found in FFS, indicating that the Palaau foraging population is

derived from the FFS nesting stock. This suggests that the forage areas throughout the Hawaiian Archipelago are from this central Pacific nesting stock and can be considered a discreet management unit. Since there are substantial differences between FFS and other Pacific nesting stocks in terms of mtDNA haplotypes and frequencies (P. Dutton unpubl. data), no further analysis using mixed stock techniques was necessary.

### FURTHER RESEARCH

Further research includes expanding this genetic survey to include other foraging areas throughout the Hawaiian Islands. A Pacific-wide analysis of genetic stock structure among nesting populations, including rookeries at other Pacific nesting sites, is also underway. Finally, microsatellites will be used in conjunction with these mtDNA data to gain a more complete understanding of the molecular ecology of green turtles in the Pacific.

*Acknowledgements.* We thank Bill Puleloa, the Medeiros family, Shandell Eames, Denise Parker and Shawn Murakawa for their fieldwork and other technical contributions and Erin LaCasella for her assistance in the laboratory.

### LITERATURE CITED

- Balazs, G.H., R.G. Forsyth, and A.K.H. Kam. 1987. Preliminary assessment of habitat utilization by Hawaiian green turtles in their resident foraging pastures. NOAA Tech. Memo. NOAA-TM-NMFS-SWFC-71, p. 107.
- Balazs, G.H., W. Puleloa, E. Medeiros, S.K.K. Murakawa and D.M. Ellis. 1998. Growth rates and incidence of fibropapillomatosis in Hawaiian green turtles utilizing coastal foraging pastures at Palaau, Molokai. In: S.P. Epperly and J. Braun, comps. Proceedings of the Seventeenth Annual Sea Turtle Symposium. NOAA Tech. Memo. NMFS-SEFSC-415. p. 130-132.
- Balazs, G.H. 1998. Sea turtles. In: S.P. Juvik and J.O. Juvik, eds. Atlas of Hawaii, Third Edition. University of Hawaii Press, Honolulu, Hawaii, p. 115.
- Fitzsimmons, N.N., C. Mortiz, C.J. Limpus, L. Pope, and R. Prince. 1997. Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and male-biased gene flow. *Genetics* 147: 1843-1854.
- Lahanas, P.N., K.A. Bjorndal, A.B. Bolten, S.E. Encalada, M.M. Miyamoto, R.A. Valverde, and B.W. Bowen. 1998. Genetic composition of a green turtle (*Chelonia mydas*) feeding ground population: evidence for multiple origins. *Marine Biology* 130:345-352.
- Roff, D.A. and P. Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms:  $\chi^2$  and the problem of small samples. *Mol. Biol. Evol.* 6:539-545.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York.
- Wetherall, J.A., G.H. Balazs, and M.Y. Yong. 1997. Statistical methods for green turtle nesting surveys in the Hawaiian Islands. In: S.P. Epperly and J. Braun, comps. Proceedings of the Seventeenth Annual Sea Turtle Symposium. NOAA Tech. Memo. NMFS-SEFSC-415. p. 278-280.



Table 1. Haplotype frequencies from turtle samples obtained at the Palaau foraging grounds and French Frigate Shoals nesting site during 1995, 1996 and 1997.

Haplotype	B	C	D	G	Fiji A	(n)
French Frigate Shoals	0.69	0.14	0.17	0	0	223
Palaau	0.72	0.14	0.14	< 0.01	< 0.01	205

Table 2. Between-year chi-square comparisons of haplotype frequencies found at Palaau in 1995, 1996, and 1997, along with French Frigate Shoals. Samples from each year were then pooled and compared. Each comparison reveals no significant differences between haplotype frequencies ( $P > 0.5$ ,  $DF=1$ ).

	Chi-Square	P-Value
Palaau Between Years	6.150	0.659
FFS Between Years	0.662	0.959
Palaau vs. FFS Pooled	2.920	0.633

## Accounting for sampling error of rare genotypes in sea turtle stock estimation

Benjamin Bolker<sup>1</sup>, Toshinori Okuyama<sup>1</sup>, Karen Bjorndal<sup>1,2</sup>, and Alan Bolten<sup>1,2</sup>

<sup>1</sup> Department of Zoology, University of Florida, P.O. Box 118525, Gainesville, Florida 32611-8525, USA

<sup>2</sup> Archie Carr Center for Sea Turtle Research, University of Florida, P.O. Box 118525, Gainesville, Florida 32611, USA

The contributions of different sea turtle rookeries to mixed-stock populations on foraging grounds can only be estimated by statistical methods using mitochondrial DNA samples from the mixed stocks and rookery populations. We evaluate methods for genetic stock estimation using simulations and data from previous studies on green and loggerhead turtles. We introduce Markov Chain Monte Carlo (MCMC) estimation, a general method of estimation which is relatively new in conservation biology and completely new to turtle studies. MCMC differs from older approaches using maximum-likelihood (ML) with non-

parametric bootstrapping in (1) using a Bayesian prior to quantify previous knowledge; (2) taking account of multiple modes in the probability distribution of contributions; and (3) incorporating sampling error more flexibly, allowing for the possibility that rare haplotypes actually present in a particular rookery were not detected in a small sample. The differences in point estimates of rookery contributions between ML and MCMC methods are relatively small, but MCMC gives more conservative and more accurate confidence limits than ML with bootstrapping, which tends to underestimate small contributions as zero.

## The cultural context of the hawksbill sea turtle (*Eretmochelys imbricata*) in Calusa society

Chuck Schaffer and Keith Ashley

University of North Florida

### INTRODUCTION

The Calusa use and symbolic representations of hawksbill turtles is revealed by archeofauna and artifacts, as well as a review of Calusa ethnohistoric and archeological documents. These findings are significant in the context of the probable ethnozoological, ceremonial, spiritual and economic meaning that the hawksbill held for them. Turtles, in general, were important to Native Americans for subsistence and their discarded remains are regularly found at archaeological sites such as middens and mortuary contexts. From a spiritual/mythological context, turtles are seen as figureheads, masks, rattles, and grave goods.

Hawksbill material and representations of a ceremonial nature, are found in association with Calusa sites and artifacts.

The Calusa inhabited southwest Florida, maintaining an estuarine economy focused on the rich aquatic bounty of the surrounding waters enveloping the area (Widmer 1988). They frequently exploited deep-water fauna and were expert seafarers. The semitropical to tropical forests of the mainland and coastal islands were also exploited for upland plant and animal resources. They were hunter-gathers with a long history of a marine-based economy extending back several millennia (Milanich 1994). The dominant tribe of southern Florida at historic contact, Spanish documents chronicle their warlike nature and an extensive trade and tribute system. Their unique and complex society exhibited an intricate hierarchical social organization and rich ceremonial life with large village and ceremonial mounds constructed of shell. Owing to the lack of workable local stone sources, Calusa material culture was comprised predominately of shell, wood, bone and textile artifacts. The recovery of which from the anaerobic muck at Key Marco and other

locations have provided a rare glimpse into their material culture.

The hawksbill sea turtle is a marine Chelonian, often found in rocky areas or coral reefs as well as the shallow waters of mangrove bays, and estuarine waters with muddy bottoms and little vegetation. Less commonly, it may be found in pelagic waters and areas of Sargassum weed (Ernst and Barbour 1989). It has a yellow and black radiating pattern on the translucent amber scutes of the carapace with overlapping scutes and four costals on each side with the first not touching the nuchal. The hawksbill nests at low densities with aggregations numbering up to several dozen. Regular observations are made in southeast Florida, predominately from Palm Beach, Martin, Monroe and the Keys (Witzell 1983). In the last century, the Keys were considered good fishing grounds for hawksbills, which are also found in Caribbean. Texas is the only other state with significant observations, but primarily of post-hatchling and juvenile individuals. Their diet consists primarily of sponges and they are generally not considered a good food source. The fatality rate from eating hawksbill flesh is high and there are no known antidotes (Pritchard 1979). Most importantly, hawksbills are the source for tortoiseshell, a thin veneer of outer shell (scute), which has been used worldwide since antiquity for decorative and other purposes.

### MATERIALS AND METHODS

This study involved an extensive literature search in conjunction with an examination of curated faunal material and artifacts. The latter material included collections at the Florida Museum of Natural History at Gainesville (FLMNH), the Univer-





NOAA Technical Memorandum NMFS-SEFSC-503

PROCEEDINGS  
OF THE TWENTY-SECOND ANNUAL  
SYMPOSIUM ON SEA TURTLE  
BIOLOGY AND CONSERVATION



**M i a m i . U S A**

4 to 7 April 2002  
Miami, Florida, USA

Compiled by:  
Jeffrey A. Seminoff

U.S. Department of Commerce  
National Oceanic and Atmospheric Administration  
National Marine Fisheries Service  
Southeast Fisheries Science Center  
75 Virginia Beach Drive  
Miami, FL 33149 USA

August 2003