



WHERE ARE THE GREEN TURTLES NESTING ON THE MAIN HAWAIIAN ISLANDS COMING FROM ?

Amy Frey¹, Peter H. Dutton¹, George H. Balazs²

¹NOAA-National Marine Fisheries Services, Southwest Fisheries Science Center, 3333 North Torrey Pines Court, La Jolla, California 92038, USA

²NOAA-National Marine Fisheries Service, Pacific Islands Fisheries Science Center, 2570 Dole Street, Honolulu, Hawaii 96822-2396, USA

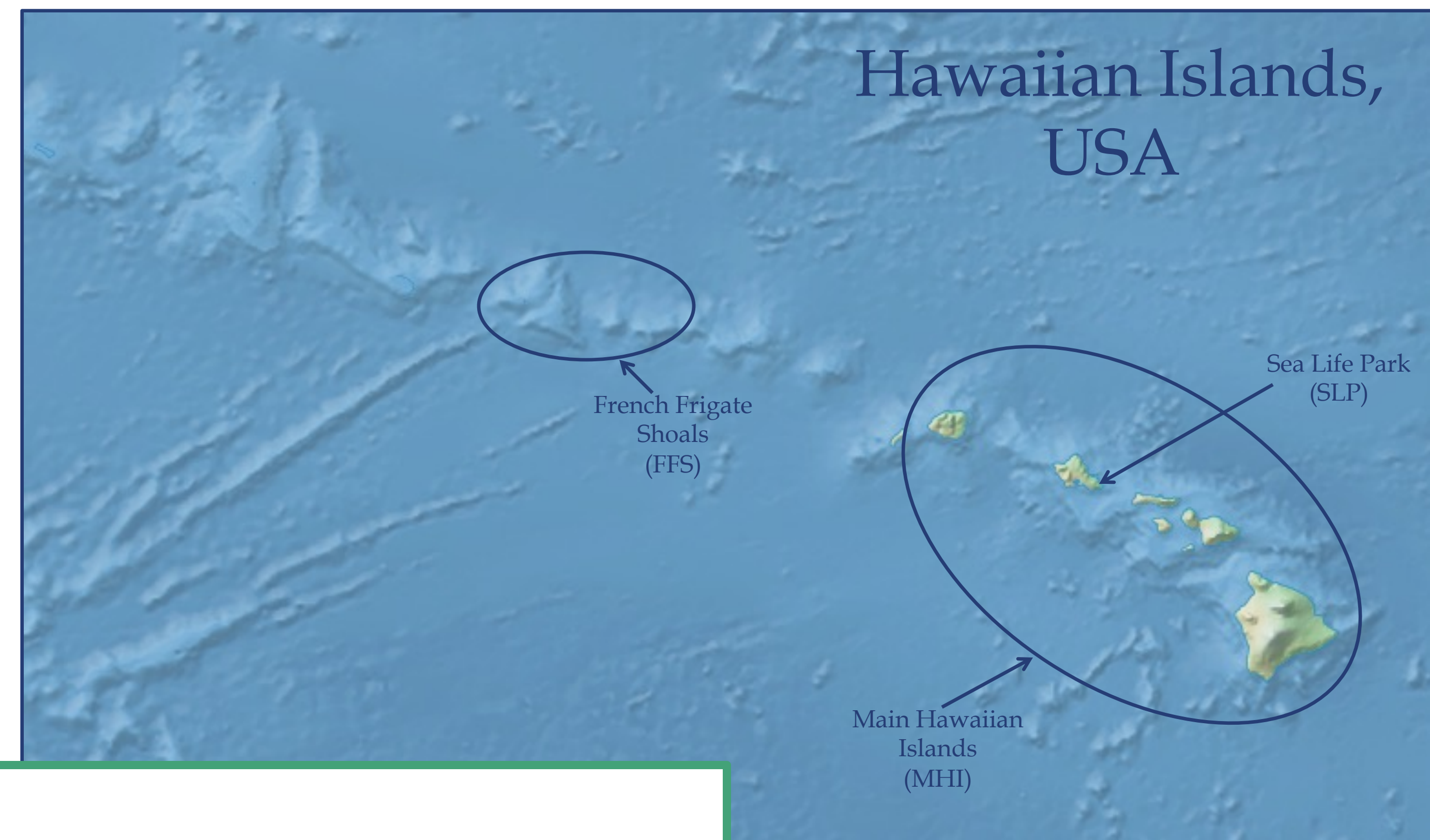
Introduction

Purpose: To determine if the green turtles nesting on the Main Hawaiian islands (MHI) are related to the captive breeders at Sea Life Park (SLP)?

- Increased nesting of green turtles has recently been observed in the MHI.
- Historically green turtle nesting occurs in the Northwestern Hawaiian Islands at French Frigate Shoals (FFS).
- Our previous genetic work determined that nests laid in the MHI belong to a relatively small (~15) and possibly relate number of females.
- Since the 1970's, there have been a few resident green turtles at Sea Life Park on Oahu, that have produced 100's of offspring that are captive-reared for ~1 year and released off the MHI.
- Recently a captive reared juvenile, originally collected from FFS, and released from SLP 19 years earlier, identified as one of the nesters on MHI¹.
- This suggests that the captive born juveniles released from SLP may be contributing to MHI nesting.
- We were able to infer genotypes of the MHI nesters from serial clutch analysis of hatchlings and examine the relatedness to the SLP vs. FFS breeding population.

Materials and Methods

- Samples: 85 nesting females from FFS
10 females from SLP
13 nesting females from MHI
- Markers: mtDNA sequencing using H950g/LCM15382³
14 microsatellite loci-All samples genotyped using 14 microsatellite loci: A6, B103, B108, B123, C102, D1, D2, D105, D108, D115⁴ and A1, B112, D102, D107⁵
- Software: ML relate⁶- perform a pairwise comparison of each individual to evaluate the relatedness of the females to each other.
Colony v2.1⁷- assign parentage if possible between SLP females, and nesting females from the MHI



Results

| | CmP1 | CmP2 | CmP3 |
|------|------|------|------|
| MHI* | 3 | 2 | 8 |
| SLP | 5 | 1 | 3 |
| FFS | 49 | 10 | 10 |

*Not all of the females from the MHI were included in this analysis

mtDNA- The most common haplotype found on MHI is not the most common found at FFS or amongst the females. Comparison of the haplotype frequencies in nesting females at FFS and the MHI (Table 9) showed the two to be highly differentiated ($F_{ST} = 0.25$, $p < 0.0001$).

| | MHI | SLP | FFS |
|-----|------|------|------|
| MHI | 0.21 | | |
| SLP | 0.07 | 0.18 | |
| FFS | 0.04 | 0.05 | 0.06 |

ML relate- The average relatedness value is highest when comparing the MHI nesters to each other, and when comparing the SLP females to each other.

Colony- Colony assigned two of the females that nested on the MHI to one the females from SLP, but did not assign a male from SLP as the father. It assigned an inferred male genotype, that was also assigned to some of the other MHI nesters. The 13 MHI nesters were assigned to 6 mothers. Five of the MHI nesters were assigned to the same mother, three were assigned to another mother, two were assigned to third mother, leaving one nester who was assigned to an additional mother.

Conclusions

The females nesting on the Main Hawaiian Islands comprise a highly related group that appear to be the result of recent colonization by a few founders. There is some suggestion of a linkage with the SLP captive turtles that warrants further analysis, but results of parentage analysis are inconclusive. They are not the offspring of any of the Sea Life Park captive females. It is possible that the routine release of captive-reared juveniles has facilitated this colonization, or alternatively that this is a natural consequence of the steady growth in numbers of the FFS nesting population over the last 35 years. Continued monitoring of the MHI population, and expansion of the sampling effort to the nesters, will provide a rare opportunity to gain insights into the processes involved in colonization of new nesting habitat, and how founder events and genetic drift influence patterns of genetic variation in marine vertebrates.

Acknowledgements

Thanks to the Molokai Turtle Trackers, Nature Conservancy community volunteers who visually monitored nesting tracks and nests. Stacy Hargrove, Kelly Stewart, Erin LaCasella, Amy Jue, and Robin LeRoux for help at the lab and with the data. This study is funded by NOAA-Fisheries.

References

- ¹Balazs, G.H. et al. (2004) Year 2000 nesting of a captive-reared Hawaiian green turtle tagged and released as a yearling. In R. Coyne, Michael, Clark, ed. *21st Annual Symposium on Sea Turtle Biology and Conservation*. Philadelphia, PA, pp. 100-101.
- ²Frey, A., et al. (in prep) Relatedness analysis of hatchlings reveals increase in nesting of green turtles (*Chelonia mydas*) in the main Hawaiian Islands is the result of a few recent founders.
- ³Abreu-Grobois A, et al (2006) New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analyses.
- ⁴Dutton, P. H. and Frey, A. (2009) Characterization of polymorphic microsatellite markers for the green turtle (*Chelonia mydas*). *Molecular Ecology Resources*, 9: 354-356.
- ⁵Frey, A., Dutton, P.H. (unpublished) Microsatellite markers for the green turtle (*Chelonia mydas*) and their utility for other marine turtle species.
- ⁶Kalinowski, ST, AP Wagner, ML Taper (2006) ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes* 6: 576-579.
- ⁷Jones O. and Wang, J. (2009) COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources* 10: 551-555.