

PATTERNS OF GENETIC DIVERSITY OF THE HAWAIIAN SPINNER DOLPHIN (*STENELLA LONGIROSTRIS*)

BY

KIMBERLY R. ANDREWS¹, LESZEK KARZMARSKI², WHITLOW W.L. AU¹, SUSAN H. RICKARDS³, CYNTHIA A. VANDERLIP⁴, and ROBERT J. TOONEN¹

ABSTRACT

We used population genetic analyses to investigate the genetic structure of the Hawaiian spinner dolphin (*Stenella longirostris*). Genetic samples were collected from spinner dolphins at locations across the Hawaiian Archipelago: Kure Atoll (n=34), Midway Atoll (n=57), Pearl & Hermes Reef (n=21), French Frigate Shoals (n=15), Ni'ihau (n=39), O'ahu (n=47), Maui/Lana'i (n=60), and the Big Island of Hawai'i (n=77). A 429-base-pair region of the mitochondrial DNA control region was used to evaluate genetic diversity and population structure. Peaks in genetic diversity were found at the Big Island of Hawai'i ($\pi=0.0082$) and French Frigate Shoals ($\pi=0.0072$), and genetic diversity was reduced at the three most northwestern Hawaiian atolls (Kure Atoll $\pi=0.0025$, Midway Atoll $\pi=0.0019$, and Pearl & Hermes Reef $\pi=0.0017$). Analysis of Molecular Variance (AMOVA) and exact tests of population subdivision indicated significant genetic structure for the spinner dolphin within Hawai'i. With few exceptions, dolphins at every island were found to be significantly genetically differentiated from dolphins at every other island for one or more tests of population subdivision (F_{ST} or $\Phi_{ST} \geq 0.02$, $p < 0.05$). Exceptions included dolphins at Kure Atoll, Midway Atoll, and Pearl & Hermes Reef, which together seemed to form one interbreeding group, distinct from the rest of the Archipelago. Dolphins at O'ahu were also an exception in that they were not differentiated significantly from dolphins at Kure Atoll, Midway Atoll, or Pearl & Hermes Reef.

INTRODUCTION

The Hawaiian spinner dolphin is a geographically isolated subgroup within *Stenella longirostris*, a species of small cetaceans found in tropical locations worldwide (Perrin, 1998). Hawaiian spinner dolphins are genetically distinct from spinner dolphins in the eastern tropical Pacific (Galver, 2000), but no genetic data are available comparing spinner dolphins from Hawai'i with spinner dolphins at nearby Pacific islands. In

¹Hawaii Institute of Marine Biology, 46-007 Lilipuna Rd, Kaneohe, HI 96744 USA

²Texas A&M University, 4700 Avenue U, Bldg. 303, Galveston, TX, 77551 USA

³Oceanic Society, San Francisco, CA USA

⁴Division of Aquatic Resources, Department of Land and Natural Resources, State of Hawaii, 1151 Punchbowl Street, Room 330, Honolulu, HI 96813 USA

Hawai‘i, spinner dolphins are found near islands and atolls, where they use calm, shallow bays and lagoons throughout most of the daylight hours (Norris et al., 1994; Karczmarski et al., 2005). Although they occur off all of the Main Hawaiian Islands, they seem to be associated with only four of the Northwestern Hawaiian Islands: Kure Atoll, Midway Atoll, Pearl & Hermes Reef, and French Frigate Shoals (Karczmarski et al., 2005) (Fig. 1). Sightings in offshore waters are not frequent, although some groups of spinner dolphins have been seen in the channels between islands and other offshore waters in the Main Hawaiian Islands (Mobley et al., 2000). There is little information on offshore distribution in the northwestern Hawaiian region (Barlow et al., 2004), and details on offshore movements at night for any location in the Hawaiian Archipelago remain meager.

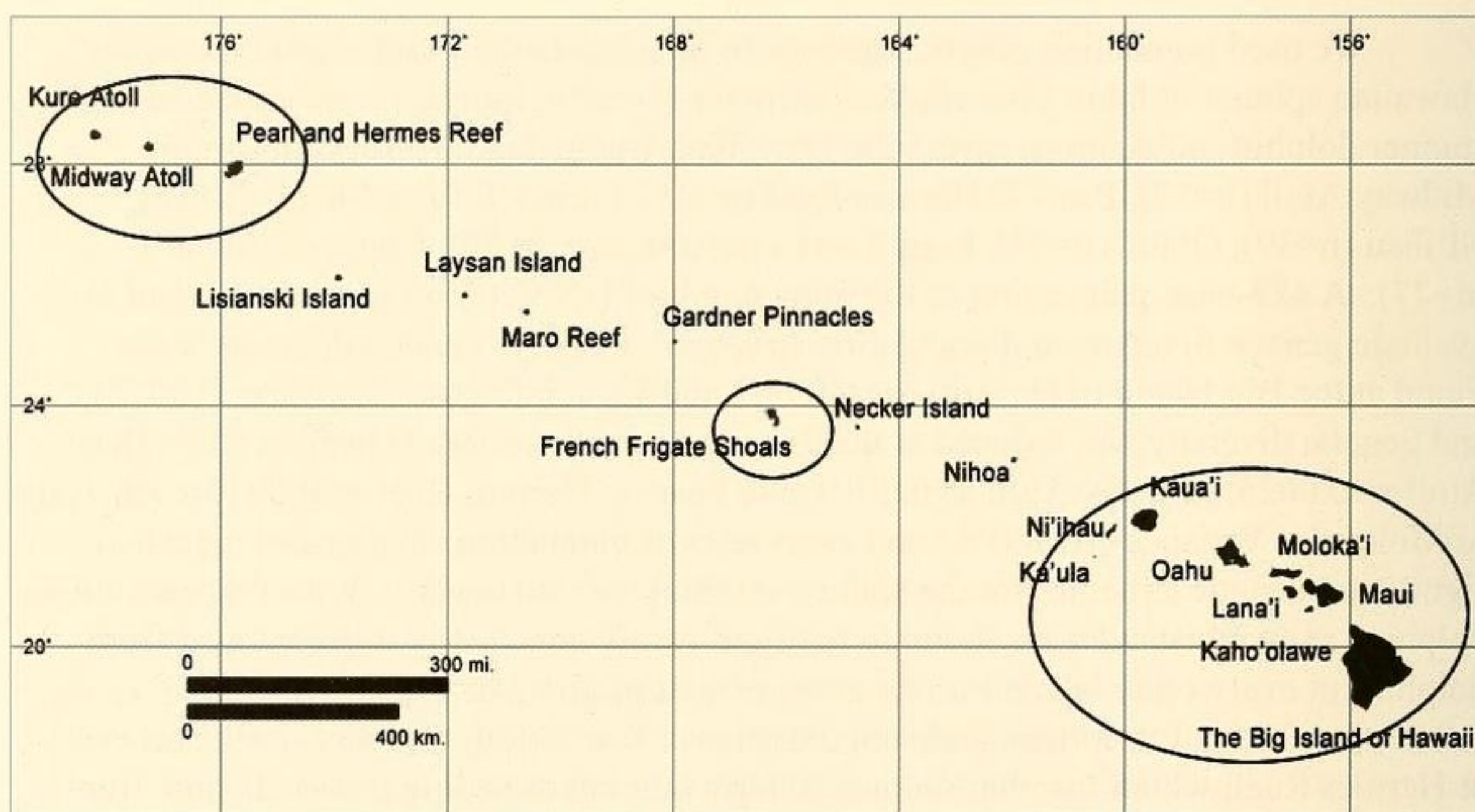


Figure 1. Map of the Hawaiian Archipelago. Circles indicate islands and atolls where spinner dolphins are regularly sighted.

Little is known about the amount of movement of the Hawaiian spinner dolphins between islands. Because spinner dolphins have a capacity for high mobility, relatively high rates of movement throughout the Archipelago might be predicted. A recent study in far northwestern Hawai‘i documented movement between Midway and Kure Atolls (Karczmarski et al., 2005) and, seemingly to a much lesser degree, between Pearl & Hermes Reef and Midway (and possibly between Pearl & Hermes Reef and Kure) (L. Karczmarski and S.H. Rickards, unpublished data). However, the overall pattern suggests that such movements are relatively infrequent, and groups show generally high geographic fidelity to their specific atoll (Karczmarski et al., 2005).

These distribution and movement data provide limited information to predict population structure of the spinner dolphin throughout the Archipelago. The fact that some spinner dolphin groups are found in the channels between the Main Hawaiian

Islands (Mobley et al., 2000) may suggest that the spinner dolphins in the Main Hawaiian Islands form one genetically homogeneous group, with considerable interbreeding between islands. Although the observed movements between Midway Atoll, Kure Atoll, and Pearl & Hermes Reef were infrequent, we would expect that these amounts of movement, if associated with successful interbreeding, would still be sufficient to result in genetic homogeneity among these three atolls. The large geographic distance between the Main Hawaiian Islands and French Frigate Shoals, and between French Frigate Shoals and the three atolls at the far-western end of the Archipelago, might limit movement and interbreeding of individuals between these locations.

To gain insight into population structure, we conducted a population genetics study using tissue samples collected from free-ranging spinner dolphins throughout the Hawaiian Archipelago. We report on preliminary analyses using the mitochondrial DNA (mtDNA) control region. Because population genetic techniques can provide valuable information for the determination of stock structure and vulnerability under the Marine Mammal Protection Act (Dizon et al., 1992; Wade and Angliss, 1997; Dizon et al., 1997), these data will have direct application to the management of the Hawaiian spinner dolphin.

METHODS

Tissue samples were collected from spinner dolphins throughout the Hawaiian Archipelago. Three sampling techniques were used: biopsy with a Paxarms air rifle (Krützen et al., 2002), biopsy with a Hawaiian sling (in which elastic propels a pole with attached biopsy tip), and a skin-swabbing technique (Harlin et al., 1999). Biopsy with a Hawaiian sling and skin swabbing involved sampling of animals riding the bow wake of a small boat, and biopsy with an air rifle involved sampling of animals between 5 and 20 meters from a boat. Skin-swab samples consisted of flakes of sloughed skin, and biopsy samples consisted of cylindrical plugs of skin and blubber about 5 mm in diameter and about 5 mm long. In addition, some extracted genomic DNA samples were provided by the National Marine Fisheries Service, Southwest Fisheries Science Center (SWFSC), including accession numbers 7185-7202, 15510, 17432, 30411-30420, 30449, 30512-30516. Numbers of samples from each location included in this study, and years samples were collected, are listed in Table 1.

Genomic DNA was extracted from tissue samples using Qiagen DNEasy extraction kits. For each sample, a polymerase chain reaction (PCR) was carried out to amplify a 489-base-pair fragment of the 5' end of the mtDNA control region. Primers used were KRA dLp 1.5t-pro modified from Pichler et al. (2001) plus an added 5' M13 tail (5'-TGTAACACGACAGCCAGTACACCCAAAGCTGGAATTC-3') and dLp5 (5'-CCATCGWGATGTCTTATTAAAGRGGAA-3') (Pichler et al., 2001). PCR reactions were 50 µl volumes containing 1X Reaction Buffer (Promega Corporation), 200 µM of each dNTP, 2.0 mM MgCl₂, 0.5 units *Taq* DNA polymerase (Promega Corporation), and 0.2 µM each primer. Cycle conditions were: 95 °C for 1 min, followed by 40 cycles of 94 °C for 30 sec, 54 °C for 30 sec, and 72 °C for 30 sec, followed by a final 72 °C extension

for 15 min. PCR products were visualized on a 1.5% agarose gel containing ethidium bromide and were cleaned prior to sequencing using Qiaquick PCR Cleanup Kits (Qiagen Corporation). Each PCR product was cycle-sequenced with both forward and reverse primers on an ABI 3730 automated sequencer. The forward and reverse sequences were aligned for each individual using Sequencher v.4.2 (Genecodes Corporation). Removal of primer sequences and ambiguous sequence resulted in a 429-base-pair consensus fragment. The resulting consensus sequences were aligned for all individuals using Sequencher v.4.2.

The computer program Arlequin v.2.000 (Schneider et al., 2000) was used to calculate standard variance components including haplotype and nucleotide diversities

Table 1. Numbers of genetic samples collected at different locations in different years and standard measures of genetic diversity of Hawaiian spinner dolphins at different locations within the Hawaiian Archipelago. The Big Island of Hawai‘i is referred to as “Big Island.”

Location	1997	2000	2001	2002	2003	2004	Total Sample Size	Nucleotide Diversity (π)	Haplotype Diversity (h)
Kure Atoll					34		34	0.0025	0.3993
Midway Atoll			47	10			57	0.0019	0.4023
Pearl & Hermes						21	21	0.0017	0.1810
French Frigate				1		14	15	0.0072	0.5333
Ni‘ihau				28	11		39	0.0065	0.6802
O‘ahu			23	6	10	8	47	0.0037	0.5402
Maui/Lana‘i		1		9	50		60	0.0042	0.4729
Big Island	17			3	57		77	0.0082	0.7163

(Nei, 1987). Haplotype diversity is calculated without taking into account the genetic distance between haplotypes, whereas nucleotide diversity does take genetic distance into account.

Arlequin was used to test for the presence of reproductively isolated subgroups at different Hawaiian islands and atolls using Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992), treating each island or atoll as an *a priori*-defined group. The Tamura and Nei model (Tamura and Nei, 1993) was found to be the best-fit model available using Modeltest v3.6 (Posada and Crandall, 1998), and this model was used to estimate genetic distances. The statistics F_{ST} and Φ_{ST} were used to evaluate the level of reproductive isolation among groups; these values range from 0 to 1 and represent

measures of the amount of genetic variation within groups versus among groups. A value of 0 indicates no genetic structure among groups, a value of 1 indicates that groups are completely reproductively isolated, and values between 0 and 1 indicate intermediate levels of isolation (Wright, 1951). The significance of F_{ST} and Φ_{ST} was evaluated using 100,000 random permutations. In addition, exact tests of population subdivision (Raymond and Rousset, 1995) were carried out with Arlequin, using 100,000 steps of a Markov chain to test for the presence of genetic structure.

RESULTS

Nucleotide and haplotype diversities for the spinner dolphin varied across the Hawaiian Archipelago (Table 1, Fig. 2). Two peaks in nucleotide diversity were observed: one at the Big Island of Hawai'i (hereafter referred to as "Big Island") and one at French Frigate Shoals. Whereas the peak in nucleotide diversity at the Big Island was due to a large percentage of individuals having unique or divergent haplotypes, the peak in nucleotide diversity at French Frigate Shoals was due to two individuals (out of a sample size of 15) that had a unique haplotype sequence which was highly divergent from any other sequence in the Archipelago. With the exception of French Frigate Shoals, nucleotide diversities at the Northwestern Hawaiian Islands were lower than at the Main Hawaiian Islands.

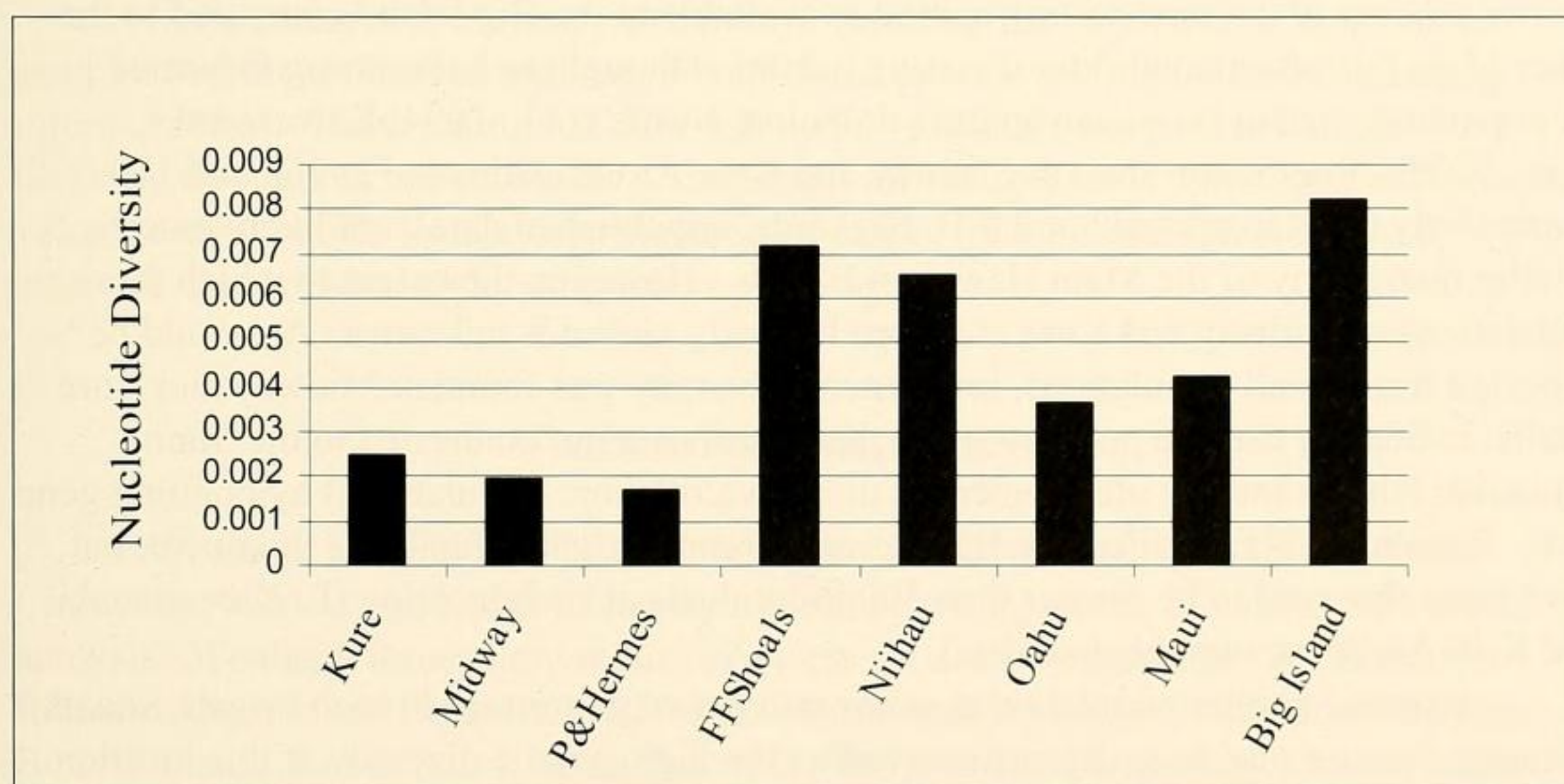


Figure 2. Nucleotide diversities at the mitochondrial DNA control region of spinner dolphins at locations across the Hawaiian Archipelago. The Big Island of Hawai'i is referred to as "Big Island."

Three tests (AMOVA pairwise Φ_{ST} using genetic distance, AMOVA pairwise F_{ST} using conventional F-statistics, and exact test of population subdivision) were used to test for the presence of reproductively isolated subgroups. With few exceptions, dolphins at every island were found to be significantly genetically differentiated from dolphins at every other island for one or more tests of population subdivision (F_{ST} or $\Phi_{ST} \geq 0.02$, $p < 0.05$). Exceptions included dolphins at Kure Atoll, Midway Atoll, and Pearl & Hermes Reef, which together seemed to form one interbreeding group, distinct from the rest of the Archipelago. Dolphins at O'ahu were also an exception in that they were not differentiated significantly from dolphins at Kure Atoll, Midway Atoll, or Pearl and Hermes Reef.

DISCUSSION

High genetic diversity at a neutral genetic locus can generally be attributed to: 1) large population size; and/or 2) intermixing of populations from more than one source. In this study, two peaks in genetic diversity of Hawaiian spinner dolphins were observed: one at the Big Island and one at French Frigate Shoals. The peak in genetic diversity at the Big Island is likely explained by population size, estimated at roughly 1,000-2,000 or more individuals (Norris et al., 1994; Östman, 1994). Although no population size estimates are available at any of the other islands, the population size at the Big Island is likely larger than populations at the other Main Hawaiian Islands because a greater amount of daytime resting habitat is available at the Big Island compared to the other Main Islands (availability of resting habitat is thought to have strong influence on population size in Hawaiian spinner dolphins; Norris et al., 1994; Karczmarski et al., 2005). Population sizes at Midway and Kure Atolls, estimated at 260 and 110 respectively (L. Karczmarski and S.H. Rickards, unpublished data), are likely much smaller than at any of the Main Hawaiian Islands. However, the extent to which these populations at Midway and Kure are reproductively closed is unknown. As would be expected from small populations, low genetic diversity was found at Midway and Kure Atolls, indicating that the populations at these atolls are not connected to the Main Hawaiian Islands (or any other potential unknown offshore populations) by ongoing gene flow. Population sizes at Pearl & Hermes and French Frigate Shoals are unknown, but have been observed to be greater than 300 individuals at each location (L. Karczmarski and K.R. Andrews, unpublished data).

Because neither population size nor movement patterns at French Frigate Shoals is known, we are unable to determine whether the high genetic diversity at this location is due to large population size or intermixing of populations. However, increased genetic diversity at French Frigate Shoals is attributed to a highly divergent haplotype in 2 out of a total of 15 individuals, making diversity due to a large population size unlikely. Instead, this pattern suggests that the high genetic diversity is likely a result of migration from another source. The divergent haplotype at French Frigate Shoals was unique among haplotypes in the Hawaiian Archipelago, further supporting the hypothesis of possible migration from outside of the Hawaiian Islands.

The genetic structure found within the Hawaiian spinner dolphin only partially matched the general expectations derived from the limited data available on movements. Whereas the distribution and movement data suggested that the dolphins at the Main Hawaiian Islands were a genetically homogeneous population with considerable levels of exchange (successful interbreeding) between islands, the genetic data reported here do not support that prediction. Rather, the data indicate that limited exchange occurs between dolphins associated with each Main Hawaiian Island. Our findings for the Northwestern Hawaiian Islands, however, did follow the initial expectations. Spinner dolphins at French Frigate Shoals were found to have limited exchange with dolphins from other islands, and dolphins at Midway Atoll, Kure Atoll, and Pearl & Hermes Reef were found to form one genetically homogeneous population that was distinct from the rest of the Archipelago.

The data indicate greater exchange rates between the three most western atolls than between the Main Islands, despite the fact that geographic distances separating these three atolls are greater than are most of the distances separating the Main Islands. These differences in exchange rates probably relate to differences between the Main Islands and the northwestern atolls in factors including population sizes and social structure (for details see Karczmarski et al., 2005), and oceanographic and physiographic features such as remoteness of habitat and availability of suitable resting sites (Karczmarski et al., 2005). These higher exchange rates may be an expression of intrinsic mechanisms related to inbreeding avoidance and preservation of genetic fitness of insular, small populations, although more research is needed to test this hypothesis.

More research is currently underway, including the collection of more tissue samples and more detailed analyses of additional genetic loci, specifically including microsatellites. These additional data will further elucidate the patterns of genetic diversity throughout the Hawaiian Archipelago for the spinner dolphin, and will provide valuable information for the determination of stock structure and vulnerability for effective conservation and management planning.

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