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Multiple Populations of Pantropical Spotted Dolphins in Hawaiian Waters

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Abstract

Understanding gene flow and dispersal patterns is important for predicting effects of natural events and anthropogenic activities on animal populations. In Hawaii, most species of odontocetes are managed as single populations. Recent exceptions include false killer whales, spinner dolphins, and common bottlenose dolphins, for which studies have shown fidelity to individual islands or groups of islands. Our study focused on pantropical spotted dolphins. We analyzed mitochondrial control region and 11 microsatellite loci from 101 individuals from 4 areas: Hawaii, Maui/Lanai, Oahu, and Kauai/Niihau. We examined F_{ST}, F'_{ST}, R_{ST}, Jost's D, and Φ_{ST} and used TESS to estimate number of populations and assignment probabilities. Our results support genetic differentiation among Hawaii, Maui/Lanai, and Oahu and suggest that pantropical spotted dolphins near Kauai/Niihau are likely transient and in low numbers. Between island regions, F_{ST} for microsatellites ranged from 0.016 to 0.045 and for mtDNA, from 0.011 to 0.282. F'_{ST}, ranged from 0.098 to 0.262 for microsatellites and 0.019 to 0.415 for mtDNA. R_{ST} and Φ_{ST} showed similar results to F_{ST} for microsatellites and mtDNA respectively, and Jost's D fell between F_{ST} and F'_{ST}. TESS supported 3 populations, and greatest mean assignment probability by island region ranged from 0.50 to 0.72. The private alleles method indicated migration rates among regions from 1.49 to 3.45, and effective population size of the island of Hawaii was estimated to be 220. There was no strong evidence to support sex-biased dispersal or group fidelity. Considering this study in the larger context of other odontocete population studies and studies of connectivity, we suggest genetic differentiation may be mediated by behavior adapted to differing habitat types and niches.

Subject areas: *Population structure and phylogeography; Conservation genetics and biodiversity*

Key words: *conservation, Hawaii, management, population genetics,* Stenella attenuata, *stock*

Population genetics has been widely applied to a variety of taxa to describe gene flow and diversity among populations and to define stocks and geographical population boundaries. Recently, population genetics studies have begun to be used to examine island-associated dolphin populations near the Hawaiian Islands (e.g., Chivers et al. 2007; Andrews et al. 2010; Martien et al. 2012). The US Marine Mammal Protection Act (1972) defines populations and stocks of marine mammals as groups of the same species or smaller taxa in a common spatial arrangement that interbreed when mature. Because of the relatively low cost of locomotion (Williams 1999) and the documentation of long distance movements (>1000 km) for some species (Wells and Gannon 2005), dolphins are typically considered capable of wide dispersal among islands and island groups. Therefore, it is often assumed for purposes of management that populations of dolphins near archipelagos interbreed randomly with each other among the regions of archipelagos. However, studies of island-associated populations, such as spinner dolphins (*Stenella longirostris*) near the Hawaiian Islands (Andrews et al. 2010) and French Polynesia (Oremus et al. 2007), common bottlenose dolphins (*Tursiops truncatus*) near the Hawaiian Islands (Baird et al. 2009; Martien et al. 2012) and the Bahamas (Parsons et al. 2006), and melon-headed whales (*Peponocephala electra*) (Aschettino et al. 2011) and rough-toothed dolphins (*Steno bredanensis*) (Baird, Webster, et al. 2008) near the island of Hawaii and in French Polynesia (Oremus 2008) have shown that dolphins may exhibit fidelity to individual island regions within archipelagos. As a result of genetic (Chivers et al. 2007) and photo-identification studies (Baird, Gorgone, et al. 2008), National Oceanic and Atmospheric Administration (NOAA) Fisheries has divided false killer whales (*Pseudorca crassidens*) into 3 Pacific Islands Region management stocks, including insular and offshore stocks within the Hawaiian Islands Exclusive Economic Zone (Carretta et al. 2013a). NOAA Fisheries has also recently divided the Hawaiian stocks of common bottlenose dolphins (*T. truncatus*) and spinner dolphins (*S. longirostris*)

into multiple stocks based on recent genetic and photo-identification studies (Baird et al. 2009; Andrews et al. 2010; Martien et al. 2012; Carretta et al. 2013a).

Our study focused on population structure of pantropical spotted dolphins (*Stenella attenuata*) near the Hawaiian Islands ranging from the island of Hawaii to the island of Niihau. We hypothesized that, based on preliminary observations using photo-identification and scar/wound pattern evidence (Baird et al. 2003), there are separate populations of pantropical spotted dolphins among the island regions of Hawaii Island, Oahu, 4-islands area (Maui, Kahoʻolawe, Molokaʻi, and Lanai), and Kauai/Niihau. We also hypothesized the potential for sex-biased dispersal and/or group fidelity to drive differentiation based on studies of other odontocete populations (e.g., Escorza-Treviño et al. 2005), recognizing that in some cases, no evidence of either driver has been found (e.g., Andrews et al. 2010). There has been little study of this species near the Hawaiian Islands (e.g., Baird et al. 2001). More extensive study has been done on their Eastern Tropical Pacific (ETP) counterparts that are impacted by the tuna purse seine fishery (e.g., Scott and Chivers 2009) and on pantropical spotted dolphins near China and Taiwan (Wang et al. 2003; Yao et al. 2004; 2008). Continued evaluation of population structure of dolphins near the Hawaiian Islands is significant not only to management but also to consideration of the broader question of what drives genetic differentiation among these different species. Studies are showing that there are not universal oceanic boundaries, such as deep channels, that isolate populations of dolphins in the Hawaiian Islands. Each species seems to have its own population structure. For example, data indicate that false killer whales are insular and pelagic (Chivers et al. 2007; Baird, Gorgone, et al. 2008); spinner dolphins assort among several island regions (Andrews et al. 2010); and melon-headed whales have an island-wide population and a small, local northwest Hawaii Island population (Aschettino et al. 2011). Understanding the basic structure of these different species' populations is an important step to evaluating the overall drivers and impacts of that structure. Further, studies such as ours contribute to the broader understanding of connectivity in the Hawaiian Islands. In addition to the studies cited above, studies that include cetaceans and other organisms have found some general barriers among the inhabited Hawaiian islands, with less connectivity than in the Northwestern Islands, despite smaller distances among the inhabited islands (Toonen et al. 2011). The larger picture of connectivity can help to inform the value of marine protected areas and other approaches to management and conservation.

One goal of assessing population structure is to define populations for management. The criteria for population delimitation for dolphins that have been used by NOAA Fisheries include F_{ST} values significantly different from zero in a priori analyses and evidence of population structure based on assignment probabilities and clustering in Bayesian analyses (Carretta et al. 2013a). Such results indicate limited gene flow among populations. Although Palsbøll et al. (2007) suggest that management units should be evaluated based on predefined threshold values for differentiation measures, it is difficult to agree on such thresholds, and pairwise fixation indices are often small for odontocetes even when significantly different from zero (e.g., Andrews et al. 2010; Martien et al. 2012). As reported in Martien et al. (2012), it may be possible to evaluate F_{ST} thresholds for marine mammals based on calculated expected values for different dispersal rates using life history characteristics. We have applied this approach to pantropical spotted dolphins near the inhabited Hawaiian Islands. We used a priori testing to compare the 4 regions described above: Hawaii Island, Oahu, 4-islands area, and Kauai/Niihau and compared our genetic differentiation values to calculated expected values and to values determined for populations of other dolphin species near the Hawaiian Islands. We also estimated migration rates for pantropical spotted dolphins among the 4 a priori regions. Further, because a priori population differentiation measures require predefined strata, we used individual assignment testing to evaluate whether there was cryptic structure. Additionally, we evaluated whether intragroup relatedness and/or sex-biased dispersal was potentially driving genetic differentiation. We also evaluated the potential effect of isolation by distance. Finally, to assist managers in developing abundance estimates for separate populations of pantropical spotted dolphins, we estimated effective population size for the population near Hawaii Island. Samples sizes were not sufficient to make these estimates for other Hawaiian populations.

In addition to providing information that serves to better understand the larger process of dolphin population structure and its drivers, this study is important because it affects the evaluation of impacts of fishing on pantropical spotted dolphins near the Hawaiian Islands. One fisheries issue is that commercial and recreational troll fisheries near the islands "fish on dolphins" to catch tuna. In this practice, fishers drive their boats through and near groups of dolphins pulling lines behind them or regularly reposition the boats at the front of groups of dolphins and deploy fishing gear. NOAA is continuing to collect data on the troll fisheries to better understand their impacts on pantropical spotted dolphins. Part of assessing these impacts is understanding pantropical spotted dolphin population structure and population sizes. If pantropical spotted dolphin populations are smaller and more island-associated than previously thought, it does not necessarily mean that fisheries bycatch has a significant impact on populations, but it does raise the question of local impacts to a higher level of consideration.

Near the Hawaiian Islands, pantropical spotted dolphins are managed as a single stock (Carretta et al. 2013a); however, Baird et al. (2001) suggested that movements among the islands may be limited based on differences in scar pattern among islands. Here, we address the question of gene flow in pantropical spotted dolphins near the Hawaiian Islands from the island of Hawaii to Kauai/Niihau using analysis of mitochondrial DNA (mtDNA) sequences and microsatellite profiles and consider this in a larger scheme of odontocete population structure and its implications in the Hawaiian Islands.

Methods and Materials

Study Site and Sample Collection

Boat-based survey effort attempted to cover a wide survey area (Baird, Gorgone, et al. 2008; Baird, Webster, et al. 2008). While all groups (individuals found swimming together) of pantropical spotted dolphins were approached for species identification and group size estimation, not all were sampled for genetic analyses (Figure 1; Table 1). For a thorough description of survey techniques, see Baird, Webster, et al. (2008) and Baird, Gorgone, et al. (2008). Four regions were defined and used for a priori genetic analyses, based on distance among islands and depths of the channels between them as per Baird, Webster, et al. (2008): the island of Hawaii; the "4-islands area" including Maui, Lanai, Kaho'olawe, and Moloka'i; the island of Oahu; and the islands of Kauai and Niihau (Figure 1). Depth was determined by taking GPS locations for each sample/sighting and overlaying the point locations on a bathymetric raster using ArcGIS 9.1 (ESRI, Redlands, CA). Distance from shore was determined using GPS locations and distance measures in ArcGIS.

Genetic samples were collected as skin biopsies from live animals encountered during surveys. Biopsy samples were taken using either a pole spear or a Barnett RX-150 crossbow (Lambertsen 1987). Biopsy tips were 25 mm in length and 8 mm in diameter with a collar to limit penetration to approximately 18 mm. Samples were stored on ice while in the field and then preserved in a dimethyl sulfoxide/saturated salt solution (Milligan 1998).

From 2002 to 2003, a total of 101 pantropical spotted dolphin samples were collected. Samples for which all microsatellite allele sizes, sex, and mtDNA haplotypes were the same were considered to be suspected duplicate samples. One sample was removed from the study as a result of suspected duplication based on these criteria, making the total samples 100 (Table 1). There were 76 additional samples collected near the island of Hawaii from 2005 to 2008 (Table 1). For this study, these 76 additional samples were used in analysis to investigate whether individuals within the same group were more

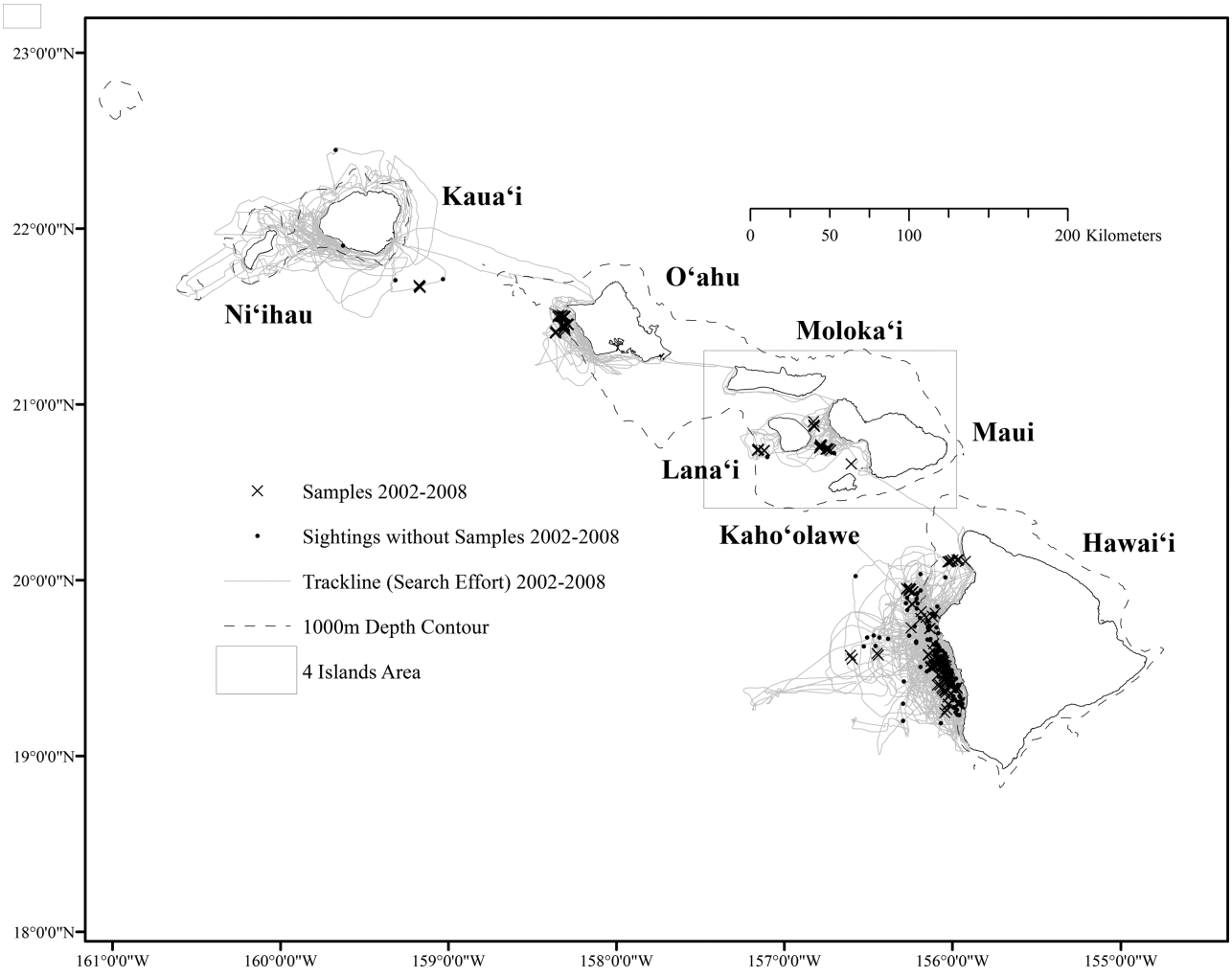

Figure 1. Locations of samples, sightings without samples, and search effort for pantropical spotted dolphins near the Hawaiian Islands 2002–2008.

Table 1 Study sites and details of genetic samples of pantropical spotted dolphins collected in Hawaiian waters in 2002 and 2003

Location	Total samples	# of Groups	Males	Females	Group size range	Depth range (m)	Distance from shore range (m)
Hawaii	38	11	15	23	40–120	633–2681	7.2–34.3
4-islands area	27	8	16	11	25–75	60–817	5.9–14.7
Oahu	27	8	19	8	4–100	839–2278	4.9–14.5
Kauai/Niihau	8	1	1	7	35–35	3477	33.9-33.9
Hawaii 2005–2008	76	40	24	53	3–194	85–4662	2.5–64.3

"4-Islands area" includes Maui, Lanai, Kaho'olawe, and Moloka'i. Additional samples from 2005 to 2008 collected near Hawaii Island were used solely to evaluate intragroup relatedness and estimate effective population size near that island.

genetically similar than individuals from different groups to examine whether group fidelity may affect gene flow. These samples were also used for estimation of effective population size near the island of Hawaii. These 76 samples were not used for the comparative analyses between regions because of the potential for temporal bias and the concern that a considerably larger sample size in one location might include more rare haplotypes and alleles thereby artificially inflate the differentiation between Hawaii Island and the other regions.

Sexing, mtDNA, and Microsatellites

The following microsatellite loci were used: EV14, EV37, EV94, (Valsecchi and Amos 1996), SL8-49, SL9-69, SD8 (Galver 2002; Escorza-Treviño et al. 2005), MK5, MK6, MK8 (Krützen et al. 2001), KWM2A, and KWM12A (Hoelzel et al. 1998). For mtDNA, the proline transfer RNA gene and hypervariable region I of the control region was amplified with primers H00034 (Rosel et al. 1994) and L15824 (Rosel et al. 1999). Sex of the individuals sampled was determined by amplification of the zinc finger gene specific for the X and Y chromosomes of cetaceans using primers ZFXY, ZFX, and ZFY following the commonly used procedures of Bérubé and Palsbøll (1996). Specific procedures for amplifying and sequencing mtDNA, microsatellites and conducting sex determination of samples are described in detail in Supplementary Material online. In fulfillment of data archiving guidelines (Baker 2013), we have deposited the primary data underlying these analyses with Dryad (or similar archive).

Statistical Analyses

Marker Evaluation

A full description of evaluation of pairwise linkage disequilibrium, null alleles and allelic dropout, and deviations from mutation-drift equilibrium for microsatellites and procedure for testing neutrality of mtDNA can be found in Supplementary Material online.

Isolation by Distance Testing

To test for isolation by distance using microsatellites, IBD 1.52 (Bohonak 2002) was used to run Mantel tests to determine whether there were correlations between population level geographic and genetic distances. To test for isolation by distance at an individual level, GENALEX 6.4.1 (Peakall and Smouse 2006) was used following the procedures of Peakall et al. (2003) to create a correlogram to compare combined genetic correlation for microsatellite loci as function of distance. To test for isolation by distance using mtDNA, IBD 1.52 was used to run Mantel tests as described above for microsatellites but using F_{ST} and Φ_{ST} as genetic distances. For a detailed description of the procedures to test for isolation by distance, see the Supplementary Material online.

Microsatellites—Fixation Indices and Jost's D

ARLEQUIN 3.0.1.2 (Excoffier et al. 2005) was used to calculate pairwise population F_{ST} and R_{ST} for microsatellite data for the 4 predefined regions (Hawaii, the 4-islands area, Oahu, and Kauai/Niihau). R_{ST} differs from F_{ST} in that it uses a step-wise mutation model rather than an infinite alleles model, which may be more appropriate for the evolution of microsatellites. Confidence intervals (CI) were calculated around microsatellite F_{ST} and R_{ST} values by running pairwise analysis of molecular variance analyses with 50000 permutations using the compute distance matrix option with "number of different alleles (FST-like)" for F_{ST} and "sum of squared differences (RST-like)" for R_{ST} and using the 2.5% bootstrap percentile values to determine CI. Bonferroni corrections were not applied to the significance level of F-statistics. Bonferroni corrections tend to increase the likelihood of type II errors. In the case of determining population structure for conservation purposes, we have greater concern about type II error than type I error.

Jost's D (Jost 2008) was also calculated for microsatellites using GENODIVE 2.0b23 (Meirmans and Van Tienderen 2004). GENODIVE does not calculate P values or CIs for pairwise Jost's D across loci. Generally, standardized F_{ST} is better suited than D for inferences of the influence of demographic processes on differentiation (Meirmans and Hedrick 2011), but Leng and Zhang (2011) recommend reporting multiple differentiation measures and comparing indices for insight into the evolutionary processes that influence population differentiation. Generally speaking, Jost's D is a better measure of differentiation, while F_{ST} is better for inferring information about migration; D is also highly affected by mutation rates, making it difficult to compare across multiple loci (Kane 2011).

Microsatellites—Assignment Testing

In order to evaluate cryptic population structure, TESS 2.3 (Chen et al. 2007; Durand et al. 2009), which uses a Bayesian clustering analysis similar to other assignment testing programs, was used to analyze microsatellite data to estimate the number of populations near the Hawaiian Islands and calculate assignment probability of each individual to each population cluster. TESS was selected because it allows for use of an admixture model and simultaneous consideration of spatial autocorrelation. Latitude and longitude in the WGS1984 geographic coordinate system were used. TESS was run using the admixture model and the linear-trend model. We used a run length of 500 000 sweeps with a burn-in of 100 000 sweeps. Log likelihood and regression coefficient graphs did not indicate a problem with convergence. The TESS manual recommends a burn-in of only 10 000 and run length of 50 000 sweeps. TESS was run with both the Conditional Auto-Regressive (CAR) and Besag, York & Mollie (BYM) models, which differ slightly, with the BYM model including noninformative priors on variance parameters (see Durand et al. 2009). Results did not differ significantly, so only the results for the BYM model are reported here. Total number of populations, K values, were set from 2 to 6 and the algorithm was run 100 times for each K. For each K, the 10 runs with the lowest Deviance Information Criterion (DIC) values were kept for further analysis. DIC can be considered as a Bayesian measure of fit or adequacy, penalized by an additional complexity term (Spiegelhalter et al. 2002). For models with negligible prior information, DIC will be approximately equivalent to Akaike's criterion (Spiegelhalter et al. 2002). The mean DIC for each K was calculated using the 10 runs for each and plotted against K. Assignment probabilities for each of the 10 runs for each K were exported from TESS into CLUMPP (Jakobsson and Rosenberg 2007), which calculated mean assignment probabilities for each individual to each cluster for each K value. In CLUMPP, the Greedy Option was used with all possible input orders repeated 1000 times. The Greedy algorithm has less extent of search than FullSearch but allows increased computational speed. The algorithm is described in Jakobsson and Rosenberg (2007) and is employed to make the computation time reasonable for datasets for which FullSearch proceeds too slowly. Mean assignment to cluster for each region was calculated by averaging the mean individual assignment probabilities for each region. To compare means for assignment to clusters, Anova analyses and calculation of means and standard errors (SEs) were performed using MINITAB 13 (Minitab, Inc., State College, PA). Tukey tests for multiple range analyses on Anova results were performed using critical Q values and q values from Zar (1999).

Microsatellites—Sex-Biased Dispersal Testing

FSTAT 2.9.3.2 (Goudet et al. 2002) was used to investigate the possibility of sex-biased dispersal. Permutations were set to 10 000, and 2-tailed tests were used to compare F_{ST} values; F_{IS}, $mAIc$, and TCS 1.21 values are also reported.

Microsatellites—Intragroup Relatedness Testing

To evaluate intragroup relatedness, KININFOR 1.0 (Wang 2006) was used to assess the informativeness of the microsatellite loci for 5 relatedness estimators. COANCESTRY 1.0 (Wang 2011) was used to calculate relatedness among pairs of individuals within and among groups. A detailed description of the methods employed to evaluate intragroup relatedness can be found in the Supplementary Material online.

Microsatellites—Migration Rate Estimation

As another indicator of gene flow, mean number of distinct alleles per locus and mean number of private alleles per locus, accounting for sample size, was calculated using AZDE 1.0 (Szpiech et al. 2008) to determine if number of private alleles was sufficient to estimate migration rates. AZDE was also used to determine mean number of private alleles per locus for pairs of putative populations to examine whether any pair of populations shared enough alleles only with each other to indicate more recent migration or founder events. Kauai/Niihau was not included in the pairwise analysis because the program can only calculate means up to the smallest population sample size, which would limit the comparisons to sample size of 8. To estimate migration rates (N_m), the private alleles method of Barton and Slatkin (1986) was applied using GENEPOP 4.0.1.0 (Raymond and Rousset 1995; Rousset 2008). This was compared to calculating N_m from F_{ST} directly using the formula $F_{ST} \approx 1/(4N_m + 1)$ (Wright 1965), although we recognize the limitations of this approach (e.g., Whitlock and McCauley 1999), and compared to results of Shannon pairwise population analysis in GENALEX 6.4.1 (Peakall and Smouse 2006). In GENALEX, sHua was set to 0 when less than 0.0001.

Microsatellites—Effective Population Size Estimation

In order to estimate effective population size to inform management, ONeSAMP 1.2 (Beaumont et al. 2002; Tallmon et al. 2004; 2008) and LDNe 1.31 (Waples and Do 2010) were used. LDNe uses linkage disequilibrium (Waples and Do 2010) and OneSamp uses a Bayesian approach (Tallmon et al. 2008) to estimate effective population size. In ONeSAMP, minimum effective population size was set to 100 and maximum effective population size was set to 10 000. In LDNe, P_{crit} of 0.05 and random mating were selected. Only the extended dataset from the island of Hawaii ($n = 113$) was used for this analysis. The other island regions did not have sufficient sample size. Tallmon et al. (2010) reported that simulations indicated that sample size should be at least 60 to make useful inferences about abundance using LDNe methods.

mtDNA—Fixation Indices

ARLEQUIN was used to calculate F_{ST} and Φ_{ST} for the mtDNA sequence data. The number of permutations in a randomization test was set to 50 000, and Φ_{ST} was calculated

using a basic pairwise comparison model without weighting mutation type because haplotype differences consisted only of transitions. TCS 1.21 (Clement et al. 2000) was used to create a haplotype network to examine the relationships among mtDNA haplotypes. A 95% connection limit was chosen in TCS, and gaps were set to a 5th state, though as there were no gaps in the aligned sequences, this did not affect the network.

Microsatellites and mtDNA—FST Standardization

Standardizations of microsatellite and mtDNA F_{ST} values were calculated using ARLEQUIN based on Meirmans and Van Tienderen (2004), Hedrick (2005), and Meirmans (2006). These standardizations help to account for high within-population variation, differences in effective population size, and markers with different mutation rates (Meirmans 2006). A full description of how standardizations were performed can be found in Supplementary Material online. Standardized F_{ST} values have been reported for spinner dolphins (Andrews 2009; Andrews et al. 2010) and common bottlenose dolphins (Martien et al. 2012) near the Hawaiian Islands, so these values are useful for comparisons among Hawaiian species. Further, standardized F_{ST} values better represent the actual magnitude of differentiation; however, they are not appropriate for evaluating migration rates (Kronholm et al. 2010).

Microsatellites and mtDNA—F_{ST} Expected Values

Expected F_{ST} can be calculated for a given dispersal rate for both nuclear and mtDNA. Given life history parameters reported in Taylor et al. (2007) and the procedure used by Martien et al. (2012), we calculated the expected F_{ST} values for pantropical spotted dolphins for a 1% dispersal rate. This dispersal rate was chosen to develop threshold F_{ST} values because Taylor (1997) suggested that marine mammal populations should be managed separately if dispersal among the populations is below a few percent per year. Martien et al. (2012) also evaluated bottlenose dolphin pairwise F_{ST} values for a 1% dispersal rate. See Supplementary Material online for a full description of the methods for estimating expected F_{ST} values.

Results

Sample Collection and Marker Evaluation

Survey effort from 2002 to 2008 near the Hawaiian Islands from Hawaii to Kauai/Niihau suggests that pantropical spotted dolphins are rarely found near Kauai/Niihau and commonly found near the island of Hawaii (Figure 1; Table 1). Samples were collected up to 40 km from shore, and depths were shallower near the 4-islands area. Total observation effort was 444 days over 48 074 km, resulting in 216 encounters (0.45 encounters/100 km) (see Supplementary Table A online for breakdown by region).

Microsatellites were successfully amplified for all samples except one sample each from Hawaii ($n-1 = 37$), the 4-islands area ($n-1 = 26$), and Oahu ($n-1 = 26$). Sex determination and mtDNA sequencing were successful for all samples. For mtDNA analyses, 571 base pairs of control region sequence from each individual were compared. For samples from 2002 to 2003, 10 haplotypes were found (GenBank accession numbers GQ852567-GQ852573, GQ852575, GQ852577, GQ852578). Seven haplotypes were unique (found in only one individual), and 77% of all samples were haplotype 3, which was found in all 4 regions (Table 2). The 8 samples from 1 group near Kauai/Niihau contained 4 of the 10 total haplotypes, 2 of which were unique to this group. Overall, 5 out of the 7 dolphins with unique haplotypes were female. All differences among mtDNA sequences were transition mutations. For the complete island of Hawaii dataset from 2002 to 2008 ($n = 113$), 10 haplotypes were found, 3 of which were not found in the other 3 regions (GenBank accession numbers GQ852574, GQ852576, GQ852579). Of these samples, 80% were haplotype 3. Four out of the 5 dolphins near Hawaii with unique haplotypes were female.

There were no indications of linkage disequilibrium, deviations from Hardy–Weinberg Equilibrium, null alleles, allelic dropout, selection on alleles, or population bottleneck for microsatellite loci, and Tajima's D results were neutral for the mtDNA control region. See Supplementary Material online for further information.

Isolation by Distance

The analyses of isolation by distance for both microsatellites and mtDNA suggested that this was not a factor in the observed population differentiation among regions. The analysis in IBD indicated that microsatellite F_{ST} and R_{ST}, as measures of genetic relatedness, did not significantly correlate with geographic distance ($Z = 43.352$, $r = -0.206$, $P = 0.750$ for F_{ST}; $Z = 37.203$, $r = -0.445$, $P = 0.790$ for R_{ST}). In individual comparisons, the correlogram of distance

Table 2 MtDNA gene diversity, nucleotide diversity, and mean pairwise differences from samples collected 2002–2003 (calculated in ARLEQUIN 3.1)

Region	Gene diversity	Nucleotide diversity	Mean pairwise differences	# Haplotypes
Hawaii ($n = 38$)	0.376 (SD ± 0.098)	0.004 (SD ± 0.002)	2.125 (SD ± 1.210)	6
4-islands area ($n = 27$)	0.527 (SD ± 0.097)	0.006 (SD ± 0.004)	3.402 (SD ± 1.796)	4
Oahu ($n = 27$)	0.145 (SD ± 0.090)	0.001 (SD ± 0.001)	0.519 (SD ± 0.450)	3
Kauai/Niihau ($n = 8$)	0.750 (SD ± 0.139)	0.008 (SD ± 0.005)	4.321 (SD ± 2.390)	4
Overall	0.450 (SD ± 0.255)	0.005 (SD ± 0.003)	2.592 (SD ± 1.429)	10

For mtDNA, nucleotide diversity is the probability that 2 nucleotides chosen at random will be different.

classes compared with the autocorrelation coefficient (r) as defined by Peakall et al. (2003) indicated that there was some positive spatial autocorrelation for individuals up to approximately 130 km, although there was no significant correlation from approximately 35 to 90 km. For pairs more than 130 km apart, correlation is not significant up to approximately 195 km, at which point the correlation becomes significantly negative and then returns to insignificant. Mean distances among island regions were all greater than 130 km except for the mean distance between Oahu and Kauai/Niihau, which was 92.9 km. The next closest pair of islands are Hawaii and the 4-islands area at 144.2 km apart. Therefore, there was no evidence of isolation by distance playing a role in differentiation among regions.

The analysis in IBD indicated that mtDNA F_{ST} and Φ_{ST}, as measures of genetic relatedness, did not significantly correlate with geographic distance ($Z = 91.584$, $r = -0.507$, $P = 0.915$ for F_{ST}; $Z = 52.641$, $r = -0.671$, $P = 0.959$ for ΦST).

Microsatellites—Fixation Indices and Jost's *D*

Most microsatellite F_{ST} and R_{ST} values were significantly different from 0 (Table 3). Overall F_{ST} was 0.033 (95% CI: 0.024–0.042), which was significantly different from zero ($P = 0.001$), as was overall R_{ST} (0.035, 95% CI: 0.005–0.074, $P = 0.001$) (Table 3). Kauai/Niihau was included in these analyses as a separate region. It is important to note the unusual situation in which few dolphins were seen despite high effort, the high diversity in mtDNA, and the ambiguity of assignment to a specific cluster in TESS; however, it also should be noted that sample size was very small for this region ($n = 8$). Gene diversity (the probability that 2 randomly chosen alleles or haplotypes are different in the sample) was similar for all regions (Table 4). Expected and observed heterozygosity was fairly high for all loci, with the number of alleles per locus ranging from 6 to 20 (Table 5). With such high within-population variation, standardized F_{ST} values can be better indicators of actual genetic differentiation because without standardization, the maximum possible value of F_{ST} varies between markers. In this case, standardized F_{ST} values were larger than nonstandardized values (Table 3). Jost's D values fell between F_{ST} and F'_{ST} (Table 3).

Expected values of F_{ST} were calculated using the process based on Martien et al. (2012) described in the Supplementary Material online section Supplementary Methods Information subheading "F_{ST} Expected Values." This process relies on using life history parameters to evaluate N_m and to determine the expected F_{ST} from the relationship $F_{ST} = 1/(4N_m + 1)$. The benefit of this approach is the use of life history and abundance information collected outside of the current project to calculate N_m. For a dispersal rate of 1%/year, and estimating population size range of 976–1428 for each island region, expected $F_{ST} = 0.002$ for microsatellites (Supplementary Table B online). Although this value may differ from the actual value because of differences in life history values for Hawaiian pantropical spotted dolphins and those reported in Taylor et al. (2007), differences in total population size, and/or violations of the assumptions of the models used, the values give a sense of the magnitude of differentiation expected for a 1% dispersal rate among 3 pantropical spotted dolphin populations near the inhabited Hawaiian Islands.

Microsatellites—Assignment Testing

A plot of K values versus DIC values from TESS indicated DIC leveled off at 3 populations (Supplementary Figure A online). Bar plots of the probability of assignment to each cluster for $K = 3$ showed clustering of assignments for Hawaii, the 4-islands area, and Oahu as 3 clusters, with Kauai/Niihau clustering equally with Hawaii and Oahu (Figure 2; see Supplementary Figure B online). Mean probability of assignment to clusters 1, 2, or 3 significantly differed within regions except Kauai/Niihau clusters 1 and 2 (Figure 2; Table 6).

Microsatellites—Sex-Biased Dispersal Testing

FSTAT results indicated no significant difference between female and male F_{ST} values (females $n = 39$, $F_{ST} = 0.021$; males $n = 50$, $F_{ST} = 0.039$; $P = 0.151$). F_{IS} and $vAIc$ values were slightly higher for females, while $mAIc$ values were lower (females $F_{IS} = 0.083$, $H_o = 0.765$, $mAIC = -0.941$, $vAIc = 11.142$; males $F_{IS} = 0.040$, $H_o = 0.778$, $mAIC = 0.734$, $vAIc = 10.570$).

Microsatellites—Intragroup Relatedness Testing

Mean pairwise relatedness comparisons for microsatellites for samples taken from the same group in comparison with samples taken from different groups indicated no significant differences in relatedness for any region or for samples pooled across regions (Supplementary Table C online). Within regions, Oahu showed higher intraregion pairwise relatedness than the other regions. See Supplementary Material online for further description of intragroup relatedness testing.

Microsatellites—Migration Rate Estimation

Mean number of distinct alleles per locus and mean number of private alleles per locus for Hawaii (0.858 SE ± 0.71 and 1.61 SE ± 0.31 respectively, $n = 37$), the 4-islands area (8.31 SE ± 0.64 and 1.40 SE ± 0.27, $n = 26$), and Oahu (7.90 SE ± 0.62 and 1.12 SE ± 0.18, $n = 26$) were within the sample size values successfully tested by Barton and Slatkin (1986) for their private alleles method of estimating migration rate (N_m). Mean number of distinct alleles per locus and mean number of private alleles per locus for Kauai/Niihau for sample size of 8 were 5.17 SE ± 0.37 and 1.31 SE ± 0.24, respectively. SEs of mean numbers of private alleles for a sample size of 26 overlapped for pairs of regions, indicating no differences (Hawaii and 4-islands area: 1.29 SE ± 0.21, Hawaii and Oahu: 1.15 SE ± 0.23, 4-islands area and Oahu: 1.10 SE ± 0.20). Using the Barton and Slatkin (1986) method, N_m was calculated to estimate pairwise migration rates for all 4 island regions (Table 7). These rates are compared to direct calculation of N_m from pairwise F_{ST} values using $F_{ST} \approx 1/(4N_m + 1)$ (Wright 1965), bearing in mind

Table 3 F_{ST} and R_{ST} for microsatellite data; F_{ST} and Φ_{ST} for mtDNA sequences; F'_{ST} (standardized F_{ST}); and Jost's D for microsatellites

	Hawaii	4-islands area	Oahu	Kauai/Niihau
(a) F_{ST} and R_{ST} for microsatellite data[a]				
Hawaii (n = 37)		**0.044 [0.019–0.079] (0.004)**	**0.055 [−0.007 to 0.132] (0.001)**	−0.014 [−0.036 to 0.003] (0.769)
4-Islands area (n = 26)	**0.028 [0.013–0.045] (<0.001)**		0.018 [−0.002 to 0.042] (0.061)	**0.047 [−0.005 to 0.091] (0.048)**
Oahu (n = 26)	**0.038 [0.023–0.053] (<0.001)**	**0.038 [0.020–0.056] (<0.001)**		0.039 [−0.032 to 0.087] (0.065)
Kauai/Niihau (n = 8)	0.016 [−0.002 to 0.026] (0.057)	**0.045 [0.023–0.064] (<0.001)**	**0.029 [0.009–0.048] (0.003)**	
(b) F_{ST} and Φ_{ST} for mtDNA sequences[b]				
Hawaii (n = 38)		0.017 (0.212)	0.005 (0.336)	0.028 (0.155)
4-Islands area (n = 27)	0.011 (0.229)		**0.105 (0.032)**	0.005 (0.387)
Oahu (n = 27)	0.016 (0.180)	**0.112 (0.010)**		**0.191 (0.012)**
Kauai/Niihau (n = 8)	0.087 (0.064)	0.018 (0.315)	**0.282 (0.013)**	
(c) F'_{ST} (standardized F_{ST})[c]				
F'_{ST}				
Hawaii		0.019	0.021	0.171
4-Islands area	0.165		0.169	0.046
Oahu	0.205	0.195		0.415
Kauai/Niihau	0.098	0.262	0.156	
(d) Jost's D for microsatellites[d]				
Hawaii				
4-Islands area	0.135			
Oahu	0.173	0.167		
Kauai/Niihau	0.062	0.219	0.119	

[a]R_{ST} is above the diagonal, and F_{ST} is below the diagonal. 95% CI for values are shown in brackets. Numbers in bold are significantly different from 0, and P values are given in parentheses. F_{ST} overall = 0.033 [0.024–0.042] (<0.001); R_{ST} overall = 0.036 [0.005–0.074] (0.001).

[b]Φ_{ST} is above the diagonal, and F_{ST} is below the diagonal. Numbers in bold are significantly different from 0, and P values are shown in parentheses. F_{ST} = 0.055 (0.023); Φ_{ST} overall = 0.039 (0.044).

[c]MtDNA F'_{ST} is above the diagonal, and microsatellite F'_{ST} is below the diagonal. F'_{ST} overall for microsatellites = 0.186; F'_{ST} overall for mtDNA = 0.089.

[d]GENODIVE does not calculate P values or CIs for pairwise Jost's D across loci.

the limitations of this approach (e.g., Whitlock and McCauley 1999), and to results of Shannon pairwise population analysis in GENALEX (Peakall and Smouse 2006) (Table 7).

Microsatellites—Effective Population Size Estimation

Two estimates of effective population size near the island of Hawaii were generated using an extended microsatellite data set of 113 individuals from that region: 156 individuals (95% CI: 132–261, Bayesian ONeSAMP method), and 373 individuals (95% CI: 209–1248, LD method of LDNe), with the CI's of the 2 methods overlapping from 209 to 261 individuals. A combined estimate of the harmonic mean was calculated following the procedure of Waples and Do (2010). The simple, unweighted harmonic mean of both methods was 220. Sample sizes were too small for other regions to estimate effective population size.

mtDNA—Fixation Indices

For mtDNA, most pairwise F_{ST} and Φ_{ST} values were not significantly different from zero except for Oahu and the 4-islands area (Table 3). Overall F_{ST} (0.055) was significantly different from zero ($P = 0.023$), as was overall Φ_{ST} (0.039, $P = 0.044$) (Table 3). CIs were not calculated because those intervals are calculated over loci and the control region is only one locus.

Table 4 Gene diversity for microsatellites from samples collected 2002–2003 (calculated in ARLEQUIN 3.1)

Region	Gene diversity	*n* Alleles
Hawaii ($n = 37$)	0.835 (SD ± 0.430)	74
4-islands area ($n = 26$)	0.826 (SD ± 0.429)	52
Oahu ($n = 26$)	0.794 (SD ± 0.413)	52
Kauai/Niihau ($n = 8$)	0.841 (SD ± 0.457)	16

The gene diversity calculation in ARLEQUIN is the probability that 2 randomly chosen alleles or haplotypes are different in the sample (equivalent to expected heterozygosity).

Standardizations of F_{ST} for mtDNA resulted in larger values than unstandardized F_{ST} (Table 3). Gene diversity, nucleotide diversity, and mean pairwise differences among mtDNA haplotypes were highest for Kauai/Niihau and lowest for Oahu (Table 2), but in general, mtDNA gene diversity among the 4 regions studied was low (0.450 standard deviation [SD] ± 0.255), with 77 out of 100 samples sharing a single haplotype. Two additional haplotypes were seen in 9 and 7 individuals, respectively, and 7 individuals had unique haplotypes. Unique haplotypes were found in all 4 island regions. A haplotype network indicated that minor haplotypes have branched off the major haplotype several times (Supplementary Figure C online). Variable sites and frequency of occurrence of each haplotype are shown in Supplementary Table D online.

For a dispersal rate of 1%/year, and estimating population size range of 976–1428 for each island region, expected F_{ST} = 0.006–0.009 for mtDNA (Supplementary Table B online).The same caveats in interpreting these values apply as to microsatellites.

Table 5 Number of alleles, and expected and observed heterozygosity at each microsatellite locus for pantropical spotted dolphin samples collected near the Hawaiian Islands 2002–2003

Locus	# of Alleles	H_e	H_o	F_{ST}	*P*
MK8	9	0.779	0.699	0.050	0.002
MK5	6	0.670	0.668	0.031	0.024
KWM12a	10	0.833	0.796	0.049	<0.001
KWM2a	18	0.916	0.850	0.013	0.051
MK6	17	0.867	0.867	0.010	0.174
EV14	20	0.929	0.903	0.017	0.011
EV37	17	0.848	0.850	0.038	0.002
SD8	19	0.912	0.810	0.046	<0.001
SL849	14	0.853	0.814	0.028	0.007
SL969	14	0.841	0.761	0.056	<0.001
EV94	20	0.916	0.889	0.031	<0.001

Overall $F_{ST} = 0.033$, $P \leq 0.001$. Total samples were 97 for each locus (194 alleles). F_{ST} and associated *P* value shown for each locus across all sample locations.

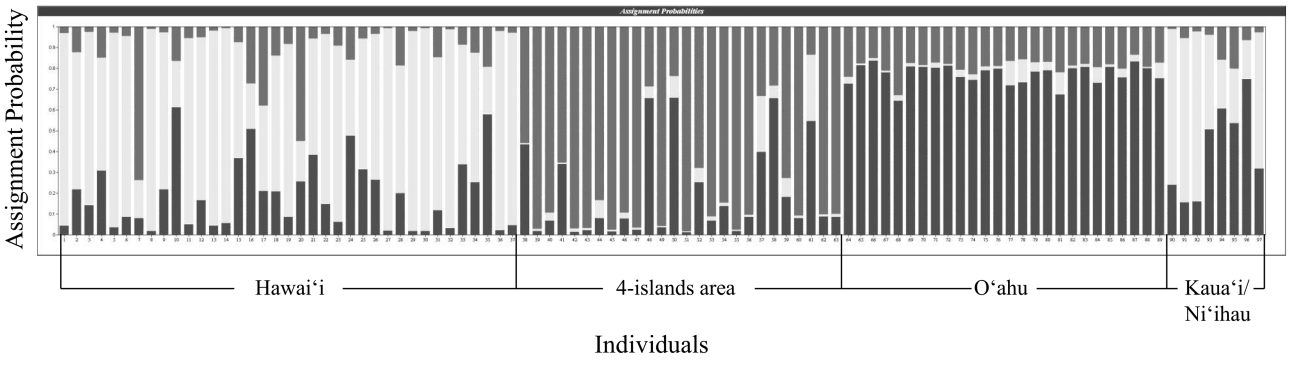

Figure 2. Example of assignment probability bar plot for $K = 3$ from TESS analysis. Note that Hawaii is predominantly light gray, the 4-islands area is predominantly medium gray, Oahu is predominantly dark gray, and Kauai/Niihau is approximately equally light gray and dark gray. These 3 shades of gray indicate assignment probability of each individual to each of the 3 clusters (a color version is available as Supplementary Figure B online).

Table 6 Assignment probability to each of 3 clusters from TESS analysis

Region	Cluster 1	Cluster 2	Cluster 3	*F* value	*P* value
Hawaii (n = 37)	0.27 (SE ± 0.02)	0.63 (SE ± 0.04)	0.10 (SE ± 0.02)	98.17	<0.001
4-islands area (n = 26)	0.24 (SE ± 0.04)	0.06 (SE ± 0.02)	0.70 (SE ± 0.05)	77.00	<0.001
Oahu (n = 26)	0.72 (SE ± 0.01)	0.11 (SE ± 0.01)	0.17 (SE ± 0.01)	2725.77	<0.001
Kauai/Niihau (n = 8)	0.43 (SE ± 0.06)	0.50 (SE ± 0.07)	0.07 (SE ± 0.02)	16.97	<0.001

Means, SEs, *F* values, and significant differences among means based on Anova analyses are shown here. The *P* values for comparing the means of the assignment probabilities for the 3 clusters in each region are shown. Tukey tests indicated that there were significant differences for all 3 clusters for each region except Kauai/Niihau Cluster 1 and Cluster 2.

Table 7 Migration rate estimations (N_m) between pairs of regions in Hawaiian Islands waters based on private alleles

Pairwise regions	Mean frequency of private alleles across both regions	N_m based on private alleles	N_m based on Wright (1965) for F_{ST}	N_m based on Shannon pairwise analysis
Hawaii-4-islands area	0.03	3.45	8.68	2.05
Hawaii-Oahu	0.04	2.90	6.33	1.54
Hawaii-Kauai/Niihau	0.05	2.63	15.38	2.49
4-islands area-Oahu	0.04	2.51	6.33	2.17
4-islands area-Kauai/Niihau	0.07	1.49	5.31	1.10
Oahu-Kauai/Niihau	0.07	1.69	8.37	0.86

These results are compared to calculation using $F_{ST} \approx 1 / (4N_m + 1)$ and results of Shannon pairwise population analysis.

Discussion

Overview

Our results suggest that pantropical spotted dolphins around the Hawaiian Islands do not form a single unstructured population. Microsatellite analyses, including genetic cluster assignments, fixation indices, standardized F_{ST} values, and Jost's D support the separation of dolphins found near Hawaii, Oahu, and the 4-islands area regions into different populations, and mtDNA analyses support splitting at least Oahu and the 4-islands area. There is also some support for a separate population near Kauai/Niihau, but these relationships should be explored further with larger sample sizes if possible. There was no support for the hypothesis of female philopatry or group fidelity driving differentiation. Local behavioral adaptations to differing environmental conditions in each region may drive genetic isolation, though further research is needed to examine this hypothesis. The pattern of pantropical spotted dolphin population structure differs from that of several other odontocete species in the archipelago that also differ from each other, lending support to behavioral isolation rather than oceanographic barriers as a driver of population differentiation among Hawaii's odontocetes.

Population Differentiation

In support of the conclusions above, the microsatellite F_{ST} and R_{ST} values among Hawaii, the 4-islands area, and Oahu were generally low but significantly different from zero (Table 3), the exception being R_{ST} of the 4-islands area compared to Oahu. These F_{ST} and R_{ST} values were similar to values obtained in studies of other dolphin species that concluded populations were differentiated (e.g., Escorza-Treviño et al. 2005; Natoli et al. 2005), including studies that found differentiation among island regions (e.g., Oremus et al. 2007; Parsons et al. 2006). Although F_{ST} values are not directly comparable across these studies because of the use of different loci with potentially differing mutation rates, effective population sizes, and/or within-population variation to influence F_{ST} (Meirmans and Hedrick 2011), the approach that has been taken by NOAA Fisheries has been to split populations for management based, in part, on F_{ST} values similar in magnitude to those found in our study (Carretta et al. 2013a). The results of analyses of the same microsatellite loci from pantropical spotted dolphins from the coastal and offshore ETP and Hawaiian Islands indicate that genetic differentiation among the Hawaiian Islands regions (Hawaii, 4-islands area, Oahu, and Kauai/Niihau) is similar to or greater than that between offshore ETP and coastal ETP, which are separate subspecies (Courbis 2011). NOAA Fisheries has split spinner dolphins in the Hawaiian Islands into Hawaii, Oahu/4-islands, and Kauai/Niihau stocks (Carretta et al. 2013a) based mainly on genetic evidence of population differentiation (Andrews 2009; Andrews et al. 2010) and split common bottlenose dolphins into Hawaii, the 4-islands area, Oahu, Kauai/Niihau, and Hawaii Pelagic stocks based on both genetics (Martien et al. 2012) and photo-identification evidence (Baird et al. 2009). F'_{ST} values for pantropical spotted dolphin microsatellites ranged from 0.098 to 0.262, and Jost's D values were intermediate between F_{ST} and F'_{ST} values (Table 3). Generally, F'_{ST} is more suited to evaluating demographic inferences, such as migration rates, while Jost's D describes allelic differentiation (Meirmans and Hedrick 2011). As Taylor (1997) suggested migration rate as the standard by which to determine population level difference among marine mammals, F'_{ST} (or other genetic statistics

that are affected by migration) are likely the more meaningful for stock assessment, but D is likely a better indicator of the magnitude of actual differentiation, particularly given the high heterozygosity in our sample.

The F'_{ST} values for microsatellites for spinner dolphin and common bottlenose dolphin stocks found in the area from Hawaii to Kauai/N'ihau, ranged from 0.004 to 0.096 for spinner dolphins (Andrews et al. 2010) and 0.019 to 0.050 for common bottlenose dolphins (Martien et al. 2012). These values are lower than those for pantropical spotted dolphins, although again it should be noted that different microsatellite loci were used in each of these studies. Again, the values of F'_{ST} are similar to those between coastal and offshore ETP pantropical spotted dolphins using the same microsatellites (Courbis 2011). The spinner and bottlenose dolphin precedents suggest that comparable action should be taken to split pantropical spotted dolphin populations into separate management stocks near the Hawaiian Islands. NOAA Fisheries recently proposed to split the pantropical spotted dolphins stock into Oahu, 4-islands, Hawaii Island, and pelagic stocks, but this proposal has not yet been finalized (Carretta et al. 2013b). Taylor (1997) suggested that marine mammal populations should be managed separately if dispersal among the populations is below a few percent per year. Based on estimates of the range of expected F_{ST} values for a 1% annual dispersal rate among 3 pantropical spotted dolphin populations for mtDNA (0.006–0.009) and for nuclear DNA (0.002), dispersal rates were less than 1%/year among Oahu, 4-islands area, and Hawaii (i.e., F_{ST} values were higher than these "threshold" ranges). Thresholds of this kind are valuable for assessing the demographic importance of F_{ST} values, but caution should be used in interpreting these results as the expected values rely on the relationship between N_m and F_{ST}, for which the model assumptions are often not met (Whitlock and McCauley 1999), potentially biasing results. This approach was useful with respect to using life history and abundance information that did not rely on the genetic analyses, but possibly, methods like private alleles or Shannon pairwise comparison are more accurate as long as genetic data are sufficient and representative of populations. In Table 7, private alleles and Shannon pairwise analyses have similar results, but N_m values based on F_{ST} tend to be 2–5 times higher.

Neigel (2002) argues that, although F_{ST} remains a useful measure and is helpful for comparisons, gene flow should be estimated by more powerful approaches when possible, including likelihood. In our study, a likelihood analysis using TESS suggested that 3 populations were present. TESS performs well at low genetic differentiation levels, with misassignment rates lower than 3.5% for F_{ST}'s greater than or equal to 0.03 and down to 2% for F_{ST} equal to 0.04 (Chen et al. 2007). Despite the clustering of Kauai/Niihau with Oahu by TESS, F_{ST} for microsatellites and F_{ST} and Φ_{ST} for mtDNA are significantly different from 0 in pairwise comparisons between these 2 regions (Table 3). However, given that only 8 samples from 1 group of dolphins from Kauai/Niihau were available (Table 1), there were not enough data to draw strong conclusions about the relationship between this region and other regions. Lack of samples from Kauai/Niihau was not due to lack of sample effort (Baird et al. 2003; 2006), rather pantropical spotted dolphins appear to be much less common in that region (Figure 1; Table 1). Baird et al. (2013) reported that pantropical spotted dolphins were likely the most abundant odontocete in the inhabited Hawaiian Islands during their 13-year study and made up over 22% of sightings near Hawaii, 4-islands area, and Oahu but only 3.9% of sightings off of Kauai/Niihau. Two of the 4 mtDNA haplotypes found in these 8 samples were not found in the other 3 regions. This shows remarkable mtDNA diversity in a small sample size from a single encounter, suggesting that pantropical spotted dolphins near Kauai/Niihau may be transients from farther west along the archipelago or from an offshore population. There are no samples of pantropical spotted dolphins from the islands further west at this time, except for one collected during the NOAA HICEAS 2010 Survey; however, spinner dolphins near Kure Atoll, Midway Atoll, and Pearl and Hermes Reef have been found to be genetically distinct from those found near other Hawaiian Islands (Andrews et al. 2010).

There was more variability in microsatellites (up to 20 alleles per locus) than mtDNA (only 10 haplotypes with 77 out of 100 individuals having haplotype 3). MtDNA F_{ST} and Φ_{ST} results supported separation of the 4-islands area from Oahu and Kauai/Niihau from Oahu, but not separation of Hawaii from the other 3 regions (Table 3). MtDNA haplotypes did not show evidence of isolation by distance, suggesting that genetic differentiation among island regions may be due to other mechanisms, such as site fidelity, behavioral isolation, etc. F_{ST} and Φ_{ST} values that were significantly different from 0 for mtDNA were low but consistent with other studies that concluded that differentiation existed among dolphin populations (e.g., Escorza-Treviño et al. 2005; Natoli et al. 2005; Mendez et al. 2007). In comparison with ETP and China/Taiwan pantropical spotted dolphins using the same section of mtDNA, mtDNA fixation indices among the Hawaiian Islands regions were similar to several comparisons among the ETP offshore and coastal subspecies and China/Taiwan (Courbis 2011). As with microsatellites, F'_{ST} values were higher than F_{ST} values with the largest values suggesting separation of Oahu from the 4-islands area and Kauai/Niihau from Oahu and Hawaii (Table 3). However, caution must be taken in interpreting results for Kauai/Niihau because of the small sample size from that region. For Hawaii compared with the 4-islands area (0.019) and compared with Oahu (0.021), F'_{ST} values tended to be lower than those found for populations of spinner dolphins (Andrews 2009; Andrews et al. 2010), but other pairwise F'_{ST} values were higher (ranging from 0.046 to 0.171) than for spinner dolphins, which ranged from −0.011 to 0.120. In comparison with ETP coastal and offshore pantropical spotted dolphin subspecies and China/Taiwan, the F'_{ST} values among regions were higher than with the Hawaiian Islands regions, except Northern Mexico in comparison with offshore ETP samples (Courbis 2011). The distinction of Oahu from the other islands is further supported by its much lower mtDNA diversity (Table 2) and higher pairwise relatedness within the region.

Absence of Sex-Biased Dispersal and Intragroup Relatedness

In cases in which sex-biased dispersal is occurring, we expect to see that the dispersing sex will have lower F_{ST} and mean assignment index values and higher F_{IS} and variation in assignment index values (Goudet et al. 2002), which was not the case for pantropical spotted dolphins near the Hawaiian Islands. Parsons et al. (2006) found that common bottlenose dolphins near the Bahamas showed site fidelity for both sexes. Likewise, Andrews et al. (2010) found no evidence of sex-biased dispersal for spinner dolphins near the Hawaiian Islands, so other mechanisms inhibiting dispersal, such as behavioral adaptations to local regions, may not be uncommon in island systems.

Comparisons of relatedness within groups and among groups in each region and across all regions for pantropical spotted dolphins suggested that group fidelity is not driving genetic differentiation. This differs from some other species, such as false killer whales, which exhibit fidelity to natal groups near the Hawaiian Islands (Martien et al. 2011). It is possible that "nongroup" samples could have been from the same groups on different days, confounding the results. However, no individuals were resampled in multiple encounters to support this. It is also possible that sampled groups were really subgroups of larger groups.

Migration Rates

Estimates of pairwise regional migration (N_m) rates based on the private alleles method of Barton and Slatkin (1986) ranged from 1.49 to 3.45 (Table 7). This method assumes no admixture and that alleles have reached an equilibrium in the populations. Bearing in mind these assumptions, which are likely violated by the populations in our study, these migration rates are relatively low. Results for N_m calculated with F_{ST} tended to be higher than the private alleles N_m values (Table 7). N_m based on Shannon pairwise comparison ranged from 0.86 to 2.49, slightly less but not notably different from the values of the private alleles method. Attempts were made to estimate migration rates using programs such as LAMARC (Kuhner 2006), but there were indications that convergence was not reached. This maximum likelihood, iterative method would calculate migration rates in both directions between populations and would provide an error factor, but given the lack of convergence, the private alleles method was applied to supply at least a sense of whether migration rates were likely high or low. These values should be used with caution given both the likelihood of admixture among populations and the fact that the values are directly related to the F_{ST} calculation rather than applying Bayesian or maximum likelihood inference. Examining private alleles shared by pairs of island regions (excluding Kauai/Niihau) did not result in any differences that would support significantly higher gene flow among some pairs or a founding event at one region with spread to other regions.

Significance

Properly defining populations is important because site fidelity, despite the physical ability to disperse, can result in local population losses when populations are stressed by anthropogenic encroachment. At this time, there are few data available regarding anthropogenic impacts on pantropical spotted dolphins near the Hawaiian Islands. Pantropical spotted dolphins with injuries from lines and boats have been photographed near the Hawaiian Islands during odontocete surveys (R. W. Baird, unpublished data), raising concerns about the level of human interactions, particularly if populations are smaller and more isolated than are recognized under the current management scheme. Effective population size (not an overall abundance estimate) for the island of Hawaii based on microsatellite data is estimated to fall between 210 and 261 with an un-weighted harmonic mean of 220, suggesting a small reproductive population in this location.

Potential Drivers of Differentiation

Differences in habitat may result in differences in prey preferences at different island regions and may inhibit dispersal and, therefore, gene flow among regions. Andrews et al. (2010) suggests that habitat differences could raise ecological barriers to gene flow that drive differentiation in spinner dolphin populations near the Hawaiian Islands. Differences in habitat features, prey types, and prey abundance across adjacent ocean areas have all been suggested as reasons for genetic differentiation in other dolphin populations (e.g., García-Martínez et al. 1999; Möller et al. 2007; Bilgmann et al. 2008; Wiszniewski et al. 2010). García-Martínez et al. (1999) suggested that dolphins preferring shallow water habitats can become isolated. This may be the case for pantropical spotted dolphins near the 4-islands area; however, additional search effort and sample collection in deeper waters near the 4-islands area would provide more evidence to assess this.

Prey behavior is thought to be a driver of genetic differentiation among short-beaked common dolphins (*Delphinus* spp.) by virtue of affecting movement patterns and association with certain oceanographic conditions (Amaral et al. 2012). Niche segregation has also been found among different dolphin species inhabiting the island area of Mayotte near Mozambique (Kiszka et al. 2011). Such findings suggest that niche segregation could be a driver of speciation for some sympatric dolphins; this process would start with population differentiation (Kiszka et al. 2011). These studies relied on stable isotope analysis, which would likely be a good approach to determine if pantropical spotted dolphins and other odontocetes near different Hawaiian Island regions tend to feed on different prey. Only one study to date has addressed habitat use of pantropical spotted dolphins near the Hawaiian Islands (Baird et al. 2001). That study focused on the 4-islands area and concluded that pantropical spotted dolphins had different habitat use patterns in that region compared with other dolphin species (Baird et al. 2001).

Odontocetes may also have different numbers of predators and competitors near different island regions. Friedlander and DeMartini (2002) found that biomass of apex predators, which included sharks, groupers, and barracuda, differed among the Hawaiian Islands. Papastamatiou et al. (2006) reported that catch per unit effort differed among shark species among the Hawaiian Islands for 4 species of shark

caught in the Hawaiian longline fishery, resulting in different estimates of the relative contribution of each species to the overall species composition near each island region. Such studies suggest possible differences in predation pressure on dolphins (and other taxa) among Hawaiian Island regions, which may contribute to driving behavioral differences that can result in differentiation.

Conclusions

In conclusion, we found evidence that pantropical spotted dolphins constitute separate populations near Hawaii, the 4-islands area, and Oahu, with some evidence to support possible differences from Kauai/Niihau as well, though further study is warranted. Other marine mammal stocks have been divided recently for management purposes based on similar levels of genetic differentiation among regions. For example, harbor porpoise on the US West Coast were split into additional stocks based on Chivers et al. (2002), and NOAA Fisheries has recently split common bottlenose dolphin and spinner dolphin stocks near the Hawaiian Islands based on genetic and photo-identification analyses (Andrews 2009; Baird et al. 2009; Andrews et al. 2010; Martien et al. 2012; Carretta et al. 2013a). We suggest the same criteria be applied to pantropical spotted dolphins near the Hawaiian Islands. Our results suggest that differentiation is not mediated by sex-biased dispersal or group fidelity, and based on our and others' research, we hypothesize that possibly behavior adapted to differing habitat types that affect strategies such as foraging and predator avoidance could be driving differentiation. Further research on these strategies is needed to confirm these differences. Tagging studies and stable isotope analyses could explore feeding habits and other behaviors that differ among the regions of the Hawaiian Islands. The implications of this study go beyond pantropical spotted dolphins to larger questions of connectivity patterns and their drivers among Hawaii's marine wildlife.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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