

Staying close to home? Genetic differentiation of rough-toothed dolphins near oceanic islands in the central Pacific Ocean

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Abstract Rough-toothed dolphins have a worldwide tropical and subtropical distribution, yet little is known about the population structure and social organization of this typically open-ocean species. Although it has been assumed that pelagic dolphins range widely due to the lack of apparent barriers and unpredictable prey distribution, recent evidence suggests rough-toothed dolphins exhibit fidelity to some oceanic islands. Using the most comprehensively extensive dataset for this species to date, we assess the isolation and interchange of rough-toothed dolphins at the regional and oceanic scale within the central Pacific Ocean. Using mtDNA and microsatellite genotyping (nDNA), we analyzed samples of insular communities from the main Hawaiian (Kaua'i n = 93, O'ahu n = 9, Hawai'i n = 57), French Polynesian (n = 70) and Samoan

(n = 16) archipelagos, and pelagic samples off the Northwestern Hawaiian Islands (n = 18). An overall AMOVA indicated strong genetic differentiation among islands (mtDNA $F_{ST} = 0.265$; $p < 0.001$; nDNA $F_{ST} = 0.038$; $p < 0.001$), as well as among archipelagos (mtDNA $F_{ST} = 0.299$; $p < 0.001$; nDNA $F_{ST} = 0.055$; $p < 0.001$). Shared haplotypes (n = 4) between the archipelagos may be a product of a relatively recent divergence and/or periodic exchange from poorly understood pelagic populations. Analyses using STRUCTURE and GENELAND identified four separate management units among archipelagos and within the Hawaiian Islands. These results confirm the presence of multiple insular populations within the Pacific and island-specific genetic isolation among populations attached to islands in each archipelago. Insular populations seem most prevalent where oceanographic conditions indicate high local productivity or a discontinuity with surrounding oligotrophic areas. Our findings have important implications for a little studied species that faces increasing anthropogenic threats around oceanic islands.

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Introduction

The lack of geographic barriers in the open ocean has long been assumed to promote relatively unrestricted gene flow for pelagic species, predicting little phylogeographic structure at the oceanic scale and negligible population structure at the local scale. However, numerous recent studies show that even species with high dispersal capabilities such as barracuda, sharks, and dolphins can show

strong population structure (Fontaine et al. 2007; Tezanos-Pinto et al. 2008; Daly-Engel et al. 2012a, b; Whitney et al. 2012). Although biogeographic barriers and distance are thought to be one of the forces influencing this structure on a large scale (Briggs and Bowen 2012; Daly-Engel et al. 2012a), population structure on a regional scale is often shaped by life history strategies, social structure, and variation in oceanographic resources (Moller 2012).

The interplay of habitat specialization, social structure, and dispersal patterns has important implications for the genetic structure of populations in dolphin communities. Coastal dolphins generally show patterns of strong genetic differentiation, low genetic diversity within populations and isolation by distance (Wiszniewski et al. 2010; Mirimin et al. 2011; Hamner et al. 2012; Richards et al. 2013), while pelagic dolphins show lower, although still significant, levels of genetic differentiation, and no particular pattern of isolation by distance (Gaspari et al. 2007; Louis et al. 2014). Genetic structure can also be influenced by highly organized social structure in both coastal (Moller 2012) and pelagic dolphins (Gaspari et al. 2007; Foote et al. 2011; Baird et al. 2012). When social structure is the main driver in population structure, random colonization and local drift are observed (Storz 1999). Such organization may increase genetic differentiation and reduce diversity, limiting the resilience of populations to recover from natural catastrophes or anthropogenic impacts.

In the open ocean of the tropics, where primary productivity is typically low and prey resources are widely scattered and less predictable, dolphins are thought to show little population structure (Gowans et al. 2007; Andrews et al. 2010). By comparison, primary productivity often increases near oceanic islands, a phenomenon known as the “island mass effect” where a combination of nutrients from the island, upwelling and sufficient light at the ocean’s surface initiate photosynthesis (Doty and Oguri 1956; Dandonneau and Charpy 1985; Longhurst 1999; Palacios 2002; Martinez 2004; Woodworth et al. 2012). The strong habitat gradient between nearshore and offshore waters caused by the island mass effect can result in resource specialization and the development of insular populations in an otherwise pelagic species. Such resource specialization has been invoked to explain the development of population structure and site fidelity around oceanic islands in several dolphin species (Oremus et al. 2007; Andrews et al. 2010; Martien et al. 2012; Oremus et al. 2012). Moreover, island topographies, and their interactions with wind and currents, create a complex system of offshore eddies that concentrate stable and abundant prey for many mesopelagic predators (Seki et al. 2002; Woodworth et al. 2012). Surveys have shown cetacean densities are greater within 10 km of shore around the Hawaiian, Samoan and French Polynesian Islands (Gannier 2000; Barlow 2006; Johnston et al. 2008; Lambert et al. 2014; Mannocci et al. 2014).

The Hawaiian Islands in the North Pacific, and the Society and Samoan Islands in the South Pacific represent some of the most isolated inhabited archipelagos in the world (Fig. 1a). They also represent high levels of diversity and endemism for marine species (Bowen et al. 2013). The Hawaiian Islands (Fig. 1b), the five archipelagos of French Polynesia including the Society Islands, and the Samoan Islands (Fig. 1c) provide habitat to one-third of the world’s cetacean species (Reeves et al. 1999; Barlow 2006; Johnston et al. 2008; Baird et al. 2013; Bowen et al. 2013) as part of the large continuous marine habitat of the Pacific Ocean. The steep volcanic slope of these islands results in deep-water species in close proximity to shore, making the islands a convenient site to study otherwise pelagic cetaceans in the wild (Poole 1995). Within the last 10 years, an increasing number of cetacean studies have provided baseline data on movement and habitat use for both near-shore and offshore species around the archipelagos in the Hawaiian Islands, French Polynesia, and Samoan Islands. Many of these studies have found fidelity to islands and genetic differentiation between islands for small odontocetes (Poole 1993; Oremus et al. 2007; Baird et al. 2008; Johnston et al. 2008; Aschettino et al. 2012; Martien et al. 2012; Baird et al. 2013; Baird 2016). The pattern of differentiation could be due to isolation by distance or episodic colonization and genetic drift, but is likely driven by the island mass effect.

Rough-toothed dolphins (*Steno bredanensis*) have a worldwide tropical and subtropical distribution (Jefferson 2008; West et al. 2011), and show site fidelity around islands. They are medium-sized dolphins generally found in depths greater than 1000 m. Although considered a pelagic species, they are also found relatively close to shore where volcanic islands provide steep bathymetric slopes (Gannier and West 2005; Baird et al. 2008; Oremus et al. 2012). The highest concentration of rough-toothed dolphin encounters is at depths of about 3000 meters around the main Hawaiian Islands (MHI) (Baird et al. 2013) and between 1000 and 2000 m in the Society Islands (Gannier and West 2005). A previous photo-identification (photo-ID) study found evidence of local populations around the MHI (Kaua’i/Ni’ihau and the Island of Hawai’i) with low levels of interchange between populations (Baird et al. 2008). Differences in behavior and habitat use suggested movement between the islands was infrequent. Moreover, frequent within- and between-year re-sightings around the Island of Hawai’i (from this point forward referred to as Hawai’i) indicated high site fidelity (43 %) and low abundance ($N < 200$; (Baird et al. 2008). Oremus et al. (2012) combined molecular markers and photo-ID to describe island communities of rough-toothed dolphins in the Leeward (Mo’orea/Tahiti) and Windward Islands (Ra’iatea/Huahine) in French Polynesia’s Society Islands.

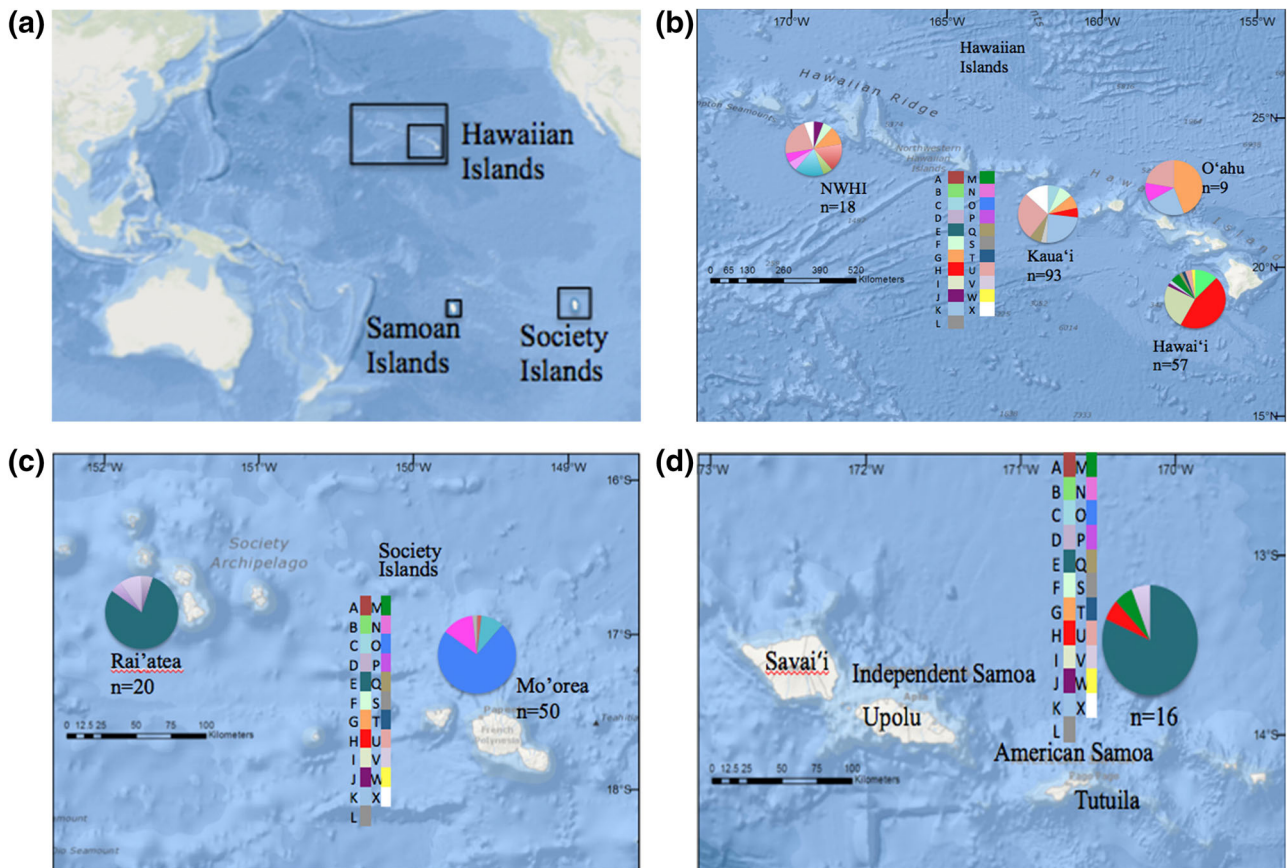


Fig. 1 *Steno bredanensis*. Each map represents one of the three archipelagos of the study area where biopsy samples (labeled with n for each population) of rough-toothed dolphins were collected. Pie charts reflect mtDNA haplotype frequencies for each island(s). **a** Pacific Ocean map showing locations where samples were collected. The *large box* for the Hawaiian Islands includes the

Northwest Hawaiian Islands. The *smaller box* includes the main Hawaiian Islands only. **b** Hawaiian Islands. **c** Society Islands of French Polynesia including Rai'atea and Mo'orea. **d** Samoan Islands including Savai'i and Upolu in Independent Samoa and Tutuila in American Samoa. (Color figure online)

They found strong site fidelity and high levels of genetic differentiation between these neighboring islands with low haplotype diversity and low abundance for Mo'orea/Tahiti ($N < 200$). Additional photo-ID studies of rough-toothed dolphins have been conducted in both the Pacific and Atlantic oceans, and indicate similar patterns of island fidelity (i.e. individuals being seen exclusively around one island in multiple years). In Tutuila, American Samoa, in the Pacific Ocean, 14 out of 41 individuals were sighted in multiple years (Johnston et al. 2008), while around Utila, Honduras, in the Atlantic Ocean, 15 of 28 individuals identified were sighted over multiple years (Kuczaj and Yeater 2007). In a separate study two populations were identified in the Southwestern Atlantic Ocean and another in the Caribbean using mtDNA (da Silva et al. 2015). Around the Canary Islands of Spain, high site fidelity (65 % of individuals seen in more than 1 year) and association patterns of individuals from different age classes observed together in multiple encounters suggested a small resident population, with strong social bonds between

individuals of different age classes and between mother/calf/juveniles (Mayr and Ritter 2005). Although these repeat sightings are relatively high for pelagic dolphins, this could also mean that some of the dolphins that are not resighted represent transients passing through an insular population.

Rough-toothed dolphins exhibit some behaviors associated with highly organized social structure including caregiving behaviors, synchronized travel in close formations, cooperative foraging, provisioning of large prey to calves, and mass stranding events (Nitta 1991; Ferrero and Hodder 1993; Poole 1993; Nekoba-Dutertre et al. 1999; Pitman and Stinchcomb 2002; Gotz et al. 2006; NMFS 2008; Fulgencio de Moura et al. 2009). These observations involved small groups (4–10 dolphins) and were generally made up of adults, subadults and occasionally a calf. These findings suggest social organization may play a role in rough-toothed dolphin population structure.

Here we present a comparative analysis of population structure in three tropical archipelagos of the Pacific. We

employ a suite of genetic markers to investigate the population structure of rough-toothed dolphins among the Hawaiian Islands, French Polynesia's Society Islands, and the Samoan Islands (Upolo and Savai'i in Samoa and Tutuila in American Samoa). We test the hypothesis suggested by Baird et al. (2008) and Oremus et al. (2012) that rough-toothed dolphins exist in independent, insular populations around oceanic islands with limited interchange or gene flow between islands and archipelagos. We interpret the observed genetic structure in relation to two hypotheses: if isolation by distance is the primary driver, we expect that populations within archipelagos may show significant genetic differentiation, but differentiation will increase with increasing geographic distance among islands within archipelagos and between archipelagos. Alternatively, if oceanographic processes such as the island mass effect or social structure is the primary driver, we expect differentiation could be driven by more episodic colonization and local genetic drift.

Methods

Tissue sample collection

Skin biopsy samples were collected from adult or subadult dolphins for genetic analyses during dedicated small boat surveys, large ship surveys or from beach-cast individuals in the MHI (Kaua'i, O'ahu, Hawai'i, Fig. 1b), the North-western Hawaiian Islands (NWHI, Fig. 1b), the Society Islands (Mo'orea/Tahiti and Ra'iatea/Huahine (from here on referred to as Mo'orea and Ra'iatea respectively, Fig. 1c), and the Samoan Islands (Fig. 1d) including Samoa (Upolo and Savai'i) and American Samoa (Tutuila). Biopsy samples were either collected with a crossbow or a modified veterinary capture rifle (Krützen et al. 2002). Samples were stored first in 70 % ethanol followed by an $-80\text{ }^{\circ}\text{C}$ freezer or stored directly in a freezer. Skin samples collected from individuals that stranded around the Hawaiian Islands and bycaught individuals around American Samoa were stored in DMSO at $-20\text{ }^{\circ}\text{C}$.

Photo-ID records and association networks were also available for many of the sampled individuals from the Hawaiian Islands (Baird et al. 2008).

DNA extraction, quantification and sex identification

Total DNA was extracted using either a Qiagen DNeasy Blood and Tissue Kit or a standard phenol:chloroform extraction protocol (Sambrook et al. 1989), modified for small samples (Baker et al. 1994). DNA was quantified with pico-green fluorescence and normalized to $15\text{ ng }\mu\text{l}^{-1}$. Sex was identified for individual dolphins by the amplification of the male-specific Sry gene multiplexed

with the ZFX gene (a positive control) as described in Gilson et al. (1998).

mtDNA sequencing

An approximately 800 bp fragment of the mtDNA control region of the 5' end was amplified via polymerase chain reaction (PCR) with primers Dlp 1.5 and Dlp 8 (Dalebout et al. 1998, 2005). The reaction was carried out in a 10 μl final volume using the protocol described in Oremus et al. (2007). Unincorporated primers were removed from the PCR product using shrimp alkaline phosphatase and exonuclease I (GE Health Care Life Sciences). Products were sequenced using the standard BigDye v.3.1 and CleanSeq was used to purify products before they were run on an ABI 3730 (Applied Biosystems) DNA automated sequencer. The sequences were aligned, manually edited, trimmed to 450 bp and haplotypes were identified using *Sequencher* 5.0 (Gene Codes Co.). Following quality control guidelines of Morin et al. (2010), sequences with a *Phred* score of <30 were repeated (Ewing et al. 1998). Variable sites with a *Phred* score of <40 were visually confirmed. If a haplotype was represented by only one sample the identity of the haplotype was confirmed by re-sequencing in both directions. Three individuals from Kaua'i showed apparent heteroplasmy at the same variable site. These sites were treated as missing values for these samples in subsequent analyses.

Microsatellite genotyping

Samples were genotyped at 14 previously published microsatellite loci (Table S1). Amplifications were carried out in a 10 μl final volume reaction containing $1\times$ Perkin-Elmer reaction buffer, 1.5–4.0 mM MgCl_2 , 0.4 μM of each primer (when fluorescent labels were pre-labeled), 0.2 mM deoxynucleotide triphosphate (dNTPs), 0.25–0.5 U Platinum *Taq* DNA Polymerase and approximately 5 ng of DNA template. Fluorescent labels were attached when loci were not labeled via an M-13 tail during amplification, changing the forward primer concentration to 0.04 μM , keeping the reverse primer concentration of 0.4 μM and adding an M-13 label concentration of 0.4 μM (Schuelke 2000). The PCR thermocycle profile was $93\text{ }^{\circ}\text{C}$ for 2 min followed by 15 cycles of $92\text{ }^{\circ}\text{C}$ for 30 s, annealing temperature (T_A) for 45 s and $72\text{ }^{\circ}\text{C}$ for 50 s. This was followed by an additional 20 cycles of $92\text{ }^{\circ}\text{C}$ for 30 s, T_A for 45 s and $72\text{ }^{\circ}\text{C}$ for 50 s and completed with a 3-min extension at $72\text{ }^{\circ}\text{C}$ for all loci except Sgui17 and GT6. These two loci followed the protocol described in the original paper, while KW12 used the profile described in Martien et al. (2012). Differences in annealing temperatures and concentrations of MgCl_2 and *Taq*, for each locus are outlined in Table S1.

PCR products were coloaded in sets of non-overlapping loci and analyzed on a 3730 sequencer (Applied

Biosystems) with formamide and 500 LIZ size standard ladder (Applied Biosystems). Following quality control guidelines (Morin et al. 2010), four internal control samples were run to compare sizes across trays, and a subset of randomly selected samples (5 %) were repeated for all loci. Alleles were called using the program *GENEMAPPER* v.3.7 (Applied Biosystems). Allele binning was manually checked before scoring loci. Samples with fewer than 10 of 15 loci were excluded from further analysis. An independent, experienced technician reviewed a subset of samples and loci at Oregon State University where analyses were conducted. *MICROCHECKER* was used to investigate the presence of null alleles, dropout and stutter that may result from errors in allele binning (Van Oosterhout et al. 2006). The program *GENEPOP* v.3.4 (Raymond and Rousset 1995) was used to evaluate heterozygote deficiency, expected heterozygosity, observed heterozygosity, the significance of deviation from Hardy–Weinberg equilibrium (HWE) expectations (each population was run separately), and test the independence of loci using linkage-disequilibrium analysis. Allelic richness was calculated using *FSTAT* v.2.9.3 (Goudet 2001). Bonferroni corrections (Rice 1989) were applied to all pairwise test results to adjust for multiple comparisons.

The program *CERVUS* v.3.0 (Kalinowski et al. 2007) was used to identify replicate samples and calculate the probability of identity (P_{ID}), or chance that a pair of randomly selected individuals will have matching genotypes. To avoid false exclusion due to potential dropout and genotyping errors (Waits et al. 2001), we initially used a relaxed criterion that allowed for mismatches at up to four loci. The electropherograms of the mismatching loci were then reviewed and either corrected based on visual inspection or repeated for confirmation. We required a minimum overlap of nine matching loci to accept samples as being replicates from the same individual. A per-allele error rate was calculated from replicate genotypes identified with the initial relaxed matching and subsequent review of near matches (Pompanon et al. 2005), as well as the random samples that were rerun in the quality control process. Identified replicate samples were validated with photo-ID, mtDNA haplotype assignment and sex identification whenever possible.

mtDNA diversity and population differentiation

Haplotype (h) and nucleotide (π) diversity for each island group and for all samples grouped by archipelago were calculated in *Arlequin* v.3.5 (Excoffier and Lischer 2010). A median-joining network of the unique haplotypes was constructed using *HapStar* (Teacher and Griffiths 2011). This construction begins by combining the minimum-spanning tree results available from the output file in

Arlequin to construct a network relating the haplotypes (Fig. 2).

Population differentiation was assessed in *Arlequin* v.3.5 with global tests followed by pairwise comparisons between ocean basins and between all pairs of island populations. The F_{ST} analogue Φ_{ST} , which accounts for the mutation process, was also used to incorporate nucleotide distances into the calculation of genetic differentiation. *PERMUT* was used to test for the contribution of mutational divergence of haplotypes by comparing the difference between F_{ST} and Φ_{ST} (Petit 2010). To test for significant differences in haplotype diversity between geographic areas in the North Pacific (NWHI, Kaua'i, O'ahu, Hawai'i) and the South Pacific (Mo'orea, Ra'iā-tea and Samoan Islands), a permutation test was generated using the standard deviation output from *Arlequin* (permutations generated in *R*, script available from A. Alexander *GitHub*).

A hierarchical AMOVA procedure calculated standard variance components, and several haplotypic correlation measures were tested using a random permutation procedure in *Arlequin* (Excoffier et al. 1992). To conduct the hierarchical analyses, the seven populations (Fig. 1; Table 1) were nested within two ocean basins, i.e. Hawaiian Islands populations in the North Pacific and Society and Samoan Islands populations in the South Pacific.

Microsatellite diversity and population differentiation

Regional differentiation in microsatellite allele frequencies was estimated as F_{ST} , using *GenAlEx* v. 6.5 (Peakall and Smouse 2012) with 10,000 permutations to assess significance. G''_{ST} , an analogue of F_{ST} that adjusts for the diversity, small sample size, and small numbers of populations (Meirmans and Hedrick 2011), was calculated in *GENODIVE* v.2 (Meirmans and Van Tienderen 2004). An hierarchical AMOVA analysis between ocean basins and among populations within ocean basins (described above) was calculated using microsatellites (F_{ST} only) in *HierFSTAT* implemented through *R*; (Goudet 2005), to examine the distribution of variation and differential connectivity between ocean basins. Taking multiple samples from one group (where 'group' refers to all dolphins sampled in a single encounter) could result in preferentially sampling closely related individuals. This could lead to an overestimate of population structure (Rodriguez-Ramilo and Wang 2012). To identify possible kin sampled together we used the relatedness program in *GenAlEx* v.6.5, calculating Queller and Goodnight's (1989) relatedness between dyads within each group in each population, and, in the instance that only two individuals were sampled, between the

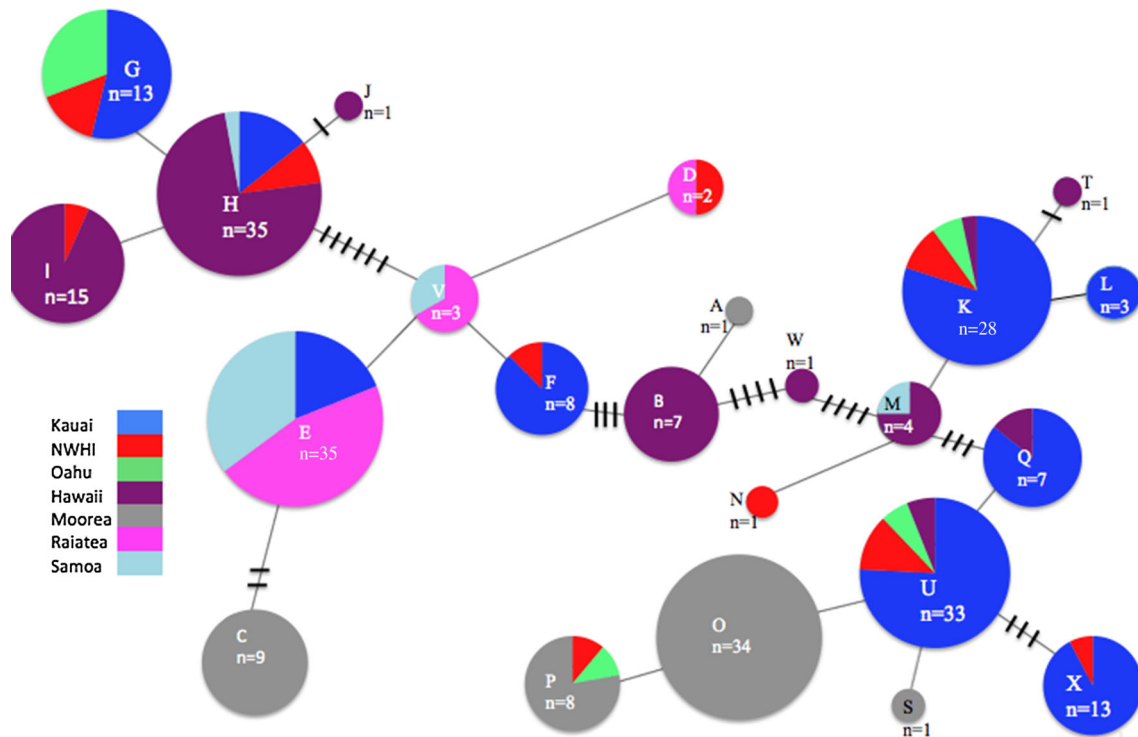


Fig. 2 *Steno bredanensis*. A median-joining network of mtDNA control region 450 bp haplotypes found in rough-toothed dolphins from the central Pacific Ocean. Each circle represents a unique haplotype and each line connecting the haplotypes indicates a single

base pair change. Small black dashed lines represent an additional substitution. The size of each circle is proportional to the sample size, and each is colored according to the region(s) where the haplotype was sampled. (Color figure online)

Table 1 *Steno bredanensis*—oceanic and island samples sizes (n) for mtDNA in the Hawaiian, Society and Samoan Islands

Geographic area	mtDNA				Microsatellites						
	n	k	<i>h</i> (SD)	π (SD)	F	M	n	K	Allelic Richness	H_O	F_{IS}
North Pacific											
NWHI	18	10	0.912 (0.059)	1.64 (0.93)	6	3	9	12.3	4.2	0.836	0.010
Kaua'i	93	10	0.798 (0.0168)	1.39 (0.75)	55	36	91	8.75	3.7	0.853	−0.064
O'ahu	9	4	0.711 (0.086)	1.38 (0.81)	7	3	10	7.3	4.4	0.736	0.079
Hawai'i	57	10	0.623 (0.046)	1.13 (0.62)	32	17	49	7.61	4.6	0.802	0.096
Hawaiian Islands total	177	18	0.799 (0.0093)	1.61 (0.85)	100	59	159	9.02	4.2	0.810	0.065
South Pacific											
Mo'orea	50	5	0.409 (0.081)	0.501 (0.31)	27	23	50	7.65	4.9	0.691	0.045
Ra'iatea	20	4	0.189 (0.108)	0.42 (0.06)	14	3	17	4.37	3.9	0.723	−0.004
Society Islands total	70	8	0.477 (0.093)	0.510 (0.19)	41	26	67	6.01	4.5	0.711	0.012
Samoan Islands total	16	4	0.350 (0.148)	0.451 (0.30)	9	4	13	4.31	3.8	0.678	−0.001
Total all islands	263	23	0.665	1.12 (0.43)	148	105	239	5.81	4.01	0.685	0.057

For mtDNA, number of haplotypes (*k*), haplotype diversity (*h*) and nucleotide diversity (in %, π) presented, with standard deviations calculated in *Arlequin*. Microsatellites numbers of individuals are given by sex (F, M) and total sample size (*n*), K is the mean number of alleles per locus across 14 loci

pair. We used Mendelian expectations (Blouin 2003) to evaluate potential relatedness between pairs, i.e. half-sibling 0.25, full sib 0.5 and parent-offspring 0.5. Therefore we considered close kin to have a relatedness value of 0.25

or higher. If a dyad was found with a relatedness value of 0.25 (the value expected for half siblings; Blouin 2003) or higher, one individual was removed from the dataset resulting in a kin restricted dataset. We then used GenAIE

v.6.5 (Peakall and Smouse 2006) to determine whether the mean pairwise relatedness within any groups that still contained three or more individuals was higher than would be expected by chance. We iteratively removed individuals from groups with higher than expected relatedness, as described in Martien et al. (2014). At each iteration we identified the individual that had the highest average relatedness to the other members of its group and removed that individual from the dataset. We continued this process until the within-group relatedness was not significantly higher than expected. If kin were sampled in independent sampling events, then this was considered to reflect the true population structure rather than a bias due to social structure, and both individuals were retained.

Population structure for all archipelagos and within the Hawaiian Islands was also evaluated in the absence of a priori divisions with a Bayesian clustering approach implemented in *STRUCTURE* v.2.3.1. (Pritchard et al. 2000). For all *STRUCTURE* analyses five independent runs (iterations) were performed for each K (number of clusters). We used a burn-in of 100,000 and a run length of 1,000,000 with all other parameters left as program defaults. We used *STRUCTURE* to cluster the entire dataset into $K = 1-7$ populations, with prior information on sampling location, using an admixture model and correlated allele frequencies. We also ran a ‘Hawaiian Islands only’ dataset including samples within the Hawaiian Islands (NWHI, Kaua‘i, O‘ahu, Hawai‘i) and let $K = 1-5$ populations using five iterations for each K. We compared estimates of likelihood across runs to confirm convergence. The results were processed in *STRUCTURE HARVESTER* v.0.6.93 (Earl and vonHoldt 2012), and evaluated for different values of K. To determine the most robust delineation of populations we evaluated where the ad hoc statistic, ΔK (Evanno et al. 2005), which estimates the rate of change in the log probability of data between successive K values, detected a clear peak. For comparison, this was then evaluated against the rate of change of the likelihood distribution (mean) calculated as $L'(K)$. The value of K is detected when there is a sharp drop in the likelihood value or a peak in ΔK .

Given the known limitations of *STRUCTURE* to detect weak differentiation (Hubisz et al. 2009) we also used the program *GENELAND* (Guillot et al. 2005b) to estimate spatial population boundaries allowing for populations to be delineated outside of our a priori hypotheses. We considered this to be informative only among the Hawaiian Islands where sample sizes were relatively large and spatial separation was relatively uniform, rather than between the large distances separating the three archipelagos. We implemented a correlated allele frequency model set with 500,000 MCMC iterations and thinning of 100. We first set K from 1 to 6 populations, and then did a second run with

K fixed at the modal value to estimate the assignment of individuals, as recommended in Guillot et al. (2005a, b). The first step was done using 10 independent runs to check for convergence, allowing K to vary from 1 to 6 populations. The runs were post-processed using a burn-in of 2000 in order to obtain posterior probabilities of population membership for each individual and each pixel of the spatial domain (400 pixels along the X axis and 400 along the Y axis).

Sex-biased dispersal

Sex-biased dispersal was assessed between each island as well as among islands in an archipelago using the microsatellite dataset in *FSTAT* (Goudet et al. 2002; Goudet 2005). Sex-specific F_{ST} , inbreeding coefficient, F_{IS} , mean assignment index (mAIC) and variance of mean corrected assignment index (vAIC) based on microsatellite genotypes were performed using two-tailed tests and 10,000 permutations of the resampling procedure. The dispersing sex is expected to have a lower F_{ST} value, but higher variance. For mtDNA, sex-specific F_{ST} values were calculated by coding the haplotypes as homozygotes and comparing the sexes for significant differences using the resampling procedure in *FSTAT*, see Oremus et al. (2007).

Isolation by distance

Mantel tests were implemented in *GenAlEx* v. 6.5 to assess the relationship between genetic differentiation and geographic distance. To generate the matrix for geographic distance we used the geographic coordinates of sample locations to measure the distance between islands and archipelagos. To generate the matrix for genetic differentiation, pairwise F_{ST} values were used for both mtDNA and microsatellite data.

Results

Genotype matching and sex ratios

Of the 296 rough-toothed dolphin samples obtained in the MHI, the NWHI, the Society Islands and the Samoan Islands chosen for the initial analysis, 251 met the quality control threshold by genotyping at a minimum of 10 of the 14 loci attempted (Table 1). The microsatellite loci were found to be moderately variable ranging from 3 to 14 alleles per locus (Tables 1, S1). The program *MICRO-CHECKER* found little evidence of null alleles, large-allele dropout or error due to stutter. We calculated an error rate of 2.9 % per allele, most of which was due to a sizing adjustment for primers with and without an M-13 extension

for labeling (Pompanon et al. 2005). The relaxed matching allowed us to detect and correct much of this error. No loci showed evidence of linkage disequilibrium. There were instances from two island groups where loci deviated significantly from HWE: two for Kaua'i (GT39 and DlrFCB1) and two for Hawai'i (GT39 and SI4, Table S8). Results were not sensitive to the inclusion of these loci (i.e. when they were removed the significance of the F_{ST} between populations did not change, and the number of populations identified by STRUCTURE and GENELAND did not change), so they were retained. Using a minimum of 10 loci for each sample, the probability that two unrelated individuals share a similar genotype was very low ($P_{ID} = 1.4 \times 10^{-15}$). From the initial relaxed matching and review of near-matches, five samples were found to have identical genotype profiles, and also matched at haplotype and sex. All of these matches were within-island recaptures, and were removed from the dataset for the purposes of the analyses. This brought the total sample size to 246 individuals to be used for analyses.

The program *GenAIE* v.6.4 identified a small number of likely close kin (five in Society Islands and four in the Hawaiian Islands, two in the Samoan Islands). Although we consider kin sampled in the same group a reflection of the true population structure of dolphins, we wanted to confirm that our sampling was not biased by the presence of related individuals. Consequently, we excluded one individual in each of these groups and reran the population level analyses. The relatedness within all of the groups was not significantly higher than expected by chance so no additional individuals were removed. Using the available photo-ID data and association network from the Hawaiian Islands (Baird, unpublished), we identified two dyads of individuals that had been seen together in over half of the encounters where they were observed (they had each been observed four times). One dyad (male/female) had different haplotypes (H and I) but a relatedness value of 0.25 (on the order of half-sibs). The other pair (female/female) had the same haplotype (H), but one individual had been removed from the microsatellite dataset due to only six loci amplifying so we were unable to determine relatedness of the pair.

mtDNA diversity and differentiation

After removing the five replicate samples, there were 266 individuals with high quality sequences for mtDNA analysis. Using 450 base pairs of the mtDNA control region, we identified 30 variable sites to resolve 23 haplotypes. Of the 23 haplotypes, seven were previously described from the Society Islands or Samoa, six by Oremus et al. (2012) and one by Olavarria et al. (2004). The 16 new haplotypes were submitted to GenBank (Accession numbers: KU745651-

KU745666), and regional haplotype frequencies were submitted to Dryad Digital Repository (<http://dx.doi.org/10.5061/dryad.29g1b>). Seven of the 16 new haplotypes (A, J, L, N, S, T, W) were unique to one island (Fig. 2). Two of the four most common haplotypes (E and H) were shared with at least three other islands and between archipelagos in low frequencies (Fig. 2). Interestingly, the most common haplotype at Mo'orea (haplotype O) was not found in Ra'iatea. In fact, there was only one shared haplotype between Mo'orea and Ra'iatea (a distance of 190 km) compared to four shared haplotypes between Kaua'i and Hawai'i (a distance of 470 km). However, it should be noted that the sampling in the Hawaiian Islands has been much more extensive than the Society Islands. Haplotype and nucleotide diversity were greatest in the Hawaiian archipelago followed by the Society Islands, with the lowest in the Samoan archipelago (Table 1). When the populations were compared separately, NWHI had the highest haplotype diversity, followed by Kaua'i, O'ahu, Hawai'i, Mo'orea, Samoan Islands, and Ra'iatea (Table 1). Despite the small sample size in the NWHI, 10 haplotypes were identified, the same number of haplotypes as Kaua'i and Hawai'i. Using the permutation test in R, we found haplotype diversity differed significantly between the North Pacific (Hawaiian Islands) and the South Pacific (Society Islands and Samoan Islands), as well as between all populations except NWHI and O'ahu; and Mo'orea and Ra'iatea (Tables S3, S4).

The haplotype network showed no obvious phylogeographic structure for the central North and South Pacific Oceans (Fig. 2). There was little geographic concordance for any archipelago. The Society Island haplotypes are not closely related to one another with the exception of haplotype O and P, which are separated by a single substitution and represent a large portion of the sampled individuals in Mo'orea. Although these haplotypes represent a high frequency for Mo'orea, no other islands in the South Pacific had these haplotypes, yet haplotype P was found in low frequencies for the NWHI and O'ahu. The Samoan Islands shared haplotypes with both the Hawaiian and Society Islands, but did not have any private haplotypes. There were several private haplotypes in other areas—four in Hawai'i and four in Mo'orea. However, only one of these haplotypes (haplotype O at Mo'orea) was found in high frequencies (71 %). Haplotype V was identified in Oremus et al. (2012) in Samoa (referenced as h6), while our extended dataset also identified this haplotype in Ra'iatea (this study) and the Eastern Pacific (Albertson unpublished). Similarly, haplotype D was identified in Oremus et al. (2012) as an Eastern Pacific haplotype (referenced as h9), while our dataset also identified this haplotype in the NWHI and Ra'iatea. Moreover, haplotype P, identified in Oremus et al. (2012) in Mo'orea, was also found in our dataset in Oahu (this study) and the Western Pacific (Albertson unpublished).

Table 2 *Steno bredanensis*—pairwise F_{ST} values for mtDNA (below diagonal) and nuclear DNA (above diagonal) between seven island populations in the Hawaiian, Society and Samoan Islands

	Kaua‘i	NWHI	O‘ahu	Hawai‘i	Mo‘orea	Ra‘iatea	Samoa Islands
Kaua‘i		0.023	0.013	<i>0.035**</i>	<i>0.039**</i>	<i>0.075**</i>	<i>0.056**</i>
NWHI	0.002		0.015	<i>0.037**</i>	<i>0.062**</i>	<i>0.073**</i>	<i>0.041**</i>
O‘ahu	0.044	0.012		<i>0.034**</i>	<i>0.059**</i>	<i>0.100**</i>	<i>0.051**</i>
Hawai‘i	<i>0.179**</i>	<i>0.139**</i>	<i>0.166**</i>		<i>0.058**</i>	<i>0.046**</i>	<i>0.021**</i>
Mo‘orea	<i>0.350**</i>	<i>0.307**</i>	<i>0.498**</i>	<i>0.429**</i>		<i>0.079**</i>	<i>0.048**</i>
Ra‘iatea	<i>0.393**</i>	<i>0.404**</i>	<i>0.511**</i>	<i>0.437**</i>	<i>0.479**</i>		<i>0.091**</i>
Samoa Islands	<i>0.300**</i>	<i>0.312**</i>	<i>0.495**</i>	<i>0.395**</i>	<i>0.410**</i>	<i>0.034</i>	

Φ_{ST} and G''_{ST} , values reported in supplementary material (Table S2). Statistically significant F_{ST} values are bolded and italicized, with * significant at $p < 0.05$; ** significant at $p < 0.001$

As reflected in the network, there were marked differences in haplotype frequencies (global $F_{ST} = 0.334$, p value < 0.001), but no clear pattern for isolation by distance was found. Tests of differentiation showed highly significant pairwise differences between archipelagos. Significant differentiation was also found between all island pairs except NWHI/O‘ahu and Kaua‘i/O‘ahu, NWHI/Kaua‘i, and Ra‘iatea/Samoan Islands (Table 2). However, unlike NWHI/O‘ahu, Kaua‘i/O‘ahu and NWHI/Kaua‘i that share several haplotypes, Rai‘atea/Samoan Islands only share one haplotype, albeit in high frequency (Fig. 1c–d). The differences were not necessarily greater between islands in different archipelagos than between islands within an archipelago, despite the large geographic distance between archipelagos, i.e. a pattern of isolation by distance would have shown increasing distance and increasing F_{ST} . The pairwise F_{ST} was greater between Mo‘orea and Samoa Islands ($F_{ST} = 0.410$) than between Kaua‘i and Samoa Islands ($F_{ST} = 0.301$). F_{ST} and Φ_{ST} values were similar (Tables 2, S2), and differences between the two indices were not significant. When the populations were pooled into archipelagos, significant genetic differentiation was found for all pairwise comparisons (Table 3). Moreover, no pattern of isolation by geographic distance was found, as larger F_{ST} values were found between the Society Islands and Samoa Islands ($F_{ST} = 0.418$) than between the Hawaiian Islands and Samoa Islands ($F_{ST} = 0.258$).

The hierarchical AMOVA used the following a priori levels: two oceans basins, North Pacific (Hawaiian Islands) and South Pacific (Society Islands and the Samoa Islands), three archipelagos (Hawaiian Islands, Society Islands and Samoa Islands) and seven island populations (NWHI, O‘ahu, Kaua‘i, Hawai‘i, Mo‘orea, Ra‘iatea and Samoa Islands). The results from the hierarchical AMOVA showed that the ocean basin level explained only 5.2 % of the haplotype diversity, among island populations within ocean basins explained 29.4 % of the diversity, and within island populations explained 65.4 % (Table 4). The permutation procedure showed that both the partitioning of variance within island populations (Φ_{ST}) and among island

populations within ocean basins (Φ_{SC}) were highly significant ($ST = 0.346$, $SC = 0.310$). Limitations on the degrees of freedom at the ocean basin level of our analysis prevented a test of this variance, but a non-hierarchical analysis where populations were pooled by archipelago and ocean basin showed this was significant (Table 5).

Microsatellite diversity and differentiation

Levels of microsatellite diversity were similar for each island with an average observed heterozygosity of 0.685 (Tables 1, S1). Significant overall genetic differentiation was found among the seven islands ($F_{ST} = 0.038$, 95 % CL 0.026–0.051; p value < 0.01 ; $F'_{ST} = 0.128$) and between most pairwise comparisons of islands (albeit with lower overall values than mtDNA data, Table 2) with the exception of the NWHI, Kaua‘i and O‘ahu (Table 2), as well as between archipelagos when populations were pooled (Table 3). In the hierarchical AMOVA analyses, 2.6 % of the variance was explained by ocean basins and 5.0 % was explained by the seven island populations (Table 5).

When all samples were included using LocPrior for sampling location in the *STRUCTURE* analysis with LocPrior, the model with the highest log-likelihood was $K = 2$ (Table S5, Fig. S1). A graphical representation of membership showed clear delineation for the North Pacific’s Hawaiian Islands, and the South Pacific’s Society Islands (Fig. 3a–d, supplementary Fig. S3a–b). However, the Samoa Islands included some individuals that clustered with Hawaii samples and some with the Society Islands (Fig. 3a). Five of these individuals were sampled during the same encounter about 10 km offshore from the island of Tutuila, American Samoa. Two of those individuals had haplotypes that were found in high frequencies in Hawai‘i and low frequencies in Kaua‘i and NWHI (haplotype H). One individual had haplotype M, found in low frequencies only in Hawai‘i. The other two had a haplotype found in all archipelagos (haplotype E), but most commonly in the South Pacific. All other Samoa samples clustered with South Pacific individuals, possessed haplotype E, and were

Table 3 *Steno bredanensis*—pairwise F_{ST} differentiation among the three archipelagos with islands pooled into the corresponding archipelago for 14 microsatellite loci above diagonal and mtDNA control region below diagonal with corresponding Φ_{ST} and G''_{ST} values in parentheses

	Hawaiian Islands	Society Islands	Samoa Islands
Hawaiian Islands		<i>0.034** (0.111**)</i>	<i>0.043** (0.158**)</i>
Society Islands	<i>0.231** (0.180**)</i>		<i>0.086** (0.112**)</i>
Samoa Islands	<i>0.255** (0.281**)</i>	<i>0.416** (0.337**)</i>	

Statistically significant values are bolded and italicized, with ** significant at $p < 0.001$

Table 4 *Steno bredanensis*—hierarchical AMOVA based on 450 bp of the mtDNA control region in the Pacific Ocean

mtDNA hierarchical AMOVA analysis	df	% of total variation	F-statistic	p value
Among ocean basins	1	5.2	CT = 0.052	n.a.
Among island populations with ocean basins	5	29.4	SC = 0.310	0.001
Within island populations	263	65.4	ST = 0.346	0.001

Ocean basins delineates the North Pacific and South Pacific; populations represent the seven island populations from Fig. 1 and Table 1

p values are based on 10,000 permutations

df degrees of freedom

Table 5 *Steno bredanensis*—hierarchical AMOVA based on 14 microsatellite loci in the Pacific Ocean

Microsatellite loci AMOVA analysis	df	% of total variation	F-statistic	p value
Among ocean basins	1	2.6	CT = 0.028	n.a.
Among island populations with ocean basins	5	5.0	SC = 0.052	0.001
Within island populations	239	92.4	ST = 0.076	0.001

Ocean basins delineates the North Pacific and South Pacific; populations represent the seven island populations from Fig. 1 and Table 1

p values based on 10,000 permutations

df degrees of freedom

collected within 2 km of shore. For higher K values the Samoan Islands show a clear distinction between both the Hawaiian Islands and the Society Islands (Fig. 3b–d, Supplementary Fig. S3a–b).

When the Hawaiian Islands were assessed exclusively in *STRUCTURE* with LocPrior for the four island regions, the highest likelihood was $K = 2$ (Figs. 4a, S2, Table S6). The island of Hawaii showed the strongest distinction from O‘ahu and Kaua‘i and this pattern is consistent at larger values of K as well (Figs. 4b, S4a–b). With three exceptions, the samples from O‘ahu and Kaua‘i showed relatively pure ancestry Fig. 4. All three had been sighted in Kaua‘i previously, and were included in the social network of other individuals frequently sighted in Kaua‘i (Baird et al. 2008).

When spatial coordinates of samples from the Hawaiian Islands were included in *GENELAND*, the highest likelihood found was $K = 5$ clusters, plus 1 “ghost population” (Fig. S5). The five clusters or populations identified were the island of Hawai‘i, the NWHI, and then three additional clusters including different samples within Kaua‘i and O‘ahu (Fig. 5). The heat maps of posterior probability

maps showed a clear cluster around the Island of Hawai‘i (Fig. S6a), a cluster near northwest Kaua‘i, a cluster around NWHI and O‘ahu (Fig. S6c), and a cluster including all Kaua‘i, O‘ahu and NWHI. So the program found population delineation for Hawai‘i, but it was unclear how Kaua‘i, O‘ahu and NWHI should be delineated. No individuals were assigned to the 1 “ghost population” and we considered these to be artifacts of the spatial distribution and number of samples (Guillot et al. 2005a).

Sex-biased dispersal and isolation by distance

There was no evidence of sex-biased dispersal identified based on the FSTAT procedure. Although mtDNA and microsatellite F_{ST} values were slightly higher for females, these differences were not significantly different from males (Table S7).

There was no evidence of isolation by distance based on the Mantel tests conducted using pairwise comparisons of islands showed no correlation between pairwise F_{ST} values and increasing geographic distance. Isolation by distance

was not significant for either Φ_{ST} or F_{ST} in the mtDNA data ($R^2 = 0.004$, $p = 0.13$; $R^2 = 0.018$, $p = 0.08$ respectively), or for the microsatellite data ($R^2 = 0.007$, $p = 0.16$).

Discussion

Despite the typically oceanic habitat of rough-toothed dolphins, our results demonstrate some degree of insular population structure for three archipelagos in the Central Pacific. These results support a growing body of evidence that pelagic species form isolated insular populations in areas with increased local productivity. Significant hierarchical partitioning of genetic variation was found between the North Pacific and South Pacific Ocean basins, and between the three archipelagos. We also found significant genetic differentiation in both mtDNA and microsatellite analyses between most islands within archipelagos with the exception of the NWHI, Kaua'i and O'ahu. The genetic partitioning resolved by STRUCTURE and GENELAND supported a separate population around the island of Hawai'i in the Hawaiian Islands, but was less clear about the partitioning of NWHI, Kaua'i and O'ahu. Together with other studies, the pattern we observe is consistent with island colonization and occasional ongoing gene flow with pelagic populations, and possibly occasional long-distance migration events between insular populations. Due to the small number of pelagic samples, we could not determine which (or how many) of these scenarios best describe the pattern of the data.

Local productivity and population structure

In the MHI, the island mass effect is responsible for the higher productivity areas directly surrounding each island. This phenomenon, combined with oligotrophic offshore waters, creates a pattern of strong population differentiation among islands for many insular species spanning trophic levels from limpets to dolphins (Polovina et al. 1994; Bird et al. 2007; Martien et al. 2012; Baird 2016). The MHIs have several causative mechanisms contributing to enhanced nearshore productivity. The tall mountains on Maui (Haleakalā) and Hawai'i (Mauna Kea) funnel the wind between them creating exceptionally strong currents. Moreover, the area around the 4-island area of Maui, Lāna'i, Kaho'olawe and Moloka'i is surrounded by relatively shallow water. Baird et al. (2008) noted sightings of rough-toothed dolphins in the 4-island area have been rare (Baird et al. 2008, 2013), and rough-toothed dolphins are typically found in depths greater than 1000 m. In addition, the leeward side is an active area of eddy generation, and these productive areas have been linked to the frequent

occurrence of large mesopelagic predators (Holland and Mitchum 2001; Seki et al. 2001) eliminating the need to travel to other islands for foraging. Finally, runoff from the islands brings nutrients to nearshore waters further enhancing productivity. The rough-toothed dolphins around the island of Hawai'i showed strong genetic differentiation and therefore minimal gene flow with other islands in the Hawaiian archipelago.

On the opposite edge of the Hawaiian archipelago, the NWHI experience a mixing zone known as the Transition Zone Chlorophyll Front resulting in a higher average productivity in this part of the island chain (Schmelzer 2000). This reduces the habitat differences between nearshore and offshore waters in that part of the archipelago (Polovina et al. 2001). Although there are several islands in the NWHI, each island is much smaller than the islands in the MHI. Without the large mass of the islands and tall mountains, the island mass effect and offshore eddies are not as prevalent in the NWHI and there is little to no runoff into the nearshore waters. With the small difference in productivity between the nearshore and offshore areas between these islands, the productive habitat could extend between islands and south to Kaua'i, the most northern island in the MHI. The lower genetic differentiation observed between the NWHI and Kaua'i could be the result of more uniform productivity throughout the area equating to lower site fidelity and larger home ranges, unlike the island mass and other effects in the MHI that may result in smaller home ranges restricted to one island. Baird et al. (2008) found lower site fidelity for rough-toothed dolphins around Kaua'i than Hawai'i and suggested a larger home range as an explanation. Therefore the home range for rough-toothed dolphins around Kaua'i could also be larger. Lastly, the lack of significant genetic differentiation among Kaua'i, O'ahu and the NWHI in our study suggests greater gene flow between these islands than between these and Hawai'i. GENELAND posterior probability maps also reflect some amount of gene flow between NWHI, Kaua'i, and O'ahu as shown with the orange and yellow coloration in Supplementary Fig. S6b–e.

Both in the Society Islands and the MHI, rough-toothed dolphins are most commonly found in depths greater than 1000 meters (Gannier 2000; Gannier and West 2005; Baird et al. 2008; Oremus et al. 2012; Baird et al. 2013). Due to the steep slope of these oceanic islands, the distance from shore at these depths is generally within a few kilometers. Similar to the MHI, the Society Islands experience increased productivity nearshore and a drop in productivity as the distance from shore increases (Longhurst 1999; Mannocci et al. 2014). It is therefore not surprising that our study, and that of Oremus et al. (2012), found genetic differentiation between the islands of Mo'orea and Rai'atea in the Society Islands despite the proximity of these islands

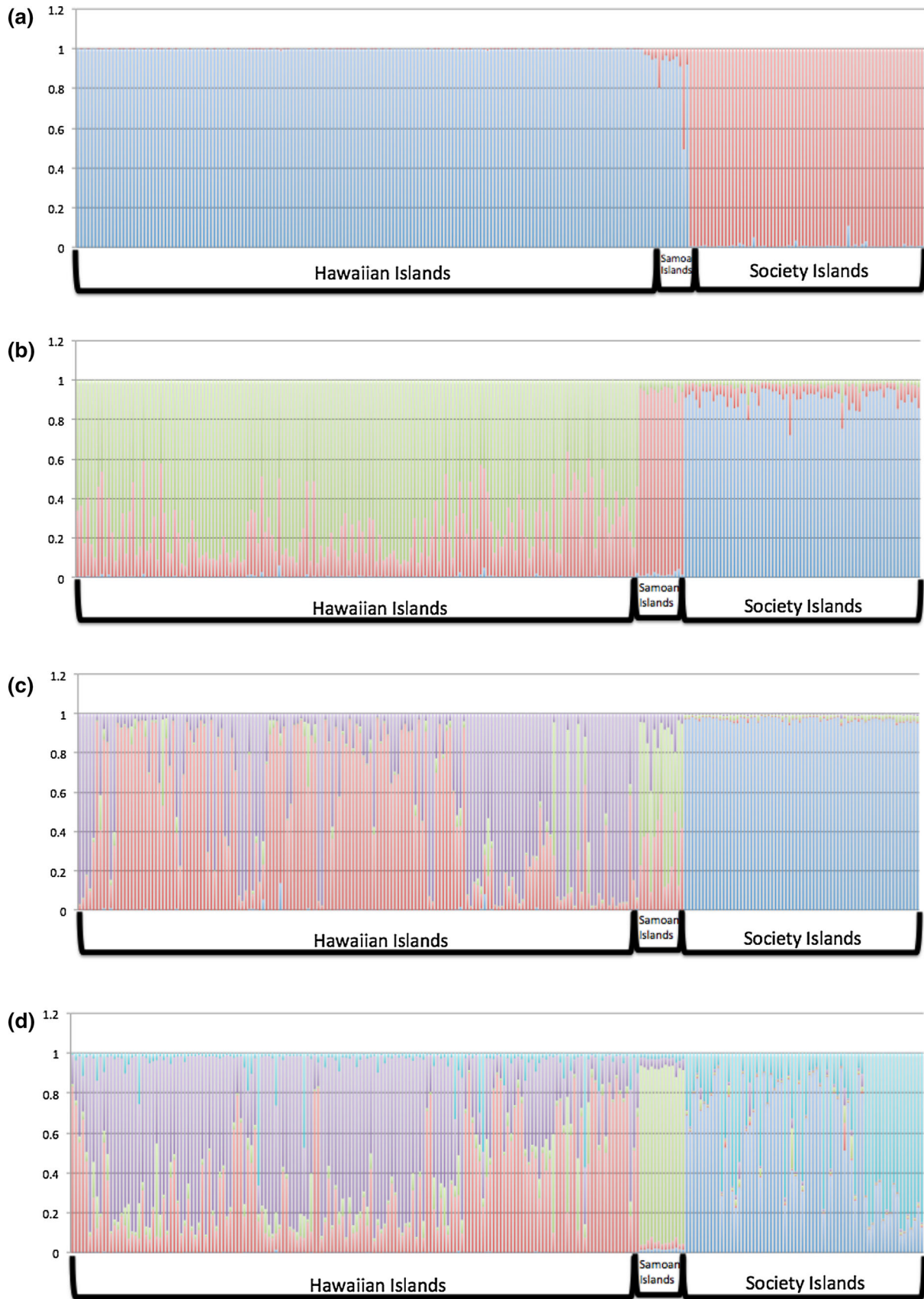


Fig. 3 Graphical representation of the results of the STRUCTURE analysis for the **a** archipelagos where the most probable model was $K = 2$ and a prior was used for sampling location. **b** Hawaiian Island populations including NWHI, O’ahu, Kaua’i, and Hawai’i where the most probable model was $K = 2$ and a prior was used for sampling location. Each vertical bar represents an individual. Bars are shaded as to the proportion of the individual’s ancestry that is attributable to groups one (blue) and two (red) as defined by STRUCTURE. (Color figure online)

(about 190 km). Rough-toothed dolphins have also been observed tens of kilometers offshore from the Society Islands (Nekoba-Dutertre et al. 1999; Gannier 2000; Mannocci et al. 2014). It is possible that the individuals observed were part of a larger, pelagic population as discussed above. However, if that is the case, and these individuals provided occasional gene flow between the Leeward (Rai’atea) and Windward islands (Mo’orea/Tahiti) we would expect that the levels of differentiation between the two islands would be lower and/or would predict a larger number of shared haplotypes and high

levels of genetic diversity. The STRUCTURE plots at $K = 5, 6$ and 7 (Figs. 3d, S3a–b) may reflect nearshore vs offshore and not just differences in the islands, but this cannot be determined. Additional sampling throughout the Society Islands would assist in determining if there are separate offshore populations, or if those dolphins are part of a nomadic population or occasional extended habitat range of the insular population, perhaps due to oceanographic and foraging patterns.

The possibility of offshore populations was also considered for the samples from the Samoan Islands. The Samoan Islands experience the island mass effect (Dandonneau and Charpy 1985), and rough-toothed dolphins have been sighted near shore and 10 km offshore (Johnston et al. 2008) The STRUCTURE analysis identified a few individuals from the Samoan Islands as ‘Hawaiian’. The field notes showed these individuals were sampled further offshore as part of a large vessel survey. Again, this could be a result of differences in nearshore versus offshore dolphins around Samoa, evidence of long distance dispersal or limitations of the STRUCTURE program.

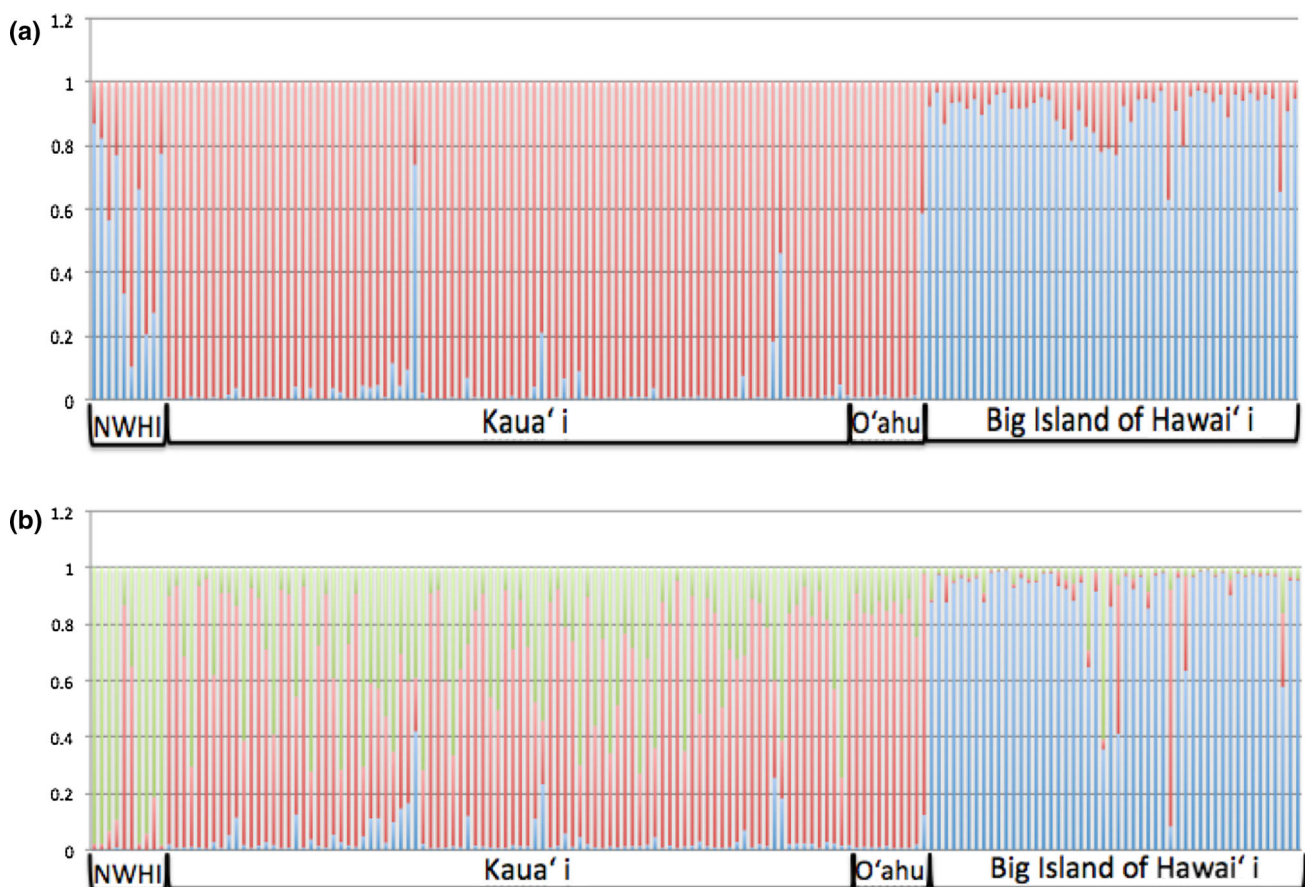


Fig. 4 *Steno bredanensis*. Hawaiian Island populations including NWHI, O’ahu, Kaua’i, and Hawai’i where the most probable model was $K = 2$ **a** and a prior was used for sampling location. $K = 3$ populations **b** is also shown. Each vertical bar represents an

individual. Bars are shaded as to the proportion of the individual’s ancestry that is attributable to groups one (blue) and two (red) as defined by STRUCTURE. (Color figure online)

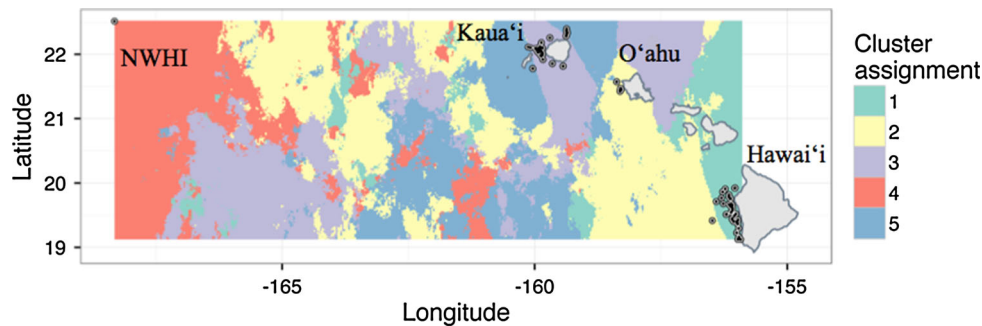


Fig. 5 *Steno bredanensis*. Map produced by *GENELAND* showing individual assignments of rough-toothed dolphins in the Hawaiian Islands of clusters for $K = 5$ where “y coordinates” are shown as latitude and “x coordinates” are shown as longitude. The black dots represent the four sampling areas, top left is NWHI, followed by

Kaua'i, O'ahu, and Hawai'i in the lower right corner. The black dots represent sampling locations. The assignment of pixels to the sixth cluster is not shown, as no individuals are assigned (“ghost cluster”, see text for further details). (Color figure online)

Gene flow throughout the Central Pacific

The significant genetic differentiation between islands within archipelagos we observed is similar to other dolphin populations that are considered isolated by insular habitat preferences (Oremus et al. 2007; Andrews et al. 2010; Martien et al. 2012; Courbis et al. 2014). However, our results suggest that the genetic differentiation of rough-toothed dolphins is most likely evidence of genetic drift, and not all islands showed a clear distinction. We found no evidence of isolation by distance, with F_{ST} values between archipelagos similar to values within archipelagos. Although the genetic data from our study and photo-ID data from previous studies in these archipelagos (Baird et al. 2008; Johnston et al. 2008; Oremus et al. 2012) support the existence of independent populations around Hawai'i, Mo'orea, Rai'atea, and the Samoan Islands, these populations could still experience low levels of gene flow from conspecifics throughout the Pacific Ocean.

The pattern we observed in the haplotype network among the archipelagos in this study of rough-toothed dolphins is consistent with the haplotype network observed in a large-scale study of population structure for the common bottlenose dolphin, *Tursiops truncatus*, in the Central Pacific. The bottlenose dolphin populations were connected by low levels of gene flow across large distances, either through occasional long-distance dispersal or gene flow with pelagic populations (Tezanos-Pinto et al. 2008). The shared haplotypes in our study among all three archipelagos ($n = 1$) and between ocean basins ($n = 4$) indicate a genetic connection between the Northern and Southern hemispheres. Moreover, the haplotypes that are found in both hemispheres were also found in the western and eastern Pacific (Oremus et al. 2012). However, it is not clear if this represents current or historic gene flow. Unlike false killer whales that showed strong phylogeographic structuring of haplotypes within the North Pacific (Martien

et al. 2014) there is no clear phylogeographic structuring of rough-toothed dolphins throughout the central Pacific. As suggested by Andrews et al. (2010) for spinner dolphins, such shared haplotypes may be remnant haplotypes from an ancestral population that colonized all three archipelagos, or they may have originated after colonization from a pelagic population that has occasional gene flow with the insular populations. Similarly, the large number of undetected intermediate haplotypes shown in the network between Kaua'i and Hawai'i could be the result of unsampled insular areas (i.e. the 4-island area or the windward side of Hawai'i). They could also be the result of “offshore” pelagic populations that have occasional influxes of gene flow into the insular populations or the result of an occasional long distance migration from another insular population. “Offshore” sightings of rough-toothed dolphin have been documented in aerial and ship surveys in French Polynesia, the Hawaiian Islands and other areas of the Central Pacific (Wade and Gerrodette 1993; Lambert et al. 2014), but it is not clear if these dolphins are a part of truly pelagic populations. Sampling from these “pelagic” populations is a priority for understanding the origins of genetic diversity in the insular populations of this species.

Social organization and foraging differences

Prey specialization and social organization would presumably enhance isolation by colonization through cultural adaptation supported by fidelity to social groups. Socially-defined population structure has the general effect of increasing the importance of genetic drift relative to other evolutionary forces (Storz 1999). However, our study and that of Oremus et al. (2012) found that the sampling of close kin in the same group was not a common occurrence, although it did occur. An association network constructed using photo-ID found four instances of rough-toothed dolphin pairs that were seen together in over half of the

encounters where they were identified (Baird unpublished) and Oremus et al. (2012) identified individuals sighted in the same group in two encounters. Differences in specializations such as foraging strategies and habitat use have been suggested as causes for genetic differentiation of populations observed in coastal bottlenose dolphins (Moller 2012), killer whales *Orcinus orca* (Hoelzel et al. 2007; Pilot et al. 2010), and pelagic dolphins such as false killer whales (Martien et al. 2014) and striped dolphins *Stenella coeruleoalba* (Gaspari et al. 2007). Rough-toothed dolphins exhibit characteristics such as care giving and cooperative foraging common in other delphinids with a highly organized social structure and cultural specialization (Lodi 1992; Pitman and Stinchcomb 2002; Fulgencio de Moura et al. 2009). To date, however, there is no evidence available to assess whether there are differences in foraging specialization at any of the islands. Further studies on foraging, diving patterns and habitat preferences would help determine the influence of social organization in structuring populations of rough-toothed dolphins.

Conservation implications

Our comparative study of rough-toothed dolphins in the central Pacific provides evidence of isolation between some insular populations in two of the three archipelagos. Three of the four island-associated populations identified in our study (i.e. Hawai'i, Mo'orea, and Rai'atea) are thought to have low abundance. The evidence for these small island-associated populations in an otherwise pelagic species has important conservation implications due to concentrated human impacts near shore. Fishing depredation by rough-toothed dolphins is increasing in the main Hawaiian Islands and the Society Islands (Poole 1993; Baird et al. 2008). Around Hawai'i, rough-toothed dolphins are known to steal fish off lines in local fisheries and there are anecdotal reports that fishermen have shot dolphins as a method of deterrent (Kuljis 1983; Nitta and Henderson 1993). Reports from local fishermen at Mo'orea indicate negative interactions are increasing between rough-toothed dolphins and local fisheries. There are unconfirmed reports that some fishermen have resorted to shooting dolphins, although most fishermen either accept the loss of bait and continue to fish in the same area, or they move to some other area to fish (Poole unpublished). In the Samoan archipelago there were two bycatch incidences (2011 and 2012), and evidence of entanglement from a beach cast animal in 2010 (J. Ward pers. comm). In another incident near Asau, Samoa, a Fisheries Division representative retrieved a longline near two groups of rough-toothed dolphins and found a number of hooks were clean and without bait or catch attached. It was suggested that the dolphins had taken the bait, and have been reported to often

approach vessels near longlines (Walsh and Paton 2003). Moreover, rough-toothed dolphins have been observed removing bait from hooks of longline fisheries operating out of Upolo (Johnston et al. 2008). These examples provide evidence that insular rough-toothed dolphins are likely to have more interactions with boats, and therefore more vulnerable to anthropogenic impacts than their pelagic conspecifics.

The significant genetic differentiation we found among most of our strata at both nuclear and mitochondrial loci supports their designation as separate management units under Moritz's (1994) criteria. However, the populations of Hawai'i, Mo'orea, Rai'atea and the Samoan Islands where we found significant differentiation fall under the delineation of separate management units (Moritz 1994). There is a growing consensus that the delineation of separate management units requires evaluation relative to the critical level of dispersal and differentiation necessary to meet the conservation objectives those units are intended to meet (Taylor and Dizon 1999; Palsboll et al. 2007). Management units under the US Marine Mammal Protection Act (MMPA) are called stocks, which are defined as demographically independent populations whose internal dynamics are more heavily influenced by births and deaths rather than immigration and emigration (Wade and Angliss 1997). Taylor (1997) used simulations to determine that, for dolphin species, the threshold dispersal rate below which two groups must be managed separately in order to meet the management objectives of the MMPA is several percent per year, with the exact value depending on the specifics of the case.

Martien et al. (2012) used Wright's (1965) formula for the relationship between F_{ST} , effective population size, and migration rate to calculate the magnitude of differentiation that would be expected between insular populations of common bottlenose dolphins that would qualify as stocks under the MMPA. They assumed a dispersal rate of 1 % per year dispersal, resulting in a per-generation dispersal rate (m) of 0.21 based on the 21 year generation time for common bottlenose dolphins from Taylor et al. (2007), and used published life history and abundance values to calculate effective population size. Courbis et al. (2014) performed a similar calculation for pantropical spotted dolphins (*Stenella attenuata*). For bottlenose dolphins, with a dispersal rate of 1 % per year, the critical values of F_{ST} above which populations should be managed as separate stocks under the MMPA were 0.028 for mtDNA and 0.007 for nuDNA, while the corresponding values for pantropical spotted dolphins were 0.006–0.009 and 0.002, respectively. Martien et al. (2012) and Courbis et al. (2014) were able to make their calculations because all of the populations they considered were of roughly equal size. However, the estimated abundance of the Kaua'i population in our study is

an order of magnitude larger than the other populations. Therefore, we cannot repeat their calculations for rough-toothed dolphins. Nonetheless, the life history parameters are similar between the three species, and the range of estimated abundances for the populations in our study is encompassed within the range of abundances from the bottlenose and pantropical spotted dolphin studies. Therefore, the expected magnitudes of differentiation calculated by Martien et al. and Courbis et al. are useful in interpreting the management significance of the differentiation we detected among rough-toothed dolphin populations. All of our F_{ST} values from statistically significant comparisons were much larger (in most cases an order of magnitude larger) than the critical values calculated for bottlenose and pantropical spotted dolphins, indicating that the Kaua'i, Hawai'i, Mo'orea, Rai'atea and the Samoan Islands populations of rough-toothed dolphins each fit the definition of a stock under the MMPA.

The situation with O'ahu and NWHI is less clear, with their estimated differentiation relative to each other and to Kaua'i falling above the threshold for nuDNA, but straddling the thresholds for common and pantropical spotted dolphins for mtDNA. With such a small sample size in O'ahu and NWHI, we currently do not have enough power to determine whether these islands warrant management units or should be combined with the Kaua'i population for management purposes. Moreover, in our study we have the influence of possible pelagic populations, and estimating the degree of mixing with pelagic populations remains elusive due to the challenges associated with sampling pelagic dolphins. With the current evidence from this study, and the evidence provided in Oremus et al. (2012), Baird et al. (2008) and Johnston et al. (2008), we recommend that Hawai'i, Mo'orea, Rai'atea and Samoan Islands be considered separate management units for the purposes addressing current population structure and short-term management issues.

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