Mitogenome and nuclear DNA differentiation in spinner (*Stenella longirostris*) and pantropical spotted dolphins (*S. attenuata*) from the eastern tropical Pacific Ocean

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Abstract:

Endemic subspecies and multiple stocks have been proposed for spinner (*Stenella longirostris*) and spotted dolphins (*Stenella attenuata*), two species with historically high mortality due to bycatch in the eastern tropical Pacific yellowfin tuna fishery. However, there has been low statistical power in tests for genetic differentiation among most of these stocks, due to large historical abundances, ongoing gene flow, and recent divergence. We tested for structure at multiple hierarchical levels by collecting whole mitochondrial genome sequences (mtDNA) and nuclear SNPs (nuDNA) from 104 spinner and 76 spotted dolphins using capture array library enrichment and highly paralleled DNA sequencing. MtDNA showed weak but significant differences between subspecies of spotted (F_{ST}: 0.0125; P = 0.0402) and spinner dolphins (F_{ST}: 0.0133; P = 0.034). NuDNA supported subspecies of spotted but not spinner dolphins. Relatively strong and significant differentiation was detected between whitebelly and eastern spinner stocks using nuDNA (F_{ST}: 0.0297; P = 0.0059). Neither mtDNA nor nuDNA supported the division of existing offshore stocks of spotted dolphins or Tres Marias spinner dolphins. This work identifies a genetic basis for biologically meaningful management units of these two species, a critical component in understanding their response in the face of historical and continued fishery interactions.

41 **Introduction:**

42

43 The study of population structure in pelagic organisms is inherently challenging. Their
44 open ocean habitats have few permanent physical barriers to gene flow, thus
45 demographic processes (*e.g.*, range expansion and metapopulation dynamics; Horne
46 2014) may be more important in structuring populations than physical separation (Norris
47 2000). In genetic terms, the dynamic nature of this environment increases the likelihood
48 of mixing between populations; even a few successful migrants per generation greatly
49 decrease the signal of population segregation (Waples 1998).

50

51 Moreover, pelagic populations often have large abundances, which can result in a large
52 amount of standing genetic variation (Norris 2000). High genetic diversity can
53 dramatically increase the time needed for populations to drift apart genetically, even in
54 the complete absence of gene flow (Taylor and Dizon 1996).

55

56 With large abundance and weak barriers, pelagic species provide challenges for using
57 genetic tools to determine population structure (Taylor and Dizon 1996, Waples 1998).
58 Spinner and spotted dolphins in the eastern tropical Pacific Ocean (ETP) typify this
59 problem and present an excellent opportunity to describe population genetic
60 differentiation at multiple levels (stocks, subspecies, global populations).

61

62 These two species historically numbered several million (Wade *et al.* 2007), but starting
63 in the 1960s, hundreds of thousands were killed annually as bycatch in the dolphin-set
64 tuna purse-seine fishery (Lo and Smith 1986, National Research Council 1992, Wade
65 1995) because both species commonly associate with one-another and with large tuna
66 (see Scott *et al.* 2012 for details). Despite over forty years of protection under the U.S.

Marine Mammal Protection Act of 1972 and specific multi-national protection under the 1999 Agreement on the International Dolphin Conservation Program (Joseph 1994, Gosliner 1999), ETP spinner and spotted dolphin populations have not recovered as expected (Wade *et al.* 2007, Gerrodette *et al.* 2008). Previous genetic studies have shown that these species retain a large amount of genetic variation (Dizon *et al.* 1994, Galver 2002, Escorza-Treviño *et al.* 2005,Andrews *et al.* 2013), which possibly obscures the detection of population genetic structure that could be used to improve recovery efforts. These species exhibit intraspecific morphometric differences supporting structured populations (Perrin *et al.* 1991, 1994). Some molecular genetics approaches have not found corresponding population genetic structure (Dizon *et al.* 1994, Galver 2002), although Andrews *et al.* (2013) found evidence for segregation in ETP spinner dolphins based on data from the Y-chromosome and Escorza-Treviño *et al.* (2005) found population structure within the coastal subspecies of spotted dolphins based on microsatellites.

Endemic spinner dolphin subspecies and stocks

There are four recognized subspecies of spinner dolphin (*Stenella longirostris*) globally. The nominate form, the pantropical or "Gray's" spinner (*S. l. longirostris*) occurs throughout the tropical waters of the world. In the central and western Pacific, Gray's spinners are found associated with islands, such as the Hawai‘ian Islands. The much smaller dwarf spinner (*S. l. roseiventris*) subspecies inhabits shallow waters of Southeast Asia (Perrin *et al.* 1989, 1999). The other two subspecies, Central American spinner dolphin (*S. l. centroamericana*) and the eastern spinner dolphin (*Stenella longirostris orientalis*) are found only in the ETP (Fig. 1, based on Perrin 1985), and were described based on differences in external coloration, body size, and skull morphology (Perrin *et al.*

1991, Douglas *et al.* 1992).

For management purposes, ETP spinner dolphin stocks correspond to the two aforementioned endemic subspecies, plus the whitebelly spinners. The Central American subspecies inhabits relatively near-shore waters off the Pacific coasts of Southern Mexico south through Panama

The eastern spinner dolphin (*S. l. orientalis*) exhibits traits indicative of a polygynous mating system (Perrin and Henderson 1979, Perrin and Mesnick 2003). Perrin and Mesnick (2003) found significant difference in testes size between the eastern subspecies and the whitebelly form, indicating differing reproductive strategies and probably different breeding behavior between the two types. Andrews *et al.* (2013) estimated high levels of gene flow between subspecies in the ETP using autosomal and mitochondrial genes and found a shared Y chromosome haplotype in the eastern and Central American subspecies that was not found in Gray's or dwarf subspecies. Interestingly, this locus was found to be polymorphic in whitebellies, supporting the hypothesis of introgression in this form (Andrews *et al.* 2013). The authors proposed that sexual selection was driving the divergence of spinner dolphins in the ETP. Finally, a distinct morphotype of the eastern spinner dolphin, known as the "Tres Marias" spinner dolphin, has been described from near the islands of the same name off the coast of Mexico. These were thought to be a distinct type based on external body morphometrics (Perryman and Westlake 1998).

The "whitebelly" spinner is proposed to represent a hybrid swarm between the eastern subspecies and the pantropical Gray's subspecies of the central and western Pacific (Perrin *et al.* 1991). Taxonomically, it is classified as part of the nominate (Gray’s) subspecies *S. l. longirostris*. Significant geographic overlap exists between the eastern

subspecies and the whitebelly form (Perrin *et al.* 1985) (See Fig. 1).

Endemic ETP spotted dolphin subspecies and stocks

Extensive analyses of cranial morphology of pantropical spotted dolphins (*Stenella attenuata*) in the ETP led to the designation of a coastal endemic subspecies (*S. a. graffmani* - Perrin 1975, Perrin *et al.* 1987). Pantropical spotted dolphins in offshore regions in the ETP and elsewhere retain the nominate trinomial, *S. a. attenuata*. In contrast to the spinner dolphins, genetic analyses of microsatellites indicated some differentiation between subspecies (Escorza-Treviño *et al.* 2005). This study identified at least four demographically independent populations within the coastal subspecies (*S. a. graffmani*) and differences between southern populations of the coastal subspecies and the pelagic subspecies. However, they found no differences between the northern populations of the coastal subspecies and the pelagic subspecies. The authors concluded that genetic diversity in the coastal subspecies is contained within demographically independent populations and that interchange is ongoing between northern populations and the pelagic subspecies. Tests for population genetic structure within the pelagic subspecies have not been conducted.

Despite the results of Escorza-Treviño *et al.* (2005) the entire coastal subspecies is currently treated as a single management stock. The offshore pantropical spotted dolphins are divided into a 'northeastern' (NE) stock, defined geographically as north of 5°N, east of 120°W and a 'western-southern' (WS) stock residing south and west of this northeastern area (Fig. 2) (Perrin *et al.* 1994). The north-south boundary between these stocks is based on a distributional hiatus around 5°N. General similarity in gross external

and cranial morphology supports a unified WS stock (see Perrin *et al.* 1994 for discussion and primary references), but genetic information has never been collected and compared for these two areas (western offshore and southern offshore).

Objectives

Spinner and spotted dolphins offer a unique opportunity to test for genetic differentiation at multiple taxonomic levels in two species with large population sizes, documented morphological differentiation, and access to tissue specimens collected *in situ* from remote pelagic regions. In this study, we apply population genetic structure analyses of whole mitochondrial genomes (mitogenomes), individual gene regions within the mitogenomes, and nuclear single nucleotide polymorphisms (SNPs) to test subspecies hypotheses and levels of differentiation between recognized and proposed management stocks, including the Tres Marias spinner dolphin and alternative stock boundaries in the offshore spotted dolphins. Testing the same hypotheses of genetic structure using new methods may seem redundant, but it is our hypothesis that previous genetic studies have lacked statistical power to detect structure. To overcome the limitations of using few genetic markers in highly abundant and genetically variable populations, we have expanded the genetic analyses to include mitochondrial genomes and sets of 51 (spinner) and 36 (spotted) nuclear SNP loci. Although still only representing a single maternally-inherited marker, our mitogenome data adds additional information to help differentiate populations based on molecular divergence (as calculated using Φ_{ST}) beyond that of the two mtDNA genes studied by Andrews *et al.* (2013) and the single mtDNA gene of Escorza-Treviño *et al.* (2005). Morin *et al.* (2012) found that a data set of 42 SNPs had similar statistical power for population genetics to that of approximately 22 microsatellite loci. Using this ratio, our 51 nuclear SNPs for spinner dolphins resulted in a data set

comparable to roughly 27 microsatellites. Therefore, if statistical power scales linearly with the number of loci, our data are expected to be over twice as powerful as the previous microsatellite data sets for spinner dolphins (12 loci; Galver *et al.* 2002). The 36 SNP data set for spotted dolphins that resulted from this work is expected to be two to three times more powerful than the previous nuDNA data set (7 microsatellite loci; Escorza-Treviño *et al.* 2005).

METHODS

Sample Collection and DNA extraction

Skin samples were collected from 104 spinner dolphins and 76 spotted dolphins *via* biopsy dart (Lambertsen 1987) on research cruises or from specimens taken as bycatch in the tuna purse-seine fishery between 1982 and 2010 (Fig. 1, 2; Supplementary Material Tables S1, S2). On research cruises it is relatively common to see some fraction of spinner dolphins of alternate morphology (*i.e.*, possibly different subspecies) within a school of spinner dolphins comprised mostly of another morphotype/subspecies. For this reason, spinner dolphin samples collected from research cruises were assigned to a stock based on the external morphology of the majority of animals in the school rather than the morphology of the individual sampled or the geographic location of the school. This approach was taken because: 1) only after observing the group (which could contain > 1,000 individuals) for some time could observers classify it to stock, 2) researchers collecting biopsies from dolphins near the bow of the research vessel found it very difficult to confidently classify fast-swimming individuals at sea, and 3) there is significant overlap in range; therefore, geography was not a reliable predictor of stock identity. Some samples were used from areas where the eastern and whitebelly spinners

197 are known to geographically overlap (see Figure 1). Spinner dolphin samples from
198 Hawai‘i spanned the breadth of the main islands and also Midway Atoll.
199
200 Spotted dolphins were assigned to subspecies and stocks based on the geographic
201 location of the sampling site. To avoid misassigned individuals near the borders of the
202 NE and WS offshore stocks, we did not use samples collected between 4°N and 6°N east
203 of 125°W. Hawai‘ian spotted dolphin samples were collected from the Kona Coast of
204 Hawai‘i and O‘ahu.
205
206 Biopsy samples were stored in salt-saturated 20% DMSO, 70% ethanol, or frozen with no
207 preservative. DNA was extracted using silica-based filter membranes (Qiagen, Valencia,
208 CA) on an automated workstation (Perkin Elmer, Waltham, MA). Starting concentrations
209 of DNA were quantified using Pico-Green fluorescence assays (Quant-it Kit, Invitrogen,
210 Carlsbad, CA) using a Tecan Genios microplate reader (Tecan Group Ltd, Switzerland).
211
212 **Library Preparation and Sequencing**
213 Next-generation sequencing libraries were generated as described by Hancock-Hanser *et*
214 *al.* (2013), using unique 6bp and 7bp index sequences for each individual to allow up to
215 100 samples to be multiplexed. Multiplexed libraries were enriched for whole
216 mitogenomes and 85 nuclear loci using Sure Select DNA Capture Arrays (Agilent
217 Technologies, Inc., Santa Clara, CA, USA) as described by Hancock-Hanser *et al.*
218 (2013). Target sequences for capture enrichment included the reference pantropical
219 spotted dolphin mitochondrial genome (Genbank No. EU557096; Xiong *et al.* 2009) and
220 a suite of 85 nuclear loci (Supplementary Material Table S3). Autosomal and sex-linked
221 conserved anchor tag loci (CATS; Lyons *et al.* 1997: Aitken *et al.* 2004) made up the
222 bulk of the nuclear loci (n=75; Supplementary Table S1 from Hancock-Hanser et al.

2013). In addition, one Y-chromosome locus (SRY Genbank No. AB275398.1) and nine nuclear exons involved in vertebrate coloration were also included (Rieder *et al.* 2001, Hoekstra *et al*. 2006, Eriksson *et al*. 2008, Hubbard *et al*. 2010). Coloration loci and the Y-chromosome locus were aligned to the common bottlenose dolphin (*Tursiops truncatus*) genome (ENSEMBL v. 61) (See Table S3 for locus details) and common bottlenose dolphin sequences (sequences in Supplemental Table S4) were used in the capture array design with the eArray software package (Agilent Technologies, Inc., Santa Clara, CA, USA). Three identical arrays were used to capture a multiplexed mix of both species. Each array contained one replicate of the mitogenome probes at a probe interval of 15bp as well as 13 replicates of probes for the nuclear loci at a probe interval of 3bp. Each enriched library was then sequenced using 1X100bp Illumina HiSeq technology (two using Illumina HiSeq2000 and one using HiSeq2500).

Mitogenome Assembly

Raw read data were filtered for quality (minimum quality score of 15) and demultiplexed by unique barcode. Consensus sequences for each sample were generated from mitogenome sequence reads using a custom pipeline (Dryad data repository doi:10.5061/dryad.cv35b) in R v2.15.0 (R Core Team, 2014). Reads were first mapped to the reference spotted dolphin sequence with the short-read alignment tool BWA (Li and Durbin, 2009). The mpileup module in SAMTOOLS (Li *et al.* 2009) was then used to convert the resulting BAM-format alignment file into a ‘‘pileup’’ text format, which was then parsed by custom R code to create the consensus sequence for each individual. The following rules were used in this process: A “N” was inserted at a position if the assembly had <3 reads, <5 reads where not all contained the same nucleotide, or >5 reads where no one nucleotide (*i.e.*, A, C, G, T) was present in >70% of the reads. All mitogenome sequences were initially aligned with MAFFT using the automatic selection

of an appropriate handling strategy ("auto") and default parameters (Katoh *et al.* 2009) followed by a refinement of alignments by eye.

NuDNA SNP Discovery and Genotyping

Using CLC Genomics Workbench v3.7 (CLC Bio, Aarhus Denmark), all sequence data were first mapped to the mitogenome references and unmapped reads were saved. We then created species-specific *de novo* references for nuDNA loci by combining reads from a subset of five individuals – representing various stocks - with the highest number of the unmapped reads. *De novo* contigs were aligned back to original nuDNA capture sequences prior to mapping reads from all animals to the *de novo* reference. For loci that did not assemble into *de novo* species-specific references, we used the original reference sequence used in the capture array assembly. Additional flanking sequence was often added with the *de novo* references relative to the original capture sequences.

All reads were assembled to the *de novo* nuDNA references with the same initial criteria outlined above for the mtDNA with one exception. Because we assembled reads from multiple individuals we did not employ the rule regarding the insertion of N's at positions with >5 reads where no one nucleotide was present in >70%. Putative SNPs were discovered using MPILEUP and variant calling tools from GATK (McKenna *et al.* 2009). We considered variable sites in nuclear loci to be potential SNPs if the population samples contained heterozygotes and/or homozygotes of the minor allele with at least 7 reads for called genotypes, and the minor allele frequency was ≥5% frequency in the sample set. Putative SNPs were screened for validation by examining BAM alignments by eye. We did not choose a SNP if it had *N*s or other ambiguities in the flanking regions or if the SNP was found in <5 individuals.

Diversity Estimates and Population Structure Analyses

Two mitogenome data sets were created for each species. First, we partitioned each species' data set into one of fifteen loci (12 coding sequences, the control region and 2 rRNA genes). ND6 and tRNA loci were removed prior to analyses because they conform to different evolutionary models and ND6 falls on the opposite strand from the remaining genes (Duchene *et al.* 2011). Sequences were aligned to the pantropical spotted dolphin reference and locus start/stop positions were annotated in GENEIOUS v5.4 (Biomatters Limited) using the GENEIOUS alignment tool and the amino acid translation tool, respectively.

Second, we removed the control region because of high variation in this region and concatenated the remaining 14 regions to make the concatenated mitogenome sequences. The final sequence lengths for the concatenated data were 13,426bp and 13,425bp for spinner and spotted dolphins, respectively. An individual was removed entirely from analyses if it contained >10% missing data across the entire concatenated sequence.

For both data sets, we estimated haplotypic diversity (*h*, Nei 1987) and nucleotide diversity (π, Tajima 1983), and assigned individual genes and whole mitochondrial genome sequences to unique haplotypes using tools from the *strataG* package in R (v. 2.3.1; Archer *et al.*2016). Two pairwise estimates of population genetic structure, F_{ST} (Wright 1949), Φ_{ST} (Excoffier *et al.* 1992), were also performed using the *strataG* package. The significance of each estimate was tested using 5000 non-parametric random permutations of the data matrix variables. For Φ_{ST}, pairwise distances were calculated using the best substitution model as identified by Akaike's Information Criterion in JModelTest version 2.1.4 (Posada 2008). Models were determined for individual gene regions and the entire concatenated dataset.

We performed a substitution rate test on each species' mitogenome data set to determine if mutations had reached a point of saturation. For this test, we generated pairwise percent differentiation and plotted this against a Jukes and Cantor (1969) correction factor generated using MEGA 5.2.2 (Tamura *et al.* 2011). We chose this model because of its simplicity; if deviations were seen here then general saturation could be assumed.

Although mitochondrial loci are assumed to be under purifying selection (Stewart *et al.* 2008) we, nonetheless, tested spinner dolphin mitochondrial genes for evidence of positive selection using both Tajima's *D* and Codon-based *Z*-Test as implemented in MEGA 5.2.2 (Tamura *et al.* 2011).

Nuclear Population Structure Analyses

Individuals with >50% of genotypes present and loci with >30% of genotypes present in the total data set were analyzed for population structure. Only the first SNP was chosen from each region sequenced to minimize issues with linkage and phasing. We then calculated pairwise F_{ST} differentiation statistics (Wright 1949) using the *strataG* package in R (Archer *et al.* 2016). Meirmans and Hedrick (2011) show that the classic F_{ST} is appropriate for biallelic SNP data, and do not recommend calculating standardized measures.

To test if SNPs associated with Y-chromosome or coloration genes contributed disproportionately to the overall patterns of population structure found using our spinner dolphin SNP data set, we conducted simulation-based F_{ST} outlier tests for selection at each locus in the selection-detection workbench *Lositan* (Antao *et al.* 2008). Using all SNPs and partitioning the samples into three putative populations (whitebelly, eastern

and Central American) we used *Lositan* to run 75,000 FDIST2 simulations (Beaumont and Nichols 1996), first calculating neutral F_{ST} (*i.e.*, removing all putative F_{ST} outliers) and then approximating a mean overall F_{ST} using bisection approximation algorithms (Antao *et al.* 2008). We chose an infinite alleles model for all simulations. All population differentiation statistics were calculated again with F_{ST} outliers removed.

Bayesian Clustering

A Bayesian clustering method implemented in STRUCTURE v 2.3.4 (Pritchard *et al.* 2000, Hubisz *et al.* 2009) was used to identify the number of populations (*K*) represented in the SNP data sets. Prior information on the origin of the samples (subspecies and geographically defined groups within subspecies) was combined with a correlated allele frequency model and an admixture model for these analyses. Data were also analyzed without location priors using the same models. We evaluated values of *K* between 1 and 5. For each assumed value of *K*, 20 independent runs were conducted. Total length of the run was set at 1,000,000 and burn-in was set at 100,000. The most likely estimate of *K* was determined by the maximum estimated mean log-likelihood of the data (*ln*P(D)) (Pritchard *et al.* 2000) and by calculating ΔK, the second-order rate of change of *ln*P(D) with respect to the *K* (Evanno *et al.* 2005).

RESULTS:

Hancock-Hanser *et al.* (2013) present information on the success rate of the DNA capture method including summary statistics of the data analyzed in this paper. As it relates to our analyses, questions might arise about how using arrays designed from closely-related species affected our results. As presented in Tables 4 and 5 of Hancock-Hanser *et al.*

(2013), spinner dolphin samples had slightly higher number of mtDNA reads per individual than spotted dolphin samples, despite use of the spotted dolphin mitogenome as the capture bait. The same pattern was found for the nuDNA capture – spinner dolphins had more reads per individual than spotted dolphins - despite all the baits being common bottlenose DNA sequence. We interpreted this consistency as an indication that inter-specific capture worked well and that any decrease in capture success (as evidenced in reads per individual for a given species) was more likely due to a combination of other factors (sample quality, multiplexing rate, sequencing technology, and/or variation in library preparation) rather than reduced capture due to inter-specific baits. The one area that might have been an issue for inter-specific capture was the hyper-variable section of the control region (see below).

Spinner dolphins

Mitogenomes

We assembled 104 complete or nearly complete (<10% missing data) concatenated spinner dolphin mtDNA data sets. The hyper-variable section of the control region had consistently lower coverage in many individuals and was removed from the concatenated data set (Supplementary Table S1). Subspecies and regional sample sizes, summary statistics and genetic diversity measures are listed in Table 1. At the subspecies level, haplotypic diversities were high and nucleotide diversity was low (>0.9722, <0.0073, respectively). The substitution rate test did not show any signs of saturation. The best nucleotide substitution model estimated by JModelTest (Posada, 2008) was JC69 (Jukes and Cantor 1969) for all individual gene regions and the entire concatenated data set. The results of F_{ST} and Φ_{ST} analyses of the mtDNA concatenated genes and Φ_{ST} of the

individual gene regions for spinner dolphins are shown in Table 2. Due to space limitations, we only discuss Φ_{ST} for the partitioned gene region analyses.

At the subspecies level, the Φ_{ST} test showed no differentiation between Central American spinners and eastern spinner dolphin subspecies in either the concatenated or partitioned data sets. F_{ST} was significant in the concatenated data set (0.0133, $P = 0.034$). Φ_{ST} comparisons of the whitebelly form and coastal Central American subspecies showed nearly significant differentiation in the concatenated data set ($\Phi_{ST} = 0.0490$; $P = 0.0542$) and seven individual gene regions. ND3 showed a significant difference at $P = 0.0054$, while all other significant comparisons between these strata were at $P < 0.05$ (Table 2).

We found no significant differences between the whitebelly and the eastern subspecies using the concatenated mitogenome data ($\Phi_{ST} = 0.0181$; $P = 0.0741$). However, eight individual mitochondrial genes showed significant differentiation. All individual gene partitions in spinner dolphins were found to be under purifying selection using Tajima's *D* tests for selection (Table S5) and Z-Test for positive selection using the Nei-Gojobori method (Nei and Gojobori 1986) (Table S6).

Φ_{ST} tests showed no differentiation between Tres Marias spinners and either ETP spinner dolphin subspecies in either the concatenated or partitioned data sets. Four individual gene regions were significantly different in the pairwise comparisons of Tres Marias and whitebelly spinner dolphins ($P < 0.05$; ND3 at $P < 0.01$). All tests involving comparisons with Hawai‘ian spinner dolphins (*S. l. longirostris*) - using the concatenated data set - were highly significant.

Four genes showed population structure (significant Φ_{ST}) in all pairwise comparisons

between Hawai‘i and ETP groups (*i.e.*, Central America, Tres Marias, eastern, and whitebelly spinner), but not in any pairwise comparisons between these ETP groups: 16S, ATP6, ND2, and ND5. Because of the low abundance and geographic isolation of the Hawai‘ian population, we presume these genetic differences between Hawai‘i and the ETP groups resulted from drift. 16S had 24 haplotypes total, but only 4 haplotypes among all 15 Hawai‘ian samples. ATP6 had many more haplotypes in total (53), but again reduced diversity in Hawai‘i (5). One of these Hawai‘ian haplotypes was common among all ETP groups, and two were exclusive to Hawai‘i. The final two Hawai‘ian ATP6 haplotypes were shared with one ETP spinner dolphin each. ND2 also had 53 haplotypes total, but only 4 spread among the 15 Hawai‘ian samples. Twelve samples from Hawai‘i had two haplotypes that were not shared with ETP populations. One individual shared a haplotype with an eastern spinner dolphin, the other two haplotypes were single samples unique to Hawai‘i. Finally, ND5 had 70 total haplotypes, but only 5 among the Hawai‘ian samples – none of which were shared with ETP populations.

Nuclear SNPs

Compared to the mitogenome NGS data, coverage for nuclear genes was low and highly variable. This was likely due to variation in sample quality. Samples were collected as far back as 30 years ago and as recently as ten years ago. Older fisheries-collected samples were stored in saturated salt and DMSO solution for many years at room temperature. In spinner dolphins, 51 SNP loci were compared across 56 samples. These data included SNPs from six coloration genes, one Y-chromosome marker (UBE1) and 44 CATS loci. F_{ST} outlier analysis indicated that four were under positive selection and five were under balancing selection (Supplemental Material Fig. S1). Once these were removed the

neutral data set included 42 SNPs: five coloration SNPs, one Y-chromosome and 36 CATS loci (See Supplemental Material Table S7 for details).

We had sufficient genotypes at the 51 SNPs for pairwise comparisons involving the ETP subspecies and the whitebelly spinner, but not the putative Tres Marias stock. Divergence metrics based on genotypes from all loci - and all neutral loci - are shown in Table 3. Comparing Central American and both whitebelly and eastern spinners, F_{ST} showed no significant differentiation. However, between eastern and whitebelly spinner dolphins, F_{ST} was significantly different ($p < 0.01$).

Analyses of Bayesian clustering in STRUCTURE estimated the most likely number of unique clusters for spinner dolphins was K=1 based on *Ln*P(K). Using the metric ΔK (Evanno *et al.* 2005), K=2 was the most likely number of clusters estimated (see Supplemental Material Fig. S2A); however, ΔK cannot evaluate K=1, so K=2 is the smallest value ΔK can estimate. Moreover, there is no obvious biological pattern to correspond to K=2 as every individual was assigned nearly 50:50 to each of the 2 clusters (Supplemental Material Fig. S2B and C). These estimates were consistent regardless of whether location priors were used.

Four loci were estimated to be subject to positive selection in spinner dolphins: beta-carotene oxygenase 1 (BCDO), glucose transporter member 2-like gene (GLUT2), myeloperoxidase-like genes (MPO), and Wilm's tumor 1-like gene (WT1) (Supplemental Material Fig. S1). GLUT2 codes for cellular membrane transporters. MPO codes for a common enzyme in blood, and WT1 is involved in the development of the urogenital system in humans. In addition, five SNPs within the following loci were estimated to be under balancing selection: COL10A, GLB79, LAPTM4A, NPPA, RHO.

455

456 **Spotted dolphins**

457

458 **Mitogenomes**

459 We assembled 76 complete or nearly complete (<10% missing data) spotted dolphin
460 mitogenomes. Sample sizes, summary statistics and genetic diversity measures are listed
461 in Table 1 (Supplementary Table S2 will include GenBank accession numbers). At the
462 level of subspecies, nucleotide diversity was higher in spotted dolphins (>0.0162) than
463 spinner dolphins. Haplotypic diversity (*h***)** is high in both species (>0.9529), but ETP
464 spotted dolphins subspecies have slightly lower levels (0.9529 and 0.9804 for the coastal
465 and offshore groups, respectively) than spinner dolphin subspecies (0.9722 and 0.9985)
466 in this region. The coastal ETP subspecies for both spinner and pantropical spotted
467 dolphins in the ETP show reduced *h* compared to their offshore ETP counterparts (Table
468 1). Similar to the spinner dolphin mitogenome data, the substitution rate test did not
469 detect any signs of saturation, and JModelTest (Posada, 2008) was JC69 (Jukes and
470 Cantor 1969) as the best substitution model for all individual gene regions and the entire
471 concatenated data set.

472

473 Results of F_{ST} and Φ_{ST} analyses of the mtDNA concatenated genes and Φ_{ST} of the
474 individual gene regions for spotted dolphins are presented in Table 4. Similar to the
475 spinner dolphins, our analyses at the subspecies level for spotted dolphins (coastal *vs.*
476 offshore) show no significant differentiation using Φ_{ST} for the concatenated or partitioned
477 data sets. F_{ST} was significant in the concatenated data set (0.0125, $P = 0.0402$).

478

479 Estimates of differentiation between the current management stocks within the offshore
480 subspecies (NE and WS stocks) using the whole mitogenome data and individual mtDNA

481 genes showed no differences. Using Φ_{ST}, no significant differences were observed
482 between the coastal subspecies and the NE offshore stock, however F_{ST} (0.0302) was
483 highly significant at $P = 0.0002$] between theses management units. Similarly, Φ_{ST} was
484 not significant for pairwise comparisons of the Coastal subspecies and WS offshore stock
485 using the concatenated data or individual genes.
486
487 Within the WS offshore stock, we found nearly significant differences between the
488 southern and western offshore regions using the concatenated mitogenome using Φ_{ST}
489 estimates ($\Phi_{ST} = 0.1666$; $P = 0.0668$). One individual mtDNA gene (ND4) had
490 significant differentiation ($p < 0.05$) and three others had nearly significant p-values (16S,
491 ND1, ND5).
492
493 Comparing separate western and southern portions of the WS stock to other partitions
494 using the mitogenome data set also yielded no significant Φ_{ST} estimates. Our comparison
495 of the NE stock to the western portion of the WS stock, however, was nearly significant
496 using the concatenated mitogenome ($\Phi_{ST} = 0.1135$; $P = 0.0517$) and four individual
497 mtDNA genes showed significant Φ_{ST} differences ($p<0.05$). Neither data set showed
498 significant differences between the NE stock and the southern portion of the WS stock for
499 either statistic.
500
501 Comparison of the coastal subspecies to just the southern portion of the WS stock
502 resulted in no significant F_{ST} or Φ_{ST} difference in the concatenated data set or individual
503 gene regions. Between the coastal subspecies and western offshore portion of the WS
504 stock, however, one individual gene region (ATP8) showed significant differentiation (P
505 < 0.05), and one (12S) showed nearly significant differentiation ($P = 0.0559$). Ideally we
506 would have partitioning the coastal subspecies south of central Mexico into the

population units described by Escorza-Triveño *et al.* (2005), but our smaller sample size prevented us from doing this.

Significant differentiation was detected between Hawai'i and the coastal subspecies, and between Hawai'i and offshore spotted dolphins, in F_{ST} and Φ_{ST} of the concatenated data set. As expected, given this result, significant differentiation was detected in many individual mtDNA genes (see Table 4). We also detected significant differences between Hawai'i and the NE stock in four genes, but not for the concatenated mtDNA data set (although it was nearly significant for Φ_{ST} at $P = 0.0645$). Hawai'i and the WS stock were significantly different in the concatentated data set using Φ_{ST}, and in nine individual genes ($P < 0.05$).

Finally, we also tested hypotheses of differences between Hawai'i and divided western and southern portions of the WS stock. Hawai'i and the western portion were differentiated using the concatenated dataset (Φ_{ST}: 0.4932; $P = 0.0645$). Ten individual genes showed differentiation between these two strata (see Table 4). Hawai'I and the southern portion of the WS stock were not differentiated based on our concatenated data sets, but did show significant differentiation in five individual genes ($P < 0.05$).

Nuclear SNPs

Similar to the spinner dolphin samples, coverage for nuclear genes was low and highly variable. For spotted dolphins, 25 samples had sufficient coverage at 36 loci to call SNP genotypes. These data enabled us to conduct pairwise comparisons between the subspecies, but insufficient samples with genotypes prevented us from conducting comparisons between the two stocks of the offshore subspecies. The 36 loci included four coloration genes and 32 CATS loci. F_{ST} outlier analysis estimated that six SNPs were

533 under positive selection and five were under balancing selection (Fig. S3). After
534 removing these loci, the neutral data set included 25 SNPs – two from coloration genes
535 and 23 from CATS loci (See Supplemental Material Table S8 for details).
536
537 Divergence metrics based on genotypes from all loci - and all neutral loci - are shown at
538 the bottom of Table 3. For spotted dolphins, we detected significant differentiation
539 between coastal spotted dolphins and offshore pantropical spotted dolphins in all three
540 statistics (P = 0.001). It is worth noting, however, that 12 of the 13 samples from the
541 offshore partition were from the WS stock, a majority of which was sampled in the
542 southern region. Unfortunately, we were unable to test for population structure between
543 the offshore stocks (or other partitions proposed by Escorza-Treviño *et al.* 2005) because
544 of too few samples and loci.
545
546 Analyses of Bayesian clustering in STRUCTURE using all SNPs estimated the most
547 likely number of unique clusters for spotted dolphins was K=2 based on LnP(K)
548 (Supplemental Fig. S4). Using the metric ΔK (Evanno *et al.* 2005), K=2 was the most
549 likely number of clusters estimated for spotted dolphins (see Supplemental Material Fig.
550 S4); however, as with the spinner dolphins, K=2 is the smallest value ΔK can estimate.
551 These estimates were consistent regardless of whether location priors were used.
552
553 Six loci were estimated to be subject to positive selection in spotted dolphins: beta-
554 carotene oxygenase 1 (BCDO), FES proto-oncogene, tyrosine kinase (FES), homeobox
555 protein Hox-C8 **(**HOXc8), myosin heavy chain 4 **(**MYH4), somatostatin (SST) and
556 tyrosinase-related protein 1 (TYRP1). BCDO produces a key enzyme in beta-carotene
557 metabolism to vitamin A, which is a vital component in processes like vision,
558 development, cell differentiation and skin color and protection. FES codes for tyrosine

559 kinase: an important component of cellular transformation. HOXc8 plays an important
560 role in morphogenesis in mammals. MYH4 is involved in building motor proteins for
561 muscle contraction. Somatostatin codes for the hormone of the same name, which is an
562 important regulator of the endocrine system. TYRP1 encodes for an enzyme in
563 melanocytes that produce melanin. In addition, five loci were estimated to be under
564 balancing selection in this species: ADH, AMBP, CHRNA, ELN, FSHB.
565
566 **Discussion**
567
568 Spinner and spotted dolphins in the eastern tropical Pacific offer a unique opportunity to
569 study genetic differentiation at multiple scales in species with high abundance and strong
570 intraspecific morphological differences. But recent divergence, large population sizes,
571 and ongoing gene flow likely contribute to low genetic divergence (Galver 2002,
572 Escorza-Treviño *et al.* 2005, Andrews *et al.* 2013) and low statistical power to detect this
573 divergence (Taylor and Dizon 1996, Waples 1998).
574
575 Using complete mitogenomes and sets of nuclear SNPs, we have provided genetic
576 support for multiple levels of biologically meaningful differentiation among subspecies
577 and populations of these two species of pelagic dolphins. However, as Table 5 shows,
578 many of the comparisons lacked a consistent pattern across these markers. We believe
579 this is useful information to report as it reflects the limitations of our data for finding
580 population structure in this unique system, but also the unique information available
581 through analysis of different markers and measures of differentiation. Our results show
582 genetic support for endemic subspecies of spinner and spotted dolphins, although the
583 strength of this support varies between markers. However, we did not find support for the

584 division of offshore stocks of spotted dolphins, nor did we find separation of the Tres
585 Marias spinner dolphins as an independent population.
586
587 F_{ST} and Φ_{ST} provide slightly different perspectives on population differentiation and we
588 believe it is important to present both measures. Our results show inconsistencies
589 between these two metrics, which does not necessarily mean analytical problems or
590 inaccuracies, but reflects something interesting about our data. F_{ST} tests for population
591 differentiation are based on allele (or haplotype) frequencies and do not provide direct
592 insights into levels of molecular divergence (Weir and Cockerham, 1984, Excoffier *et al.*
593 1992, Meirmans and Hedrick 2011). In cases where haplotypes are similar within
594 population and different between populations (such as those that would result via drift in
595 small populations), F_{ST} is good at detecting frequency differences that indicate genetic
596 structure. However, when haplotype diversity is high within and among populations, very
597 large sample sizes are needed to characterize haplotype frequencies to detect differences
598 using F_{ST}. In this situation, F_{ST} point values will be underestimated. Moreover, sampling
599 effects can become important drivers of F_{ST} beyond the base frequency of alleles present
600 and result in false positive results. We collected more sequence data to examine
601 haplotypic similarities in other parts of the mitochondrial genome to help resolve close
602 population relationships. One risk of adding more data is that the haplotype discovery
603 curve never plateaus - more unique haplotypes are added thereby increasing the difficulty
604 of characterizing haplotype frequencies among and between populations. Sequencing
605 additional samples will help rectify this issue.
606
607 Φ_{ST} is certainly not immune to these issues of high heterozygosity, but Φ_{ST} estimates
608 capture more information regarding the differentiation due to sequence divergence (or
609 nucleotide diversity) in addition to differences in haplotype frequencies. Although we

chose to focus the bulk of the discussion on Φ_{ST}, we do report statistically significant measures of F_{ST} and briefly compare and contrast the two metrics. One down side of focusing on Φ_{ST} (and another reason it is important to report F_{ST} as well) is that Φ_{ST} may be more indicative of older, long-term processes, whereas F_{ST} can show recent differences among populations. In addition, given that the test for significance is determined by an arbitrary cut-off ($P = 0.05$), we also present results that are "nearly significant". Given the difficulty of distinguishing these groups in previous works, we felt it important not to focus too intensely on the arbitrary cut-off, but rather overall patterns of indicators.

The discordance we observed between the mtDNA and nuDNA markers (Table 5) could result from a number of factors. Despite the increase in power gained over other studies, it could be that our SNPs still do not have enough statistical power to detect differences in some cases because of recent divergence, continued low-level interbreeding, and/or high diversity and historical abundance. Differences in the rate of evolution in the two genomes (*i.e.*, mitogenome evolving faster than the nuDNA genome) could have resulted in more signal from drift appearing in allele frequencies due to the smaller N_e of the mitogenome (Moritz 1994). Alternatively, the discordance we observed could be a result of male-mediated exchange diluting the signal of structure in nuDNA or female site-fidelity increasing structure in the mtDNA. Although there is some evidence from tagging studies that some dolphins move substantial distances (Perrin *et al.* 1979), a thorough investigation into the differences between sexes is lacking. We believe it is therefore most likely that the SNP data set lacks statistical power, and suggest increasing the number of SNPs in future studies. Moreover, our sample sizes were low in some partitions (n=7). This could result in the allele frequencies of populations being under-characterized, which could skew results in over- or under-classification. Efforts should be

made to collect more samples for future studies and efforts should be made to increase the number of SNP loci genotyped for each individual. Studies using simulations have shown that F_{ST} can be precise when using a small sample size (4-6) using >1,000 SNPs (Willing *et al.* 2012). Given the known evolutionary and demographic factors that could obscure population genetic structure in these species, and the relative difficulty in obtaining representative sample sets, we recommend targeting >1,000 SNPs for future studies. Of course, differences between groups will eventually be found as additional markers are added using hypothesis-testing methods (*i.e.*, *P*-values), even if the differences are not biologically meaningful. Conversely, non-significant results could occur with low levels of geneflow between strata that are demographically independent populations (Avise 1995; Taylor and Dizon 1996). Because management decisions rely on them, results must be interpreted within the context of all available information and with recognition of the caveats of the data used to generate them.

Endemic Spinner Dolphin Subspecies: Eastern and Central American

No differences were detected between the two endemic subspecies using Φ_{ST} on the concatenated spinner dolphin mitogenomic data set, but traditional F_{ST} was significantly different – supporting endemic subspecies distinction (Central American and eastern). F_{ST} was very low as expected due to high abundance and haplotype diversity. The difference in these two metrics, on the same data set, results from Φ_{ST} including differences in nucleotide divergence in the calculation; thus we conclude that haplotypes within these two subspecies are very similar, but that haplotype frequencies are significantly different. Nuclear SNP data did not support differences between these endemic subspecies, either with traditional F-statistics or Bayesian analysis in STRUCTURE. The lack of detectable structure could be because STRUCTURE's clustering algorithm attempts to maximize Hardy-Weinberg and linkage equilibrium

within clusters of samples. Data sets such as our spinner dolphin data, that represent a relatively small proportion of the overall variation of the true populations, have been shown to lack power to detect population clusters defined by Hardy-Weinberg and linkage (Kalinowsky 2010).

Despite the lack of population structure from our nuDNA SNP analyses, our results provide evidence of genetic differentiation between the accepted ETP endemic subspecies (Perrin *et al.* 1991) concordant with morphology and results from Andrews *et al.* (2013) who used data from the nuclear Actin gene. Differences in ecological, distributional, morphological, nuDNA, and now mtDNA data support the recognition of these distinct subspecies.

Whitebelly Spinner Dolphins

Our comparisons involving the putative intergrade, the whitebelly spinner, also revealed discordant patterns between the mtDNA and nuDNA data sets (Table 5). Every whitebelly sample had a unique mitogenome haplotype, and as a result, frequency-based measures of differentiation such as *F-statistics* will be underestimated. Despite low point values, mitochondrial F_{ST} *and* Φ_{ST} estimates between the Central American and whitebelly forms were nearly significant, indicating possible separation. Nuclear SNP data, however, failed to show differences between these groups.

Although we used a slightly different set of samples from their study, Andrews *et al.* (2013) also found differentiation between Central American and whitebelly spinners using mtDNA genes (control region and cyt*b*). We recovered the same pattern for those genes and several others (Table 2). Andrews *et al.* (2013) used a similar sample set to ours, but included 10 samples of Central American spinners that had questionable

688 subspecific identity (based on further investigation of the sample collection records at
689 SWFSC by MSL). Samples were initially identified as Central American spinners, but the
690 confidence in the identification was low and they should have been recorded as
691 "unidentified". These samples could have been eastern spinner dolphins. Unfortunately,
692 our sample size was low for the Central American spinners after removal of these
693 questionable samples (mtDNA: *n*=9; nuDNA: *n*=7), and we might not expect to find
694 intraspecific structure with so few samples. The Central American subspecies, with lower
695 relative abundance, might be expected to show higher levels of structure due to drift, and
696 comparisons between this subspecies and the eastern subspecies showed evidence of
697 weak structure based on allele frequencies.
698
699 Two explanations for the possible differentiation between Central American and
700 whitebelly spinners in the mtDNA are isolation by distance and admixture between
701 whitebellies and Hawai'ian spinners. These are the two most geographically distant
702 putative populations of ETP spinner dolphins; therefore, isolation by distance could
703 contribute to population genetic structuring. Admixture between the whitebelly and
704 Hawai'ian spinners would bring novel genotypes from the Gray's subspecies (Hawaii)
705 into the whitebellies resulting in genetic structure.
706
707 Andrews *et al.* (2013) inferred high migration rates between whitebelly and eastern
708 spinner dolphins (30.1 migrants per generation from whitebelly to eastern and 57.9
709 migrants from eastern to whitebelly). Despite this high rate of migration, we detected
710 weak differentiation. We found nearly significant differences between the whitebelly
711 spinner and the eastern spinner using the concatenated mitogenome data, although the
712 Φ_{ST} estimate was not significant ($P = 0.0741$). We also saw significant differences
713 between these strata in eight individual mtDNA genes.

Nuclear SNP data also showed significant differences between the eastern subspecies and the whitebelly intergrade. These results are concordant with phenotype and Y-chromosome differences (Andrews *et al.* 2013) where the most noticeable differences occur between the geographically overlapping whitebelly and eastern. We agree with these authors that there is likely a porous barrier to gene flow across the eastern Pacific basin, as mixed groups are common and interbreeding probably occurs with some regularity between eastern and Central American subspecies and between eastern and whitebelly spinners. However, we feel that this 'introgression zone' between whitebelly spinners and eastern spinners deserves further investigation. We hypothesize that either divergence with gene flow is ongoing in this area or the whitebelly spinner is the result of a recent reconnection in an area of historical separation across a known biogeographic boundary, the east Pacific basin.

Breeding biology and movement patterns could also affect the patterns we see between the whitebelly and eastern spinner dolphins. In particular, assortative mating can decrease N_e, which could serve to amplify signal of structure in the nuDNA genome. The eastern spinner dolphin is thought to have a more polygynous mating system than the whitebelly form (Perrin and Mesnick, 2003). These authors concluded that relatively few males are involved with mating, serving to reduce N_e and potentially increase genetic structure (Perrin and Mesnick, 2003). Conversely, however, a skewed breeding system might also increase dispersal, as adult male dominance might promote movements of juvenile males which then become established breeder outside their natal range. Unfortunately, very little is known about the movement patterns of individual dolphins in the ETP, and less is known about differences in movement based on sex. High site fidelity in males could also restrict male-mediated geneflow between groups and increase relative signal in nuDNA

740 analyses.

741

742 The statistical power to estimate levels of migration between very large populations with
743 low relative sample sizes is weak (Waples 1998, Taylor *et al.* 2000). For this reason, we
744 did not estimate levels of migration for these data. Andrews *et al.* (2013) did estimate
745 migration in ETP spinner dolphins and found lower, but significantly different from zero,
746 rates of migration per generation between populations of Gray's (Hawai'ian and other
747 Pacific Island groups) spinners and the whitebelly spinners (3.22 migrants per generation
748 into Gray's and 1.6 into whitebelly spinners). The rate of migration into Gray's spinner
749 populations from the eastern population was estimated to be less than one (0.82), but
750 significantly different from zero. Although this was not a major focus of our study, the
751 differences we detected between the Hawaiian population and the ETP pelagic
752 populations were higher than any comparisons within the ETP, supporting the hypothesis
753 that this is an insular population.

754

755 **Alternative spinner dolphin stocks:**

756 We found no support for a Tres Marias population that differs from the eastern or Central
757 American subspecies (*e.g.*, Perryman and Westlake 1998) using the concatenated or
758 individual mitochondrial gene data sets. This is unsurprising given the weak genetic
759 differences we found between the accepted endemic subspecies with much more marked
760 morphological differences. We found statistically significant differences in four
761 individual mtDNA genes when comparing the Tres Marias group to the whitebelly
762 spinners and several nearly significant genes. Unfortunately, we were unable to test
763 hypotheses that Tres Marias differs from other groups with the nuDNA due to small
764 sample sizes. We do not feel confident making taxonomic recommendations for the "Tres
765 Marias" spinners based on these analyses. Additional studies should approach this

766 question using larger sample sets and additional data.

767

768

769 **Spotted dolphin subspecies:**

770 Spotted dolphin mitogenomes have lower haplotypic diversity but higher nucleotide
771 diversity than spinner dolphins, despite extremely high historical population sizes in the
772 former. The two main reasons for lower haplotypic diversity could be a recent and/or
773 prolonged population bottleneck, such as the decrease caused by mortalities in the tuna
774 purse-siene fishery, or an extremely matrifocal social structure (Hoelzel *et al*. 2007).
775 Although matrifocal social structure is known in several species of odontocetes (*e.g.*,
776 killer whales and sperm whales), it is not a known characteristic of spotted dolphins, and
777 thus is an unlikely cause of low genetic diversity.

778

779 Similar to our findings for spinner dolphins, traditional F_{ST} calculated for the
780 mitogenome data set supports differentiation of the offshore *S. a. attenuata* and the
781 endemic coastal *S. a. graffmani* subspecies, whereas Φ_{ST} failed to indicate any difference
782 - either for the entire genome or within any single gene. Our results show the NE stock
783 being strongly differentiated from the coastal subspecies (based on allele frequency
784 alone), counter to the results found by Escorza-Treviño *et al.* (2005) showing connection
785 between the NE stock and the coastal subspecies using seven microsatellite loci. In that
786 study, the authors inferred that there was a strong connection between the coastal and
787 offshore subspecies in northern Mexico. The differences between our results and those of
788 Escorza-Treviño *et al.* (2005) could be due to sampling; the previous study more samples
789 from the northern portion of the coastal spotted dolphin range than we did. Additionally,
790 the differences could be attributed to the unique evolutionary patterns of the different
791 markers examined in Escorza-Treviño *et al.* (2005) (*i.e.*, microsatellites) vs. the

792 mitogenomes used in our study.

793

794 In contrast to our results for spinner dolphin subspecies, our analyses of divergence using
795 36 SNP loci showed highly significant differentiation between the two spotted dolphin
796 subspecies based on traditional *F*-statistics and cluster analyses, supporting the
797 hypothesis that two spotted dolphin subspecies exist in the ETP.

798

799 **Spotted dolphin stocks:**

800 A main objective of this work was to test for difference between existing (NE, WS, and
801 Coastal) and proposed (independent W and S) management stocks. Using the whole
802 mtDNA genome data set, we found no evidence for differentiation between the two
803 current stocks (NE and WS). This could be because the two stocks are genetically
804 connected or because our data lack power to detect differentiation at this fine scale. The
805 large mtDNA found weak evidenced for splitting up the current WS stock - a high Φ_{ST}
806 value (0.1666) and nearly significant (P = 0.0668). Similarly, we detected nearly
807 significant differences between the NE stock and the western group of the WS stock
808 using the whole mtDNA genome. Four mtDNA loci had significant Φ_{ST} estimates for this
809 partition. The NE and the offshore southern group were not significantly different in any
810 test, suggesting that the distributional hiatus at 5° north is not a barrier to gene flow. We
811 cannot say with any certainty if this is the case, however, because of the low sample size
812 for the southern portion of WS stock (n=9); a larger sample size is necessary to
813 convincingly investigate this hiatus. Overall, the whole concatenated mtDNA genome
814 was not as useful as anticipated for delimiting stock structure, possibly because it
815 introduced more variation (*via* novel haplotypes) into an already highly variable system.
816 Whole mtDNA genomes have been useful for clarifying subspecific boundaries where
817 information in single mtDNA genes has shown low variability (Archer *et al.*2013, Morin

et al. 2010), including in this paper, but testing population-level boundaries in highly abundance cetaceans using mtDNA genomes may be less feasible.

The SNP data set indicated differences between the offshore and coastal spotted dolphin subspecies, but did not include data from individuals from the NE offshore stock of spotted dolphins. Therefore, this comparison includes animals from the most geographically separate portions of the offshore (WS) and coastal subspecies range. Additional nuclear data from the NE stock are needed to determine whether proximate populations of these two subspecies are also as genetically divergent.

Drift in mtDNA loci as indicated by comparisons with Hawai‘i

Because of the greater divergence observed between Hawai‘ian and ETP populations of these two dolphins, we thought it would be informative to highlight genes showing structure (Hawai‘i vs. ETP), likely due to neutral drift acting on a small insular population, that might be useful for studying other Hawai‘ian populations of cetacean species. Four genes showed population structure (significant Φ_{ST}) in all pairwise comparisons between Hawai‘i and ETP groups (*i.e.*, Central America, Tres Marias, eastern, and whitebelly spinner), but not in any pairwise comparisons between these ETP groups: 16S, ATP6, ND2, and ND5. Because of the low abundance and geographic isolation of the Hawai‘ian population. We tested for positive selection in ETP spinner dolphin mitochondrial genes with significant Φ_{ST} estimates. All of the mtDNA regions with significant Φ_{ST} were found to be under purifying selection ((negative Tajima’s *D* - Table S5; and non-significant Z-tests – Table S6) indicating that the within-mitogenome differences are accumulating by neutral drift rather than via positive selection in ETP spinner dolphins. Significant differences between ETP groups and the Hawai‘ian insular

844 population of spotted dolphins were found in all but five of the mtDNA genes. We note
845 however that the low sample sizes for Hawai'ian spotted dolphins may explain some of
846 the non-significant differences observed with respect to ETP stocks.
847
848 **Positive Selection in ETP Spinner Dolphin mtDNA**
849 In general selection should affect linked loci equally. However, selection can act on
850 individual mtDNA genes, such as in the case of cytochrome *b* in Antarctic killer whales
851 (Foote *et al.* 2010). These authors posit that the positive selection measure is in response
852 to need for increased metabolic performance for residence in cold waters. We tested for
853 positive selection in spinner dolphin mitochondrial genes and found none. We did not test
854 for positive selection in spotted dolphins because there was no existing hypothesis of
855 positive selection, but moreover, because there were no individual mtDNA genes that
856 supported differentiation between the two ETP subspecies.
857
858 **NuDNA Loci Under Selection**
859 Although finding four of the 51 SNP loci (7.8%) in spinner dolphins and six of the 36
860 SNP loci (16.7%) in spotted dolphins exhibiting evidence of positive selection was
861 unexpected, similar results have been reported in other studies. Russello *et al.* (2011)
862 found eight of their 52 loci (15.4%) to be outliers and useful for detecting ecotype
863 divergence in Okanagan Lake kokanee (*Oncorhynchus nerka*). Bay and Palumbi (2014)
864 found 2807 of 15399 SNPs (18.2%) to be F_{ST} outliers (before further filtering for
865 analyses) in a population of tabletop corals. We removed all outlier loci (positive and
866 balancing selection) for all population divergence statistics and the results remained the
867 same as with all the data included (Table 3). The FDIST2 (Beaumont and Nichols 1996)
868 method implemented in *Lositan* can be prone to false positives, although it generally
869 performs better than ARLEQUIN (Excoffier and Lisher 2010) at minimizing type I error

when detecting F_{ST} outliers (Narum and Hess 2011).

We hypothesized that positive selection on nuclear loci coding for skin pigment coloration could be contributing to the marked differences in coloration between the forms of spinner dolphins in the ETP. Simulation-based tests for selection at each locus using the program *Lositan* (Antao *et al.* 2008) found two coloration genes that were subject to positive selection: BCDO and TYRP1. In both species, BCDO was under positive selection, while spotted dolphins also exhibited positive selection in TYRP1. Pairwise F_{ST} estimates were significantly different between coastal and offshore spotted dolphin subspecies ($P < 0.006$ for both loci) and between ETP endemic spinner dolphin subspecies ($P < 0.05$) (Table S9). Thus, these loci may be part of the molecular basis for differences in coloration observed in this region for these species. Further analysis with larger sample sizes will be needed to verify this finding.

Conclusions:

Defining population genetic structure is challenging for species with large historical population sizes and high mobility. These populations can retain high genetic variation even as abundance becomes relatively low, which could obscure signals of genetic structure used to designate stock boundaries for estimating population abundance and setting stock-specific mortality limits. Ultimately, without information on structure, populations could be under-classified and unique evolutionary units and populations could go extinct as we may fail to take appropriate conservation action. Alternatively, there is a cost to managing populations as separate when there is no biological basis to do so. Such errors can have economic, social, and political consequences resulting from

unnecessary restrictions on human activity. Furthermore, a consistent pattern of these errors will "stiffen the resolve of skeptics and make it difficult to accomplish sound resource management in the future" (Waples 1998).

This unique system of two delphinids, with available samples collected *in situ* from remote offshore environments encompassing extensive geographic and morphological variation, was used to test for population genetic structure at multiple hierarchical levels in species with high historical abundance and high intra-specific morphological variation. Our results show a complex pattern of genetic structure in the two different data sets for each species. Although complex, we believe the structure observed in our results is biologically meaningful. Given the aforementioned difficulties with detecting structure using genetic techniques in this system – and the supporting morphometric results - even subtle signatures of structure are significant findings. The mitogenome data show support for the endemic ETP spinner and spotted dolphin subspecies. The nuclear SNP data show strong support for spotted dolphin subspecies but failed to find segregation in morphologically divergent spinner dolphin subspecies, although small sample size for the coastal subspecies limited power to detect genetic divergence.

A lack of differentiation in the SNP data between the two most geographically distant groups (the whitebelly and the Central American spinner dolphins) was unexpected, especially since strong differences were detected between the more proximate eastern and whitebelly forms.

We found very little support for the division of offshore stocks of spotted dolphins and no support for the unique form of Tres Marias spinner dolphins as compared to the eastern or Central American subspecies. This is not to say that these biological entities do not

922 exist, just that our data do not support them or may not have sufficient power to detect the
923 subtle genetic differences between them. Efforts are being made to collect more SNP data
924 (>1,000) from specimens collected throughout the range of these animals to perform
925 high-resolution population structure analyses (Leslie and Morin, *In Review*). Further, we
926 suggest the collection and analysis of additional samples from the Central American
927 subspecies to compare to existing offshore subspecies samples collected from fisheries
928 bycatch and research cruises. In addition, we highly recommend additional studies of
929 nuDNA and studies of population structure that incorporate environmental variables as
930 potential population boundaries in this area. Finally, placing these populations within a
931 global phylogeographic context will help provide a better context for our results by fully
932 characterizing intraspecific diversity and establishing the evolutionary process that led to
933 ETP endemism.

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Table 1. Summary statistics for ETP spinner (A) and spotted (B) dolphin mitogenome data. n_H: number of haplotypes; PS: polymorphic sites; h: haplotype diversity; π: nucleotide diversity; %: percent of unique haplotypes.

A. Spinner dolphins *Stenella longirostris* (n = 104)						
Subspecies/Stock	**n:female/male/unk**	**n_H**	**PS**	***h***	**π**	**%**
Central American *S. l. centroamericana*	9:4/4/1	8	648	0.9722	0.0057	0.7778
eastern~ *S. l. orientalis*	53:28/19/6	51	648	0.9985	0.0073	0.9245
Putative Stocks						
whitebelly *S. l. longirostris*	27:16/11/0	27	457	1	0.0043	1
Tres Marias^~ *S. l. orientalis*	21:8/10/3	20	373	0.9952	0.0078	0.9048
Hawai'i *S. l. longirostris*	15:1/4/10	9	104	0.9921	0.0068	0.8260

B. Spotted dolphins *Stenella attenuata* (n = 76)						
Subspecies	**n:female/male/unk**	**n_H**	**PS**	***h***	**π**	**%**
Coastal *S. a. graffmani*	24:11/13/0	16	234	0.9529	0.0162	0.5000
ETP offshore[§] *S. a. attenuata*	47:20/19/8	43	519	0.9804	0.0198	0.7222
Offshore Stocks (*S. a. attenuata*) - Current and Putative^						
northeastern	25:10/8/7	22	400	0.9867	0.0238	0.8000
western-southern	17:9/7/1	17	298	1	0.0096	1
Offshore western^	8:7/1/0	8	191	1	0.0087	1
Offshore southern^	9:2/6/1	9	253	1	0.0092	1
Hawai'i	5:1/3/1	3	36	0.7000	0.0244	0.4000

^ Stocks that are not recognized for management purposes. ~ The Tres Marias spinner samples are part of the eastern stratum. [§] Includes data for five samples that were omitted from stock comparisons because they were sampled too close to geographic stock boundaries.

Table 2: Pairwise divergence estimates for subspecies and stocks of spinner dolphins based on concatenated mitogenome data (F_{ST}, Φ_{ST} and χ^2) and partitioned mitogenomic data (Φ_{ST} only). Light gray backgrounds for p<0.05; medium gray for p<0.01; darker gray backgrounds for p<0.001 (p-values in parentheses).

Taxon 1 (n) vs. Taxon 2 (n)	**Concatenated mitogenome**		**Partitioned mitogenome Φ_{ST} (p-value)**														
	F_{ST} (p-value)	**Φ_{ST} (p-value)**	**12s** n_H=24	**16s** n_H=24	**ATP6** n_H=53	**ATP8** n_H=11	**COI** n_H=65	**COII** n_H=39	**COIII** n_H=47	**CYTB** n_H=61	**CR** n_H=50	**ND1** n_H=59	**ND2** n_H=53	**ND3** n_H=21	**ND4** n_H=56	**ND4L** n_H=22	**ND5** n_H=70
Central Amer. (9) vs. eastern (53)	0.0133 (0.034)	-0.0127 (0.5235)	-0.0120 (0.5265)	0.0061 (0.4977)	-0.0076 (0.4001)	0.0590 (0.0801)	-0.0276 (0.8640)	-0.0199 (0.6988)	-0.0158 (0.5766)	-0.0094 (0.4711)	0.0017 (0.3983)	0.0148 (0.2501)	-0.0260 (0.7376)	0.0338 (0.1325)	-0.0287 (0.6950)	-0.0268 (0.7444)	-0.0139 (0.5368)
Central Amer. (9) vs. whitebelly (27)	0.0128 (0.056)	0.0490 (0.0542)	-0.0165 (0.5882)	0.0217 (0.1947)	0.0311 (0.1277)	0.1279 (0.0189)	0.0351 (0.0903)	0.0936 (0.0144)	0.0086 (0.2995)	0.0601 (0.0456)	0.0555 (0.0412)	0.0844 (0.0362)	0.0113 (0.2833)	0.1505 (0.0054)	0.0870 (0.0464)	0.0478 (0.0931)	0.0273 (0.1203)
eastern (53) vs. whitebelly (27)	0.0007 (0.2867)	0.0181 (0.0741)	0.0307 (0.0414)	0.0159 (0.0835)	0.0051 (0.2421)	-0.0065 (0.5546)	0.0264 (0.0288)	0.0342 (0.0152)	-0.0020 (0.4501)	0.0154 (0.1165)	0.0270 (0.0059)	0.0260 (0.0468)	0.0104 (0.1687)	0.0638 (0.0018)	0.0464 (0.0422)	0.0343 (0.0214)	0.0026 (0.2859)
Tres Marias (21) vs. Central Amer. (9)	0.0155 (0.0914)	-0.0345 (0.7576)	0.0113 (0.2921)	-0.0283 (0.7240)	-0.0436 (0.7284)	-0.0082 (0.2863)	-0.0393 (0.8636)	-0.0318 (0.7150)	-0.0301 (0.6752)	-0.0238 (0.5872)	-0.0102 (0.5328)	-0.0158 (0.5219)	-0.0451 (0.8698)	0.0022 (0.4025)	-0.0558 (0.8900)	-0.0638 (0.9470)	-0.0383 (0.7888)
Tres Marias (21) vs. eastern (32)	0.0009 (0.4107)	-0.0116 (0.7084)	-0.0109 (0.6474)	-0.0217 (0.9462)	-0.0088 (0.5169)	0.0019 (0.3119)	-0.0124 (0.7654)	-0.0182 (0.8772)	-0.0150 (0.8116)	-0.0062 (0.5291)	-0.0135 (0.8454)	-0.0117 (0.6898)	-0.0031 (0.4447)	-0.0206 (0.8894)	-0.0185 (0.7898)	-0.0049 (0.4887)	-0.0105 (0.6442)
Tres Marias (21) vs. whitebelly (27)	0.0024 (0.1934)	0.0263 (0.0807)	0.0421 (0.0643)	0.0111 (0.1979)	0.0086 (0.2423)	0.0175 (0.2421)	0.0323 (0.0519)	0.0406 (0.0362)	-0.0005 (0.3907)	0.0311 (0.0765)	0.0413 (0.0168)	0.0359 (0.0636)	0.0243 (0.1087)	0.0676 (0.0052)	0.0859 (0.0789)	0.0485 (0.0448)	0.0124 (0.1807)
Hawaii (15) vs, whitebelly (27)	0.0456 (0.0001)	0.1964 (0.0002)	0.0236 (0.1667)	0.3590 (0.0002)	0.2560 (0.0002)	-0.0127 (0.6582)	0.1964 (0.0002)	0.1885 (0.0006)	0.0154 (0.1363)	0.1031 (0.0004)	0.0771 (0.0036)	0.0818 (0.0026)	0.3302 (0.0002)	0.4467 (0.0002)	0.1324 (0.0002)	-0.0137 (0.2197)	01858 (0.0002)
Hawaii (15) vs. eastern (53)	0.0449 (0.0001)	0.1849 (0.0002)	0.0428 (0.0605)	0.3293 (0.0002)	0.2268 (0.0002)	-0.0002 (0.4031)	0.2061 (0.0002)	0.2104 (0.0002)	0.0338 (0.0625)	0.1182 (0.0026)	0.2050 (0.0002)	0.1406 (0.0012)	0.3090 (0.0002)	0.3283 (0.0002)	0.1339 (0.0025)	0.0170 (0.1643)	0.1494 (0.0007)
Hawaii (15) vs. Central Amer. (9)	0.0636 (0.0219)	0.3284 (0.0002)	-0.0083 (0.4045)	0.5265 (0.0002)	0.3328 (0.0004)	0.1600 (0.0631)	0.3983 (0.0002)	0.4280 (0.0002)	0.1415 (0.0034)	0.2474 (0.0002)	0.2464 (0.0002)	0.3091 (0.0002)	0.4352 (0.0004)	0.3863 (0.0002)	0.2728 (0.0002)	0.1597 (0.0701)	0.2854 (0.0004)
Hawaii (15) vs. Tres Marias (21)	0.0487 (0.0004)	0.2260 (0.0002)	0.0796 (0.0478)	0.3900 (0.0002)	0.2552 (0.0002)	0.0339 (0.2507)	0.2576 (0.0002)	0.2351 (0.0002)	0.0703 (0.0272)	0.1398 (0.0004)	0.1533 (0.0002)	0.1958 (0.0002)	0.3454 (0.0002)	0.3608 (0.0002)	0.1667 (0.0004)	0.0630 (0.0669)	0.1828 (0.0002)

Table 3. Pairwise divergence estimates (F_{ST}) for spinner and spotted dolphin subspecies, respectively, using all nuclear SNPs, and using only neutral SNPs. Light gray backgrounds for p<0.05; Medium gray for p<0.01; darker gray backgrounds for p<0.001.

Spinner dolphins		**All 51 SNPs**	**42 Neutral SNPs**
Taxon 1 n:female/male/unk	**Taxon 2** n:female/male/unk	**F_{ST}** **(p-value)**	**F_{ST}** **(p-value)**
Central American 7:3/3/1	eastern 28:15/7/6	-0.0023 (0.4485)	-0.0066 (0.5148)
Central American 7:3/3/1	whitebelly 21:12/9/0	0.0148 (0.2607)	0.0082 (0.3216)
eastern 28:15/7/6	whitebelly 21:12/9/0	0.0297 (0.0059)	0.0282 (0.0099)
Spotted dolphins		**All 36 SNPs**	**25 Neutral SNPs**
Offshore 13:6/6/1	Coastal 12:5/7/0	0 .1711 (0.001)	0.1493 (0.0005)

Table 4: Pairwise divergence estimates for subspecies and stocks of spotted dolphins using concatenated mitogenome data (F_{ST}, Φ_{ST} and χ^2) and partitioned mitogenomic data (Φ_{ST} only). n_H listed below each gene name is the number of haplotypes for that gene. Light gray backgrounds for p<0.05; medium gray for p<0.01; darker gray backgrounds for p<0.001 (p-values in parentheses). "*NA*" indicates comparisons where Φ_{ST} could not be estimated because all individuals in both strata share the same haplotype. "*" are where one stratum was n<5.

	Concatenated mitogenome		**Partitioned mitogenome Φ_{ST} (p-value)**														
Taxon 1 (n) vs. Taxon 2 (n)	**F_{ST} (p-value)**	**Φ_{ST} (p-value)**	**12S** n_H=6	**16S** n_H=7	**ATP6** n_H=20	**ATP8** n_H=5	**COI** n_H=21	**COII** n_H=13	**COIII** n_H=11	**CYTB** n_H=20	**CR** n_H=20	**ND1** n_H=15	**ND2** n_H=17	**ND3** n_H=10	**ND4** n_H=23	**ND4L** n_H=2	**ND5** n_H=29
Coastal (24) vs. Offshore (52)	0.0125 (0.0402)	-0.0091 (0.4961)	0.0089 (0.2553)	-0.0316 (0.9932)	-0.0149 (0.6788)	-0.0169 (0.7536)	0.0018 (0.3265)	-0.0133 (0.6182)	-0.0243 (0.8890)	-0.0143 (0.6067)	-0.0122 (0.7336)	-0.0099 (0.5357)	-0.0198 (0.7610)	-0.0222 (0.9006)	-0.0042 (0.4085)	0.0041 (0.3023)	-0.0056 (0.4217)
Northeastern (25) vs. western-southern (17)	0.0045 (0.2099)	-0.0076 (0.4111)	-0.0014 (0.3779)	0.0079 (0.2841)	-0.0156 (0.5332)	0.0057 (0.2691)	-0.0194 (0.6164)	0.0003 (0.3637)	-0.0193 (0.5067)	-0.0211 (0.5423)	-0.0091 (0.5552)	0.0086 (0.2585)	-0.0021 (0.3473)	-0.0068 (0.4139)	0.0039 (0.3077)	-0.0038 (0.3771)	-0.0187 (0.5574)
Coastal (24) vs. northeastern (25)	0.0302 (0.0002)	-0.0082 (0.4405)	0.0032 (0.3375)	-0.0326 (0.8096)	-0.0204 (0.7070)	-0.0061 (0.4689)	-0.0007 (0.3651)	-0.0060 (0.4325)	-0.0271 (0.7540)	-0.0201 (0.6148)	-0.0041 (0.4653)	0.0031 (0.3041)	-0.0119 (0.4797)	-0.0105 (0.4947)	0.0055 (0.2923)	0.0016 (0.3249)	-0.0125 (0.5309)
Coastal (24) vs. western-southern (17)	0.0144 (0.0884)	-0.0342 (0.8102)	-0.0186 (0.5621)	-0.0356 (0.6598)	-0.0297 (0.7402)	0.0081 (0.3153)	-0.0313 (0.8118)	-0.0355 (0.8624)	-0.0449 (0.8950)	-0.0385 (0.8666)	-0.0024 (0.8060)	-0.0392 (0.9142)	-0.0335 (0.7224)	-0.0393 (0.9112)	-0.0311 (0.7582)	-0.0360 (0.7624)	-0.0285 (0.6812)
Offshore southern (9) vs. offshore western (8)	0.0771 (0.2249)	0.1666 (0.0668)	-0.1717 (0.0781)	0.2129 (0.0618)	0.1167 (0.1039)	0.1117 (0.0801)	0.1229 (0.0743)	0.1361 (0.0939)	0.1816 (0.0767)	-0.0471 (0.4611)	0.0683 (0.1177)	0.1767 (0.0575)	0.1771 (0.0743)	0.1155 (0.1183)	0.2148 (0.0394)	0.1382 (0.1231)	0.1895 (0.0529)
Northeastern (25) vs. offshore western (8)	0.0027 (0.4291)	0.1135 (0.0517)	0.0853 (0.0945)	0.1848 (0.0352)	0.0728 (0.1223)	0.1128 (0.0252)	0.0575 (0.1397)	0.1164 (0.0504)	0.1117 (0.0749)	0.0064 (0.3259)	0.0348 (0.1635)	0.1525 (0.0372)	0.1179 (0.0775)	0.1142 (0.0697)	0.1497 (0.0394)	0.1309 (0.0689)	0.0894 (0.0957)
Northeastern (25) vs. offshore southern (9)	0.0073 (0.3755)	-0.0400 (0.7446)	-0.0242 (0.5728)	-0.0537 (0.8008)	-0.0468 (0.8162)	-0.0691 (0.9552)	-0.0291 (0.6287)	-0.0387 (0.7512)	-0.0491 (0.7828)	-0.0509 (0.8168)	-0.0300 (0.7886)	-0.0379 (0.7394)	-0.0392 (0.7150)	-0.0551 (0.9540)	-0.0238 (0.5626)	-0.0694 (0.9756)	-0.0327 (0.6092)
Coastal (24) vs. offshore southern (9)	0.0255 (0.0762)	-0.0130 (0.4065)	-0.0323 (0.5874)	-0.0277 (0.4713)	-0.0279 (0.5721)	-0.0147 (0.4071)	-0.0122 (0.4423)	-0.0079 (0.3971)	-0.0227 (0.4611)	-0.0579 (0.8690)	-0.0418 (0.8558)	-0.0012 (0.3477)	-0.0419 (0.6714)	-0.0309 (0.5854)	0.0051 (0.3209)	-0.0160 (0.4301)	-0.0030 (0.3453)
Coastal (24) vs. offshore western (8)	0.0049 (0.4321)	0.0749 (0.1331)	0.1368 (0.0559)	0.1366 (0.0855)	0.0594 (0.1583)	0.1363 (0.0167)	0.0751 (0.1281)	0.0406 (0.1953)	0.0769 (0.1535)	-0.0089 (0.3361)	0.0488 (0.1359)	0.0609 (0.1541)	0.0901 (0.1101)	-0.0372 (0.2059)	0.1067 (0.0881)	0.0239 (0.2425)	0.0841 (0.1269)
Hawaii (5) vs. Coastal (24)	0.1430 (0.0026)	0.2773 (0.0208)	0.4166 (0.0019)	0.2176 (0.0572)	0.2767 (0.0174)	-0.0502 (0.5687)	0.4032 (0.0028)	0.1687 (0.0762)	0.2175 (0.0585)	-0.2859 (0.0254)	0.2037* (0.0326)	0.3643 (0.0049)	0.1575* (0.0947)	0.3085 (0.0042)	0.2660 (0.0252)	0.2811 (0.0202)	0.2541 (0.0244)
Hawaii (5) vs. Offshore (47)	0.1181 (0.0006)	0.1582 (0.0389)	0.1806 (0.0422)	-0.1282 (0.1107)	0.1882 (0.0352)	-0.0459 (0.6156)	-0.2138 (0.0124)	-0.0818 (0.1323)	0.1361 (0.0632)	-0.0849 (0.1481)	0.1485* (0.0475)	0.2598 (0.0082)	0.1146* (0.1449)	0.2609 (0.0054)	0.1239 (0.0593)	0.1689 (0.0545)	0.1303 (0.0517)
Hawaii (5) vs. northeastern (25)	0.0576 (0.2709)	0.1308 (0.0645)	0.1153 (0.0962)	0.0809 (0.1793)	(0.1584 (0.0353)	-0.0198 (0.4695)	-0.1981 (0.0206)	0.0638 (0.1739)	0.1099 (0.1123)	0.1478 (0.0714)	0.1372* (0.0567)	0.2499 (0.0102)	0.0869* (0.1279)	0.2751 (0.0051)	0.0984 (0.1029)	0.1446 (0.0843)	0.0951 (0.1355)
Hawaii (5) vs. western-southern (17)	0.2474 (0.0702)	0.2273 (0.0238)	0.2942 (0.0284)	0.2139 (0.0774)	0.2670 (0.0297)	-0.0542 (0.8308)	0.2583 (0.0244)	0.1353 (0.1133)	-0.1992 (0.0547)	0.2259 (0.0286)	0.1704* (0.0547)	0.3089 (0.0196)	0.1793*(0.1473)	0.2751 (0.0234)	0.1925 (0.0356)	0.2673 (0.0342)	0.2062 (0.0366)
Hawaii (5) vs. offshore western (8)	0.4958 (0.0732)	0.4932 (0.0179)	0.4558 (0.0318)	0.5298 (0.0168)	0.4984 (0.0148)	0.0285 (0.3925)	0.4640 (0.0119)	0.4572 (0.0364)	0.5036 (0.0352)	0.0013 (0.4061)	0.3598* (0.0771)	0.5523 (0.0039)	0. 4484* (0.0328)	0.4915 (0.0033)	0.5093 (0.0114)	0.1309 (0.0689)	.0924 (0.1393)
Hawaii (5) vs. offshore southern (9)	0.1509 (0.2207)	0.1274 (0.1167)	0.3012 (0.0268)	0.0306 (0.2757)	0.1750 (0.0718)	0.0443 (0.1961)	0.2126 (0.0202)	0.0036 (0.4437)	0.0161 (0.3045)	0.1384 (0.0872)	-0.1260* (0.0865)	0.2138 (0.0178)	0.0039* (0.2641)	-0.0551 (0.0206)	0.0808 (0.1389)	0.1382 (0.1231)	0.5038 (0.0198)

Table 5. Summary table of pairwise comparisons using both mtDNA and nuDNA data sets (sample sizes in parentheses). A "□" denotes significance of whole mtDNA based on at least one measure (see Tables 2-4). 'ns' = non-significant. '*NA*' = not tested due to insufficient data. " ~ " = indicating possible structure with *P*-value between 0.05 and 0.1. "# Genes" is the number or significant mtDNA genes ("~" + # for low *P*-value genes).

			mtDNA		nuDNA
Spinner dolphins	**Taxon 1 (n_{mt}/n_{nuc})**	**Taxon 2 (n_{mt}/n_{nuc})**	**Whole**	**# Genes**	
Test of endemic subspecies	Central American (9/7)	eastern (53/28)	□	0	ns
Testing whitebelly intergrade	Central American (9/7)	whitebelly (27/21)	~	7: ~1	ns
Testing whitebelly intergrade	eastern (54/28)	whitebelly (27/21)	~	8; ~1	□
Alternative stock hypotheses	Tres Marias (21/0)	Central American (9/0)	~	0	*NA*
""	Tres Marias (21/0)	Eastern (32/0)	ns	0	*NA*
""	Tres Marias (21/0)	Whitebelly (27/0)	~	4; ~5	*NA*
""	Hawaii (15/0)	Whitebelly (27/0)	□	11	*NA*
""	Hawaii (15/0)	Eastern (32/0)	□	11; ~1	*NA*
""	Hawaii (15/0)	Central American (9/0)	□	12; ~1	*NA*
""	Hawaii (15/0)	Tres Marias (21/0)	□	13; ~1	*NA*

Spotted dolphins	**Taxon 1 (n_{mt}/n_{nuc})**	**Taxon 2 (n_{mt}/n_{nuc})**	**Whole**	**# Genes**	**nuDNA**
Testing subspecies	Offshore (52/13)	Coastal (24/12)	□	0	□
Testing existing stocks	Offshore NE (25/0)	Off. western-southern (17/0)	ns	0	*NA*
Testing existing stocks	Offshore NE (25/0)	Coastal (24/0)	□	0	*NA*
Testing existing stocks	Offshore WS (17/0)	Coastal (24/0)	~	0	*NA*
Alternative stock hypotheses	Offshore southern (9/0)	Offshore western (8/0)	~	1: ~9	*NA*
""	Offshore NE (25/0)	Offshore western (8/0)	~	4; ~7	*NA*
""	Offshore NE (25/0)	Offshore southern (9/0)	ns	0	*NA*
""	Offshore southern (9/0)	Coastal (24/0)	~	0	*NA*
""	Offshore western (8/0)	Coastal (24/0)	ns	1; ~3	*NA*
""	Hawaii (5/0)	Coastal (24/0)	□	10; ~4	*NA*
""	Hawaii (5/0)	Offshore (52/0)	□	6; ~4	*NA*
""	Hawaii (5/0)	Offshore NE (25/0)	~	4; ~4	*NA*
""	Hawaii (5/0)	Offshore WS (17/0)	□	9: ~3	*NA*
""	Hawaii (5/0)	Offshore western (8/0)	□	10; ~2	*NA*
""	Hawaii (5/0)	Offshore southern(9/0)	ns	5; ~3	*NA*

Figure 1. Sampling localities and range map for spinner dolphins within the ETP. Subspecies and stock boundaries based on Perrin *et al*. 1985. Red dots indicate Central American spinners. Blue symbols indicate eastern spinners - boxes are the proposed Tres Marias form. Green dots indicate whitebelly spinners, a proposed intergrade between the

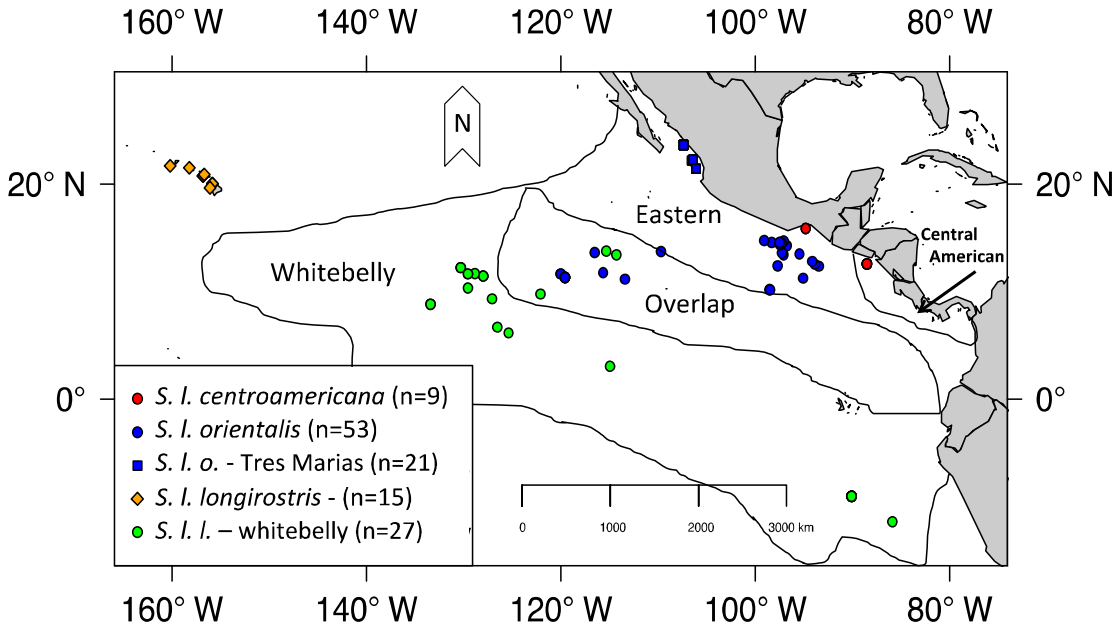

Figure 2. Sampling localities for spotted dolphins with ETP subspecies and stock boundaries based on Perrin *et al.* 1985. Coastal spotted dolphins (*S. a. graffmani*) are in red and offshore (*S a. attenuata*) are in blue. Blue circles indicate sampling locations for the northeastern stock of offshore spotted dolphins. Blue triangles indicate samples from Hawaii. Inverted triangles indicate southern offshore samples that were removed from analyses of offshore stocks because they were collected between 4°N and 6°N; these samples were included in subspecies-level analyses. Animals that represent the western substock were the group of blue squares west of 120°W and animals representing the southern sub-stock were the group of blue squares taken from south of the 5°N stock boundary. Samples sizes for mtDNA analyses presented in the key; for nuDNA sample sizes, see Table 3.