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

Leslie, Matthew S.,[1,2,3*] Archer, Frederick I., [2,1] Morin, Phillip A.[2,1]

1. Scripps Institution of Oceanography, University of California San Diego, mail-code 0208, 9500 Gilman Dr. La Jolla, CA, 92093 USA

2. Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA, 8901 La Jolla Shores Dr., La Jolla, CA 92037, USA

3. Current Affiliation: National Museum of Natural History, Smithsonian Institution, PO Box 37012, MRC 121, Washington, DC 20013-7012

*Author for correspondence: lesliem@si.edu

Running Title: Mitogenomic population structure of pelagic dolphins

Keywords: mitochondrial DNA, conservation genetics, pelagic dolphins

Abstract:

Spinner dolphins (*Stenella longirostris*) and spotted dolphins (*S. attenuata*) show high intraspecific morphological diversity and endemic subspecies in the eastern tropical Pacific Ocean (ETP). Previous studies of mitochondrial DNA (mtDNA) have found low genetic differentiation among most of these groups, possibly due to demographic factors, ongoing gene flow, and/or recent divergence. These species were heavily depleted due to bycatch in the ETP yellowfin tuna fishery. Because population structure is important for accurate management of the recovery of these species, we collected whole mitochondrial genome sequences from 104 spinner and 76 spotted dolphins to test structure hypotheses at multiple hierarchical levels. Our results showed significant differences between subspecies of spotted (F_{ST}: 0.0125; P = 0.0402) and spinner dolphins (F_{ST}: 0.0133; P = 0.034), but no support for the division of existing offshore stocks of spotted dolphins or Tres Marias spinner dolphins. We compare these results to previous results of genome-wide nuclear SNP data and suggest high haplotype diversity, female dispersal, male philopatry, or relative power of the two datasets explains the differences observed. Our results further support a genetic basis for biologically meaningful management units at the subspecies level, and provide a critical component to mitigating historical and continued fishery interactions.

Introduction:

Determining population genetic structure is important for accurately managing protected wildlife species (Taylor 2005). Because mitochondrial DNA (mtDNA) is more abundant in cells and has a higher rate of mutation - thus accruing variability on a time-scale typical of population divergence – it has been the preferred marker for population genetic studies of wildlife (Moritz 1994; Allendorf 2017). Moreover, because of the strictly maternal inheritance of mtDNA, comparing the strength of genetic structure between mtDNA and nuclear DNA (nuDNA) can provide valuable insights into maternal genetic structure and sex-bias dispersal in wildlife populations (Moritz 1994). Mitochondrial DNA data are particularly useful for species with strong matrilineal social structure – such as several toothed whale species (i.e., killer whales, sperm whales, and pilot whales). In cetaceans, whole mtDNA genome (mitogenome) sequencing has provided additional clarity species-level population structure and phylogeographic patterns where single mtDNA markers have not (Archer *et al.* 2013; Morin *et al.* 2010). We expanded upon previous mtDNA datasets and include the whole mitochondrial genome to test for population structure in two species of dolphin.

Fisheries bycatch is arguably the largest threat facing cetaceans today (Read *et al.* 2006). One of the largest fisheries bycatch events in history occurred in the eastern tropical Pacific Ocean (ETP) and heavily impact pelagic spinner and spotted dolphins in this area. These two species were abundant (numbering in the low millions) in the ETP (Wade *et al.* 2007), but because both species commonly associate with one-another and with large tuna (see Scott *et al.* 2012 for details), bycatch in the dolphin-set tuna purse-seine fishery starting in the 1960s killed hundreds of thousands annually (Lo and Smith 1986, National Research Council 1992, Wade 1995). Despite protection under the U.S. Marine Mammal Protection Act of 1972 and multi-national protection under the 1999 Agreement on the

68 International Dolphin Conservation Program (Joseph 1994, Gosliner 1999), ETP spinner
69 and spotted dolphin population abundances remain low (Wade *et al.* 2007, Gerrodette *et*
70 *al.* 2008). Determining how populations are naturally structured in the ETP is critical to
71 accurately managing the recovery of these populations.
72
73 **ETP Spinner Dolphins**
74 Globally, there are four subspecies of spinner dolphin (*Stenella longirostris*). The
75 nominate form, the pantropical spinner (*S. l. longirostris*) inhabit in all tropical waters of
76 the world outside the ETP. Pantropical spinners are usually associated with islands in the
77 central and western Pacific, such as the Hawai‘ian Islands. In shallow waters of Southeast
78 Asia, there is a much smaller dwarf spinner subspecies (*S. l. roseiventris*) (Perrin *et al.*
79 1989, 1999). The Central American spinner dolphin (*S. l. centroamericana*) and the
80 eastern spinner dolphin (*Stenella l. orientalis*) are endemic to the ETP (Fig. 1, based on
81 Perrin 1985). Analyses of external coloration, body size, and the extensive analyses of
82 cranial morphology lead to the erection of these ETP subspecies (Perrin *et al.* 1991,
83 Douglas *et al.* 1992). The Central American subspecies is found off the Pacific coasts of
84 Southern Mexico south through Panama, in relatively near-shore waters. The eastern
85 spinner dolphin (*S. l. orientalis*), on the other hand, inhabits offshore waters that extend
86 from Baja California, Mexico, south to Ecuador (Perrin 1990).
87
88 For management purposes, the two ETP endemic spinner dolphin subspecies are
89 considered stocks, plus a third stock - the whitebelly spinner. The "whitebelly" spinner is
90 proposed to represent a hybrid swarm between the eastern subspecies and the pantropical
91 subspecies of the central and western Pacific (Perrin *et al.* 1991). Taxonomically, it is
92 classified as part of the nominate (pantropical) spinner subspecies *S. l. longirostris*.
93 Significant geographic overlap exists between the eastern subspecies and the whitebelly

form (Perrin *et al.* 1985) (See Fig. 1). 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).

Some molecular genetics approaches have not found genetic structure corresponding to the subspecific morphological differences (Dizon *et al.* 1994, Galver 2002). 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 the pantropical 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. Recently, Leslie and Morin (2016) found strong population structure within both species using genome-wide SNP data.

ETP Spotted Dolphins

The pantropical spotted dolphins (*Stenella attenuata*) in the ETP is split into two subspecies based on morphometric analyses: a coastal endemic subspecies (*S. a. graffmani* - Perrin 1975, Perrin *et al.* 1987) and an offshore pantropical subspecies (*S. a. attenuate*). Genetic analyses of microsatellites show high genetic diversity in spotted dolphins and support 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. Escorza-Treveño *et al.* (2005) identified demographically independent populations within the coastal subspecies and posited that interchange continues between the northern *S. a. graffmani* populations and the offshore pantropical subspecies.

Although the results of Escorza-Treviño *et al.* (2005) indicate substructure, the entire coastal subspecies is currently a single management stock. Offshore pantropical spotted dolphins in the ETP are divided into two stocks: 1) the 'northeastern' (NE) stock is defined geographically as north of 5°N, east of 120°W, and 2) the 'western-southern' (WS) stock is defined as south and west of this northeastern area (Fig. 2) (Perrin *et al.* 1994). A distributional hiatus along 5°N is the basis for the north-south boundary between NE and WS stocks (Perrin *et al.* 1994), and this has recently been supported by SNP analyses (Leslie and Morin 2016).

Given the morphological differentiation between subspecies and recent evidence of nuclear DNA genetic differentiation, we assume the previous results from single mtDNA loci lacked power to resolve these close intraspecific relationships. In addition, studies of other cetacean species have shown mitochondrial genomes to be a useful tool for resolving intraspecific relationships when single mtDNA genes cannot (Archer *et al.*2013, Morin *et al.* 2010.

Objectives

We used DNA capture array library enrichment and highly paralleled DNA sequencing to collect whole mitochondrial genome sequence data from 104 spinner and 76 spotted dolphins to test hypotheses of population genetic structure at multiple hierarchical levels

in the eastern tropical Pacific Ocean. We performed analyses of whole mtDNA genomes (mitogenomes) and individual mtDNA genes to test observed levels of differentiation between recognized and proposed management stocks. We also tested for structure supporting the Tres Marias spinner dolphin and alternative stock boundaries in the offshore spotted dolphins. Although still only representing one locus, mitogenomes allow us to examine matrilineal population structure and contrast our findings with those found in previous studies using nuclear DNA (Escorza-Treviño *et al.* 2005; Andrews *et al.* 2013; Leslie and Morin 2016) to infer sex-biased dispersal.

METHODS

Sample Collection and DNA extraction

Skin samples used in this study were collected from free-ranging animals *via* biopsy dart (Lambertsen 1987) on research cruises or from dead specimens killed as bycatch in the tuna purse-seine fishery between 1982 and 2010 (104 spinner dolphins and 76 spotted dolphins 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 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 method was preferable because: 1) only after observing the group (which could contain > 1,000 individuals) for some time could observers classify it to stock, 2) the external characters distinguishing subspecies are subtle, therefore researchers collecting biopsies from the bow of the research vessel could

not confidently classify fast-swimming individuals in real time, and 3) the ranges of ETP spinner dolphin subspecies overlap making geography an unreliable predictor of stock identity. Some samples were used from areas where the eastern and whitebelly spinners are known to geographically overlap (see Figure 1). Spinner dolphin samples from Hawai‘i spanned the breadth of the main islands and also Midway Atoll.

Because there is little overlap of subspecies distribution in ETP pantropical spotted dolphins, geographic location of the sampling site was used to assign samples to subspecies and stocks. To avoid misassigned individuals near the borders of the NE and WS offshore stocks, we did not use samples collected between 4°N and 6°N east of 125°W. Hawai‘ian spotted dolphin samples were collected from the Kona Coast of Hawai‘i and O‘ahu.

Biopsy samples were stored in salt-saturated 20% DMSO, 70% ethanol, or frozen with no preservative. We extracted DNA using silica-based filter membranes (Qiagen, Valencia, CA) on an automated workstation (Perkin Elmer, Waltham, MA). DNA was quantified using Pico-Green fluorescence assays (Quant-it Kit, Invitrogen, Carlsbad, CA) and a Tecan Genios microplate reader (Tecan Group Ltd, Switzerland).

Library Preparation and Sequencing

Next-generation sequencing libraries were generated as described by Hancock-Hanser *et al.* (2013), using unique 6bp and 7bp index sequences for each individual to allow up to 100 samples to be multiplexed. Multiplexed libraries were enriched for whole mitogenomes and 85 nuclear DNA loci using Sure Select DNA Capture Arrays (Agilent Technologies, Inc., Santa Clara, CA, USA) as described by Hancock-Hanser *et al.* (2013). Sequence data from the 85 nuclear loci were not used in this study. Target

198 sequences for capture enrichment included the reference pantropical spotted dolphin
199 mitochondrial genome (Genbank No. EU557096; Xiong *et al.* 2009) and a suite of 85
200 nuclear loci (not included in this study). Three identical arrays - designed with the eArray
201 software package (Agilent Technologies, Inc., Santa Clara, CA, USA) - were used to
202 capture a multiplexed mix of both species. Each array contained one replicate of the
203 mitogenome probes at a probe interval of 15bp as well as 13 replicates of probes for the
204 nuclear loci at a probe interval of 3bp. Each enriched library was then sequenced using
205 1X100bp Illumina HiSeq technology (two using Illumina HiSeq2000 and one using
206 HiSeq2500).
207
208 **Mitogenome Assembly**
209 Raw read data were filtered for quality (minimum quality score of 15) and demultiplexed
210 by unique barcode. Consensus sequences for each sample were generated from
211 mitogenome sequence reads using a custom pipeline (Dryad data repository
212 doi:10.5061/dryad.cv35b) in R v2.15.0 (R Core Team, 2014). Reads were first mapped to
213 the reference spotted dolphin sequence with the short-read alignment tool BWA (Li and
214 Durbin, 2009). The mpileup module in SAMTOOLS (Li *et al.* 2009) was then used to
215 convert the resulting BAM-format alignment file into a ‘‘pileup’’ text format, which was
216 then parsed by custom R code to create the consensus sequence for each individual. The
217 following rules were used in this process: A “N” was inserted at a position if the
218 assembly had <3 reads, <5 reads where not all contained the same nucleotide, or >5 reads
219 where no one nucleotide (*i.e.*, A, C, G, T) was present in >70% of the reads. All
220 mitogenome sequences were initially aligned with MAFFT using the automatic selection
221 of an appropriate handling strategy (“auto”) and default parameters (Katoh *et al.* 2009)
222 followed by a refinement of alignments by eye.
223

Diversity Estimates and Population Structure Analyses

Two mitogenome data sets were created for each species. First, we partitioned each species' mitogenome into 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) and 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).

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 dolphin 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

We assembled 104 complete or nearly complete (<10% missing data) concatenated spinner dolphin mtDNA data sets (Genbank accession numbers in Supplementary Table S1). 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 each individual gene region 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 (Φ_{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 S3) 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 primarily from drift in the Hawai‘ian population, though some unique haplotypes in Hawai‘i also suggest sequence divergence between the subspecies. 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.

Spotted dolphins

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

Results of F_{ST} and Φ_{ST} analyses of the mtDNA concatenated genes and Φ_{ST} of the individual gene regions for spotted dolphins are presented in Table 4. Similar to the

354 spinner dolphins, our analyses at the subspecies level for spotted dolphins (coastal *vs.*
355 offshore) show no significant differentiation using Φ_{ST} for the concatenated or partitioned
356 data sets. F_{ST} was significant in the concatenated data set (0.0125, $P = 0.0402$).
357
358 Estimates of differentiation between the current management stocks within the offshore
359 subspecies (NE and WS stocks) using the whole mitogenome data and individual mtDNA
360 genes showed no differences. Using Φ_{ST}, no significant differences were observed
361 between the coastal subspecies and the NE offshore stock, however F_{ST} (0.0302) was
362 highly significant at $P = 0.0002$ between theses management units. Similarly, Φ_{ST} was
363 not significant for pairwise comparisons of the Coastal subspecies and WS offshore stock
364 using the concatenated data or individual genes.
365
366 Within the WS offshore stock, we found nearly significant Φ_{ST} differences between the
367 southern and western offshore regions using the concatenated mitogenome (0.1666; $P =$
368 0.0668). One individual mtDNA gene (ND4) had significant differentiation ($p < 0.05$) and
369 three others had nearly significant p-values (16S, ND1, ND5).
370
371 Comparing separate western and southern portions of the WS stock to other partitions
372 using the mitogenome data set also yielded no significant Φ_{ST} estimates. Our comparison
373 of the NE stock to the western portion of the WS stock, however, was nearly significant
374 using the concatenated mitogenome ($\Phi_{ST} = 0.1135$; $P = 0.0517$) and four individual
375 mtDNA genes showed significant Φ_{ST} differences ($p<0.05$). Neither data set showed
376 significant differences between the NE stock and the southern portion of the WS stock for
377 either statistic.
378

379 Comparison of the coastal subspecies to just the southern portion of the WS stock
380 resulted in no significant F_{ST} or Φ_{ST} difference in the concatenated data set or individual
381 gene regions. Between the coastal subspecies and western offshore portion of the WS
382 stock, however, one individual gene region (ATP8) showed significant differentiation (P
383 < 0.05), and one (12S) showed nearly significant differentiation (P = 0.0559). Ideally we
384 would have partitioned the coastal subspecies south of central Mexico into the population
385 units described by Escorza-Triveño *et al.* (2005), but our smaller sample size prevented
386 us from doing this.
387
388 Significant differentiation was detected between Hawai‘i and the coastal subspecies, and
389 between Hawai‘i and offshore spotted dolphins, in F_{ST} and Φ_{ST} of the concatenated data
390 set. As expected, given this result, significant differentiation was detected in many
391 individual mtDNA genes (see Table 4). We also detected significant differences between
392 Hawai‘i and the NE stock in four genes, but not for the concatenated mtDNA data set
393 (although it was nearly significant for Φ_{ST} at P = 0.0645). Hawai‘i and the WS stock
394 were significantly different in the concatentated data set using Φ_{ST}, and in nine individual
395 genes (P < 0.05).
396
397 Finally, we also tested hypotheses of differences between Hawai‘i and divided western
398 and southern portions of the WS stock. Hawai‘i and the western portion were
399 differentiated using the concatenated dataset (Φ_{ST}: 0.4932; P = 0.0179). Ten individual
400 genes showed differentiation between these two strata (see Table 4). Hawai‘i and the
401 southern portion of the WS stock were not differentiated based on our concatenated data
402 sets, but did show significant differentiation in five individual genes (P<0.05).
403
404

Discussion

Spinner and spotted dolphins in the eastern tropical Pacific offer a unique opportunity to study genetic differentiation at multiple scales in species with strong intraspecific morphological differences. Recent divergence, high genetic diversity, large population sizes, and ongoing gene flow likely contribute to low detectability of genetic divergence (Galver 2002, Escorza-Treviño *et al.* 2005, Andrews *et al.* 2013, Taylor and Dizon 1996, Waples 1998). Using complete mitogenomes, we found some genetic support for endemic subspecies of spinner and spotted dolphins, although the strength of this support varies between markers (see Table 5). We did not find support, however, for the division of offshore stocks of spotted dolphins; nor did we find separation of the Tres Marias spinner dolphins as an independent population. In contrast, nuclear SNP analysis recovered these stock-level differences (Leslie and Morin 2016). The difference in our findings compared to those of Leslie and Morin (2016) could reflect the limitations of our mtDNA data or something biologically meaningful about the populations.

First, we will discuss the individual comparisons for each species, then finish with an overall discussion comparing our findings with those of others.

Spinner Dolphins

Traditional F_{ST} was very low as expected, but supported endemic subspecies distinction (Central American and eastern). We found non-significant results from Φ_{ST} – a metric that includes differences in nucleotide divergence. Thus we conclude that haplotypes within these two subspecies are very similar, but that haplotype frequencies are significantly different.

Nevertheless, our results provide evidence of genetic differentiation between the accepted ETP endemic subspecies concordant with morphology (Perrin *et al*. 1991) and results from Andrews *et al*. (2013) who used data from the nuclear Actin gene, and Leslie and Morin (2016) who used restriction-associated nuDNA sequencing. Differences in ecological, distributional, morphological, nuDNA, and now mtDNA data support the recognition of these distinct subspecies.

Breeding biology and movement patterns could also affect the patterns we see between the Central American 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 polygynous mating system (Perrin and Mesnick, 2003). Perrin and Mesnick (2003) 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 breeders 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 analyses.

F_{ST} and Φ_{ST} tests between the whitebelly spinner and the Central American endemic ETP subspecies revealed nearly significant differentiation (Table 5), indicating possible separation. Nuclear SNP data support differences between these two groups (Leslie and Morin 2016). In our mitogenome data set, every whitebelly sample had a unique haplotype. As a result, frequency-based measures of differentiation such as *F*-statistics

were likely underestimated.

Using slightly different samples, 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 cyt*b* and several others (Table 2). Moreover, Andrews *et al.* (2013) included 10 samples of Central American spinners that had questionable subspecific identity (based on further investigation of the sample collection records at SWFSC by MSL). These samples were initially identified as Central American spinners, but the confidence in the identification was low and they should have been labeled as "unidentified". Given the uncertainly, these samples could have been from eastern spinner dolphins. Removal of these samples reduced our representation of Central American spinners (n=9), which may have impacted our ability to detect intraspecific structure. However, the Central American subspecies has lower relative abundance, and therefore might be expected to show higher levels of structure due to drift.

Two biological explanations for the possible differentiation between Central American and whitebelly spinners in the mtDNA are isolation by distance and admixture between whitebellies and Hawai'ian spinners. These are the two most geographically distant putative populations of ETP spinner dolphins; therefore, isolation by distance could contribute to population genetic structuring. Admixture between the whitebelly and Hawai'ian spinners would bring novel haplotypes from the Gray's subspecies (Hawai'i) into the whitebellies resulting in genetic structure.

We found nearly significant Φ_{ST} between the whitebelly spinner and the eastern spinner using the concatenated mitogenome data. In addition, we also found significant differences between these strata in eight individual mtDNA genes. Leslie and Morin's

(2016) SNP analysis supported differentiation of these two groups. Andrews *et al.* (2013) inferred high migration rates between whitebelly and eastern spinner dolphins (30.1 migrants per generation from whitebelly to eastern and 57.9 migrants from eastern to whitebelly). Despite this high rate of migration, we detected evidence of differentiation.

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

Differences in breeding systems could help drive or maintain differentiation between eastern and whitebelly spinner dolphins (Perrin and Mesnick 2003). A polgynous system in eastern spinners could result in higher site-fidelity and lower male *Ne* – both of which would accentuate signal in nuDNA population structure. Alternatively, as discussed above, admixture between whitebelly and Hawai'ian spinners could also result in novel whitebelly genotypes resulting in higher apparent population structure between whitebelly and eastern spinners.

Alternative spinner dolphin stocks:

We found no support for a Tres Marias population that differs from the eastern or Central American subspecies (*e.g.,* Perryman and Westlake 1998) using the concatenated or individual mitochondrial gene data sets. Given the weak genetic differences we found between the accepted endemic subspecies with much more marked morphological differences, this result may not be surprising. We found statistically significant differences in four individual mtDNA genes when comparing the Tres Marias group to the whitebelly spinners and several nearly significant genes. We do not feel confident making taxonomic recommendations for the "Tres Marias" spinners based on these analyses. Additional studies should approach this question using larger sample sets and additional data.

Spotted dolphins:

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

Similar to our findings for spinner dolphins, traditional F_{ST} calculated for the spotted dolphin mitogenome data set supports differentiation of the offshore *S. a. attenuata* and the endemic coastal *S. a. graffmani* subspecies, whereas Φ_{ST} failed to indicate any difference - either for the entire genome or within any single gene. Our results show the

NE stock being strongly differentiated from the coastal subspecies (based on allele frequency alone), counter to the results found by Escorza-Treviño *et al.* (2005) showing connection between the NE stock and the coastal subspecies based on seven microsatellite loci. In that study, the authors inferred that there was a strong connection between the coastal and offshore subspecies in northern Mexico. The differences between our results and those of Escorza-Treviño *et al.* (2005) could be due to sampling; the previous study included more samples from the northern portion of the coastal spotted dolphin range than we did. Additionally, the differences could be attributed to the unique evolutionary patterns of the different markers examined in Escorza-Treviño *et al.* (2005) (*i.e.*, microsatellites) vs. the mitogenomes used in our study.

Spotted dolphin stocks:

A main objective of this work was to test for difference between existing (NE, WS, and Coastal) and proposed (independent W and S) management stocks. Using the whole mtDNA genome data set, we found no evidence for differentiation between the two current stocks (NE and WS). This could be because the two stocks are genetically connected or because our data lack power to detect differentiation at this fine scale. The concatenated mtDNA indicated weak evidenced for splitting up the current WS stock - a high Φ_{ST} value (0.1666) and nearly significant ($P = 0.0668$). Similarly, we detected nearly significant differences between the NE stock and the western group of the WS stock using the concatenated mtDNA genome. Four mtDNA loci had significant Φ_{ST} estimates for this partition. The NE and the offshore southern group were not significantly different in any test, suggesting that the distributional hiatus at 5° north is not a barrier to gene flow. We cannot say with any certainty if this is the case, however, because of the low sample size for the southern portion of WS stock (n=9); a larger sample size is necessary to convincingly investigate this hiatus. Overall, the whole

561 concatenated mtDNA genome was not as useful as anticipated for delimiting stock
562 structure, possibly because it introduced more variation (*via* novel haplotypes) into an
563 already highly variable system. Whole mtDNA genomes have been useful for clarifying
564 subspecific boundaries where information in single mtDNA genes has shown low
565 variability (Archer *et al.*2013, Morin *et al.* 2010), including in this paper, but testing
566 population-level boundaries in highly abundant cetaceans using mtDNA genomes may be
567 less feasible.
568
569 Leslie and Morin (2016) found divergence between the offshore and coastal spotted
570 dolphin subspecies, but did not include data from individuals from the NE offshore stock
571 of spotted dolphins. Therefore, this comparison includes animals from the most
572 geographically separate portions of the offshore (WS) and coastal subspecies range.
573 Additional nuclear data from the NE stock are needed to determine whether proximate
574 populations of these two subspecies are also as genetically divergent.
575
576 **Overall Patterns Observed**
577 Despite the increase in data over other mtDNA studies, it is likely that whole
578 mitogenomes still do not provide enough statistical power to detect differences given the
579 recent divergence, continued low-level interbreeding, and/or high diversity and historical
580 abundance. We collected whole mitogenomic data to help resolve close population
581 relationships. However, one risk of adding more data in this situation – with populations
582 with high genetic diversity – is that haplotype discovery may not plateau within a sample
583 set. In other words, more unique haplotypes are added thereby increasing the difficulty of
584 characterizing haplotype frequencies among and between populations. Sequencing
585 additional samples could help rectify this issue.
586

587 Similarly, there are also limitations to using frequency-based *F*-statistics. F_{ST} is good at
588 detecting frequency differences that indicate genetic structure in cases where haplotypes
589 are similar within populations and different between populations (such as those that
590 would result via drift in small populations). However, when haplotype diversity is high
591 within and among populations, very large sample sizes are needed to characterize
592 haplotype frequencies to detect differences using F_{ST}. In this situation, F_{ST} point values
593 will be underestimated. Moreover, sampling effects can become important drivers of F_{ST}
594 beyond the base frequency of alleles present and result in false positive results. Our initial
595 hypothesis was that sequencing more of the mitogenome would result in more shared
596 differences within populations which would be reflected in both the frequency-based
597 statistics; this was not the case.
598
599 Instead, we found significant differences between subspecies of both spinner and spotted
600 dolphins using F_{ST}, but not Φ_{ST}. F_{ST} and Φ_{ST} provide slightly different perspectives on
601 population differentiation and we believe it is important to present both measures. Our
602 results show inconsistencies between these two metrics, which does not necessarily mean
603 analytical problems or inaccuracies, but reflects something interesting about our data. F_{ST}
604 tests for population differentiation are based on allele (or haplotype) frequencies and do
605 not provide direct insights into levels of molecular divergence (Weir and Cockerham,
606 1984, Excoffier *et al.* 1992, Meirmans and Hedrick 2011). Φ_{ST} estimates capture more
607 information regarding the differentiation due to sequence divergence (or nucleotide
608 diversity) in addition to differences in haplotype frequencies. The high heterozygosity
609 issues mentioned above can still impact Φ_{ST}. Although we chose to focus the bulk of the
610 discussion on Φ_{ST}, we do report statistically significant measures of F_{ST} and briefly
611 compare and contrast the two metrics. Φ_{ST} may be more indicative of older, long-term
612 processes, whereas F_{ST} can show recent differences among populations, indicating

613 another reason it is important to report both metrics. In addition, given that the test for
614 significance is determined by an arbitrary cut-off ($P = 0.05$), we also present results that
615 are "nearly significant". Combined with given the difficulty of distinguishing these
616 groups in previous works, we felt it important not to focus too intensely on the arbitrary
617 cut-off, but rather overall patterns of indicators.
618
619 Moreover, our sample sizes were low in some partitions (n=7). This could result in the
620 allele frequencies of populations being under-characterized, which could skew results in
621 over- or under-classification. Efforts should be made to collect more samples for future
622 studies.
623
624 Alternatively, non-significant results could occur with low levels of geneflow between
625 strata that are demographically independent populations (Avise 1995; Taylor and Dizon
626 1996). Because management decisions rely on them, results must be interpreted within
627 the context of all available information and with recognition of the caveats of the data
628 used to generate them.
629
630 The discordance we observed between the mitogenome results and those using nuDNA
631 data (Leslie and Morin 2016) could also reflect biological factors. One possibility is
632 female-mediated exchange diluting the signal of structure in mtDNA or male site-fidelity
633 increasing structure in the mtDNA. Although there is some evidence from radio tagging
634 studies that spinner and spotted dolphins can move relatively large distances (Perrin *et al.*
635 1979), a thorough investigation into the differences between sexes is lacking. At least for
636 spinner dolphins it is likely that the polygynous breeding system described by Perrin and
637 Mesnick (2003) would contribute to increased signal of structure in the nuclear genome.
638

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 (16S, ATP6, ND2, and ND5) showed population structure (significant Φ_{ST}) in all pairwise comparisons between Hawai'i and ETP spinner dolphin groups (*i.e.*, Central America, Tres Marias, eastern, and whitebelly spinner), but not in any pairwise comparisons between these ETP groups. All of the mtDNA regions with significant Φ_{ST} were found to be under purifying selection (negative Tajima's *D* - Table S3; and non-significant Z-tests – Table S4) 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 population of spotted dolphins were found in all but five of the mtDNA genes. We note however that the low sample sizes for Hawai'ian spotted dolphins may explain some of the non-significant differences observed with respect to ETP stocks.

Positive Selection in ETP Spinner Dolphin mtDNA

Selection should affect linked loci equally; however, selection can act on individual mtDNA genes, such as in the case of cytochrome *b* in Antarctic killer whales (Foote *et al.* 2010). We tested for positive selection in spinner dolphin mitochondrial genes and found none. We did not test for positive selection in spotted dolphins because there were no individual mtDNA genes that supported differentiation between the two ETP subspecies.

Conclusions:

665 Defining population genetic structure is challenging for species with large historical
666 population sizes and high mobility. These populations may retain high genetic variation
667 even as abundance becomes relatively low, which could obscure signals of genetic
668 structure used to designate stock boundaries for estimating population abundance and
669 setting stock-specific mortality limits. Ultimately, without information on structure,
670 populations could be under-classified and unique evolutionary units and populations
671 could go extinct as we may fail to take appropriate conservation action. Alternatively,
672 there is a cost to managing populations as separate when there is no biological basis to do
673 so. Such errors can have economic, social, and political consequences resulting from
674 unnecessary restrictions on human activity. Furthermore, a consistent pattern of these
675 errors will "stiffen the resolve of skeptics and make it difficult to accomplish sound
676 resource management in the future" (Waples 1998).
677
678 This unique system of two delphinids, with available samples collected *in situ* from
679 remote offshore environments encompassing extensive geographic and morphological
680 variation, was used to test for population genetic structure at multiple hierarchical levels
681 in species with high historical abundance and high intra-specific morphological variation.
682 Our results show a complex pattern of genetic structure in the two different data sets for
683 each species. Although complex, we believe the structure observed in our results is
684 biologically meaningful. Given the aforementioned difficulties with detecting structure
685 using genetic techniques in this system – and the supporting morphometric results - even
686 subtle signatures of structure are significant findings. The mitogenome data show support
687 for the endemic ETP spinner and spotted dolphin subspecies.
688
689 We found very little support for the division of offshore stocks of spotted dolphins and no
690 support for the unique form of Tres Marias spinner dolphins as compared to the eastern

691 or Central American subspecies. This is not to say that these biological entities do not
692 exist, just that our mtDNA data do not support them or may not have sufficient power to
693 detect the subtle genetic differences between them. Further, we recommend the collection
694 and analysis of additional samples from the Central American subspecies to compare to
695 existing offshore subspecies samples collected from fisheries bycatch and research
696 cruises. In addition, we recommend additional studies of population structure that
697 incorporate environmental variables as potential population boundaries in this area.
698 Finally, placing these populations within a global phylogeographic context will help
699 provide a better context for our results by fully characterizing intraspecific diversity and
700 establishing the evolutionary process that led to ETP endemism.

Acknowledgements:

MSL was supported by a National Science Foundation (NSF) Graduate Research Fellowship, a NSF Integrative Graduate Education and Research Traineeship Fellowship, the Ralph A. Lewin Graduate Fellowship in Marine Biology at Scripps Institution of Oceanography, the Lerner-Grey Memorial Foundation at the American Museum of Natural History, and the Edna Bailey Sussman Foundation. We would especially like to thank Dr. William F. Perrin who provided guidance and support throughout the development and completion of this study. Danielle Davila, Brittany Hancock-Hanser, Gabriela Serra-Valente, Victoria Pease, Kelly Robertson, Dr. Louella Dolar, Nicole Beaulieu and Morgane Lauf for help in the laboratory and tissue archive. Dr. Karen Martien provided a thoughtful critique of this manuscript. Al Jackson provided copies of original field datasheets. We thank Dr. Robin Baird of Cascadia Research Collective and Dr. Erin Oleson of the NOAA Pacific Islands Science Center for permission to use Hawai‘ian spinner dolphin samples. The Scripps Research Institute provided assistance with sequencing, especially Drs. Steve Head, Lana Shaffer and John Shimashita. Support for this research was also provided from Drs. Lisa Ballance and Barbara Taylor of the Marine Mammal and Turtle Division of SWFSC. We are grateful to William Perrin, Karen Martien, Kim Andrews, Patricia Rosel and three anonymous reviewers for their careful and constructive reviews of the manuscript.

721 **Literature Cited:**

722

723 Aitken, N., S. Smith, C. Schwarz, and P. A. Morin. 2004. Single nucleotide
724 polymorphism (SNP) discovery in mammals: a targeted-gene approach. Molecular
725 Ecology 13:1423-1431.

726

727 Allendorf, F. W. 2017. Genetics and the conservation of natural populations: allozymes
728 to genomes. Molecular Ecology 26(2):420-430.

729

730 Andrews, K. R., W. F. Perrin, M. Oremus, *et al*. 2013. The evolving male: spinner
731 dolphin (*Stenella longirostris*) ecotypes are divergent at Y chromosome but not mtDNA
732 or autosomal markers. Molecular Ecology 22:408-2423.

733

734 Antao, T., A. Lopes, R. J. Lopes, A. Beja-Pereira, and G. Luikart. 2008. LOSITAN: A
735 workbench to detect molecular adaptation based on a F_{ST}-outlier method. BMC
736 Bioinformatics, 9(1):323.

737

738 Archer, .F I., P. E. Adams, B. B. Schneiders. 2016. STRATAG: An R package for
739 manipulating, summarizing and analysing population genetic data. Molecular Ecology
740 Resource. doi:10.1111/1755-0998.12559.

741

742 Archer, F. I., P. A. Morin, B. L. Hancock-Hanser, K. M. Robertson, M. S. Leslie, M.
743 Berube, S. Panigada, B. L. Taylor. 2013. Mitogenomic phylogenetics of fin whales
744 (*Balaenoptera physalus spp*.): genetic evidence for revision of subspecies. PLoS ONE
745 8(5): e63396.

746

747 Avise, J. C. 1995. Mitochondrial DNA polymorphism and a connection between genetics
748 and demography of relevance to conservation." Conservation Biology 9(3): 686-690.
749
750 Beaumont M. A., and R. A. Nichols. 1996. Evaluating loci for use in the genetic analysis
751 of population structure. Proceedings of the Royal Society of Londong B 263:1619-1626.
752
753 Dizon, A.E., W. F. Perrin, and P. A. Akin. 1994. Stocks of dolphins (*Stenella* spp. and
754 *Delphinus delphis*) in the eastern tropical Pacific: a phylogeographic classification.
755 NOAA Technical Report NMFS 119, 20pp.
756
757 Dornburg, A., M. C. Brandley, M. R. McGowen, and T. J. Near. 2012. Relaxed clocks
758 and inferences of heterogeneous patterns of nucleotide substitution and divergence time
759 estimates across whales and dolphins (Mammalia: Cetacea). Molecular Biology and
760 Evolution 29:721-736.
761
762 Douglas, M.E., G. D. Schnell, D. J. Hough, and W. F. Perrin. 1992. Geographic variation
763 in cranial morphology of spinner dolphins *Stenella longirostris* in the eastern tropical
764 Paciic Ocean. Fisheries Bulletin 90:54-76.
765
766 Drummond, A., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by
767 sampling trees. BMC Evolutionary Biology 7:214.
768
769 Duchene, S., F. I. Archer, J. Vilstrup, S. Caballero, and P. A. Morin. 2011. Mitogenome
770 phylogenetics: The impact of using single regions and partitioning schemes
771 on topology, substitution rate and divergence time estimation. PLoS ONE 6(11): e27138.
772 doi:10.1371/journal.pone.0027138.

Eriksson, J., G. Larson, U. Gunnarsson, B. Bed'hom, M. Tixier-Boichard, *et al.* 2008. Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken. PLoS Genetics 4(2): e1000010 10.1371/journal.pgen.10000010.

Escorza-Treviño, S., F. I. Archer, M. Rosales, A. Dizon, and A. Lang. 2005. Genetic differentiation and intraspecific structure of Eastern Tropical Pacific spotted dolphins, *Stenella attenuata*, revealed by DNA analyses. Conservation Genetics 6(4): 587-600.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611-2620.

Excoffier, L. and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564-567.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131(2):479.

Foll, M. and O. E. Gaggiotti. 2008. A genome scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. Genetics 180:977-993.

798 Galver, L. 2002. The molecular ecology of spinner dolphins, *Stenella longirostris*:
799 genetic diverstiy and population structure. Ph.D. Dissertation, University of California.
800
801 Gerrodette, T., G. Watters, W. Perryman, and L. T. Balance. 2008. Estimates of 2006
802 dolphin abundance in the eastern tropical Pacific, with revised estimates from 1986-2003.
803 NOAA Tech. Memo. NMFS-SWFSC-422.
804
805 Gosliner, M. L. 1999. The tuna-dolphin controversy. Pages 120-155 *in* J. R. Twiss Jr. and
806 R. R. Reeves, eds. Conservation and management of marine mammals. Smithsonian
807 Institution Press, Washington DC.
808
809 Hancock-Hanser, B., A. Frey, M. S. Leslie, P. H. Dutton, and P. A. Morin. 2013.
810 Targeted multiplex next-generation sequencing: Advances in techniques of mitochondrial
811 and nuclear sequencing for population genomics. Molecular Ecology Resources 13:254-
812 268.
813
814 Hoekstra, H. E., R. J. Hirschmann, R. A. Bundey, P. A. Insel, and J. P. Crossland. 2006.
815 A single amino acid mutation contributes to adaptive beach mouse color pattern. Science,
816 313(5783):101-104.
817
818 Hoelzel, A. R., J. Hey, M. E. Dahlheim, C. Nicholson, V. Burkanov, and N. Black. 2007.
819 Evolution of population structure in a highly social top predator, the killer whale.
820 Molecular Biology and Evolution 24 (6):1407-1415.
821
822 Horne, J. B. 2014. Thinking outside the barrier: neutral and adaptive divergence in Indo-
823 Pacific coral reef faunas. Ecology and Evolution 28(6):991-1002.

824

825 Hubbard, J. K., J. A. C. Uy, M. E. Hauber, H. E. Hoekstra, and R. J. Safran. 2010.
826 Vertebrate pigmentation: from underlying genes to adaptive function. Trends in Genetics,
827 26(5):231-239.

828

829 Hubisz, M., D. Falush, M. Stephens, and J. Pritchard. 2009. Inferring weak population
830 structure with the assistance of sample group information. Molecular Ecology Resources
831 9:1322-1332.

832

833 Joseph, J. 1994. The tuna-dolphin controversy in the eastern Pacific Ocean: Biological,
834 economic and political impacts. Ocean Development and International Law 25:1-30.

835

836 Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules. Pages 21-132 in H.
837 M. Munro, ed. Mammalian protein metabolism. Academic Press, New York.

838

839 Kalinowsky, S. T. 2010. The computer program STRUCTURE does not reliably identify
840 the main genetic clusters within species: simulations and implications for human
841 population structure. Heredity 106(4):625-632.

842

843 Katoh, K., G. Asimenos, and H. Toh. 2009. Multiple alignment of DNA sequences with
844 MAFFT. Methods in Molecular Biology 537:39–64.

845

846 Lambertsen, R. H. 1987. A biopsy system for large whales and its use for cytogenetics.
847 Journal of Mammalogy 68:443-445.

848

849 Leslie, M. S., and P. A. Morin. In press. Using genome-wide SNPs to detect structure in
850 high-diversity and low-divergence populations of severely impacted eastern tropical
851 Pacific spinner (*Stenella longirostris*) And pantropical spotted dolphins (*S. attenuata*).
852 Frontiers in Marine Science 3:253. doi: 10.3389/fmars.2016.00253.
853
854 Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows -
855 Wheeler transform. Bioinformatics 25:1754-1760.
856
857 Li, H., B. Handsaker, A. Wysoker, *et al.* 2009. The sequence alignment/map format and
858 SAMtools. Bioinformatics 25:2078-2079.
859
860 Lo, N., and T. D. Smith. 1986. Incidental mortality of dolphins in the eastern tropical
861 Pacific, 1959-72. Fishery Bulletin 84: 27-34.
862
863 Lyons, L., T. Laughlin, N. Copeland, *et al.* 1997. Comparative anchor tagged sequences
864 (CATS) for integrative mapping of mammalian genomes. Nature Genetics 15:47-56.
865
866 McKenna, A., M. Hanna, E. Banks, *et al.* 2009. The Genome Analysis Toolkit: a
867 MapReduce framework for analyzing next-generation DNA sequencing data. Genome
868 Research 20(9):1297-1303.
869
870 Meirmans, P. G. and P. W. Hedrick 2011. Assessing population structure: FST and
871 related measures. Molecular Ecology Resources 11(1):5-18.
872

873 Morin, P. A., F. I. Archer, A. D. Foote, *et al.* 2010. Complete mitochondrial genome
874 phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species.
875 Genome Research 20: 908-916.
876
877 Morin, P. A., F. I. Archer, V. L. Pease, *et al.* 2012. Emperical comparison of single
878 nucleotide polymorphisms and microsatellites for population and demographic analyses
879 of bowhead whales. Endangered Species Research 19:129-147.
880
881 Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical
882 review. Molecular Ecology 3:401-411.
883
884 Narum, S. R., and J. E. HESS. 2011. Comparison of F_{ST} outlier tests for SNP loci under
885 selection. Molecular Ecology Resources 11:184–194.
886
887 National Research Council. 1992. Dolphins and the tuna industry. National Academy
888 Press, Washington DC.
889
890 Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press.
891
892 Nei, M., and Gojobori T. 1986. Simple method for estimating the number of synonymous
893 and nonsynonymous nucleotide substitutions. Molecular Biology and Evolution 3:418-
894 426.
895
896 Norris, Richard D. 2000. Pelagic species diversity, biogeography, and evolution.
897 Paleobiology 26.sp4: 236-258.
898

899 Perrin, W. F. 1990. Subspecies of Stenella longirostris (Mammalia: Cetacea:
900 Delphinidae). Proceedings of the Biological Society of Washington 103:453-463.
901
902 Perrin, W. F., P.A. Akin, and J. V. Kashiwada. 1991. Geographic variation in external
903 morphology of the spinner dolphin *Stenella longirostis* in the eastern Pacific and
904 implications for conservation. Fisheries Bulletin 89(3):411-428.
905
906 Perrin, W. F., M. L. L. Dolar, and D. Robineau. 1999. Spinner dolphins (*Stenella*
907 *longirostris*) of the western Pacific and Southeast Asia: pelagic and shallow-water forms.
908 Marine Mammal Science 15(4): 1029-1053.
909
910 Perrin, W. F., W. E. Evans and D. B. Holts. 1979. Movements of pelagic dolphins
911 (*Stenella* spp.) in the eastern tropical Pacific as indicated by results of tagging, with
912 summary of tagging operations, 1969-76. NOAA Technical Memo NMFS 737:14 pp.
913
914 Perrin, W. F., and J. R. Henderson, 1979. Growth & reproductive rates in two populations
915 of spinner dolphins, *Stenella longirostris*, with different histories of exploitation. Reports
916 of the International Whaling Commission Special Issue 6:417–430.
917
918 Perrin W. F., and S. L. Mesnick. 2003. Sexual ecology of the spinner dolphin, *Stenella*
919 *longirostris*: Geographic variation in mating system. Marine Mammal Science 19:462-
920 483.
921
922 Perrin, W. F., N. Miyazaki, and T. Kasuya. 1989. A dwarf form of the spinner dolphin
923 (*Stenella longirostris*) from Thailand. Marine Mammal Science 5:213-227.
924

925 Perrin, W. F., G. D. Schnell, D. J. Hough, J. W. Gilpatrick Jr., and J. V. Kashiwada. 1994.
926 Reexamination of geographic variation in cranial morphology of the pantropical spotted
927 dolphin, *Stenella attenuata*, in the eastern Pacific. Fisheries Bulletin 92(2):324-346.
928
929 Perrin, W. F., M. D. Scott, G. J. Walker, and V. L. Cass. 1985. Review of geographical
930 stocks of tropical dolphins (*Stenella* spp. and *Delphinus delphis*) in the eastern Pacific.
931 NOAA Technical Report NMFS 28. 28 pp.
932
933 Perrin, W. F., E. D. Mitchell, J. G. Mead, D. K. Caldwell, M. C. Caldwell, P. J. H. Van
934 Bree, and W. H. Dawbin. 1987. Revision of the spotted dolphins, *Stenella* spp. Marine
935 Mammal Science 3:99–170.
936
937 Perryman, W. L., and R. L. Westlake. 1998. A new geographic form of the spinner
938 dolphin, *Stenella longirostris*, detected with aerial photogrammetry. Marine Mammal
939 Science 14:38-50.
940
941 Posada, D. 2008. jModelTest: Phylogenetic model averaging. Molecular Biology and
942 Evolution 25:1253-1256.
943
944 Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure
945 using multilocus genotype data. Genetics 155.2:945-959.
946
947 R Core Team. 2014. R: A language and environment for statistical computing. R
948 Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
949

950 Read A. J., P. Drinker, S. Northridge. 2006. Bycatch of Marine Mammals in U.S. and
951 Global Fisheries. Conservation Biology 20(1):163-169.
952
953 Rieder, S., S. Taourit, D. Mariat, B. Langlois, and G. Guérin. 2001. Mutations in the
954 agouti (ASIP), the extension (MC1R), and the brown (TYRP1) loci and their association
955 to coat color phenotypes in horses (*Equus caballus*). Mammalian Genome, 12(6):450–
956 455.
957
958 Russello, M. A., S. L. Kirk, K. K. Frazer, and P. J. Askey. 2012. Detection of outlier loci
959 and their utility for fisheries management. Evolutionary Applications 5:39–52.
960
961 Scott, M. D., S. J. Chivers, R. J. Olson, P. C. Fiedler, and K. Holland. 2012. Pelagic
962 predator associations: Tuna and dolphins in the eastern tropical Pacific Ocean. Marine
963 Ecology Progress Series 458:283-302.
964
965 Stamatakis, A., T. Ludwig, and H. Meier. 2005. RAxML-III: a fast program for
966 maximum likelihood-based inference of large phylogenetic trees. Bioinformatics 21:456-
967 463.
968
969 Stewart, J. B., C. Freyer, J. L. Elson, A. Wredenberg, Z. Cansu, A. Trifunovic, N.
970 Larrson. 2008. Strong purifying selection in transmission of mammalian mitochondrial
971 DNA. PLoS Biol 6(1): e10. doi: 10.1371/journal.pbio.0060010
972
973 Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations.
974 Genetics 105(2):437-460.
975

976 Tamura, K. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the
977 control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology
978 and Evolution 10:512–26.
979
980 Tamura, K., D. Peterson, N. Peterson, *et al.* 2011. MEGA5: Molecular Evolutionary
981 genetics analysis using maximum likelihood, evolutionary distance, and maximum
982 parsimony methods. Molecular Biology and Evolution 28:2731-2739.
983
984 Tavare, S. 1986. Some probabilistic and statistical problems on the analysis of DNA
985 sequences. Lectures on Mathematics in the Life Sciences 17:57-86.
986
987 Taylor, B. L. 2005. Identifying Units to Conserve. Marine Mammal Research:
988 Conservation Beyond Crisis. Baltimore, MD. The Johns Hopkins University Press. 149-
989 164.
990
991 Taylor, B. L., and Dizon A. E. 1996. The need to estimate power to link genetics and
992 demography for conservation. Conservation Biology 10:661–664.
993
994 Taylor, B. L., Chivers, S. J., Sexton, S., and Dizon, A. E. 2000. Evaluating dispersal
995 estimates using mtDNA data: Comparing analytical and simulation approaches.
996 Conservation Biology 14:1287–1297.
997
998 Wade, P. R. 1995. Revised estimates of incidental kill of dolphins (Delphinidae) by the
999 purse-seine tuna fishery in the eastern tropical Pacific, 1959-1972. Fishery Bulletin
1000 93:345-354.
1001

1002 Wade, P. R., G. M. Watters, T. Gerrodette, and S. B. Reilly. 2007. Depletion of spotted
1003 and spinner dolphins in the eastern tropical Pacific: Modelling hypotheses for their lack
1004 of recovery. Marine Ecology Progress Series 343:1-14.
1005
1006 Waples, R. S. 1998. Separating the wheat from the chaff: patterns of genetic
1007 differentiation in high gene flow species. Journal of Heredity 89(5):438-450.
1008
1009 Willing, E.-M., C. Dreyer, and C. van Oosterhout. 2012. Estimates of genetic
1010 differentiation measured by F_{ST} do not necessarily require large sample sizes when using
1011 many SNP markers. PLoS ONE 7(8): e42649.
1012
1013 Weir, B.S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of
1014 population structure. Evolution 38(6):1358-1370.
1015
1016 Wright, S. 1949. The genetical structuring of populations. Annals of Eugenics 15(1):323-
1017 354.
1018
1019 Xiong, Y., M. C. Brandley, S. Xu, K. Zhou, and G. Yang. 2009. Seven new dolphin
1020 mitochondrial genomes and a time-calibrated phylogeny of whales. BMC Evolutionary
1021 Biology 9:20.
1022
1023

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 mtDNA and nuDNA data sets (sample sizes in parentheses). In the mtDNA column, a "□" denotes significance of whole mtDNA based on at least one measure (see Tables 2-4), 'ns' = non-significant, " ~ " = indicating possible structure with *P*-value between 0.05 and 0.1. "# Genes" is the number of significant mtDNA genes ("~" + # for nearly significant genes). For the nuDNA column, a "□" denotes significance and '*NA*' denotes not tested (Leslie & Morin 2016; *Escorza-Treviño *et al*. 2005).

			mtDNA		nuDNA
Spinner dolphins	**Taxon 1 (n_{mt}/n_{nuc})**	**Taxon 2 (n_{mt}/n_{nuc})**	**Whole**	**# Genes**	
Test of endemic subspecies	Central American (9/9)	eastern (53/36)	□	0	□
Testing whitebelly intergrade	Central American (9/7)	whitebelly (27/15)	~	7: ~1	□
Testing whitebelly intergrade	eastern (54/36)	whitebelly (27/15)	~	8; ~1	□
Alternative stock hypotheses	Tres Marias (21/12)	Central American (9/9)	~	0	□
""	Tres Marias (21/12)	Eastern (32/36)	ns	0	□
""	Tres Marias (21/12)	Whitebelly (27/12)	~	4; ~5	□
""	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 (24/27)	□	0	□*
Testing existing stocks	Offshore NE (25/15)	Off. western-southern (17/16)	ns	0	□
Testing existing stocks	Offshore NE (25/15)	Coastal (24/27)	□	0	□
Testing existing stocks	Offshore WS (17/16)	Coastal (24/27)	~	0	□
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

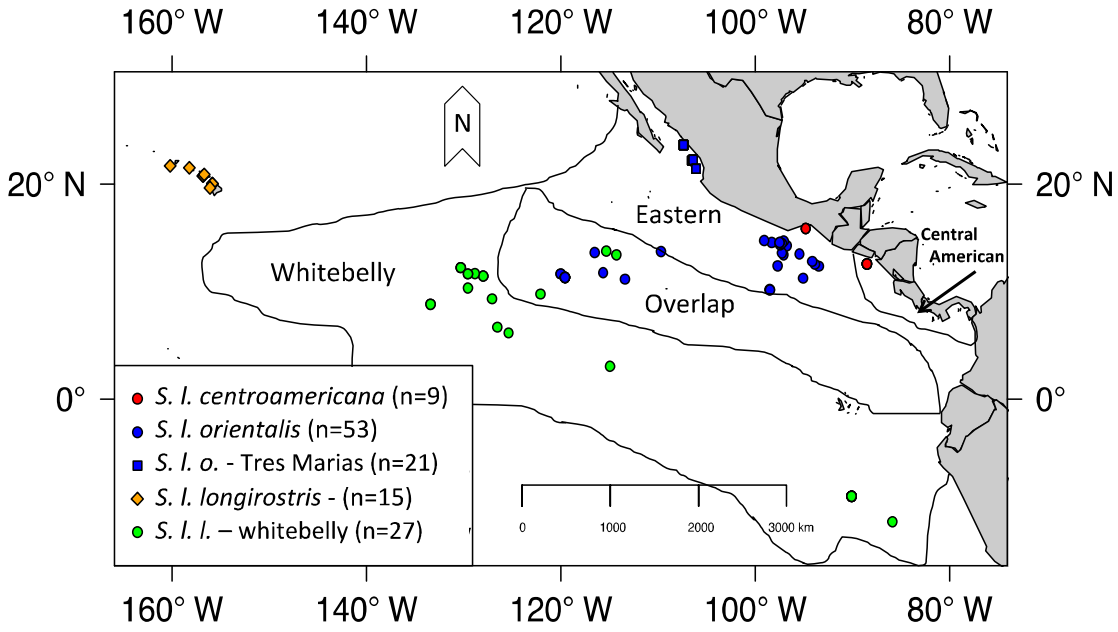

Figure 2. Sampling localities for spotted dolphins with ETP subspecies and stock boundaries based on Perrin *et al.* 1985. Coastal spotted dolphins (*S. a. graffmani*) are in red and offshore (*S a. attenuata*) are in blue. Blue circles indicate sampling locations for the northeastern stock of offshore 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 legend.