A FEASIBILITY STUDY TO EVALUATE USING MOLECULAR GENETIC DATA TO STUDY POPULATION STRUCTURE OF EASTERN NORTH PACIFIC *DELPHINUS DELPHIS*

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ABSTRACT

Only one management unit of *Delphinus delphis* is recognized off the coasts of California, Oregon and Washington: the CA/OR/WA stock. However, available data on dorsal fin coloration patterns, contaminant concentrations and reproductive seasonality suggest there may be more than one stock. To assess the feasibility of using a molecular genetic marker to test the hypothesis of a panmictic CA/OR/WA stock, we analyzed mitochondrial DNA control region sequence data for 63 *D. delphis* specimens assigned to five putative populations: the central eastern tropical Pacific, northern eastern tropical Pacific, and southern, central and northern California. Evidence of genetic distinctness was detected for putative populations within the CA/OR/WA stock, which supports the biological evidence suggesting multiple stocks. These results, which are based on a relatively small data set representing a large population (> 350,000 animals) with high haplotypic diversity (98%), led us to conclude that a molecular genetic study of population structure was feasible for the eastern North Pacific *D. delphis* population.

INTRODUCTION

Our objective was to evaluate whether molecular genetic data could be used to study population structure of *Delphinus delphis* (short-beaked common dolphin) in the eastern North Pacific, because ultimately, we would like to determine whether the CA/OR/WA stock designated for managing *D. delphis* represents a single panmictic stock. One advantage of using molecular genetic markers is that they reveal patterns of gene flow, and thus, evidence of population structure. However, molecular genetic markers have inherently low power to detect low, but demographically significant dispersal rates (Dizon et al., 1995; Hudson et al., 1992; Taylor et al., 1997), and the CA/OR/WA stock of *D. delphis* has three characteristics that may preclude the use of genetic data to study population structure: (1) high abundance (*i.e*., 373,573; CV = 0.19; Barlow 1997), (2) high haplotypic diversity (*i.e*., 93% haplotypic diversity; Rosel et al., 1994) and (3) an essentially continuous distribution within the region. Therefore, we thought a feasibility study was warranted. The genetic marker we chose for analyses was the mitochondrial DNA (mtDNA) control region, because it is haploid and maternally inherited, and therefore has an effective population size approximately a quarter of that for diploid nuclear markers, which results in relatively rapid differentiation of population subunits, primarily due to genetic drift, when gene flow is limited (*i.e*., negligible movement of breeding females). To interpret the results from this feasibility study, we interpreted our statistical results with $\alpha = 0.1$ and considered evidence of low P-values (*i.e*., $P < 0.1$) sufficient to warrant further study.

The CA/OR/WA stock of *D. delphis* is affected by the California drift gillnet fishery, which targets common thresher shark (*Alopias vulpinus*), short-finned mako shark (*Isurus oxyrinchus*) and swordfish (*Xiphias gladius*). The observed incidental kill of marine mammals in this fishery has been documented since 1988 (Herrick and Hanan, 1988; Hanan *et al.*, 1993; Barlow *et al.*, 1994), and annual estimates of mortality for each species impacted by the fishery have been made since 1990 when the National Marine Fisheries Service implemented an observer program for the fishery (Lennert *et al*., 1994; Julian and Beeson, 1998; Cameron, 1998; Cameron and Forney, 1999; 2000; Carretta, 2001; 2002). Even though *D. delphis* has the highest mortality rates of all cetacean species affected by this fishery, best available data indicate that the incidental fishery mortality is less than the estimated number of 'potential biological removals' (PBR) the population can withstand. Average annual mortality estimates have ranged from 79 for 1994-98, which is the number used in the current stock assessment report, to 280 for the period 1990 to 1993. The current estimate of PBR is 3,188, which is much greater than the current estimate of average annual mortality (Carretta *et al*., 2002). However, this assessment would be incorrect if the current management unit does not accurately reflect the structure of the population (see Taylor, 1997).

D. delphis is widely distributed in the eastern North Pacific (Fig. 1) and, in addition to the California drift gillnet fishery, this species is incidentally killed in the yellowfin tuna purse-seine fishery operating in the eastern tropical Pacific (ETP) and the high seas driftnet fishery operating in the central North Pacific. Four management units are recognized in the region: the southern, central and northern ETP, and CA/OR/WA stocks (Perrin *et al*., 1985; Carretta *et al*., 2002). Research vessel surveys have documented the distribution of *D. delphis* in the ETP, and hiatuses in distribution define the boundaries of

the southern, central and northern stocks of *D. delphis* in the ETP (Fig. 1). On the other hand, the CA/OR/WA stock boundaries are geo-political. The southern boundary is the U.S.A./Mexico border, which separates the northern ETP stock and the CA/OR/WA stock, the western boundary is the 200 nm exclusive economic zone of the U.S.A., and the northern boundary is the U.S.A./Canada border. However, research vessel surveys off the coasts of California, Oregon and Washington have documented that the distribution of *D. delphis* extends beyond these boundaries (Fig. 2; Barlow, 1995; 1997). In addition to the discrepancy between the CA/OR/WA stock boundaries and the distribution of animals in the region, most of the drift gillnet fishing effort is off southern CA (Julian and Beeson, 1998). Thus, aligning the stock boundaries to reflect population structure would improve our ability to interpret the impact of fisheries on these dolphins and to develop appropriate management plans.

Differences in overall adult size and reproductive seasonality have been documented for the central and northern ETP stocks of dolphin (Perryman and Lynn, 1993), and differences in dorsal fin coloration patterns (Farley, 1995), contaminant loading (NWFSC and SWFSC unpublished data) and reproductive seasonality (SWFSC unpublished data) have been documented for animals inhabiting the waters off northern and southern California (*i.e.*, within the CA/OR/WA stock). These studies subdivided data sets for analyses north and south of 35° N latitude (*i.e.*, the approximate latitude of Point Conception, CA), which separates "warm-temperate" from "cool-temperate" oceanographic regions. On the basis of these results, we hypothesized that molecular genetic markers may also reveal limited dispersal of animals between northern and southern California. In this report, we present our analyses of a preliminary mtDNA control region sequence data set to test hypotheses of population structure within the eastern North Pacific population of *D. delphis* and our assessment of whether an expanded study would be feasible and warranted.

MATERIALS AND METHODS

Samples used in this study were collected between 1989 and 2000 from animals incidentally killed in the California driftnet fishery (n=35) or biopsied at sea (n=28). Sixty (60) of the 63 samples were collected between September and January, hopefully minimizing any confounding effects of seasonal movements. All tissues (*i.e.*, 58 skin, 5 muscle or internal organ) were preserved in a 20% dimethylsulphoxide solution saturated with NaCl (Amos and Hoelzel, 1991; Amos, 1997) and are archived at the Southwest Fisheries Science Center (SWFSC; contact author SJC for information).

The 5' end of the hypervariable mtDNA control region was amplified from extracted genomic DNA (Gemmell and Akiyama, 1996) using primers H16498[1] (5'-cctgaagtaagaaccagatg- 3') (Rosel *et al*., 1994) and L15812 (5'-cctccctaagactcaaggaag- 3') (developed at the SWFSC) (Saiki *et al.*, 1988; Palumbi *et al.*, 1991). Both strands of the amplified DNA product for each specimen were sequenced independently as mutual controls using standard protocols on the Applied Biosystems Inc. (ABI) model 377 sequencer. All sequences were aligned using SEQED, version 1.0.3 software (ABI, 1992), and the final sequences were 401 base pairs long.

The computer program Arlequin, version 2.0 was used to calculate haplotypic diversity, to estimate genetic divergence between putative populations as expressed by Φ_{ST} (*i.e.*, a genetic distance-based statistic) and to generate a minimum spanning network to examine the phylogenetic and geographic concordance among haplotypes (Schneider *et al*., 2000). We also compared putative populations using χ^2 (*i.e.*, a frequency-based statistic) and examined the evolutionary relationships among haplotypes using the program Phylogenetic Analysis Using Parsimony, version 4.0 (PAUP; Swofford, 1993).

We tested the null hypothesis of a panmictic population of *D. delphis* in the eastern North Pacific using both Φ_{ST} and χ^2, because each statistic provides a different measure of genetic distinctness. The Φ_{ST} statistic detects differences in the relatedness of haplotypes between strata, which change due to drift and mutation when there is essentially no gene flow between groups. That is, statistically significant Φ_{ST} values mean that haplotypes within a stratum are more closely related (*i.e.*, have a smaller genetic distance

[1] Primer names reference their location within the fin whale sequence published in Árnason *et al*., 1991.

or be more genetically homogenous) to each other than to those found in other strata. This statistic uses genetic distance to quantify relatedness, and we used the number of homologous nucleotide differences between two individuals as the measure of genetic distance. Φ_{ST} is analogous to the more familiar F-statistic but is modified for pairwise comparisons of genetic distance data and tests significance with a non-parametric permutation method in an analysis of variance framework (AMOVA; Excoffier *et al*., 1992). On the other hand, χ^2 detects differences in haplotype frequencies between strata, which are expected to be different due to the complicated interplay of dispersal (albeit low) and genetic drift, and makes no assumptions about the evolution or relatedness of haplotypes (Rolf and Bentzen, 1989).

Conventional analyses designed to detect intra-specific structure are based on *a priori* stratification of the samples using non-genetic criteria (e.g. a distributional hiatus or geographic barrier). Our *a priori* stratification of samples was made primarily on the basis of sampling discontinuities and recognized the following distinct sampling sites: central ETP, northern ETP, southern CA, central CA and northern CA. However, for the southern CA strata we defined the northern boundary on the basis of oceanographic habitat rather than latitude. *D. delphis* inhabit cool temperate (*i.e*., California Current) waters off central and northern California, and warm sub-tropical water off southern California (Forney, 2000). However, the Southern California Bight is influenced by both cool temperate and warm sub-tropical waters, and in this area (*i.e*., between 30^o and 35^o N latitude) a mix of dorsal fin coloration patterns was found (Farley, 1995). Therefore, we defined the northern boundary of our southern CA putative population as the intersection of the cool and warm temperate water masses that characterize the region (Fig. 3). We interpreted our statistical tests for genetic differentiation between putative populations with $\alpha = 0.1$. We considered rejecting H_o when $P<0.10$ as evidence of genetic differentiation between *a priori* strata, because our data set was small, the statistics have low power and we were primarily interested in assessing the feasibility of using genetic data to study intra-specific structure.

RESULTS

There were 48 haplotypes among the 63 sequences generated (Table 1). Haplotypic diversity was 0.977 (+/- 0.0118), and the mean number of pairwise differences between haplotypes was 7.6324 (+/- 3.607) (Table 2). Within the 401 base pair sequences, there were 62 polymorphic sites including 58 transitions, 4 transversions and 4 indels.

Phylogenetic reconstructions revealed no geographic concordance among haplotypes. However, statistically significant genetic divergence was revealed between putative populations: central ETP, northern ETP, southern CA, central CA and northern CA, by Φ_{ST} or χ^2. The overall Φ_{ST} statistic was statistically significant in the AMOVA ($\Phi_{ST}= 0.041$; $p \leq 0.002$), and several pairwise comparisons of neighboring putative populations were also statistically significant. Specifically, the southern CA stratum was significantly different from both the northern ETP and the central CA strata. Using χ^2, we also found statistically significant evidence of genetic differentiation for the southern and central CA pairwise comparison (Table 3). Three strata were not statistically distinguishable from their neighboring putative populations: the northern and central ETP strata and the northern CA stratum. However, we would not expect to be able to statistically distinguish these strata from their neighbors because each sample sequenced represented a different haplotype (Table 4). Rather than pooling these strata with their nearest neighbor for analyses, we kept them separate and present the results for pairwise comparisons of all putative populations. We decided on this approach because this was a feasibility study with small sample sizes and high haplotypic diversity in each stratum (*i.e*., there are few common haplotypes; Table 4).

DISCUSSION

The apparent genetic distinctness of the southern CA stratum suggests that the CA/OR/WA stock likely contains multiple stocks. Given the small number of samples in our preliminary data set, the apparent high

haplotypic diversity of the species and the inherently low power of the test statistics: Φ_{ST} and χ^2 (Dizon *et al*., 1995; Taylor *et al*., 1997), detecting evidence of genetic distinctness was not expected. However, when these results are considered together with the available information suggesting limited movement of animals between southern and northern CA: dorsal fin coloration patterns, contaminant loading and reproductive seasonality, the preponderance of evidence suggests that *D. delphis* off southern CA are a separate stock. The approach of combining evidence from several disparate data sets (e.g., morphological, contaminant and genetic studies) has been applied in studies of intra-specific structure as a means to make an inference about animal movement patterns based on a preponderance of evidence (Dizon *et al*., 1992). When this is done, one would not necessarily expect or demand each contrast to be significant for each criterion, but a significant finding in any marker provides information that animal movement may be limited.

We have highlighted the apparent genetic distinctness of the southern CA stratum because we are specifically interested in whether the CA/OR/WA stock of *D. delphis* is panmictic, but these results are also the first look at genetic markers for evidence of intra-specific population structure of *D. delphis* in the eastern North Pacific. Although the central and northern ETP stocks were not statistically distinguishable from each other in our analyses, the recognition of these stocks is well supported by other data and our sample sizes were small (*i.e*., n = 6/stratum). Specifically, there is a hiatus in distribution between the stocks (Fig. 1; Perrin *et al*., 1985) as well as documented differences in average adult size and reproductive seasonality. That is, the mean total body length of northern ETP adults was 179.0 cm and the mean for central ETP adult females was 191.2 cm. Furthermore, reproductive seasonality differed between stocks. The northern ETP stock had a spring peak in reproduction with births occurring between January and July, while the central ETP stock had births occurring throughout the year (Perryman and Lynn, 1993). We would expect that additional samples for genetic analyses would reveal genetic differentiation between these stocks.

Our interpretation of results from these analyses is that a molecular genetic study of population structure in eastern North Pacific *D. delphis* is feasible. Furthermore, the molecular genetic evidence that the CA/OR/WA stock is not panmictic warrants further study, and we recommend expanding the study to more appropriately examine the question of intra-specific structure within eastern North Pacific *D. delphis*.

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Figure 1. Sightings of short-beaked common dolphin (*Delphinus delphis*) made on Southwest Fisheries Science Center (SWFSC) research cruises in the eastern North Pacific from 1974 to 2002 (+). Four stocks or management units are recognized within the region: (1) southern, which is south of 3° N latitude, (2) central, which is between 3° N and 15° N latitude, (3) northern, which is north of 15° N to the U.S.A./Mexico border, and (4) CA/OR/WA, which is the 200 nm exclusive economic zone of the west coast of the U.S.A. and is shown in more detail in Figure 2.

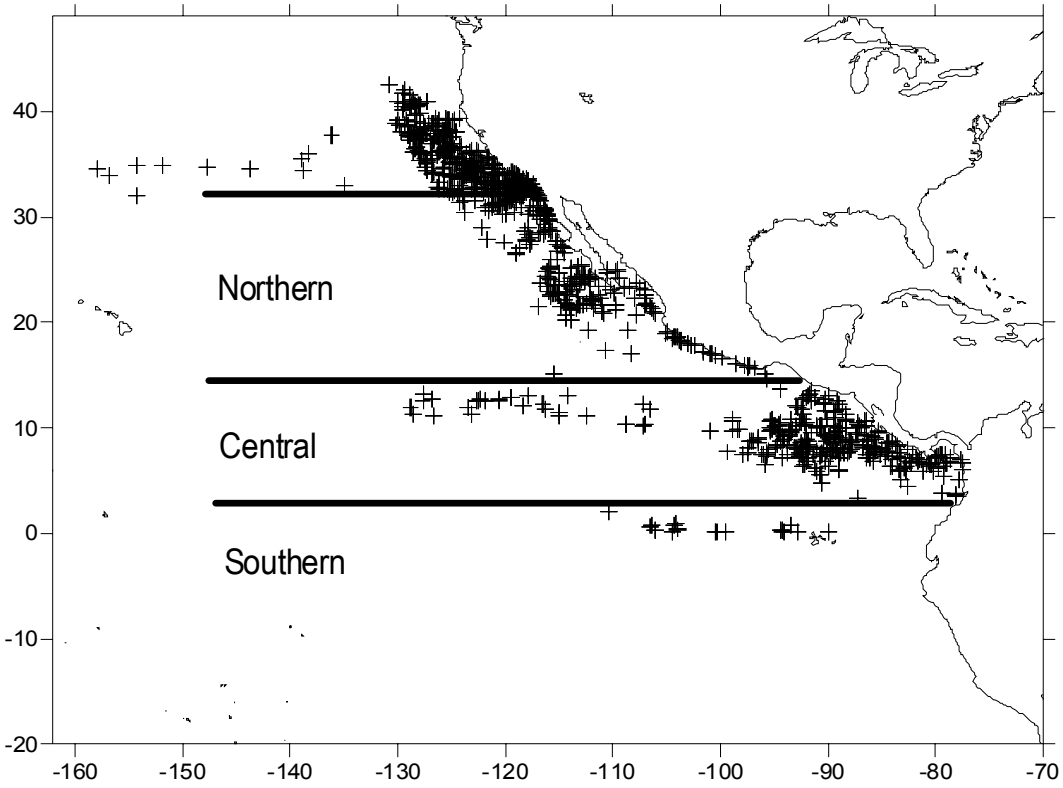


Figure 2. At-sea sightings of *D. delphis* recorded during SWFSC research cruises. The outer bold line is the study area border, and the dashed line is the 200 nm exclusive economic zone of the U.S.A.

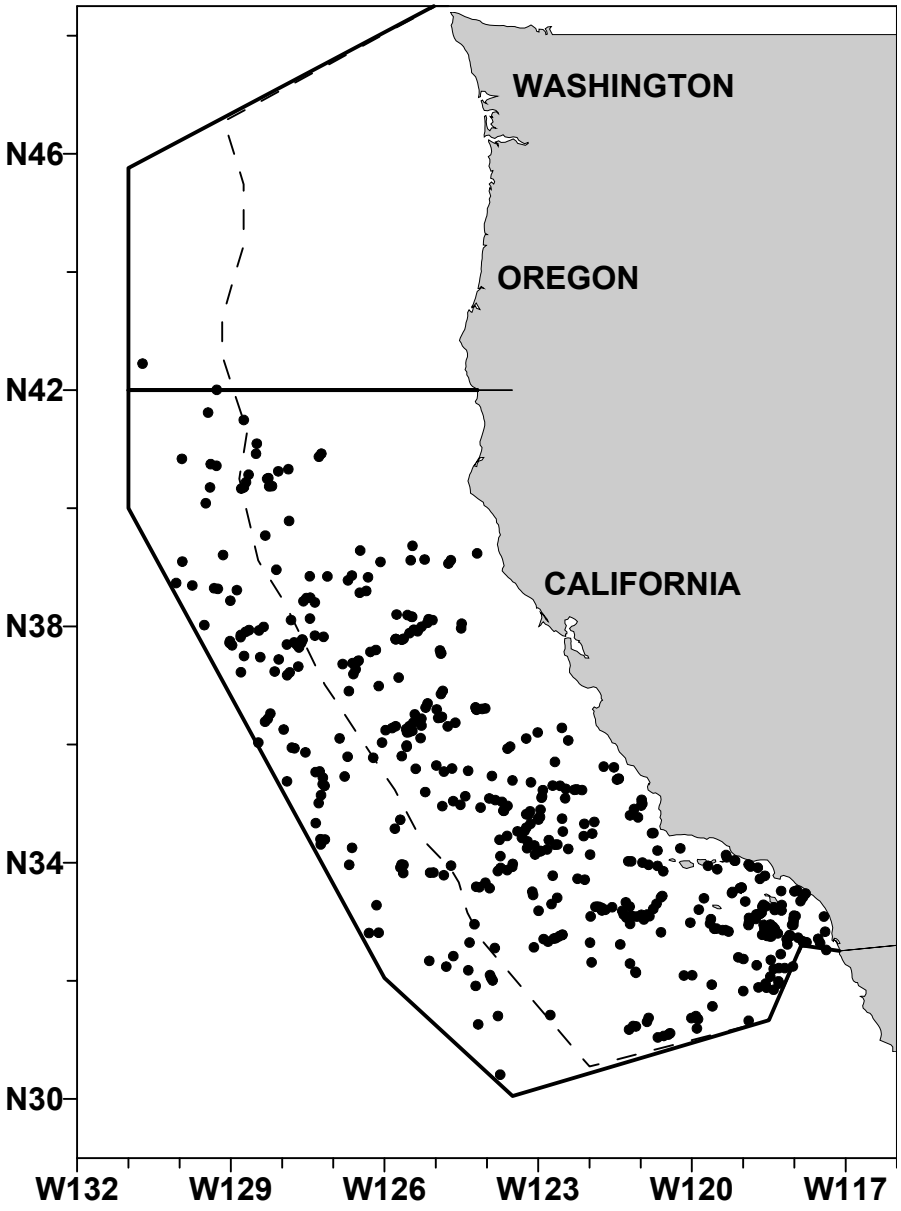
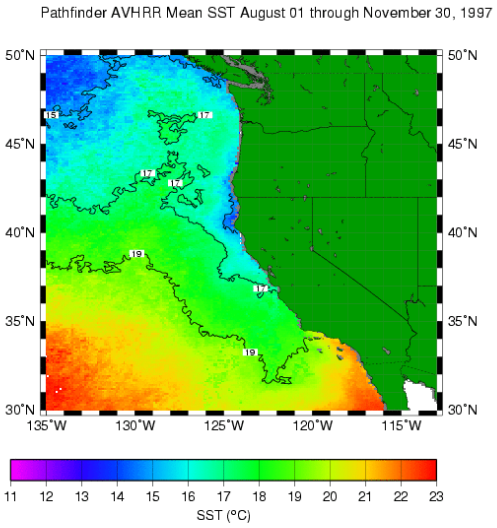


Figure 3. Sea surface temperature (SST) contours averaged for August through November, which is the period when most *Delphinus delphis* specimens have been collected. (A) The influence of warm subtropical waters in the Southern California Bight (SCB) is most evident in 1997 when there was an El Niño event occurring. (B) The overall influence of the subtropical water mass in the SCB is evident in the 1996-2002 average of SST data.

(A)



(B)

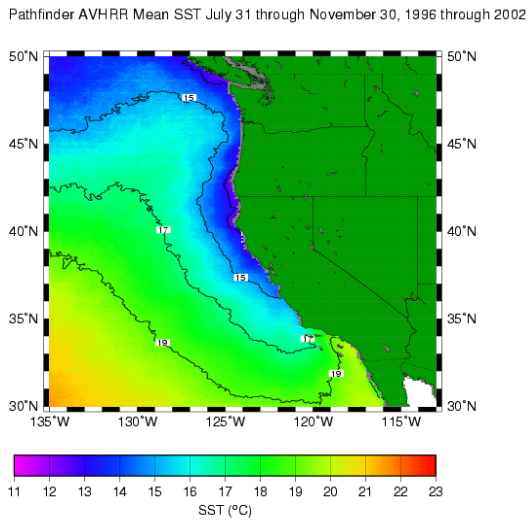


Figure 4. The collection locations for samples collected off the coast of California and Oregon that were used in this study. The circles group samples considered as putative populations in analyses: southern CA, central CA and northern CA. The circle between 30^{o} and 35^{o} N latitude is the southern CA stratum. The next circle north is the central CA stratum, and north of that circle is the northern CA stratum. The two other strata represented in the analyses were the central and northern ETP stocks of *Delphinus delphis*. Samples from these stocks were collected within the range of these stocks as described in Figure 1.

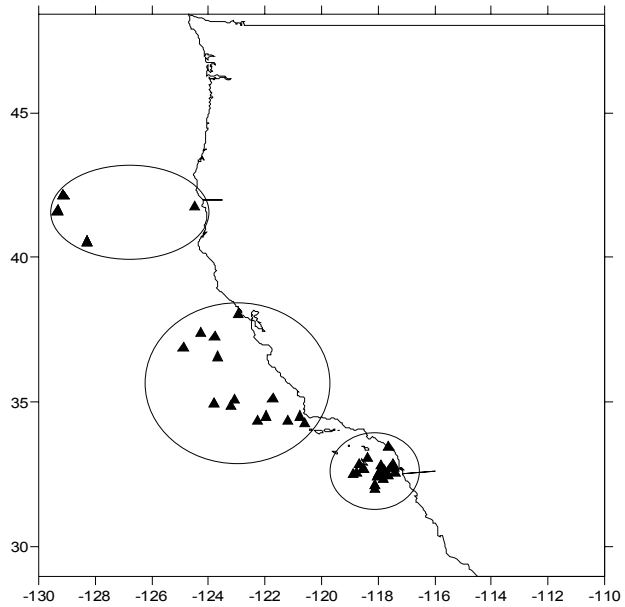


Table 1. — The variable sites of the 48 haplotypes are identified by position in the 4012 base pair mitochondrial DNA control region sequence. The haplotypes are listed by their Southwest Fisheries Science Center (SWFSC) Genetics Archive accession number, and the haplotypes represented by more than one sample in the data set have the frequency of occurrence in parentheses after the accession number.

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                  1 1111111111 1112222222 2222222222 2222333333 333333333
           4556688990 0001115556 7890111445 5667778888 8999000112 224567999
           3474978193 6790460268 1335178581 4594590235 9145346686 899982345
146        CAGGAACTCC ATTAGTATGT GTATTTCCGC AACCATCCTT TACTCCTTAA TCACTTTCT
159 (9)    .......... ......G... ........A. .........C .....A.... ..GT....C
175        .......... ......G... ........A. .......TCC .....G.... ..GT.....
689 (3)    .......... .......... ........A. .......TC. ...C.G.... .TGTC....
694        .......... ......G... ........A. .......T-C .....A.... ..GT....C
1929       .......... .C........ ...C....A. .......T.C .....G.... ..GT.....
2892       .......... ........A. ........A. .........C. .....G.... ..GT.....
4819       .......... ....A.G... ........A. .......... .....G.... ..GT.....
4886       .......... ...G...... ...C....A. ........CC .....G.... ..GT.....
4888 (3)   .......... .......... .......... .G.....TC. ...C.G.... ..GT.....
4998       ...A...... ...G....A. ........A. .......T.. ..TC.G.... ..GT.CC..
5000       .........T .......... .......TA. .......TCC ..TC.G.... ..GT.....
6166       .......... ....A..... .......... .......TCC .....G.... ..GT.....
6172       ....G..... .......... .......... .......TC. .....G.... ..GT.....
6175       .......... .......... ........A. .......TCC .....G.... ..GT..CA.
6176       .......... .......... .......TA. .G.T...TCC ...C.G.... ..GT..C..
6211       .......... .......... ........A. ....G...C. .....A.... ..GT..CA.
6212       ......T... .......... ........A. .......T.. .....G.... ..GT.....
6215       .......... .......... ........A. .......TC. ...C.G.... ..GT.....
6216       .......... ....A..... .......... .......TCC C..C.A.... ..GT.....
6217       .....G.... ..C...G... ...C...... .......TC. C....G...G ..G......
6218       ..A....... .......... ........A. ........C. .....G.... C.G......
6219 (2)   .......... .......... .......TA. .......TCC ..TC.G.... ..GT.....
6222       .......... .......... .......... .......TCC ...C.G.... ..G......
11509      .......... .......... .......... ..T....TC. C..C.G.... ..GT.....
11720      T......... .......... .......... .......TC. ..TC.G.... ..GT.....
11723      T.......T. .......... .......... .......T.A ...C.GC.G. ..G......
11722      .......C.T .......A.. ......T.A. ...T.C..C. ..T..G...G ..GT..C..
11946      .......... .......... .........T .......TC. .....G.... ..GT.....
11986      .......... .......... ..G....... .......T.. ...C.G.... ..GT.C...
11996      .......... .......... .......... GG.....TC. ...C.A.... ..GT..CT.
11997      ..A....... G......... ....C..... .......TC. .....G.... C.GT.....
12101      .......... .........C A......... .G.....T.. ...C.G.... ..G......
12102      T......... ...G...... .......... .......TCA C..C.G.... ..GT.....
14960      .......... .......... .......TA. .......T.C ...C.G.... ..GT.....
14964      .......... .......... ........A. ........C. ...C.G.... ..GT.....
14967      .......... .......... ...C....A. .......T.. ..TC.GC... ..GT.C...
14969      .G......T. .......... .......... .......TCC C..C.A.... ..G...CT.
14970 (2)  .......... .......... ........A. .......TCC ...C.A.... ..GT...A.
14972      .......... .....C.... .......... ........C. ..TC.A.... ..GT.....
14975      .......... .......... .....C.... .....C.TC. .......... ..GT.....
14976 (2)  .......... .......... ........A. .......TCC .....G.C.. ..GT...A.
14979      .......... .......... ........A. .........C .G..TG.... ..GT..CA.
14985      .......... .....G.... ........A. .......T.C .....A.... ..GT....C
14992      .......... .........C .C......A. .G....TT.C ...C.G.... ..GT.....
14994      .......... .......... .......... .......T.C ..TC.A...G ..GT.....
15113      .......... .......C.. .......T.. .......TCC .....G.... ..GT.....
23183      ...A...... ...G....A. ........A. .......TC. ..TC.G.... ..GT.C...
```

Table 2. — Sequence statistics for *Delphinus delphis* (short-beaked common dolphin) mitochondrial DNA control region sequences.

Sequence characteristics	**Eastern North Pacific (n=63)**	Central eastern tropical Pacific (n=6)	Northern eastern tropical Pacific (n=6)	Southern California (n=27)	Central California (n=14)	Northern California (n=10)
# of haplotypes	**48**	6	6	18	14	9
Haplotype diversity	**0.977**	1.0	1.0	0.929	1.0	0.978
Polymorphic sites (n)	**62**	21	25	34	27	25
Observed indels (n)	**4**	0	1	2	1	2
Nucleotide diversity	**0.0190 (+/- 0.0099)**	0.0190 (+/- 0.0119)	0.0230 (+/- 0.0142)	0.0175 (+/- 0.0094)	0.0177 (+/- 0.0099)	0.0189 (+/- 0.0109)
Average pairwise differences	**7.6324 (+/- 3.6072)**	7.6000 (+/- 4.1352)	9.2000 (+/- 4.9376)	6.986 (+/- 3.387)	7.099 (+/- 3.545)	7.556 (+/- 3.855)

Table 3. P-values for pairwise comparisons of *a priori* strata. Results of statistical comparisons with Φ_{ST} are below the diagonal and with χ^2 are above the diagonal. P-values < 0.1 are printed in bold text.

Putative Populations	1. Central ETP (n=6)	2. Northern ETP (n=6)	3. Southern CA (n=27)	4. Central CA (n=14)	5. Northern CA (n=10)
1. Central ETP	*	1.0	**0.0007**	0.9960	0.4990
2. Northern ETP	0.9378	*	0.1704	0.9960	0.4940
3. Southern CA	**0.0066**	**0.0304**	*	**0.0448**	**0.0327**
4. Central CA	**0.0033**	**0.0526**	**0.0793**	*	0.2917
5. Northern CA	**0.0473**	0.5012	0.3336	0.6873	*

Table 4. — Haplotype frequency distributions for each stratum represented in the analyses.

Haplotype	TOTAL	Central ETP	Northern ETP	Southern California	Central CA	Northern CA
1	9			7	1	1
2	3			3		
3	3		1	2		
4	2		1	1		
5	2			1	1	
6	2					2
7	1			1		
8	1			1		
9	1			1		
10	1			1		
11	1			1		
12	1			1		
13	1			1		
14	1			1		
15	1			1		
16	1			1		
17	1			1		
18	1			1		
19	1			1		
20	1				1	
21	1				1	
22	1				1	
23	1				1	
24	1				1	
25	1				1	
26	1				1	
27	1				1	
28	1				1	
29	1				1	
30	1				1	
31	1				1	
32	1					1
33	1					1
34	1					1
35	1					1
36	1					1
37	1					1
38	1					1
39	1	1				
40	1	1				
41	1	1				
42	1	1				
43	1	1				
44	1	1				
45	1		1			
46	1		1			
47	1		1			
48	1		1			