Conservation Genetics (2005) 6:587–600
DOI 10.1007/s10592-005-9013-9

Genetic differentiation and intraspecific structure of Eastern Tropical Pacific spotted dolphins, *Stenella attenuata*, revealed by DNA analyses

Sergio Escorza-Treviño[1,2,*], Frederick I. Archer[2], Maria Rosales[1], Aimee Lang[2] & Andrew E. Dizon[2]

[1]*Department of Biology, California State University, 5151 State University Dr. Los Angeles, Los Angeles, CA, 90032, USA;* [2]*Southwest Fisheries Science Center, 8604 La Jolla Shores Dr. La Jolla, La Jolla, CA, 92038, USA*

*(*Corresponding author: phone: +1-323-343-2086; fax: +1-323-343-5795; e-mail: sescorz@calstatela.edu.)*

Received 27 July 2004; accepted 11 October 2004

Key words: microsatellites, mtDNA, population structure, spotted dolphin, stocks

Abstract

Mitochondrial DNA (mtDNA) control region sequences and microsatellite loci length polymorphisms were used to investigate genetic differentiation in spotted dolphins (*Stenella attenuata*) in the Eastern Tropical Pacific and to examine the intraspecific structure of the coastal subspecies (*Stenella attenuata graffmani*). One-hundred and thirty-five animals from several coastal areas and 90 offshore animals were sequenced for 455 bp of the mitochondrial control region, resulting in 112 mtDNA haplotypes. Phylogenetic analyses and the existence of shared haplotypes between the two subspecies suggest recent and/or current gene flow. Analyses using χ^2, F_{ST} (based on haplotype frequencies) and Φ_{ST} values (based on frequencies and genetic distances between haplotypes) yielded statistically significant separation (randomized permutation values $P < 0.05$) among four different coastal populations and between all but one of these and the offshore subspecies (overall $F_{ST} = 0.0691$). Ninety-one coastal animals from these four geographic populations and 50 offshore animals were genotyped for seven nuclear microsatellite loci. Analysis using F_{ST} values (based on allelic frequencies) yielded statistically significant separation between most coastal populations and offshore animals, although no coastal populations were distinguished. These results argue for the existence of some genetic isolation between offshore and inshore populations and among some inshore populations, suggesting that these should be treated as separate units for management purposes.

Introduction

Understanding the processes of genetic subdivision in cetaceans is especially challenging because most species inhabit vast geographic ranges with few geographic boundaries. Yet, populations of these highly mobile animals adapt to local conditions and differentiate and species evolve. Sound marine mammal management argues for the protection of locally adapted populations (Taylor 1997). Pan-tropical spotted dolphins (*Stenella attenuata*) represent a good example. They are distributed globally in tropical and warmer temperate waters (Rice 1998). Details on the species distribution are best known for the eastern and central Pacific (Dizon et al. 1994), where the species is killed incidentally during yellow-fin tuna purse-seine fishing operations. In the Eastern Tropical Pacific Ocean (ETP), two subspecies are distinguished: the coastal spotted dolphin (*Stenella attenuata graffmani*) and the offshore spotted dolphin (*S. a. attenuata*). The coastal subspecies can be recognized by its relatively larger body and heavier spotting (Perrin et al. 1994), heavier skulls and larger teeth (Schnell et al. 1982).

Two stocks of offshore spotted dolphins are recognized in the ETP, based on morphological and tagging data (Perrin et al. 1985; Schnell et al.

1986; Perrin et al. 1994): Northeastern and Western-Southern (Dizon et al. 1994). Only one stock of coastal spotted dolphins is currently recognized (Dizon et al. 1994), although morphological differences had been described between Gulf of California and Central American coastal animals (Douglas et al. 1984). This coastal stock has been recognized as "depleted" under the Marine Mammal Protection Act (MMPA) since 1980, along with the northeastern stock of offshore spotted dolphins. In 1997, the US. Congress passed the International Dolphin Conservation Program Act (Public Law 105-42) as part of an international agreement to address the dolphin bycatch problem (AIDCP 1998; Gosliner 1999), directing the National Marine Fisheries Service to determine if the chasing and encirclement of dolphins in the fishery was having a significantly adverse impact on depleted dolphin stocks. The status of coastal spotted dolphins is a part of the tuna-dolphin issue that remains unresolved. Little is known about the separation of coastal and offshore stocks or potential for further population subdivision within the coastal subspecies. Our study uses variation in the mitochondrial DNA (mtDNA) and seven nuclear short tandem repeat (microsatellite) loci to investigate genetic differentiation. Specifically, in order to evaluate if the potential for great distance dispersal in these animals translates into broad gene flow that prevents the development of detectable population subdivision, this paper investigates: (1) the existence of genetic differentiation between coastal and offshore spotted dolphins and (2) genetic structure within the coastal region.

Materials and methods

Samples

One-hundred and thirty-five samples from the coastal region and 90 samples from the recognized Northeastern offshore stock (Dizon et al. 1994) were used in this study. The geographic location and number of samples are summarized in Figure 1. Skin from coastal spotted dolphins was obtained from biopsies of free-ranging animals collected during research cruises between 1995 and 2000, while samples of offshore animals were obtained from fisheries bycatch between 1985 and 1993. Samples were stored in an aqueous solution of 20% (v/v) dimethyl sulfoxide (DMSO) saturated with sodium chloride (NaCl) (Amos and Hoelzel 1991) or kept frozen until DNA extraction. Coastal samples were collected throughout the ETP, from the southern Gulf of California to Ecuador. Coastal animals were identified based on morphology by experienced observers during biopsying. Offshore individuals were identified based on both morphology and distance from the coast (Dizon et al. 1994). Each coastal individual was assigned to one of 29 sampling sites (Figure 1). Single animals were assigned to the nearest sampling site so that all sites contained two or more samples.

DNA extraction

Tissue (100–300 mg) was digested in cetyltrimethylammonium bromide (CTAB; Winnepennickx et al. 1993) extraction buffer, and DNA was purified by standard phenol/chloroform/isoamyl alcohol (25:24:1) extractions (modified from Sambrook et al. 1989). The precipitate was resuspended in TE buffer to an average concentration of 1.5 μg/μl. The quality of the DNA was examined via electrophoresis on 1% or 2% agarose gels using approximately 1.5 μg of DNA.

Mitochondrial DNA

A DNA fragment of about 650 base pairs (bp) comprising the proline transfer RNA gene and the hypervariable region I of the control region was amplified using the Polymerase Chain Reaction (PCR). Reactions were performed in 25 μl volumes, containing 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 150 μM each dNTP, 0.3 μM each primer, 1.25 units of *Taq* DNA polymerase (Perkin Elmer Cetus, Promega, or Gibco BRL), and approximately 50 ng of genomic DNA. The thermal cycling profile included an initial hot start of 2 min and 30 s at 90 °C, followed by 35 amplification cycles. Each of these cycles consisted of denaturation for 45 s at 94 °C, annealing for 1 min at 48 °C, and extension for 1 min and 30 s at 72 °C. An additional 5-min interval at 72 °C was added at the end of the cycle series to ensure complete extension of the PCR products. The following primers, which anneal between the tRNA threonine gene and the tRNA proline gene

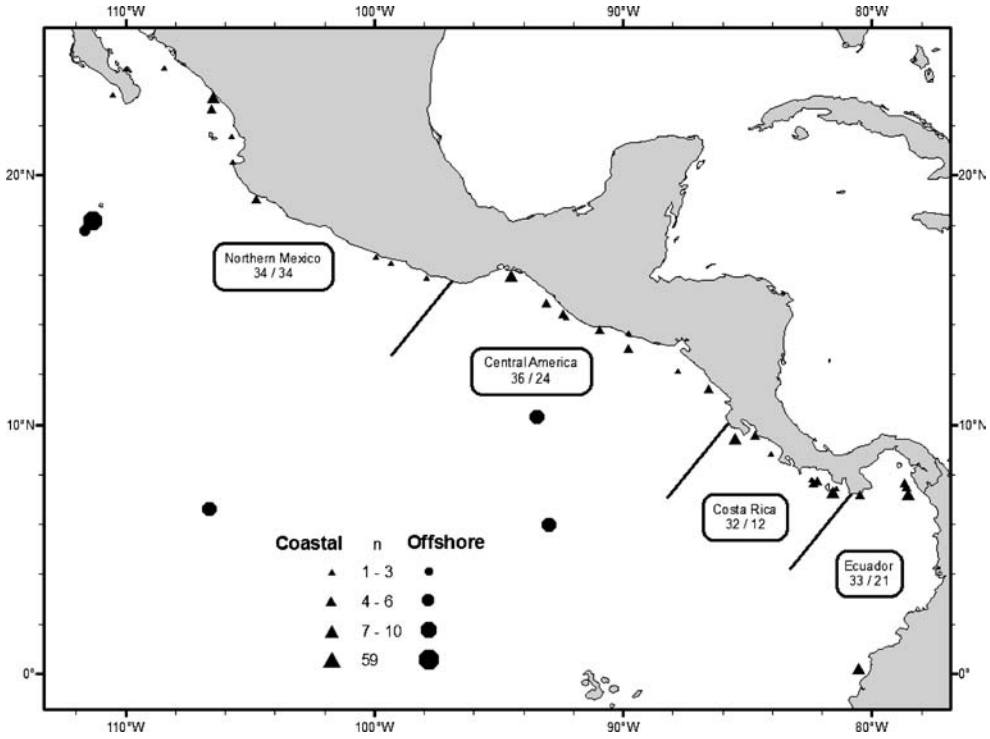

Figure 1. Geographic location of coastal (triangles) and offshore (circles) spotted dolphin samples collected for this study. Thick lines show boundaries between the final four coastal populations. Numbers in boxes indicate sample size for mitochondrial (first number) and microsatellite (second number) analyses for each coastal population.

(L-strand) and the B region (H-strand), were used (numbers refer to the 3′ base of the primer with reference to the human mtDNA sequence of Anderson et al. (1981): L15965 5′-CCTCCCTAAGACTCAAGG-3′ (developed at our laboratory) and H00034 5′-TACCAAATCTATGAAACCTCAG-3′ (Rosel et al. 1994).

Successful amplification products were then cleaned by filtration through purification columns (QIAquick® 250, QIAGEN) according to the manufacturer's specifications. Both heavy and light strands were cycle-sequenced using the PRISM® DyeDeoxy Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems Inc.). The primers used for sequencing 455 bp of the mitochondrial control region were L15965 (described above) and H16498 5′-CCTGAAGTAAGAACCAGATG-3′ (Rosel et al. 1994). Sequencing chemistry was optimized for 20 μl reactions containing 60–200 ng double stranded PCR product, 0.2 μM primer, and 6 μl terminator ready reaction mix. The cycling profile was 10 s denaturation at 96 °C, 5 s annealing at 50 °C, and 4 min extension at 60 °C, for 25 cycles. Sequenced products were purified by ethanol precipitation and then run on an ABI 377 DNA automated sequencer. Editing of opposing strands was done simultaneously using SeqEd v. 1.0.3 software, designed to deal with the output files of the automated sequencer. Because of CITES export permit issues, some coastal samples were processed aboard research vessels while in territorial waters. In those instances, only sequencing reactions were performed, and thus, no microsatellite data are available for a portion of the coastal dataset.

Microsatellite genotyping

Seven microsatellite loci (dinucleotide repeats) shown to be polymorphic in several cetacean species were used in this study: EV14, EV37, EV94, and EV104 (Valsecchi and Amos 1996); and Sl849, Sl969, and Sl1026 (Galver 2002). None of these loci had been originally screened on spotted dolphin.

DNA fragments encompassing the target microsatellite regions were amplified from 91 coastal

and 50 offshore individuals using PCR. Reactions were performed individually in 25 μl volumes, containing 10–100 ng of genomic DNA, 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 150 μM each dNTP, 0.3 μM each primer (one fluorescently labeled), and 1.25 units of *Taq* DNA polymerase (Perkin Elmer Cetus, Promega, or Gibco BRL). The thermal cycling profile included an initial hot start of 3 min at 97 °C, followed by 35 amplification cycles. Each of these cycles consisted of denaturation for 1 min at 90 °C, annealing for 1 min at a locus-specific temperature, and extension for 1:30 min at 72 °C. Annealing temperatures were for EV14, 64 °C; EV37, 50 °C; EV94, 55 °C; EV104, 43 °C for 10 cycles and 46 °C for 25 cycles; Sl849, 49 °C for 10 cycles and 52 °C for 25 cycles; Sl969, 54 °C; and Sl1026, 55 °C. For every locus, an additional 5-min interval at 72 °C was added at the end of the cycle series to ensure complete extension of the PCR products.

The fragment sizes of the successful amplification products were measured with an ABI 377 DNA automated sequencer running in the "genotyping" mode. Allele sizes were determined by using both an internal standard marker (Genescan-500 ROX; Applied Biosystems) and a standard set of samples for calibration between gels.

No statistically significant linkage disequilibrium was observed between any pair of loci, so it was assumed all seven loci were unlinked (Raymond and Rousset 1995).

Sex determination

Sex was genetically determined by differential amplification of the zinc finger gene regions present in the X and Y chromosomes (ZFX and ZFY, respectively). Multiplex PCR reactions were performed using the three primers reported by Bérubé and Palsbøll (1996). PCR products were separated by electrophoresis on 2% NuSieve® gels, and sex determined from the resulting banding pattern: males present two bands, females one.

Data analysis

Phylogeny

Both parsimony and genetic distance-based methods were used to reconstruct the phylogenetic relationships among the haplotypes. The heuristic search algorithm of PAUP (Phylogenetic analysis Using Parsimony, Version 3.1.1, Swofford 1993) was used and 1000 minimum trees were saved. This algorithm uses the criterion of maximum parsimony to find the tree(s) that require the least number of evolutionary changes. The genetic distance between haplotypes, measured as the proportion of differences, was used to construct a neighbor-joining tree (Saitou and Nei 1987) with the aid of the computer program MEGA version 2.1 (Kumar et al. 2001). Neighbor-Joining operates on the principle of finding pairs of OTUs in consecutive stages of clustering that result in the minimum total branch length (Saitou and Nei 1987).

Population differentiation and structure

For samples from the coastal region, initial population strata were defined using the Monmonier maximum difference algorithm (Manel et al. 2003) on a Delaunay network (Brassel and Reif 1979) connecting adjacent sampling sites. The algorithm was performed by hand using values of F_{ST} for mtDNA haplotypes as a measure of genetic distance among the 29 sampling sites (non-significant values of F_{ST} were considered to be zero). The resultant strata defined by these boundaries were then tested using an analysis of molecular variance (AMOVA, Excoffier et al. 1992) and χ^2 tests (Roff and Bentzen 1989). Boundaries which resulted in non-statistical pairwise comparisons were removed, and the strata on either side were combined to produce the final populations.

The average pairwise distance within groups (nucleotide diversity, d, Nei 1987) and between groups (nucleotide divergence) was estimated using the SENDBS program, written by N. Takezaki (National Institute of Genetics, Mishima, Shizuoka, Japan), using the proportion of differences among haplotypes and 1000 bootstraps for the computation of standard errors. The extent of population subdivision was examined using an analysis of molecular variance on both the mitochondrial and the microsatellite data, and χ^2 tests on mitochondrial data. The AMOVA analyses were performed using the program Arlequin v. 2.000 (Schneider et al. 2000), which calculates F_{ST} (Wright's fixation index, Wright 1965; Cockerham and Weir 1993), for both mitochondrial and

microsatellite data, and their analogs (Φ_{ST}) in the case of mitochondrial data. F_{ST} and Φ_{ST} indicate the proportion of the genetic variance that is due to subdivision into *a priori* determined populations. The genetic distance between a pair of haplotypes was estimated as the proportion of the nucleotide differences between them, and the null distribution of pairwise F_{ST} and Φ_{ST} values under the hypothesis of panmixia was obtained by 10,000 permutations of the original data set. In order to distinguish between isolation and migration as explanations for observed patterns of genetic divergence among coastal populations, the program MDiv (Nielsen and Wakeley 2001) was used to estimate the posterior distribution of the scaled migration rate ($M = 2 \times \text{Ne} \times \text{migration rate}$) and the scaled divergence time ($T = \text{divergence time}/(2 \times \text{Ne})$), using 5,000,000 cycles for the Markov Chain, 500,000 cycles for burn-in time, and the HKY model of mutation.

For microsatellite loci, the number of alleles per locus, observed heterozygosity (H_o), expected heterozygosity (H_e) and allelic richness were used to estimate the level of polymorphism. Allelic richness controls for variation in sample size and was calculated using the program FSTAT 2.9.3 (Goudet 2001). The program Genepop 3.1 (Raymond and Rousset 1995) was used to evaluate the existence of deviations from the expected Hardy–Weinberg genotypic frequencies and linkage disequilibrium using Fisher's exact test and 1000 cycles in the Markov chain.

Results

Mitochondrial genetic diversity

Two-hundred and twenty-five specimens were sequenced and 112 different haplotypes identified (deposited in GenBank database under accession numbers: XXX–XXX), 75 of which were found in just one individual (Figure 2). Of the 37 haplotypes common to more than one individual, 14 were common to offshore and coastal animals. No heteroplasmy, either in the length or in the nucleotide sequence of the amplified fragment, or indels were detected. In total, 63 sites were variable and 41 were phylogenetically informative.

The average pairwise distance (nucleotide diversity, d, Nei 1987) was 1.36% (S.E. = 0.72%), while the overall haplotype diversity (h, Nei 1987) was 98.12% (S.D. = 0.35%). Nucleotide diversities for coastal and offshore animals were 1.35% (S.E. = 0.30%) and 1.39% (S.E. = 0.29%) respectively, while nucleotide divergence between the two was 1.38% (S.E. = 0.30%).

Microsatellite genetic variation

Varying levels of polymorphism were observed in all microsatellite loci used. The number of alleles per locus ranged from four for locus EV104 to 32 for locus EV37. Allelic richness, expected (H_e) and observed (H_o) heterozygosity are shown in Table 1. None of these measures of diversity were significantly different when compared among populations (Table 1). Deviations from HW equilibrium were tested for each locus at each population. Both the Central America population (at locus SL1026) and the offshore population (at locus EV37) showed a statistically significant heterozygote deficiency ($P < 0.001$, $\alpha = 0.001$ when the Bonferroni correction is applied to an $\alpha = 0.05$). The pattern of population differentiation did not change when these loci were excluded from the analysis, and therefore were kept in all analyses. No significant heterozygote excess was observed. Allele frequencies are shown in Tables 2 and 3.

Phylogeny

Because of the very low number of informative characters ($n = 41$) relative to the number of unique haplotypes ($n = 112$), maximum-parsimony analysis resulted in numerous polytomies joining a large number of the haplotypes. Over 1000 most parsimonious trees were found, and the strict consensus tree provided no phylogenetic resolution (not shown). A neighbor-joining tree (Saitou and Nei 1987) showed no strict concordance between clades and geographic origins or morphotype of the samples, with haplotypes found in offshore and in coastal animals present in nodes throughout the tree. Except for a single terminal node, bootstrap support values for all nodes were below 70%. There were 11 nodes with bootstrap support between 50 and 70%, of which only 6 were not terminal (included more than two haplotypes). The largest of these lineages contained 11 haplotypes representing five of the six populations, including both coastal and offshore samples.

	Polymorphic Sites							Haplotype Frequencies				
	111	1111111222	2222222222	2222333333	3333333333	4444444444	444	NM	CA	CR	Ec	Off
	5667799033	3345588001	2233444788	9999001111	1222334469	0001122224	444	34	36	32	33	90
	2797968201	2991559016	2608789814	2689270256	7134467920	2368934671	247					
01	CCCTTTGTTT	CGTACGGTTT	TAGTTTACAC	TAATCCTTCC	TTATTGTGCC	CTTCCGTTCA	TAA	1				
02	....	....T.....	.......T..	........T.	.......AT.	.....AC...	CG.	1				
03	...C......	....TA....	.......TG.	....	.....A.A..	.....A....	...	3				3
04	....	....TA....	.......TG.	....T...T.	.......A..	.C...AC...	...	1				2
05	....	....TA....	.......TG.	....T...T.	.......A..	.C...A....	...	1				
06	....	....T.....	.......T..	........T.	.......A..	.C...A....	...	5	2		1	9
07	....	....T.....	.......TG.	..GCT.....	.......A..	.C...AC...	...	1				
08	....C.....	....	....	....	.....A.A..	....	...	1				
09	....	....T...C.	.......T..	.G......TT	.......A..	.C...AC...	...	1				
10	....C.....	....	....	....	.....A.A..	.......C..	...	1				
11	...C......	....TA...C	........G.	....	C......A.T	.....A....	...	1				
12	....	....	....	....	.......A..	....	...	2	1			3
13	....	....T.....	.......T.T	........T.	.......A..	.CC..A....	...	1				
14	...C......	....TA....	.......T.T	....	.......A..	.....A....	...	3				
15	....	....T.....	.......T..	........T.	.......A..	.C...AC...	CG.	1				3
16	.......C..	.A..T.....	.......TG.	........T.	.....A.A..	.C...A....	...	1				
17	...C......	T...TA....	.......TG.	....	..G....A..	.....A....	...	1				
18	...C......	....TA....	.......TG.	.....T....	.....A.A..	.....AC...	...	1				
19	....	.A..T.....	.......T.T	........T.	.......A..	.C...AC...	CG.	1				
20	...C......	....TA....	.......T..	........TT	.......A..	.....A....	...	2				
21	....	....T.....	.......T..	........T.	.......A..	.C...AC...	...		8			
22	...C......	....TA....	.......T..	......C...	.......A..	.....A....	...		2			
23	....	....T.A...	.......T..	........T.	.......A..	.C...AC...	...		1			
24	....	....TA....	.......T..	....T...T.	.......A..	.C...AC...	...		1			
25	...C......	..C.TA....	.......T..	....	.......A..	.....A....	...		2			1
26	....	....T.....	C......TG.	..G......T	.......A..	.C...AC...	...		2			
27	...C......	....TA....	.......T..	.....T....	.....A.A..	.....A....	...		1			
28	.......C..	....T.....	.......TG.	........TT	.......A..	.C...A....	...	1				
29	....	.A.....C..	.......T..	........T.	.......A..	.C...AC...	.G.	1				
30	....	....T.....	.......T..	........TA	.......A..	.C...AC...	CG.	1				
31	...C......	....TA....	.......T..	....	.......A..	.....A....	...	1		1		3
32	.......C..	.A..T.....	.......TG.	........T.	.......A..	.C...A....	...		1			
33	....	....T.....	.......T..	....T...TT	.......A..	.C........	...		1			
34	....	....T.....	.......T..	........T.	.......AT.	.C...A....	...		1			
35	....	....T.....	.......T..	....T...T.	.......A..	.C...A....	...		1			1
36	....	....	....	....T.....	.......AT.	....	...			4	2	1
37	....	....T.....	.......T..	........T.	....	.C...A....	...			2		1
38	....	....	....	....T.....	.......A..	....	...			2	4	1
39	...C......	....TA....	.......T..	....	.......A..	.....AC...	...			18		
40	.T.C......	....TA....	.......TG.	..T...C.TT	..G....A..	.C...A....	...			1		
41	....	....T.....	.......T..	........T.	.......A..	.....A....	...		1	1	3	
42	....	.A..T.....	.......T..	C.......T.	.......A..	.....AC...	.G.				1	
43	...C......	....TA....	.G.....T..	....	.......A..	.....A....	...		1		1	
44	........C.	....T.....	.......T..	........TT	..G....A..	.C...A....	...				2	
45	....	.......C..	....	....T.....	.......AT.	....	...				1	
46	....	.A..T.....	.......T..	....T...T.	.......A..	.....A....	.G.				1	
47	....	....	....	....	.......A..	......C...	...				2	1
48	...C......	....TA....	.......T..	....	.......A..	.....A.C..	...				1	
49	....	.A..T.....	.......T..	C.......TT	.......A..	.....AC...	.G.				3	
50	...C......	..C.TA...C	........G.	....	.....A.A..	.....A....	...		1			
51	...C......	....TA....	........G.	....	.......A..	.....A....	...		1			
52	....	....T.....	.....C.T..	........T.	.......A..	.C...AC...	...		1			
53	....	....T.....	.......T..	.........T	.......A..	.C........	...		1			
54	....	....T.....	.......T..	........TT	.......A..	.C........	...		1			1
55	...C.....C	...GTA....	.......TG.	..T.T...TT	.......A..	.....AC...	...			2	1	
56	....	....T.....	.......T..	........T.	....C..A..	.CC..A....	...				1	
57	....	.A..T.....	.......T.T	........T.	.......A..	.C...AC.T.	.G.				1	
58	....	....T.....	.......T..	........T.	.......A..	.....AC...	...				1	
59	...C......	....TA...C	........G.	....	.......A..	.....A....	...				2	4
60	........C.	....T.....	.......T..	........TT	.......A..	.....A....	...					2
61	...C......	....TA....	.......T..	........T.	.......A..	.....A....	...					1
62	....	....	....	....	.......A..	....	..C					1
63	T..C......	.A..TA....	.......T..	....	.......A..	.....A....	...					2
64	...C......	..C.TA....	.......T..	....	.......A..	...GGA....	...					1

Figure 2. Variable sites of 112 spotted dolphin mtDNA control region haplotypes are shown on the left. Dots indicate identity with the reference sequence. Haplotype frequencies for each putative population are shown on the right.

```
65  ......... ....T..... .......TG. .......CT. .......A.. .C...A.... ...
2
66  ......... ....T..... .......T.. ........TT C......A.. .....AC... ...                    2
67  ...C...... ....TA...C ........G. ......... .....A.A.. .....A.... ...                    1
68  ......... ....T..... .......T.. ........T. .....A.A.. .C...AC... CG.                    3
69  ...C...... ..C.TA.... .C.....T.. ........T. .......A.. .....A.... ...                    1
70  ...C...... T...TA.... ......GTG. .......... ..G..A.A.. .....A.... ...                    1
71  ......... ....T..... C..C...TG. ........TT .......A.. .C...A.... ...                    2
72  ...C...... ....TA.... .......T.. ......... .......AT. .C...A.... ...                    1
73  ......... ......... ..T..... ......... .......A.. ......... ...                        1
74  ...C...... .....A.... .......T.. ......... .......A.. .....AC... ...                    1
75  ......... ....TA.... .......TG. ....T...T. .....A.A.. .C...AC... ...                    1
76  ......... ....T..... .......T.. ......... .......A.. .C...A.... ...                    1
77  T.....A... ....T..... .......T.. ........T. .......A.. .C...AC... CG.                    2
78  ...C...... ....TA.... .......TG. ......... .......A.. .....A.... ...                    2
79  ......... ....T..... .......T.. .......CT. .......A.. .C...AC... CG.                    1
80  ..AC...... T...TA.... .......TG. ........T. ..G..A.A.. .....A...G ...                    1
81  ...C...... ..C.TA.... .......T.. ........T. .......A.. .....A.... ...                    1
82  T......... ....TA.... .......TG. ....T...T. .......A.. .C...AC... ...                    1
83  ...C...... ....TA.... .......T.. ......... .....A.A.. .....A.... ...                    1
84  ...C...... T...TA.C.. .......TG. ......... ..G..A.A.. .....A.... ...                    1
85  .......C.. ....T..... .......TG. .........T .......A.. .C...AC... ...                    1
86  ......... .A..T..... .......T.. ........T. .......A.. .C...A.... .G.                    1
87  ...C...... T...TA.C.. .......TG. ......C... ..G..A.A.. .....A.... ...                    1
88  ......... .A..T..... .......T.T ........T. .......A.. .C...AC... .G.                    1
89  ......... ....T..... .......TG. ........T. .......A.. .C...A.... ...                    1
90  ...C...... ....TA.... .......T.. ......... .......A.. .C...A.... ...                    1
91  ......... ....T..... .......T.. ........T. .......A.. .C...A.... CG.                    1
92  ......... ....T..... ...C...T.. ......... .......A.. .....A.... .G.                    1
93  ...C...... ....TA.... .G.....T.. ......... .......A.. T....A.... ...                    1
94  ......... ....T..... .......T.. ........T. .......A.. .C...ACC.. CG.                    1
95  ......... ....T..... .......T.. ........T. .......AT. .....A.... CG.                    1
96  ......... ....T..... .......T.. ..G....... ...C...A.. .C...A.... ...                    1
97  ...C...... ....TA.... ....C..T.. ..G...C... ......CA.. .....A.... ...                    1
98  .....C.... ....T..... .......T.. ........T. .......AT. .C...AC... CG.                    1
99  ......... ....T..... .......T.. ........T. .....A.A.. .C...A.... ...                    1
100 ......... ....TA.... .......TGT .........T .....A.A.. .....AC... ...                    1
101 ....C..... ......... ......... ......... .......A.. ......... ...                        2
102 ...C...... ....TA.C.. .......T.. ........T. .......A.. .....A.... ...                    1
103 ...C...... ....TA.... .......T.. ........T. .C...A.A.. .....A.... ...                    1
104 ......... ....T..... .......T.. ........T. .......A.. .CC..A.... ...                    1
105 ......... ....TA.... .......T.. ......... .....A.A.. .....A.... ...                    1
106 ......... ....T..... .......T.. ........T. .......A.. .C...AC... .G.          1     2
107 ......A... ....T..... .......T.. ........T. .......A.. .C...A.... CG.                1
108 ......... ....T..... ......... ........T. .......A.. .....AC... CG.                2
109 ......... ....T..... .....C.TG. ........T. .......A.. .C...A.... ...    1
110 ......... ....T..... C......TG. ..GCT....T .......A.. .C...AC... ...    2
111 ...C...... ....TA.... ......... ......... .......A.. .....A.... ...    1
112 ......... .....A.... .......TG. ....T...T. .......A.. .....AC... ...    1
```

Figure 2. Continued

Table 1. Measures of genetic diversity (± SD) within populations of spotted dolphins for microsatellites

Population	Number of individuals sampled	Mean allelic richness per Locus	Mean observed heterozygosity	Mean expected heterozygosity
Northern Mexico	34	4.17 (1.22)	0.767 (0.077)	0.784 (0.249)
Central America	24	3.67 (1.35)	0.683 (0.149)	0.694 (0.303)
Costa Rica	12	3.88 (1.39)	0.762 (0.135)	0.726 (0.281)
Ecuador	21	4.00 (1.12)	0.694 (0.111)	0.778 (0.212)
Offshores	50	4.28 (1.39)	0.718 (0.074)	0.780 (0.284)

Population structure

Mitochondrial results

Statistically significant genetic differentiation was detected when a comparison was made between all coastal and the offshore samples ($F_{ST} = 0.0132$, $P < 0.001$; $\Phi_{ST} = 0.0105$, $P < 0.05$; $\chi^2 = 158.752$, $P < 0.001$).

Within the coastal samples, the Monmonier algorithm delineated five boundaries, defining six

Table 2. Allele frequencies for microsatellite loci (locus name in upper left corner of each matrix), expressed as percentage

EV14	NM	CA	CR	Ec	Off
1	1.52				3.45
2	3.03				5.17
3					8.62
4					3.45
5	3.03				1.72
6	7.58				3.45
7	4.55		12.50		5.17
8	3.03	3.85	4.17	12.50	3.45
9	13.64	6.35	8.33	17.50	8.62
10	12.12	19.04	8.33	7.50	5.17
11					3.45
12	7.58			5.00	5.17
13	13.64	20.19	29.17	20.00	17.24
14	1.52	6.35		5.00	
15	4.55	1.93		2.50	5.17
16	6.06	22.12	16.67	12.50	12.07
17	10.61	8.85	4.17	5.00	6.90
18	7.58	4.43	12.50	7.50	1.72
19				2.50	
20		6.93		2.50	
21			4.17		

EV37	NM	CA	CR	Ec	Off
1				5.00	
2					1.28
3		2.78			2.56
4					1.28
5		2.28			
6	5.36		8.33	2.50	3.85
7	3.57				
8	1.79		4.17		3.85
9			4.17		5.13
10	12.50	23.74	4.17	20.00	
11	1.79			2.50	
12	12.50	17.43	8.33	22.50	7.69
13				2.50	7.69
14	1.79	4.55		2.50	7.69
15	5.36				
16	1.79	5.56	12.50		5.13
17		2.78		2.50	3.85
18		2.28	8.33	7.50	7.69

EV37	NM	CA	CR	Ec	Off
19	1.79				6.41
20	3.57	2.78	4.17		6.41
21	10.71	2.28		2.50	2.56
22		5.56	4.17	2.50	6.41
23	7.14				2.56
24	3.57	2.28		2.50	2.56
25	1.79	2.28	8.33	5.00	3.85
26	1.79				2.56
27	10.71	12.38	8.33	5.00	3.85
28	1.79				2.56
29	5.36	8.34	16.67	7.50	
30	1.79		8.33	2.50	2.56
31	3.57			2.50	
32		2.78		2.50	

EV94	NM	CA	CR	Ec	Off
1					1.14
2	21.21	20.98	33.33	16.67	18.18
3	24.24	31.64	20.83	21.43	22.73
4	22.73	20.98	25.00	30.95	10.23
5	4.55	16.09	8.33	9.52	6.82
6	9.09	2.28	4.17	2.38	1.14
7	1.52	1.93		2.38	9.09
8	4.55			2.38	11.36
9	1.52		4.17	2.38	4.55
10	3.03			2.38	
11				2.38	4.55
12					1.14
13					1.14
14	1.52		4.17	2.38	1.14
15		4.20			2.27
16					1.14
17				2.38	
18	1.52				1.14
19	4.55	1.93		2.38	2.27

EV104	NM	CA	CR	Ec	Off
1	86.96	91.43	83.33	78.57	90.91
2	2.17			7.14	4.55
3	6.52	8.57	16.67	14.29	4.55
4	4.35				

NM = Northern Mexico, CA = Central America, CR = Costa Rica, Ec = Ecuador, and Off = offshore.

strata. Pairwise comparisons among these strata resulted in non-significant F_{ST} and χ^2 values across two of the boundaries, which were then removed (data not shown). The remaining three boundaries created four populations: Northern Mexico, Central America, Costa Rica, and Ecuador (names are approximations to geographic areas and do not imply territorial waters – Figure 1).

For F_{ST}, there were statistically significant levels of genetic subdivision for all pairwise comparisons of coastal populations, and for three of the four comparisons between coastal and offshore populations (Table 4, upper matrix). In the case of Φ_{ST}, all but one of the pairwise comparisons between coastal populations and three of the four comparisons between coastal and offshore popu-

Table 3. Allele frequencies for microsatellite loci (locus name in upper left corner of each matrix), expressed as percentage

SL849	NM	CA	CR	Ec	Off	SL969	NM	CA	CR	Ec	Off
1					1.00	1	1.52	5.00		8.33	4.55
2	2.00				2.00	2	4.55	8.85	5.56	12.50	1.52
3					1.00	3	46.97	80.39	83.33	66.67	12.12
4	4.00				1.00	4	15.15	5.77	5.56	4.17	54.55
5	30.00	29.45	25.00	28.95	31.00	5	4.55				9.09
6				2.63	3.00	6	15.15				1.52
7	6.00	7.50	25.00	13.16	13.00	7	4.55				4.55
8		10.84		2.63	4.00	8	3.03		5.56		
9	10.00					9					1.52
10	2.00	7.78	12.50	15.79	6.00	10	4.55				3.03
11	10.00	5.00	12.50	7.89	7.00	11					3.03
12					3.00	12					1.52
13	8.00		12.50	7.89	1.00	13				8.33	3.03
14	4.00										
15					3.00	SL1026	NM	CA	CR	Ec	Off
16	2.00	2.50			2.00	1					1.02
17	2.00	5.00	4.17	5.26	2.00	2				2.63	1.02
18	2.00				5.00	3			4.55		
19	2.00	13.34	4.17	2.63		4	4.55			5.26	2.04
20					1.00	5	13.64	13.94	4.55	5.26	7.14
21		10.56		2.63	1.00	6	1.52				2.04
22		5.28		5.26		7	7.58			2.63	1.02
23					2.00	8	1.52				3.06
24	4.00					9	10.61	24.52	13.64	13.16	5.10
25					3.00	10					1.02
26	2.00			2.63		11		1.93	9.09	2.63	9.18
27					3.00	12	1.52	6.25	9.09	23.68	5.10
28	6.00	2.78	4.17		2.00	13	1.52	5.05	4.55	7.89	5.10
29					1.00	14	18.18	9.62	4.55	5.26	12.24
30	4.00			2.63		15	13.64	1.93	9.09	10.53	7.14
31					2.00	16	3.03	8.90	9.09	5.26	5.10
						17	4.55	8.18	4.55	2.63	4.08
						18	1.52		13.64		3.06
						19	1.52	1.93	4.55	5.26	2.04
						20				2.63	5.10
						21	1.52	1.93			5.10
						22					4.08
						23	1.52	6.97			
						24	1.52	3.13	4.55	2.63	1.02
						25	3.03	3.85	4.55		3.06
						26	1.52				1.02
						27	1.52				
						28	1.52	1.93		2.63	2.04
						29	1.52				
						30	1.52				2.04

NM = Northern Mexico, CA = Central America, CR = Costa Rica, Ec = Ecuador, and Off = offshore.

lations were statistically significant (Table 4, lower matrix). Statistically significant levels of genetic subdivision for χ^2 resulted for all pairwise comparisons among coastal populations, as well as for all but one pairwise comparison between coastal and offshore populations (Table 5). Overall, between 6 and 7% of the total molecular variance was accounted for by stratifying the sample into

Table 4. Pairwise comparisons between coastal and offshore populations of spotted dolphins based on mtDNA

	Northern Mexico	Central America	Costa Rica	Ecuador	Offshores
Northern Mexico		0.0328***	0.1776***	0.0288***	0.0020
Central America	0.0163		0.1869***	0.0384***	0.0264***
Costa Rica	0.0990***	0.1402***		0.1616***	0.1541***
Ecuador	0.0441**	0.0739***	0.1367***		0.0168***
Offshores	−0.0126	0.0214*	0.0988***	0.0538***	

F_{ST} values are shown in the upper matrix and Φ_{ST} values in the lower matrix. Statistically significant results (calculated from 10,000 random permutation tests) are outlined in grey. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 5. Pairwise comparisons of χ^2-values between coastal and offshore populations of spotted dolphins based on mtDNA

	Northern Mexico	Central America	Costa Rica	Ecuador
Central America	d.f. = 45 χ^2 = 61.61***			
Costa Rica	d.f. = 31 χ^2 = 64.00***	d.f. = 31 χ^2 = 65.99***		
Ecuador	d.f. = 42 χ^2 = 63.67***	d.f. = 40 χ^2 = 61.32***	d.f. = 23 χ^2 = 45.99***	
Offshores	d.f. = 77 χ^2 = 83.40	d.f. = 78 χ^2 = 106.14***	d.f. = 64 χ^2 = 107.10***	d.f. = 74 χ^2 = 100.77***

Statistically significant results (calculated from 10,000 random permutation tests) are outlined in grey. $^{***}P < 0.001$.

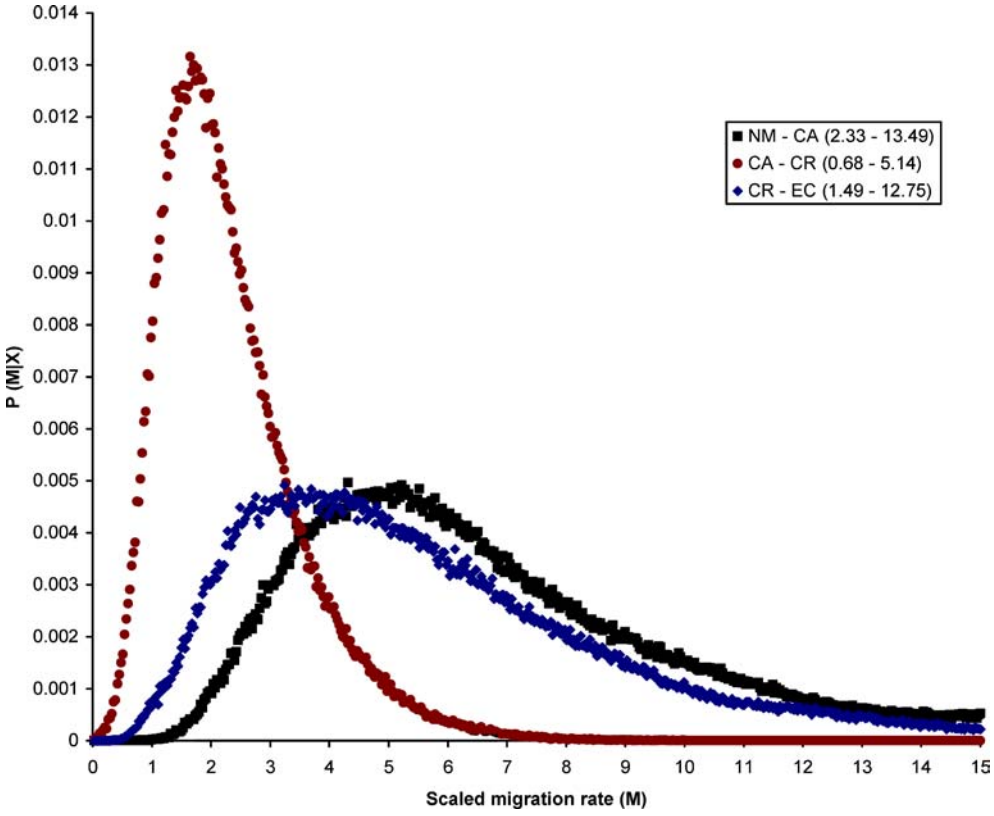

Figure 3. Posterior distributions of the scaled migration rate ($M = 2 \times \text{Ne} \times \text{migration rate}$) between pairs of adjacent coastal populations. NM: Northern Mexico; CA: Central America; CR: Costa Rica; and Ec: Ecuador. 95% credibility intervals are shown in parenthesis.

Table 6. Pairwise comparisons of F_{ST}-values between coastal and offshore populations of spotted dolphins based on seven microsatellite loci

	Northern Mexico	Central America	Costa Rica	Ecuador
Central America	0.0057			
Costa Rica	−0.0017	−0.0009		
Ecuador	−0.0124	0.0046	−0.0018	
Offshores	−0.0032	0.0416***	0.0352***	0.0202***

Statistically significant results (calculated from 10,000 random permutation tests) are outlined in grey. ***$P < 0.001$.

the four coastal and one offshore populations, which was highly significant ($F_{ST} = 0.0691$, $P < 0.001$; $\Phi_{ST} = 0.0556$, $P < 0.001$). When tests were conducted for each sex independently, statistical significance was lost for pairwise comparisons involving males in the case of Central America (male $n = 14$) and females for Costa Rica (female $n = 8$).

The posterior distributions of the scaled migration rate ($M = 2 \times \text{Ne} \times \text{migration rate}$) between pairs of adjacent coastal populations showed different modes, ranging from $M = 1.7$ between Central America and Costa Rica to $M = 4.3$ between Northern Mexico and Central America. However, there was overlap within the 95% credibility intervals for all posterior distributions (Figure 3).

Microsatellite results

Genetic differentiation between coastal and offshore samples was highly significant ($F_{ST} = 0.0180$, $P < 0.001$).

Pairwise comparisons using AMOVA were made among the four coastal and the offshore populations. Although only 0.8% of the total molecular variance overall was accounted for by the stratification, the result was statistically significant ($F_{ST} = 0.0085$, $P < 0.001$). Results from pairwise comparisons are shown in Table 6. Statistically significant results for F_{ST} were found for all but one of the four pairwise comparisons between offshore and coastal populations (Northern Mexico), while none of the comparisons between coastal populations resulted in a statistically significant F_{ST} value (Table 6).

Discussion

mtDNA diversity

Due to the existence of a very high proportion of unique haplotypes that differ by only a small number of bases, high levels of haplotypic diversity were observed in the present study. This low nucleotide diversity, similar for both coastal and offshore animals as well as for the nucleotide divergence between the two, was smaller than divergence values reported between closely related species in cetaceans within the same ocean basin, such as 2.1% for common dolphins, *Delphinus delphis* and *D. capensis* (Rosel et al. 1994) or 4.4% for bottlenose dolphins, *Tursiops truncatus* and *T. aduncus* (Wang et al. 1999). This, together with the lack of signal in phylogenetic trees and, above all, the existence of shared haplotypes between both types, suggests a long history of gene flow between coastal and offshore populations. This gene flow might still be taking place, or, if already interrupted, insufficient time for lineage sorting would have elapsed since isolation.

Population structure

Analysis of variance in the mitochondrial control region within and among populations uncovered substantial levels of genetic partitioning. When coastal samples were divided into four populations (Figure 1), significant differences were found for all pairwise comparisons (Tables 4 and 5), revealing structure in the currently recognized single coastal stock.

Pairwise differences were larger between populations in the southern part of the range (Central America – Costa Rica, and Costa Rica – Ecuador), as compared to the north (Northern Mexico – Central America). One explanation for these differences could be that the southern populations (especially Costa Rica, which has the largest F_{ST} and Φ_{ST} values in all pairwise comparisons) were the first to diverge, while Northern Mexico is the most recently diverged. The different nature of F_{ST} and Φ_{ST} values would further support this conclusion. F_{ST} is based solely on haplotype fre-

quencies, while Φ_{ST} takes into consideration both haplotype frequencies and genetic distances among haplotypes. In the case of the northern populations, only F_{ST} values are statistically significant, indicating that there has not been enough time for haplotypes to diverge, even if the frequencies of these haplotypes differ.

An alternative explanation for this pattern could be that there are different rates of gene flow among northern and southern populations. If this was the cause of differences between F_{ST} and Φ_{ST} values, then comparisons between Central America and Costa Rica, and Costa Rica and Ecuador should present the lowest migration rates. However, the posterior distributions of the scaled migration rate (M) between adjacent coastal populations (Figure 3) do not support this. While we observed the lowest value of M for the comparison of Central America and Costa Rica, there was overlap within the 95% credibility intervals for all posterior distributions, suggesting similar migration rates between northern and southern population comparisons.

Posterior distributions for the scaled divergence time (T) were so broad as to be virtually uninformative (results not shown). As explained in Nielsen and Wakeley (2001), this results from a large Monte Carlo variance in the parameter and can occur for very large values of T. Thus, we were unable to make direct inferences regarding differential divergence times using the MDiv algorithm on our data.

Mitochondrial results also revealed genetic differentiation between the offshore and the coastal populations. Statistically significant differentiation was detected when comparing overall coastal and offshore samples. Additionally, all the pairwise comparisons between offshore and coastal populations were statistically significant, except for Northern Mexico (Tables 4 and 5), supporting a strong subdivision throughout the study area.

Contrasting with the mitochondrial results, analysis of variance of microsatellite loci uncovered very low levels of genetic partitioning among the coastal populations, but showed differentiation when overall offshore and coastal samples were compared, as well as between each of these and the offshore population, except for Northern Mexico. Still, although genetic differentiation between offshore and coastal animals was highly significant, no structure was detected within the coastal region (Table 6).

This pattern of greater structure in mitochondrial than in nuclear markers appears to be common in marine mammals (Hoelzel et al. 2002). A possible explanation for the differential structure could be the lower effective population size for mitochondrial markers in relation to nuclear markers. In our study, structure based on microsatellite loci may have gone undetected due to the low sample sizes, which would cause population differences to be statistically non-significant (Waples 1998), resulting in an underestimate of subdivision. A third explanation would be the existence of differential dispersal rates between sexes. However, when a separate analysis of genetic structure for each sex was undertaken, loss of significance was not correlated to either sex but, rather, to sample size. Therefore, although sex-biased dispersal could not be ruled out, no evidence for its existence was found.

The Northern Mexico population showed some special characteristics. Despite having the second highest sample size for any coastal population, no statistically significant differentiation was shown when compared to offshore animals, either for mitochondrial or microsatellite markers. Morphological differences between coastal spotted dolphins from this area and other coastal and offshore animals have been previously described (Schnell et al. 1982; Douglas et al. 1984). Reduced gene flow, in comparison with more southerly areas, between both subspecies was suggested as the likely cause (Douglas et al. 1984). This is in contrast to our results, which indicate a higher level of gene flow between Northern Mexico and offshore animals than for any other coastal population.

It should be noted that low sample sizes could lead to errors in the assessment of population differentiation. Small sample sizes can inadequately capture the true haplotypic frequency of a population and severely limit the power of an analysis to detect differentiation, (Peterman 1990; Dizon et al. 1995), generally causing real population differences to be statistically non-significant (i.e., an increase in the type II error – Waples 1998) and tending to underestimate overall structure. The fact that population differentiation was detected despite the relatively small sample sizes indicates that some genetic isolation exists, both

between offshore and coastal populations as well as among coastal populations, as long as sampling is not biased. While the spatial and temporal coverage of the collected samples made sampling bias towards particular haplotypes unlikely, future analyses with more samples will be an important test of these results.

Even with the small sample sizes for some populations in this study, F_{ST} values among these populations, though relatively small, were highly significant, and fall well within the range observed for other cetaceans, such as Pacific white-sided dolphins ($F_{ST} = 0.055$, Hayano et al. 2004), bottlenose dolphins from the Mediterranean Sea, Western North Atlantic, Western Africa, and the Gulf of Mexico ($F_{ST} = 0.072$–0.103, Natoli et al. 2004), or Dall's porpoises ($F_{ST} = 0.033$–0.210, Escorza-Treviño and Dizon 2000). Nonetheless, the number of populations could increase, or the boundaries be reassessed, upon examination of more samples. For instance, in the present study, only seven samples were available from Ecuador, and these were pooled with samples from Panama to attain a higher sample size in the southern-most population. It is possible, though, that some structure exists within this area, and could be unveiled as more samples are added and the sampling gaps are filled.

Despite these limitations, these results bear great importance for the conservation and management of coastal delphinids both in the ETP and elsewhere. While population subdivision between coastal and offshore forms has been documented in several other delphinid species (Rosel et al. 1994; Hoelzel et al. 1998), this paper presents evidence of significant structure within what was previously considered to be a single panmictic coastal population. Based on our findings, we would encourage investigations of coastal forms of other species for similar levels of structuring.

Although listed as depleted, the status of coastal spotted dolphins in the ETP is an important, but unresolved component of the tuna-dolphin issue. The magnitude of the early kill in this stock is uncertain, but was probably quite high relative to population size (Gerrodette pers. comm.). Because the results shown here indicate significant population subdivision in coastal spotted dolphins, the historical impact of the tuna fishery may have been much higher than previously believed. Our results suggest that these populations should be treated as separate units for management purposes.

Finally, the finding that the Northern Mexico population is indistinguishable from the offshore animals suggests that the current geographic and morphological criteria for distinguishing *Stenella attenuata attenuata* from *S. a. graffmani* in this region warrants re-evaluation. This effort rests on the ability to collect more biopsy samples as well as detailed information on external morphology in the future.

Acknowledgements

We would like to thank Sarah Mesnick and Rick LeDuc for their spirited and helpful discussions and four anonymous reviewers for their comments, which greatly improved the manuscript. Thanks to Carrie LeDuc for her technical help. We are also grateful to those who provided samples for this study.

References

AIDCP (1998) Agreement on the International Dolphin Conservation Program. International agreement signed May 21, 1998 in Washington, DC. Text of agreement available at http://www.iattc.org/PDFFiles/AIDCP%20Oct%202001%20ENG.pdf.

Amos B, Hoelzel AR (1991) Long-term preservation of whale skin for DNA analysis. In: The Genetic Ecology of Whales and Dolphins (eds. Hoelzel AR), pp. 99–103. Special publication No. 13 of the IWC, Cambridge, England.

Anderson S, Bankier AT, Barrell BG, et al. (1981) Sequence and organization of the human mitochondrial DNA. *Nature*, **290**, 457–465.

Bérubé M, Palsbøll P (1996) Identification of sex in cetaceans by multiplexing with three ZFX and ZFY specific primers. *Mol. Ecol.*, **5**, 283–287.

Brassel KE, Reif D (1979) A procedure to generate *Thiessen polygons*. *Geogr. Anal.*, **11**, 289–303.

Cockerham CC, Weir BS (1993) Estimation of gene flow from *F*-statistics. *Evolution*, **47**, 855–863.

Dizon AE, Perrin WF, Akin PA (1994) Stocks of dolphins (*Stenella* spp. and *Delphinus delphis*) in the Eastern Tropical Pacific: A phylogeographic classification. NOAA Technical Report NMFS, 119 pp.

Dizon AE, Taylor BL, O'Corry-Crowe GM (1995) Why statistical power is necessary to link analysis of molecular variation to decisions about population structure. *Am. Fish. Soc. Symp.*, **17**, 288–294.

Douglas ME, Schnell GD, Hough DJ (1984) Differentiation between inshore and offshore spotted dolphins in the eastern tropical Pacific Ocean. *J. Mammol.*, **65**, 375–387.

Escorza-Treviño S, Dizon AE (2000) Phylogeography, intraspecific structure, and sex-biased dispersal of Dall's porpoise, *Phocoenoides dalli*, revealed by mitochondrial and microsatellite DNA analyses. *Mol. Ecol.*, **9**, 1049–1060.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Galver LM (2002) The molecular ecology of spinner dolphins, stenella longirostris: genetic diversity and population structure. Ph.D. dissertation. University of California, San Diego.

Gosliner ML (1999) The tuna-dolphin controversy In: Conservation and Management of Marine Mammals (eds. Twiss JR Jr., Reeves RR), pp. 120–155. Smithsonian Institution Press, Washington, DC.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Hayano A, Yoshioka M, Tanaka M, Amano M (2004) Population differentiation in the Pacific white-sided dolphin *Lagenorhynchus obliquidens* inferred from mitochondrial DNA and microsatellite analyses. *Zool. Sci.*, **21**, 989–999.

Hoelzel AR, Goldsworthy SD, Fleischer RC (2002) Population genetics. In: Marine Mammal Biology: An Evolutionary Approach (eds. Hoelzel AR), pp. 325–352. Blackwell Science, Oxford, England.

Hoelzel AR, Potter CW, Best PB (1998) Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. *Proc. R. Soc. Lond. B*, **265**, 1177–1183.

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) *MEGA2: Molecular Evolutionary Genetics Analysis Software*, Arizona State University, Tempe, Arizona, USA.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends Ecol. Evol.*, **18**, 189–197.

Natoli A, Peddemors VM, Hoelzel R (2004) Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. *J. Evol. Biol.*, **17**, 363–375.

Nei M (1987) *Molecular Evolutionary Genetics*, Columbia University Press, New York, USA.

Nielsen R, Wakeley JW (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics*, **158**, 885–896.

Perrin WF, Scott MD, Walker GJ, Cass VL (1985) Review of geographical stocks of tropical dolphins (*Stenella* spp. and *Delphinus delphis*) in the eastern Pacific. NOAA Technical Report NMFS 28, 28 p.

Perrin WF, Schnell GD, Hough DJ, Gilpatrick JW Jr, Kashiwada JV (1994) Reexamination of geographic variation in cranial morphology of the pantropical spotted dolphin, *Stenella attenuata*, in the eastern Pacific. *Fish. Bull.*, **92**, 324–346.

Peterman RM (1990) Statistical power analysis can improve fisheries research and management *Canad. J. Fish. Aqua. Sci.*, **47**, 2–15.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *J. Hered.*, **86**, 248–249.

Rice DW (1998) *Marine Mammals of the World Systematics and Distribution*, Special publication No. 4 of the Society for Marine Mammology, Lawrence, Kansas, USA.

Roff DA, Bentzen P (1989) The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Mol. Biol. Evol.*, **6**, 539–545.

Rosel PE, Dizon AE, Heyning JE (1994) Genetic analysis of sympatric morphotypes of common dolphins (genus *Delphinus*). *Mar. Biol.*, **119**, 159–167.

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406–425.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN ver. 2.000: A Software for Population Genetics Data Analysis*, Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Schnell GD, Douglas ME, Hough DJ (1982) Geographic Variation in Morphology of Spotted and Spinner Dolphins (*Stenella attenuata and S. longirostris*) from the Eastern Tropical Pacific. SWFSC Admin Report LJ-82–15C. 213 p.

Schnell GD, Douglas ME, Hough DJ (1986) Geographic patterns of variation in offshore spotted dolphins (*Stenella attenuata*) of the Eastern Tropical Pacific Ocean. *Mar. Mammal Sci.*, **2**, 186–213.

Swofford P (1993) *PAUP: Phylogenetic Analysis Using Parsimony, Version 31*, Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois, USA.

Taylor BL (1997) Defining "Population" to meet management objectives for marine mammals In: Molecular Genetics of Marine Mammals (eds. Dizon AE, Chivers SJ, Perrin WF), pp. 49–65. Society for Marine Mammology, Lawrence, KS(Special Publication Number 3.).

Valsecchi E, Amos W (1996) Microsatellite markers for the study of cetacean populations. *Mol. Ecol.*, **5**, 151–156.

Wang JY, Chou LS, White BN (1999) Mitochondrial DNA analysis of sympatric morphotypes of bottlenose dolphins (genus: *Tursiops*) in Chinese waters. *Mol. Ecol.*, **8**, 1603–1612.

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species *J. Hered.*, **89**, 438–450.

Winnepenninckx B, Backeljau T, De Wachter R (1993) Extraction of high molecular weight DNA from mollusks. *Trends Genet.*, **9**, 407.

Wright S (1965) The interpretation of population structure by *F*-statistics with special regard to systems of mating *Evolution*, **19**, 395–420.