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RESEARCH ARTICLE

Conservation genetics of the franciscana dolphin in Northern Argentina: population structure, by-catch impacts, and management implications

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Abstract Evaluating population structure in the marine environment is a challenging task when the species of interest is continuously distributed, and yet the use of population or stock structure is a crucial component of management and conservation strategies. The franciscana dolphin (*Pontoporia blainvillei*), a rare endangered coastal cetacean, suffers high levels of by-catch all along its distribution range in the Western South Atlantic, and questions have been raised about boundaries or divisions for population management. Here we apply genetic tools to better understand population structure and migration, sex-biased dispersal, and to assess potential genetic and demographic impacts of by-catch. Our analyses, based on mtDNA control region sequences, reveal significant genetic division at the regional level and fine-scale structure within our study area. These results suggest that the population in northern Buenos Aires is the most isolated population in Argentina. We found no significant departure from an equal sex ratio among the by-caught animals. A few cases of multiple entanglements appeared to be mother–calf pairs based on field observations and individuals sharing the same mtDNA control region lineage. The distribution of haplotype frequencies observed could imply that some maternal lineages are more prone to be subject to higher rates of by-catch, although biopsy sampling is necessary to fully evaluate whether maternal lineage distributions are the same for biopsy sampled and by-caught animals. A genetic indication of population size disequilibrium was detected for all populations in Argentina, which is consistent with available rates of by-catch and abundance estimates. Collectively, our findings support the current scheme of larger recognized Franciscana Management Areas (FMA), but argue for a finer-scale subdivision within Northern Buenos Aires region (FMA IV). Finally, an integrated approach to promote conservation of this endangered small cetacean has to involve identification of genetic and demographic threats, a more sustainable fishery strategy to reduce by-catch, and designation of protected areas that are supported by underlying population structure for franciscana dolphins.

Keywords Franciscana dolphin · By-catch impacts · Population structure · Conservation genetics · Management

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Introduction

Detecting population structure among continuously distributed groups of animals is one of the most challenging aspects for practitioners in conservation genetics (Amos and Balmfored 2001; Hey and Machado 2003). In the marine environment, this problem is further complicated by the fact that many species are continuously distributed over large and (apparently) homogeneous areas, therefore exhibiting virtually no a priori signs of population subdivision. Yet understanding population structure is a key component of management efforts (King and Burke 1999; Palumbi 2003; Waples 1998). In cases where there is absolute certainty about a species' population structure, specific protected areas could be set up to help conserve as much of the genetic diversity of the species as possible, or minimally for each identified population or distinct population segment (DPS) (Buonaccorsi et al. 2005).

Conceptually, the problem of defining populations and their associated boundaries is equally challenging. There are no fully objective, quantitative, or unequivocal population definitions, but instead qualitative and operational definitions are often used that are context dependent. In an attempt to provide guiding principles that could inform the discussion of population definition in the context of management, Waples and Gaggiotti (2006) proposed two types of population definitions: an evolutionary one that centers around reproductive interactions among individuals, and an ecological one framed by demographic forces and mainly concerned with co-occurrence in space and time. Evolutionary speaking, populations could be considered isolated for management purposes when derived statistical estimators approach certain values (i.e., $N_e m < 25$ or $F_{ST} > 0.01$ (Waples and Gaggiotti 2006).

Prior to such suggestions for a more quantitative operational approach to define population structure, a degree of genetic structure (primarily in the form of lack of panmixia) had been considered sufficient indication of population discreteness and has served to delimit management units for small cetaceans (Brennin et al. 1997; Chivers et al. 2002; Rosel et al. 1995, 1999; Wang et al. 1996). Management schemes for harbor porpoises (*Phocoena phocoena*) in the North Atlantic and North Pacific designed based on population genetic data are examples of this approach (Chivers et al. 2002; Rosel et al. 1999; Wang et al. 1996). Despite their methodological differences, these studies proposed the recognition of independent management units when genetic differentiation among a priori designated populations yielded significant results for any of the parameters used (F_{ST}, Φ_{ST}, or X^2). Nonetheless, defining management units based on genetic data remains situational and context dependent. An appropriate evaluation of threats corresponding to analyses of population structure and delimitation of management units is often essential (Hoelzel 1998; Taylor and Dizon 1999), and permits an informed allocation of resources that can be devoted to management strategies.

Using a combination of genetic, demographic, and other relevant biological information represents a more comprehensive approach for resolving population boundaries in continuously distributed cetaceans (Feral 2002; Taylor and Dizon 1999). Four types of data are particularly relevant and could be used in combined analyses of population structure: (i) Oceanographic data may serve to construct a priori hypotheses regarding the placement of subpopulation boundaries for genetic analyses or to corroborate evidence of genetic structure, since environmental variables such as depth and temperature may have importance in the development of ecological niches (Hoelzel 1998; Natoli et al. 2005). (ii) Ecological partitioning, derived from satellite telemetry data, parasite and contamination loads or feeding habits, may provide evidence of shallow population structure related to dispersal and movement patterns (Andrade et al. 2000; Aznar et al. 1994, 1995; Bordino and Wells 2005; Ott 1994; Pinedo 1982; Pinedoet al. 1989). (iii) Morphological characters served to propose and or define cetacean stocks and populations, and have been typically used before molecular techniques were commonly available (Messenger and McGuire 1998; Pinedo 1991; Rosenbaum et al. 1995). (iv) Molecular genetic data provide a wide range of estimators that serve to define and characterize demographic groups or populations with a higher degree of resolution as compared to morphological data (Amos and Balmford 2001; Avise 1995, 2000; Frankham 1995).

For molecular genetic data, mitochondrial DNA (mtDNA) has been commonly analyzed to estimate genetic diversity in cetacean populations (Hoelzel 1994, 1998; Hoelzel et al. 1991), and to evaluate patterns of association among maternal lineages with anthropogenic effects, reproductive rates and mortality, and behavioral characteristics (Baker et al. 1994; Weinrich et al. 2006). The frequency distribution of maternal lineage haplotypes among populations and their molecular distances were used to assess gene flow patterns and population structure (Excoffier 1992; Hartl and Clark 1997; Weir and Cockerham 1984). In addition, recently developed software packages allow robust estimations of population parameters such as migration using a likelihood approach (see ''Methods'' section) (Nielsen and Wakeley 2001; Wakeley 1996).

The franciscana dolphin (*Pontoporia blainvillei*) is a coastal species endemic to the southwestern Atlantic Ocean from Itaunas, Brazil (17°25′ S) to the Golfo Nuevo, Argentina (43° S approx, see Fig. 1) (Crespo et al. 1998). Its coastal distribution makes it vulnerable to anthropogenic activities; coastal cetaceans are mainly threatened by incidental

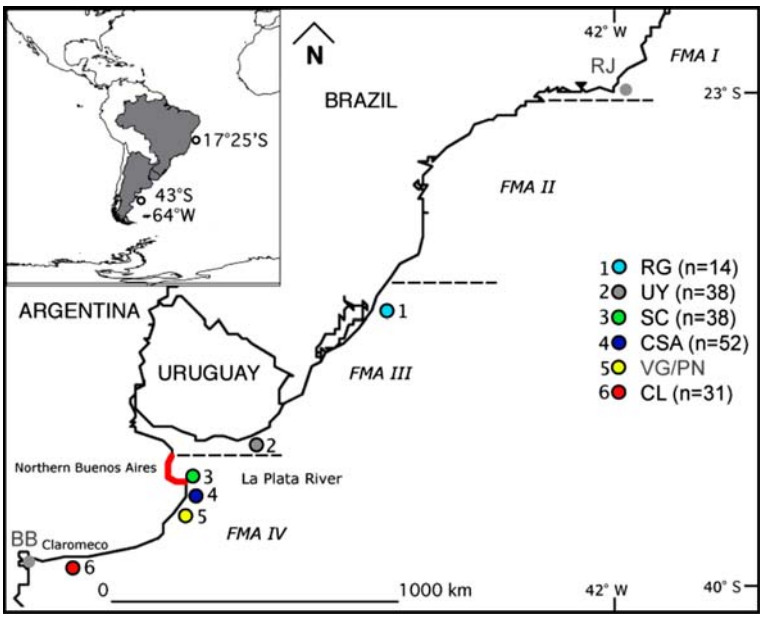

Fig. 1 Study area map with sampling sites in color and numbered. *A priori defined* geographic locations RJ, VG/PN and BB (*in grey*) were not included in the genetic structure analysis. Sample size for each of the populations in the genetic structure analysis is in parenthesis. Colored sampling sites were included in the statistical parsimony network. The red line marks the Bahia Samborombon and the dotted lines delimit the four FMAs (adapted from Secchi et al. 2003)

entanglement or by-catch (Berggren et al. 2002; Bordino et al. 2002; Dawson et al. 1998; Dawson and Slooten 1993; Majluf et al. 2002; VanWaerebeek and Reyes 1990; VanWaerebeek et al. 1997), which severely impacts population size and connectivity, as well as other demographic parameters such as the sex ratio, age structure and social status. This dolphin is no exception, and due to its incidental entanglement in gillnets, is currently suggested as the most threatened cetacean along the east coast of South America (Bordino et al. 2002; Secchi et al. 1998, 2003; Secchi and Wang 2002). The franciscana is listed in the Appendix II of the Convention in International Trade of Endangered Species (CITES), in the Appendix I of the Convention on Migratory Species (CMS), appears as ''Data Deficient'' in the International Union for the Conservation of Nature and Natural Resources (IUCN) Red Data Book, and is designated as a ''species of concern'' by the IUCN Cetacean Specialist Group. Franciscana dolphins are incidentally captured by fishermen in shallow waters, where small and mid-scale fishing activities take place (Corcuera et al. 1994). An annual catch of 500 dolphins was estimated from the fisheries of the Buenos Aires coastal area in Argentina (Corcuera et al. 1994). However, it has been suggested that by-catch in Argentina has been underestimated during the last decade (Bordino et al. 2004; Bordino et al. 2002).

Although some basic information regarding the behavioral ecology, abundance, and incidental mortality of the species in Argentina has been reported (Bordino et al. 2004; Bordino et al. 2002; Bordino et al. 1999; Crespo et al. 2004), additional knowledge concerning population structure is needed for the implementation of locally based management plans (Secchi et al. 2003). Because franciscana dolphins experience different levels of threats throughout their range, management authorities require information about the distribution, structure, and connectivity among populations in order to most effectively evaluate and minimize impacts from these threats. As part of these efforts, Secchi et al. (2003) proposed the creation of four Franciscana Management Areas (FMAs), the southernmost (FMA IV) including all areas to the south of the Bahia Samborombon in Argentina (Fig. 1).

There have been attempts to identify biological populations for this species within the context of the current FMA definitions. Based on osteological differences, Pinedo (1991) was able to distinguish two different stocks of franciscanas, one at each side of the Santa Catarina State in Brazil. Secchi and colleagues (Secchi et al. 1998) performed a genetic analysis based on control region mtDNA data from 10 individuals from Rio de Janeiro and 10 individuals from Rio Grande do Sul, and suggested that these were two genetically distinct populations, supporting Pinedo (1991). Subsequently, Lazaro and colleagues (Lazaro et al. 2004) used control region mtDNA data from 94 individuals sampled in Uruguay, in Buenos Aires, and from those previously studied by Secchi et al. (1998) in Brazil. This recent study agreed with Secchi et al. (1998) findings and further suggested the presence of two additional populations, one in what they called Rio de la Plata (Uruguay and northern Buenos Aires combined) and the other in Claromeco, Argentina.

Here we apply genetic data from 190 individuals to further assess the degree of population structure within this coastally distributed endangered small cetacean in South America. In particular, by analyzing 107 specimens from previously unsampled areas within the distribution of this species, we attempt to evaluate if the current FMAs are still valid in light of the new genetic data and analyses. At a finer geographical

scale, our analysis of genetic and demographic information for this endemic and endangered coastal cetacean provides needed information that can be incorporated into management strategies involving the creation of marine protected areas in coastal regions proximate to Buenos Aires.

Methods

Sample collection and molecular analyses

Tissue samples of 107 individuals were obtained from incidentally entangled (also termed by-catch or by-caught) franciscana dolphins (*Pontoporia blainvillei*) in coastal-fishery gillnets in San Clemente (SC), Cabo San Antonio (CSA) and other localities in the Buenos Aires area between 2000 and 2005 (Fig. 1). These samples were collected as part of a necropsy procedure, and were preserved in ethanol (96%). The 83 remaining mtDNA control region sequences were obtained from Genbank, and represent haplotypes with known geographic locations previously identified by Secchi et al. (1998) and Lazaro et al. (2004).

Total genomic DNA was extracted from muscle tissue samples following the procedures in a commercially available kit (QIAamp Tissue Kit from QiaGen, California). A fragment of 560 bp of the mtDNA control region was amplified (primers L159256 and H00651 (Kocher et al. 1989). PCR amplifications were carried out in a 50 µl reaction volumes using the following conditions: 1 pM of each primer, 10 mM Tris, 50 mM KCl, 3 mM MgCl, 1 unit AmpliTaq^{TM} Polymerase, 1 mM each NTP, and 1 µl (1–2 ng) template DNA. Thermal profiles were as follows: initial denaturation for 3 min at 94°C followed by 32 amplification cycles [30 s at 94°C, 30 s at 52°C, 1 min at 72°C] and a final 5 min extension at 72°C. PCR products were cycle-sequenced (both forward and reverse) with dye-labeled terminators using conditions recommended by the manufacturer (Applied Biosystems). The thermal profile used was as follows: 30 cycles [10 s at 96°C, 5 s at 50°C, and 4 min at 60°C]. The samples were then cleaned up by filtration in a matrix of Sephadex^{TM} /water or alternatively by ethanol precipitation, and analyzed in a 3730 DNA Analyzer (Applied Biosystem®, Foster City, CA).

Determination of sex was accomplished by PCR amplification and subsequent Taq I digestion of homologous regions on the X and Y chromosomes (ZFX/ZFY) (Palsboll et al. 1992).

Haplotyping and diversity estimates

DNA sequence variation was characterized into mtDNA haplotype definitions following the nomenclature in Secchi et al (1998) and Lazaro et al (2004). From the 560 bp mtDNA fragment, a 407 bp consensus region containing most variation was examined within our samples and compared with those obtained by Secchi et al. (1998) and Lazaro et al. (2004). Matching of sequences to a haplotype was done using Collapse 1.2 (available from http://www.darwin.uvigo.es) and MacClade v. 4.01. We further verified this ''haplotyping'' procedure with the DNAsp software (Rozas et al. 2003). Haplotypic diversity, Hd (Nei 1987), the mean number of pairwise differences among sequences, K (Kimura 1980; Tajima 1983), and the nucleotide diversity, π (Kimura 1980; Nei et al. 1975; Tajima 1983) in our sample were assessed using Arlequin 2.0 (Raymond and Rousset 1995) and DNAsp.

Analysis of population structure

We tested for population differentiation between groups of individuals in Rio Grande (RG) Brazil, Uruguay (UY), the samples we obtained in San Clemente (SC) and Cabo San Antonio (CSA) in Argentina, and those previously reported for the locality of Claromeco (CL), also in Argentina (Secchi et al. 1998, Lazaro et al. 2004, see Table 1). Individuals in Rio de Janeiro (RJ) were not included in our study, as they have shown very significant divergence from any other studied population to the north of our study area (Lazaro et al. 2004). Samples we collected in the localities of Villa Gessell (VG) (n = 2), Pinamar (PN) (n = 2) and Bahia Blanca (BB) (n = 3), all in Argentina, were not included in this analysis of population structure due to their small sample size. Samples with unknown geographic location (UN) (n = 10) were also excluded.

The diversity and geographic variation of mitochondrial DNA control region haplotypes was quantified using the Analysis of Molecular Variance procedure (AMOVA,

Table 1 Geographic distribution of the 190 franciscana dolphin samples used in this study with corresponding study area and year if samples were presented in previous studies

Area	FMA	n	Authors	Analysis (this study)
RG	III	14	Lazaro et al, 2004	1, 2, 3
UY	III	38	Lazaro et al, 2004	1, 2, 3
SC	IV	38	this study	1, 2, 3
CSA	IV	52	this study	1, 2, 3
CL	IV	31	Lazaro et al, 2004	1, 2, 3
VG	IV	2	this study	3
PN	IV	2	this study	3
BB	IV	3	this study	3
UN	–	10	this study	3

Analyses 1, 2, and 3 correspond to the present work and refer to population genetic analysis (1), maximum likelihood migration parameter estimations (2) and haplotype network (3)

Excoffier et al. 1992) as implemented in the software Arlequin 2.0. F_{ST} and Φ_{ST} statistics were computed. The significance of the observed Φ or F-statistics was tested using the null distribution generated from 5,000 non-parametric random permutations of the data matrix variables. The extent of geographical heterogeneity in haplotype frequency distributions was further assessed through a χ^2 analysis, conducted in DNA SP v3.0 (Rozas et al. 2003). Chi-square statistics have often been shown higher power than sequence based statistics for detecting population structure (Hudson et al. 1992). To test for a random distribution of individuals between pairs of populations, we conducted an exact test of population differentiation using Arlequin 2.0. The significance of this test (analogous to a Fisher's test) is an indication of non-random association of individuals among populations for both global and pairwise comparisons. In order to test for significant patterns of isolation by distance we carried out a Mantel test of autocorrelation between genetic and geographic distances using Arlequin 2.0. The significance of this test was assessed through 10,000 random permutations of the variables.

Patterns of genetic variation between the identified haplotypes were depicted using Median Joining networks (Bandelt et al. 1999) as implemented in Network (http://www.fluxus-engineering.com). We also constructed statistical parsimony haplotype networks for comparison (Posada and Crandall 2001; Templeton et al. 1992) using the software TCS (Clement et al. 2000). All samples, including those from VG/PN, BB, and UN, were included in the haplotype networks.

Migration rates

A Markov Chain Monte Carlo (MCMC) procedure was used to produce migration rate estimates among populations. This procedure, implemented in the program MDIV (Nielsen and Wakeley 2001); available from the Computational Biology Service Unit at Cornell University at http://www.cbsuapps.tc.cornell.edu/mdiv.aspx), jointly estimates multiple parameters for pairs of populations in a Maximum Likelihood framework. This approach differs from most classical models of population subdivision in that the latter make one of two assumptions: either migration is constant and time of population divergence infinite (equilibrium migration model), or populations are assumed to have diverged a finite amount of time in the past and to have become effectively isolated since this time (isolation model) (Nielsen and Wakeley 2001; Wakeley 1996). We employed the likelihood approach to simultaneously estimate migration rates and divergence times between RG–UY, UY–SC, SC–CSA, and CSA–CL. This partitioning scheme of contiguous populations resembles a stepping stone pattern of population connectivity. We also estimated the abovementioned parameters between areas CSA and UY in order to contrast these results with the fixation indices in the population structure analysis.

MDIV (Nielsen and Wakeley 2001) estimates $M = (m/\mu)$ or the migration rate per gene per generation between populations scaled by the mutation rate, $T = (t\mu)$ or the time since the two populations diverged scaled by the mutation rate, and the parameter $\theta = (4N\mu)$, where N is the effective population size, and μ the mutation rate of the studied gene region. We also calculated the population migration rate per generation $M_G = (2\ Nm)$, which is the effective rate at which genes come into a population, as well as divergence time in units of $2N$ generations ($t/2N$). No estimates of mutation rate are necessary for these conversions.

Run parameters were set to default values, excepting the maximum-scaled migration rate (see Table A1 in the Appendix for a detailed description of all run parameters). We performed a minimum of 10 independent runs of 1×10^7 iterations each, and a burn-in of 500,000 iterations for each of our parameter estimations. We compared these with runs using 1×10^6 iterations of burn-in and observed no differences in the parameter probability distribution, indicating that 500,000 iterations were appropriate for the burn-in stage in our runs. In three out of the ten runs of the M estimation for CSA-UY, the scaled migration rate was reported being > 30, as these were cases of lack of convergence to a most likely value, resulting probably from high similarity between some of the sequences being compared by the algorithm. Although distinguishing between M estimates greater than 30 is irrelevant from an evolutionary point of view, it may be important from a demographic perspective in small or medium size populations (Waples 1998). Since our three mentioned runs did not result in a most likely value for M we decided to exclude them from our calculations (see Appendix).

Testing sex-biased genetic structure in Cabo San Antonio

We tested for sex-biased population structure among the samples obtained in Argentina, estimating the previously used parameters (F_{ST}, Φ_{ST}, X^2 and the exact test of population differentiation) for males, females and all individuals. These analyses were performed including areas SC and CSA, since we have no information on the sex of animals from Brazil, Uruguay or Claromeco.

Assessing genetic and demographic impacts of by-catch in coastal Buenos Aires

We tested for departures from a balanced sex ratio for each area and all areas combined listed in Table 1 and Fig. 1, as well as for each year and all years combined. We also computed the sex ratio for samples representing high frequency haplotypes, in order to test for sex-bias among samples of those haplotypes. In addition, we determined the mitochondrial lineage identity of animals that were entangled in the same gillnet, during the same 24 h period (the average time between deploying and retrieving fishing gear). Lastly, we tested for population size equilibrium in this area by calculating the raggedness index (Harpending 1994) from the mismatch distribution using DNAsp software. The raggedness index and mismatch distribution are used to test hypotheses of population expansion and population stability (Rogers and Harpending 1992; Weber et al. 2000), irrespective of the assumed mutational process of the genetic region being analyzed (Rogers et al. 1996). The raggedness index is directly related to the degree of mismatch between the observed and expected distributions.

Results

Haplotype reconstruction and genetic diversity

The genetic analysis of the mtDNA control region from franciscana dolphins in the Buenos Aires region resulted in the identification of 19 new haplotypes for the species (Genbank accession numbers EF394099–EF394117), almost equal to what was previously known for all FMAs and bringing the total of haplotypes known in Franciscana to 42. Overall, 29 of these haplotypes were detected in our study. Secchi et al. (1998) and Lazaro et al. (2004) reported six and 22 haplotypes, respectively.

Following the naming conventions of Secchi et al. (1998) and Lazaro et al. (2004), the new haplotypes in our study come from animals sampled in SC and the CSA areas (Fig. 1). Haplotypes previously identified by Lazaro et al. (2004) and Secchi et al. (1998) were found in most of our sampling locations (see ''Population genetic structure and migration'' section below).

Population SC exhibits the lowest value for every estimate of genetic diversity that was conducted (Table 2 and Fig. 2), indicating maternal lineages sampled from this areas were similar in terms of molecular distance from one another (π and K). Population CSA showed the highest haplotype diversity (highest Hd) although these maternal lineages also had similar molecular distance values when compared to one another. Populations CL, RG and UY show intermediate levels of genetic diversity at both the haplotype and molecular levels.

Median-Joining and Statistical Parsimony networks show comparable spatial patterns of genetic diversity, therefore only the latter is shown. The identified haplotypes are widely distributed in the entire region, with the exception of two haplotypes found in the areas of CSA and CL (Figs. 1 and 3). As depicted in the network, most of the differences between geographic locations are in haplotype frequencies rather than haplotype identity. The two most frequent haplotypes are still those reported as highest by Lazaro et al. (2004), while the rest of the haplotypes occur at lower frequencies.

Population genetic structure and migration

The among-groups component of the Analyses of Molecular Variance analysis was significant when both the haplotype frequencies as well as molecular distances were considered ($F_{ST} = 0.05692$, $p < 0.005$; $\Phi_{ST} = 0.05692$, $p < 0.005$). The χ^2-test also showed significant differentiation ($\chi^2 = 201.617$, $p < 0.001$, df = 116).

In the pairwise comparisons, we found significant population structure between SC and all other populations when both the haplotype frequencies as well as molecular distances were considered (Table 3). CL is differentiated from all other populations only when haplotype frequencies are used, a result that is in agreement with Lazaro et al. (2004). The exact test of population differentiation

Table 2 Genetic diversity indices, where n is the sample size, H (n) is the number of haplotypes, Hd is the haplotipic diversity, K is the mean number of pairwise differences among sequences, and π is the nucleotide diversity

Population	n	H(n)	Hd	π	K
RG	14	5	0.8242 (0.0567)	0.0099 (0.0059)	4.0110 (2.1318)
UY	38	13	0.8208 (0.0436)	0.0136 (0.0074)	5.5249 (2.7156)
SC	38	13	0.7923 (0.0567)	0.0076 (0.0045)	3.0654 (1.6305)
CSA	52	21	0.8944 (0.0277)	0.0106 (0.0059)	4.3341 (2.1787)
CL	31	11	0.8602 (0.0400)	0.0120 (0.0067)	4.8688 (2.4397)

Standard errors are given in parenthesis

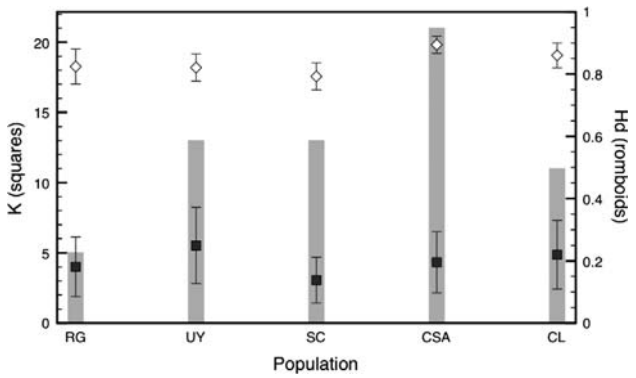

Fig. 2 Intra-population genetic diversity estimates. Haplotipic diversity values (Hd = romboids) are plotted along the left ordinate axis. Mean number of pairwise differences among sequences values (K = squares) for each population, with standard errors is plotted along the right ordinate axis. The number of haplotypes is represented by the gray vertical bars and is plotted along the right ordinate axis

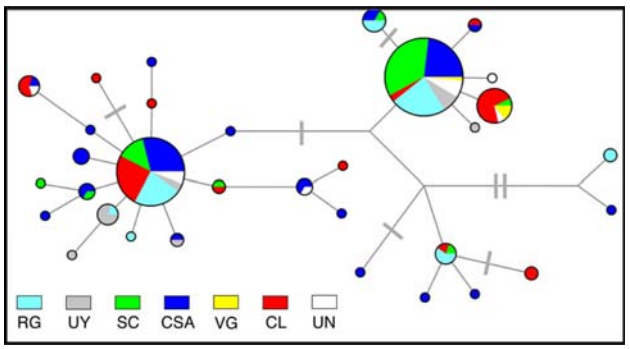

Fig. 3 Statistical parsimony network. Haplotype colors denote their geographic location. Circle size is proportional to haplotype frequency. Hatch marks indicate additional steps to a single mutation separating haplotypes. The two most frequent haplotypes are also most frequent in Lazaro et al. (2004). The color-coding used in this figure matches Fig. 1. 'UN' is used for haplotypes from dolphins with unknown precise geographic location

supports both these results (Table 3). CSA is significantly different from all other populations with the exception of UY when haplotype frequencies are considered. In contrast, CSA is only significantly different from SC when molecular distances are employed, which is supported by the exact test of population differentiation (Table 3). Pairwise comparisons of genetic distance as a function of geographical distance are shown in Fig. 4a and comparisons between contiguous sampling sites are shown in Fig. 4b. The Mantel test of autocorrelation between genetic and geographic distance was not significant ($p = 0.44$).

Likelihood estimates of population migration rates per generation were lowest between populations UY and SC (Table 4 and Fig. 5). The highest migration rates were estimated between UY and CSA and between SC and CSA, respectively; the population migration rate per generation

Table 3 Fixation indices calculated for the different proposed populations

	RG	UY	SC	CSA	CL
RG	–	0.0321	**0.1077**	**0.0448**	**0.1156**
UY	0.0133	–	**0.0808**	0	**0.0776**
SC	**0.1623****	**0.0516****	–	**0.0528**	**0.1002**
CSA	0.0288	0.0034	**0.1227****	–	**0.0414**
CL	0.0560**	0.0356**	**0.1505****	0.0032**	–

F_{ST} values are above diagonal and Φ_{ST} values below diagonal. Statistically significant F_{ST} and Φ_{ST} values ($p < 0.05$) are in bold. Double asterisks (**) below the diagonal indicate significant values ($p < 0.05$) for the exact test of population differentiation

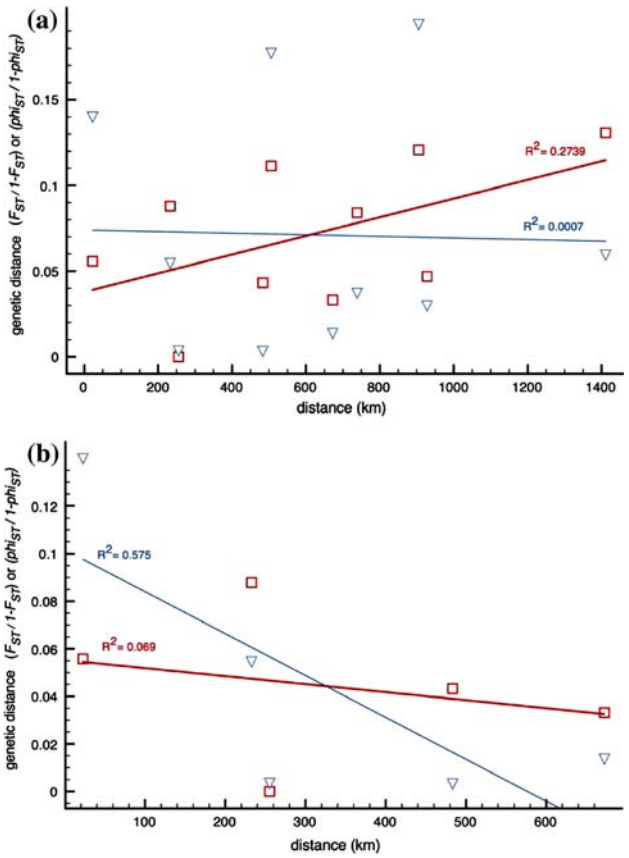

Fig. 4 (**a**) Pairwise estimates of genetic distance plotted against values of geographical distances for the same pairs of populations. The Mantel test of correlation between genetic and geographical distances was not significant (see "Results" section). Thick red squares and line represent the F_{ST} data and regression line, whereas thin blue triangles and line represent the Φ_{ST} data and regression line, respectively. Regression values are shown above each regression line. (**b**) Estimates of genetic distance plotted against values of geographical distances for contiguous pairs of geographic locations. Thick red squares and line represent the F_{ST} data and regression line, whereas thin blue triangles and line represent the Φ_{ST} data and regression line, respectively. Regression values are shown above each regression line

Table 4 Likelihood based estimation of migration rates and divergence time between population pairs, performed with software MDIV

	θ (4Nμ)	M (m/μ)	T (tμ)	MG (2 Nm)	TG (t/2N)	Distance (km)
RG-UY	2.594 (0.017)	6.273 (0.317)	0.098 (0.013)	8.135	0.076	672.48
UY-SC	3.089 (0.027)	2.58 (0.085)	0.168 (0.020)	3.984	0.109	232.69
SC-CSA	4.752 (0.031)	15.774 (1.257)	0.039 (0.008)	37.478	0.016	22.61
CSA-CL	5.833 (0.056)	5.191 (0.194)	0.071 (0.006)	15.140	0.024	483.30
UY-CSA	5.574 (0.041)	19.799 (1.703)	0.029 (0.006)	55.182	0.010	255.30

At least ten runs were averaged to obtain the shown values (see Appendix). Standard errors are in parenthesis. M_G and T_G represent the population migration rate and population divergence time per generation scaled by population size, respectively

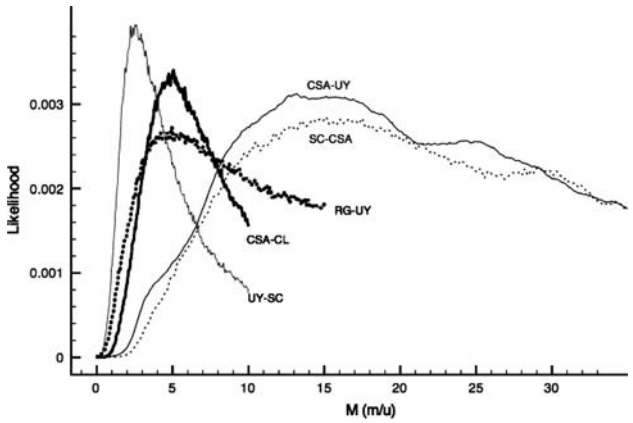

Fig. 5 Likelihood based estimates of migration rate M for each population pair. Only one representative run per population comparison is shown for illustrative purposes. Although not shown, all five distributions had a long tail off to the right, indicating a low likelihood of obtaining higher estimates of migration rates. Actual number of runs per population comparison are detailed in the Appendix

between RG and UY was comparable to the value estimated between CSA and CL. These estimated values of migration rates do not correlate with their corresponding geographical distance (Table 4). Estimated scaled divergence times exhibit the opposite behavior to migration rates as expected. The highest scaled divergence time was observed between populations UY and SC, whereas UY–CSA and SC–CSA presented the lowest value for the same estimator (Table 4).

Sex-biased genetic structure

Significant genetic structure was observed between SC–CSA for males (F_{ST} = 0.06375, $p < 0.005$; Φ_{ST} = 0.28158, $p < 0.005$), and for males and females combined (F_{ST} = 0.05279, $p < 0.005$; Φ_{ST} = 0.12269, $p < 0.005$) when we partitioned the dataset according to sex (Table 5). Females compared between SC and CSA exhibited no significant structure in mtDNA haplotypes (F_{ST} = 0.02565, $p > 0.005$; Φ_{ST} = 0, $p > 0.005$). In all comparisons, χ^2 analyses were not significant ($p > 0.05$), whereas significance of the exact test of population differentiation supported the fixation indices estimates (Table 5).

Consequences of by-catch—genetic perspective

Sample composition

All molecular sex determinations for the samples we obtained in Argentina were successful, and the sex ratio was nearly 1:1 (51 females, 56 males). Similarly, there were no significant differences between sexes when we considered all areas and years together, or when data were partitioned by areas and/or years. Lastly, there were no significant deviations from an equal sex ratio for common haplotypes (Fisher's tests, $p > 0.05$). Despite this lack of statistically significant differences, males were consistently captured in greater proportions than females (67% of the captured animals in 2001 were males, 56% in 2002, 60% in 2003, and 64% in 2004).

Simultaneous entanglements

We recorded seven cases of more than one animal entangled in the same gillnet over the period of one day. In four of these cases, the pair of animals shared the same haplotype and there was always an adult female in the pair. In the three remaining cases, the animals had different haplotypes. Two of these cases were male–female pairs and one was a male–male pair (Table 6).

Table 5 Sex-biased population structure indices

Gender	Fst	Φst	X^2 (p)	Exact test (p)
Both	**0.05279**	**0.12269**	>0.05	**<0.05**
Females	0.02565	0	>0.05	>0.05
Males	**0.06375**	**0.28158**	>0.05	**<0.05**

Statistically significant values ($p < 0.05$) for the F_{ST} and Φ_{ST} estimators and for the exact test of population differentiation are highlighted in bold

Table 6 Simultaneous entanglements of pairs of individuals in Buenos Aires

Event #	Haplotypes	Genders	Locality
1	G, G	F, F	SC
2	J, J	F, F	SC
3	G, J	M, M	SC
4	J, J	F, F	SC
5	J, M17	F, M	CSA
6	X, M19	F, M	CSA
7	G, G	F, M	CSA

Haplotypes are designated according Secchi et al. (1998) nomenclature. M17 and M19 are new haplotypes found in this study. Males are designated as 'M' and females as 'F'

Population size equilibrium

All populations in Buenos Aires showed significant mismatches between the observed and expected distributions of the average number of nucleotide differences between sequences. The raggedness index resulted greatest for population CSA (r_{SC} = 0.0582, r_{CSA} = 0.0725, r_{CL} = 0.0382).

Discussion

Although our analysis is limited in that it uses a single mitochondrial marker, this study provides new insights into the application of genetic data for broad and fine scale management strategies of populations of franciscana dolphins. While additional information (i.e., additional mitochondrial genes, or nuclear or microsatellite loci) might increase the resolution of our analyses, the results from mtDNA control region sequences alone were highly informative for the utility of conservation genetics of franciscana dolphins.

Population genetic structure and migration

Population structure

Our results show strong quantitative evidence for the presence of at least two genetically recognizable populations of franciscana dolphins (SC and CL) within FMA IV in Argentina and suggest the possibility of a third population (CSA) in the same area. Our analyses also support the previously identified populations in Uruguay and Rio Grande that correspond to FMA III (Secchi et al. 1998; Lazaro et al. 2004).

Highly significant and consistent values for the various genetic differentiation estimators support the consideration of SC as a genetically distinct population based on the criteria described above. The fact that both fixation indices (F_{ST} and Φ_{ST}) as well as the exact test of population differentiation are concordant strongly supports our conclusion. Statistical support for CL as a distinct population management unit is almost as strong as that for SC, since the F_{ST} indices and the exact test of population differentiation were statistically significant for all comparisons involving the CL group. Lazaro et al. (2004) found the same result for CL without including samples from SC or CSA in their analysis. Significant differences in haplotype frequencies, and no statistical support for molecular distances, suggest genetic divergence at the level of a demographic aggregation of breeding individuals with small evolutionary divergence (Chivers et al. 2002). Recent genetic divergence in continuously distributed cetacean aggregations is common (Cassens et al. 2003; Dalebout et al. 2001), producing haplotypes that differ by only one or two mutations as we show in Fig. 3. We found a lack of statistical significance for the comparison CSA and UY. While CSA shows significant genetic differentiation in other pairwise comparisons, in total, our results are not conclusive enough to justify that it is a genetically distinct population using only the mtDNA data presented here.

It is not unusual to find a lack of statistically significant values for these genetic estimators between geographically neighboring or nearby populations of cetaceans, like those between CSA and UY (Chivers et al. 2002; Rosel et al. 1999; Wang et al. 1996), as genetic isolation is sometimes a function of geographical distance. It is interesting to observe, however, strong evidence of genetic differentiation among populations that are geographically closer to one another, as is the case for UY and SC, or SC and CSA. This observed differentiation of population SC may be similar to what is observed between neighboring inshore and offshore populations of bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Mexico (Sellas et al. 2005). In the case of franciscana dolphins, population isolation and differentiation may be taking place between more sheltered and open waters along the coast, with the Samborombon area (population SC) being an example of the former.

Despite the conspicuous genetic structure presented between geographic locations in northern Argentina, in particular for populations SC and CL, there are no clear phylogeographic patterns among the haplotypes analyzed, as depicted in the haplotype network (Fig. 3). This result is in agreement with the conclusions in Lazaro et al. (2004), since the only phylogeographic signal they found was introduced by the samples from the Rio de Janeiro area, which were genetically differentiated from any other area where franciscana dolphins occur. Other studies of continuously distributed small cetaceans have reported an absence of phylogeograhic signal as well (Chivers et al. 2002; Rosel et al. 1999; Wang et al. 1996). The two most frequent haplotypes we identified occupy basal positions in

the haplotype network, and these same haplotypes were also the highest in frequency in Lazaro et al. (2004), suggesting that they might be ancestral haplotypes (Clement et al. 2000; Posada and Crandall 2001). Most of the derived haplotypes (i.e., those separated from the most frequent ones by one or two mutations, see Figs. 1 and 3) were sampled in CSA and were detected in only one or two individuals. This further supports our suggestion of very recent divergence for CSA group of animals in FMA IV, since 'young' populations generally exhibit numerous derived haplotypes in low frequencies (Waples 1998). However, additional evidence is needed to more conclusively define the population structure and relationships of dolphins in the CSA area compared to other nearby franciscana populations.

Although there are only slight differences among most of the estimated values of genetic diversity, the fact that population SC presents the lowest genetic diversity values despite being a relatively abundant population (Bordino et al. 2004; Crespo et al. 2004) suggests that low diversity levels need to be evaluated in light of population size, sample size and origin of by-catch.

Sex-biased dispersal

The reported results of local genetic structure when samples of both sexes were analyzed separately are suggestive of a lack of sex-biased dispersal. This is not an unusual finding in coastally distributed small cetaceans (Natoli et al. 2005; Rosa et al. 2005; Sellas et al. 2005), although cases of male-biased dispersal are more frequently reported (Adams and Rosel 2006; Cassens et al. 2005; Escorza Treviño and Dizon 2000; Moller and Beheregaray 2004; Rosel et al. 1995, 1999). It has been suggested that mtDNA can be used to infer patterns of differential migration between sexes by identifying cases of significant female genetic structure (Escorza Treviño and Dizon 2000). In the case of female structure, it is assumed that females are more phylopatric and males tend to show more likely characteristics associated with wide-ranging dispersal patterns (Hoelzel 1994).

In our analysis, the absence of female structure gives no evidence of sex-biased structure. Lack of support of female genetic structure for the F_{ST} and Φ_{ST} indices or by the exact test of population differentiation is strong support for our hypothesis of no sex-biased dispersal at the local level. We should highlight, however, that these results only apply, in principle, to the two studied areas of SC and CSA in Argentina. Additional analyses for the rest of the populations within this species' distribution are needed before generalizing about sex-biased migration on franciscana dolphins at the regional scale. In addition, the study of nuclear genetic markers would add resolution to this issue by assessing male genetic structure explicitly (Goudet et al. 2002).

Migration

The estimated population migration rates per generation do not exactly match expectations from the results of the fixation indices. However, likelihood-based migration estimations involve quite different assumptions than the procedures used to calculate fixation indices, so we do not expect precise or exact concordance between the datasets. All scaled migration values should be interpreted as relative intensities of gene flow, with the assumption that population size does not vary significantly between our a priori defined populations. Bordino et al. (2004) estimated coastal population densities of 0.48 and 0.40 ind/km^2 for the SC and CSA areas, respectively, and concluded that these values were not significantly different. Assuming that these two areas represent hypothetical bounded areas of 10,000 km^2 bordering the coast, the estimated densities would translate into abundances of 4,800 and 4,000 individuals for the SC and CSA areas, respectively (the estimated abundance for the entire Buenos Aires region was 31,350 individuals, according to Bordino et al 2004). Although there are no abundance estimates for the CL or UY areas, Bordino et al. (2004) determined that population densities in the southern areas of Buenos Aires did not differ significantly compared to those from the northern areas. Given the reported lack of significant differences in population densities in the different areas, we argue that the estimated relative measures of gene flow can be directly compared to one another.

The estimated migration rate and divergence time values suggest that groups UY and SC have historically had the lowest rate of gene flow when contrasted with the relatively high migration rates observed for SC–CSA, CSA–CL and CSA–UY. There are several equally plausible hypotheses that can account for significant population structure and high migration rates among dolphins in these areas. For example, a recent isolation of individuals originally from CSA that dispersed into the Samborombon Bay would explain the high migration rates CSA and SC. Population CL could have been also recently founded by CSA, which would account for their high migration rate and small divergence time, as well as for CLs genetic differentiation from any other population. Alternatively, very high historical levels of gene flow between CSA and UY may be producing the observed migration and divergence time values. While the current mechanisms of gene flow are not fully resolved, the degree of population structure and migration shed new light on the connectivity of these areas for population management (Frankham 1995; Hoelzel 1998).

The estimated migration rate values for franciscana dolphins in Argentina are comparable to those obtained for other small cetaceans using an analogous likelihood-based statistical framework. Natoli and colleagues (Natoli et al. 2005) suggested that migration rate values between $2N_em = 22$ and $2N_em = 30$ among populations of bottlenose dolphins in the Mediterranean are ''relatively high''. Similarly, Sellas and colleagues (Sellas et al. 2005) reported migration rate values between $2N_em = 2$ and $2N_em = 7$ among bottlenose populations within the Gulf of Mexico as being ''relatively low''. Although rigorous comparisons of migration rates between these species and the franciscana dolphins are not straightforward given their different ecology and behavior, cualitative comparisons could serve illustrative purposes. With these considerations in mind, our reported migration rate values between RG and UY ($2N_em = 8.135$) and those between UY and SC ($2N_em = 3.894$) could be viewed as relatively low, whereas all other migration rate values among franciscana dolphins in Argentina would be relatively high (see Table 4).

Isolation by distance?

Evidence of clinal variation of morphological and molecular characters in the terrestrial and marine environment is common in the literature (Arnason et al. 2000; Brumfield 2005; Godoy et al. 2004; Houlden et al. 1999; Jefferson and Van Waerebeek 2002). Lazaro et al. (2004) proposed a model of isolation by distance to explain the distribution of genetic distances between populations of franciscanas in Brazil, Uruguay and Southern Buenos Aires (with group CL being the only sample for this southern area) in light of their results. However, this model does not seem to hold when we include populations SC and CSA in our analysis, with the non-significant Mantel test ($p = 0.44$) for correlating genetic and geographical distances. Low regression values confirm this result when all pairwise combinations are considered (Fig. 4a). If we consider only comparisons between contiguous sampling sites, a weak (and non-significant) pattern of increasing genetic distance as geographical distance decreases is shown (Fig. 4b). In addition, our likelihood-based estimations of population migration rates per generation do not suggest an isolation by distance pattern among contiguous populations (see Table 4 in ''Results'' section).

Our findings counter the idea of a nearest-neighbor or stepping-stone model as the main driver for gene flow within this species, and in particular for the SC population. This point seems especially appropriate for a species in the marine environment, where there are expectations about gene flow and dispersal in the absence of obvious geographic barriers but different behavioral, ecological and environmental cues could account for gene flow estimations (Hoelzel 1998).

Behavior is an important driver of reproduction and dispersal, and hence of gene flow. Bordino and Wells (2005) used radio-telemetry to follow individuals captured and released in the Bahia Samborombon, in the San Clemente area (SC). The radio telemetry study followed three female franciscanas for 6 weeks and showed a maximum dispersal of 20 km from the capture location. In agreement with these results, posterior satellite tracking efforts (Bordino and Wells, pers. comm.) monitored two male and two female franciscanas for over a year in the same area. Both studies show a resident behavior for the tagged animals without dispersing significantly from that location, suggesting a clear resident pattern for the studied animals in SC.

Ecology and behavior are tightly linked in natural systems. A type of resident-like behavior may be influenced, in turn, by ecological cues related to feeding or breeding strategies. The Bahia Samborombon is one of the areas in the Buenos Aires Province coast where common prey items for franciscana dolphins are more abundant (Lasta 1995). This suggests that this resident-like behavior may be associated with feeding strategies. On the other hand, given the frequency with which we observe adult–calf pairs in the area, we cannot rule out that animals may be using the area to breed and/or calve. The Bahia Samborombon, to the north of San Clemente, is an enclosed habitat with shallow waters and high prey abundance (Bezzi and Boschi 2000; Lasta 1995; Lasta and Acha 1996), which would make it suitable for calving. Our hypothesis is supported by other case studies involving two small cetacean species in which ecological features seem to play important roles in maintaining population differentiation (Cassens et al. 2005; Rosa et al. 2005).

There is additional evidence that supports our contention that the ecological niche plays an important role for influencing population structure in this species. Diet was found to be more similar between animals inhabiting areas in Rio Grande and Uruguay than between any of these areas and Argentina (Ott 1994; Pinedo 1982; Pinedo et al. 1989). Aznar and colleagues (Aznar et al. 1994, 1995) and Andrade and colleagues (Andrade et al. 2000) showed that parasite infection levels in franciscana dolphins from Rio Grande and Uruguay are significantly different to those sampled in Argentina.

Environmental factors have been previously proposed as drivers of population structure in small coastal cetaceans (Hoelzel 1994; Natoli et al. 2005). The La Plata estuary has a high concentration of sediments, produced by the discharge of the Parana River. This, in combination with the predominant winds and currents in the area (O'connor 1991), the water chemical composition (Colombo et al. 2005, 2006) and physical features of the estuary (Guerrero et al. 1997), makes it a distinct aquatic environment (Huret

et al. 2005; Jaureguizar et al. 2004) and play an important role for franciscana dolphins.

In light of the abovementioned interaction among behavioral, ecological, and environmental features in relation to the presence of population structure, there is a clear need to allocate future efforts to explicitly address some of these issues in more depth for the franciscana. In particular, a comprehensive approach combining quantitative assessments of relevant oceanographic variables, data on resource availability and distribution and cetacean genetic and demographic information would certainly contribute to addressing some of the likely mechanisms influencing population partitioning. Finally, detailed behavior studies in combination with satellite telemetry data are instrumental in linking genetic and demographic partitioning with environmental and ecological factors in an explicit spatial and temporal framework.

Demographic and genetic impacts of by-catch

The detrimental consequences of accidental entanglement in gillnets are not only reflected in reductions of population size, as by-catch also affects important demographic and genetic aspects of populations (Harwood 1999; Morizur et al. 1999). We find empirical evidence that these impacts are occurring among Argentina's franciscana dolphins based on the genetic results effecting population size and impacts to particular maternal lineages, and social groupings.

Population size fluctuations

The mismatch distributions suggest that populations in Buenos Aires are not in population size equilibrium (Harpending 1994; Rogers and Harpending 1992). However, these changes in population size have occurred over evolutionary time and it is therefore not possible to date them based on the genetic data alone. Additional data reporting high levels of by-catch along the entire species distribution (Albareda and Albornoz 1994; Bordino et al. 2002; Corcuera et al. 1994; Pinedo et al. 1989; Secchi et al. 2003; Secchi and Wang 2002) and the abundance estimates to date (Crespo 2004), suggest that these changes in population size equilibrium may actually be recent reductions in population size.

Differential impacts to females with calves

We reported seven cases of simultaneously captured franciscana dolphins during the summer seasons. The four cases of an adult female and a juvenile sharing the same mitochondrial haplotype are suggestive of mother–calf pairs, although a microsatellite analysis is needed for parent-offspring confirmation. Valsecchi and colleagues (Valsecchi and Zanelatto 2003) reported a similar case with four simultaneously entangled franciscana dolphins sharing the same mitochondrial haplotype. In that case, a microsatellite analysis suggested that the group might have consisted of a mother, its two calfs and possibly the father of one of the calfs. The presence of mother–calf pairs being entangled would signify a serious threat to the population, and further confirm the possibility of a significant breeding activity in San Clemente and surrounding areas.

Differential impacts to maternal lineages

Rates of by-catch appear to show an association with certain maternal lineages when compared to the genetic composition of the sampled populations. Having found that multiple entanglements impacted mainly haplotypes G and J (which are in highest frequencies in the total sample) out of the 29 haplotypes present in the study area is an indication that some maternal lineages may be suffering differentially from by-catch, especially considering the possibility that matrilineal groups may move together and may be affected by similar phenomena (Baker et al. 1994; Weinrich et al. 2006). Since all the samples in our analysis result from by-catch, we do not know if the accidentally entangled animals truly represent the full extent of the franciscana dolphin genetic variability and population composition. To provide more insight into these results, a sampling scheme that combines by-caught and biopsy-sampled animals would be needed.

Management implications

When significant genetic structure is found between populations, which may or may not be defined a priori, such populations are typically recognized as separate management units (Avise 1995, 2000; Frankham 1995; Hoelzel 1994, 1998; Palumbi and Cipriano 1998). Based on results from the franciscana populations identified in the current and previous studies (Secchi et al. 1998; Lazaro et al. 2004), levels of genetic differentiation provide strong evidence for managing the broad-scale populations as FMA separately throughout their range (Secchi et al. 2003).

The Franciscana Management Area IV in Argentina is supported by our analysis, since it encompasses populations (SC, SCA, and CL) that are genetically isolated from those found in Brazil (RG) and Uruguay (UY). Moreover, we provide behavioral, ecological and environmental information that is consistent with and supports our genetic findings. Furthermore, we found evidence of a fine-scale genetic structure within Argentina, which could merit further subdivision within the FMA IV. Specifically, at

least two genetically distinct populations are recognized in the southern tip of the species distribution: SC and CL.

Following the traditional approach in studies of cetacean population structure, we base our conclusions regarding genetic differentiation on *significance* of the fixation indices rather than their specific value (Hoelzel 1994, 1998). However, some reference to these values may be useful for some management schemes that compare or contrast these values across differentiated populations. Among of the population comparisons involving SC, we found significant values of $F_{ST} > 0.5$ and two comparisons exhibit significant values of $F_{ST} > 0.1$. This pattern is even stronger when we look at the Φ_{ST} values (Table 3), suggesting that SC would be a population under the evolutionary paradigm definition proposed by Waples and Gaggiotti (2006). In addition, satellite telemetry data for this population suggests fidelity to this location during the entire year, which is consistent with the demographic paradigm of population definition. This situation is somewhat similar for the CL population, but is mainly supported by the F_{ST} values that are concordant with the evolutionary paradigm of population definition in Waples and Gaggiotti (2006). The status for the CSA assemblage should be revisited with additional data and analyses (i.e., a combination of mtDNA with microsatellites).

In this effort to conserve cetacean populations, marine protected areas (MPAs) have worked as effective tools in a number of cases (Hooker and Gerber 2004; Lundquist and Granek 2005). An effective MPA requires a sound design aimed at protecting particularly important evolutionary, ecological, and demographic factors such as: (i) maintenance of genetic diversity by enhancing migration from other populations; (ii) protection of feeding grounds when these are identified; and (iii) protection of areas thought to be important for reproduction. The Bahia Samborombon and surrounding areas, where population SC was sampled, seem to involve all three mentioned factors. Our initial genetic investigations coupled with ecological studies indicate that further protection of this area for dolphins is merited. To this end, implementing a sustainable fishery management scheme where fishing techniques that are proved to minimize by-catch are used, would be a firm step to the conservation of at least some franciscana dolphin populations.

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Appendix

Appendix A

Table A1. Initial parameters and results for *each* of the MDIV runs

Run	Run parameters			Estimated parameters		
	# of cyc	Burn-in	max *M*	Theta	*M*	*T*
RG-UY						
1	1×10^7	5×10^5	10	2.581789	8.520	0.040
2	1×10^7	5×10^5	10	2.581789	5.420	0.100
3	1×10^7	5×10^5	10	2.688475	4.880	0.250
4	1×10^7	5×10^5	10	2.752486	9.480	0.120
5	1×10^7	5×10^5	10	2.709812	5.960	0.040
6	1×10^7	5×10^5	10	2.645801	5.080	0.100
7	1×10^7	5×10^5	10	2.475104	7.080	0.120
8	1×10^7	5×10^5	10	2.581789	5.260	0.020
9	1×10^7	5×10^5	10	2.603126	5.140	0.040
10	1×10^7	5×10^5	10	2.624463	6.720	0.100
			Average (10)	*2.624463*	*6.354*	*0.093*
11	1×10^7	5×10^5	15	2.581789	7.650	0.050
12	1×10^7	5×10^5	15	2.667138	5.310	0.150
13	1×10^7	5×10^5	15	2.517778	5.280	0.040
14	1×10^7	5×10^5	15	2.603126	4.980	0.080
15	1×10^7	5×10^5	15	2.475104	7.650	0.120
16	1×10^7	5×10^5	15	2.645801	7.080	0.120
17	1×10^7	5×10^5	15	2.539115	7.110	0.210
18	1×10^7	5×10^5	15	2.496441	7.530	0.150
19	1×10^7	5×10^5	15	2.603126	4.740	0.060
20	1×10^7	5×10^5	15	2.496441	4.590	0.050
			Average (15)	*2.562585*	*6.192*	*0.103*
			Average (10–15)	*2.593524*	*6.273*	*0.098*
UY-SC						
1	1×10^7	5×10^5	10	3.136666	3.100	0.120
2	1×10^7	5×10^5	10	3.111370	2.700	0.040
3	1×10^7	5×10^5	10	3.086074	2.400	0.190
4	1×10^7	5×10^5	10	3.161961	2.220	0.150
5	1×10^7	5×10^5	10	3.237848	2.740	0.250
6	1×10^7	5×10^5	10	3.060799	2.340	0.220
7	1×10^7	5×10^5	10	3.035483	2.540	0.190
8	1×10^7	5×10^5	10	3.010187	2.800	0.240
9	1×10^7	5×10^5	10	3.111370	2.320	0.130
10	1×10^7	5×10^5	10	2.934300	2.640	0.150
			Average (10)	*3.088606*	*2.580*	*0.168*

Table A1 continued

Run	Run parameters			Estimated parameters		
	# of cyc	Burn-in	max *M*	Theta	*M*	*T*
SC–CSA						
1	1×10^7	5×10^5	35	4.432267	16.450	0.020
2	1×10^7	5×10^5	35	4.875494	17.500	0.020
3	1×10^7	5×10^5	35	4.812176	14.280	0.070
4	1×10^7	5×10^5	35	5.002130	13.440	0.080
5	1×10^7	5×10^5	35	4.812176	14.140	0.020
6	1×10^7	5×10^5	35	4.748858	16.450	0.030
7	1×10^7	5×10^5	35	4.843835	8.820	0.030
8	1×10^7	5×10^5	35	4.717199	15.050	0.160
9	1×10^7	5×10^5	35	4.590563	14.700	0.020
10	1×10^7	5×10^5	35	4.717199	13.090	0.020
			Average (35)	*4.755190*	*14.392*	*0.047*
11	1×10^7	5×10^5	50	4.843385	21.350	0.020
12	1×10^7	5×10^5	50	4.622222	17.500	0.030
13	1×10^7	5×10^5	50	4.875494	33.900	0.060
14	1×10^7	5×10^5	50	4.527245	10.700	0.020
15	1×10^7	5×10^5	50	4.622222	14.300	0.030
16	1×10^7	5×10^5	50	4.875494	17.300	0.030
17	1×10^7	5×10^5	50	4.812176	23.800	0.030
18	1×10^7	5×10^5	50	4.748858	10.900	0.020
19	1×10^7	5×10^5	50	4.748858	10.900	0.020
20	1×10^7	5×10^5	50	4.812176	10.900	0.040
			Average (50)	*4.748813*	*17.155*	*0.030*
			Average (35-50)	*4.752001*	*15.774*	*0.039*
CSA-CL						
1	1×10^7	5×10^5	10	5.926629	4.240	0.100
2	1×10^7	5×10^5	10	5.701983	4.980	0.090
3	1×10^7	5×10^5	10	5.740257	5.020	0.070
4	1×10^7	5×10^5	10	5.814806	5.040	0.050
5	1×10^7	5×10^5	10	6.150276	4.590	0.040
6	1×10^7	5×10^5	10	5.852081	6.240	0.080
7	1×10^7	5×10^5	10	5.553885	5.200	0.090
8	1×10^7	5×10^5	10	5.740257	5.860	0.060
9	1×10^7	5×10^5	10	5.777532	4.920	0.050
10	1×10^7	5×10^5	10	6.075727	5.820	0.080
			Average (10)	*5.833343*	*5.191*	*0.071*
UY-CSA						
1	1×10^7	5×10^5	35	5.610075	>30	0.050
2	1×10^7	5×10^5	35	5.788741	24.990	0.050
3	1×10^7	5×10^5	35	5.610075	23.800	0.010
4	1×10^7	5×10^5	35	5.753007	>30	0.030
5	1×10^7	5×10^5	35	5.645808	16.800	0.030
6	1×10^7	5×10^5	35	5.431411	>30	0.030
7	1×10^7	5×10^5	35	5.538609	17.300	0.010
8	1×10^7	5×10^5	35	5.431411	23.600	0.010
9	1×10^7	5×10^5	35	5.467142	12.800	0.060
10	1×10^7	5×10^5	35	5.467143	19.300	0.010
			Average (35)	*5.574342*	*19.799*	*0.029*

Each run comprised three likelihood distributions, one for each of the parameters estimated by the model: theta, the migration parameter between populations (*M*), and the divergence time between populations (*T*). Each of the resulting theta values, migration parameters (*M*) and divergence time parameters (*T*) were obtained as the maximum likelihood values from each of the distributions. We reported the *average* value for each of the three parameters, calculated from all sets of runs between each pair of populations. Migration parameters that did not converge to a most likely value were reported as > 30 and were not taken into account in the average calculations

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