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RESEARCH ARTICLE

Fine-scale matrilineal population structure in the Galapagos fur seal and its implications for conservation management

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Abstract Females of many pinniped species generally exhibit strong fine-scale philopatry, but it is unclear over what spatial scale this behavior may translate into genetic population structure. We conducted a population genetic survey in the Galapagos fur seal, *Arctocephalus galapagoensis*, an endangered pinniped endemic to a small geographic range in the northwest of the Galapagos archipelago. To assess patterns of genetic diversity levels and population differentiation, we analyzed part of the mitochondrial control region (mtDNA) and 18 microsatellites DNA markers. We detected similar levels of genetic diversity to many other pinniped species ($h = 0.86$, $\pi = 0.012$, $A = 7.44$) despite severe anthropogenic exploitation in the nineteenth century and recurrent population crashes due to recent climatic perturbations associated with El Niño Southern Oscillation events. We further found remarkably strong fine-scale matrilineal population structure, with 33.9 % of the mtDNA variation being partitioned among colonies separated by as little as 70 km swimming distance. In contrast, population structure inferred from nuclear markers was weak. Our findings provide further evidence that natal philopatry can translate into fine-scale genetic population structure in highly mobile species. We discuss the relevance of our results for the fine-scale conservation management of this species with a very restricted geographic range.

Keywords *Arctocephalus galapagoensis* · Philopatry · Genetic diversity · Galapagos Islands · Pinnipeds

Introduction

Natal philopatry is widespread among animals (e.g., Greenwood 1980). Returning to the location where one was successfully raised to reproduce may allow individuals to benefit from locally suitable habitat (Shields 1982) and from interactions with known neighbours or kin (Greenwood 1980; Shields 1982; Wolf and Trillmich 2008). However, philopatry may also increase competition among related individuals and may lead to inbreeding, thereby reducing genetic variation and adaptive potential. This in turn may contribute towards negative population dynamics (Forcada and Hoffman 2014) and eventually increase the risk of extinction (Shields 1982).

Despite its importance, natal philopatry is difficult to study via direct observation and requires long-term mark-recapture studies. One indirect solution is to apply genetic markers, either to genetically identify individuals across multiple years (Hoffman et al. 2006a) or to indirectly quantify the intensity and spatial scale of homing

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behaviour by evaluating population genetic structure. Philopatry will in general reduce gene flow among populations and increase the effects of genetic drift. In species with male-biased dispersal, the reduction will be more pronounced in the maternally inherited mitochondrial genome. A striking example of this comes from a study that identified remarkably strong fine-scale structuring for Australian sea lions (*Neophoca cinerea*), with colonies as close as ~100 km apart characterised by unique mitochondrial DNA (mtDNA) haplotypes (Campbell et al. 2008). The implication of this observation is that female recruitment occurs mainly from within the colony, leading to higher risk of localized extinction such as may be caused by human perturbations or demographic and/or environmental stochasticity (Goldsworthy and Page 2007), and a lower propensity to recolonize previous breeding areas. This pattern follows the common observation in pinnipeds that maternally inherited mtDNA markers are more differentiated than nuclear markers (Stanley et al. 1996; Hoffman et al. 2009; Wolf et al. 2008), consistent with the expectation of male-biased dispersal (Perrin and Mazalov 2000).

Uniparental nonrecombining mtDNA has shorter coalescent times than nuclear DNA and is thus well suited to delineate young evolutionarily significant units—ESUs (Corl and Ellegren 2012), that should ideally be reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies of nuclear loci (e.g., microsatellites) (sensu Moritz 1994). ESUs objectively define units below the level of species that should be prioritized for protection when they are under threat (Ryder 1986; Moritz 1994; Chan et al. 2006; Hedrick et al. 2006; Robalo et al. 2007; Bottin et al. 2007) in face of limited resources (Avise 1989). Characterization of population structure and identification of ESUs permit adoption of more effective conservation management strategies, mainly related to the translocation and reintroduction of endangered species, in order to maximize the overall viability of a metapopulation (McCarthy et al. 2004; Akçakaya et al. 2007).

In addition to the implications of female philopatry for ESU delineation, extreme philopatry can be of concern where it is associated with a polygynous mating system, as is characteristic for otariid seals (Boness 1991). In this case, declines in female numbers will strongly affect local operational sex ratios, impact local genetic diversity and make local populations vulnerable to genetic (e.g., inbreeding, drift, bottlenecks), environmental (e.g., *El Niño*, global climate change) or anthropogenic (e.g., hunting) impacts that may increase extinction risk (Frankham et al. 2002). According to Cornuet and Luikart (1996), many populations around the world are suffering demographic bottlenecks (reduction of census size) and genetic bottlenecks (reduction of effective population size, N_e) as a result of increasing habitat fragmentation and isolation. The analysis of genetic diversity can be used to test the hypothesis that a population recently experienced a genetic bottleneck by comparing the empirical heterozygosity (H_e) of a sampled population with the heterozygosity expected in an equilibrium population (H_{eq}). In nonbottlenecked populations that are near mutation-drift equilibrium, H_{eq} will be equivalent to the observed heterozygosity under Hardy–Weinberg equilibrium, HWE (H_e). However, if a population has suffered a recent bottleneck, the mutation-drift equilibrium is transiently disrupted and H_e will exceed H_{eq} computed from the number of alleles in the sample (Luikart and Cornuet 1998).

The Galapagos fur seal (GFS), *Arctocephalus galapagoensis*, is a non-migratory species that is endemic and resident of the Galapagos Islands, Ecuador (00°35′ S, 91°00′ W, Fig. 1a), where it is mainly distributed across the northern and western parts of the archipelago (mainly Fernandina and Isabela islands). The species' geographic range is unusually small for a pinniped species, covering an area smaller than the Galapagos Marine Reserve (less than 140,000 km^2). This limited distribution reflects the highly localized influence of an upwelling of cold, nutrient-rich waters from the Humboldt and Cromwell currents, which provide sufficient food resources (Alava and Salazar 2006; IUCN 2014). The species' small range and its mobility set the stage to study the potential impact of philopatry at a high spatial resolution.

The GFS is also an important species from a conservation perspective. It was driven to the brink of extinction by human hunting and no well-defined GFS breeding colonies remained in the archipelago towards the end of the nineteenth century (Heller 1904 *apud* Trillmich 1987). Protective measures were put in place in 1930, but the population only began to recover after 1959, when the Galapagos archipelago was declared a National Park (Seal Conservation Society 2010). Nowadays, the population size is believed to range between 10,000 and 15,000 individuals (IUCN 2014). Currently, the GFS is listed in the appendix II of CITES, delineating species that are not necessarily threatened by extinction but may become so unless trade is closely controlled (CITES: Convention on International Trade in Endangered Species of Wild of Fauna and Flora 2013). The GFS is also categorized as Endangered by IUCN Red List and falls into the A2a category, which applies to species that have suffered a 50 % or greater population decline over the last 10 years or three generations (IUCN 2014).

The GFS population is also subject to natural fluctuations due to *El Niño Southern Oscillation* (ENSO) events (Wyrtki 1982; Philander 1983; Trillmich and

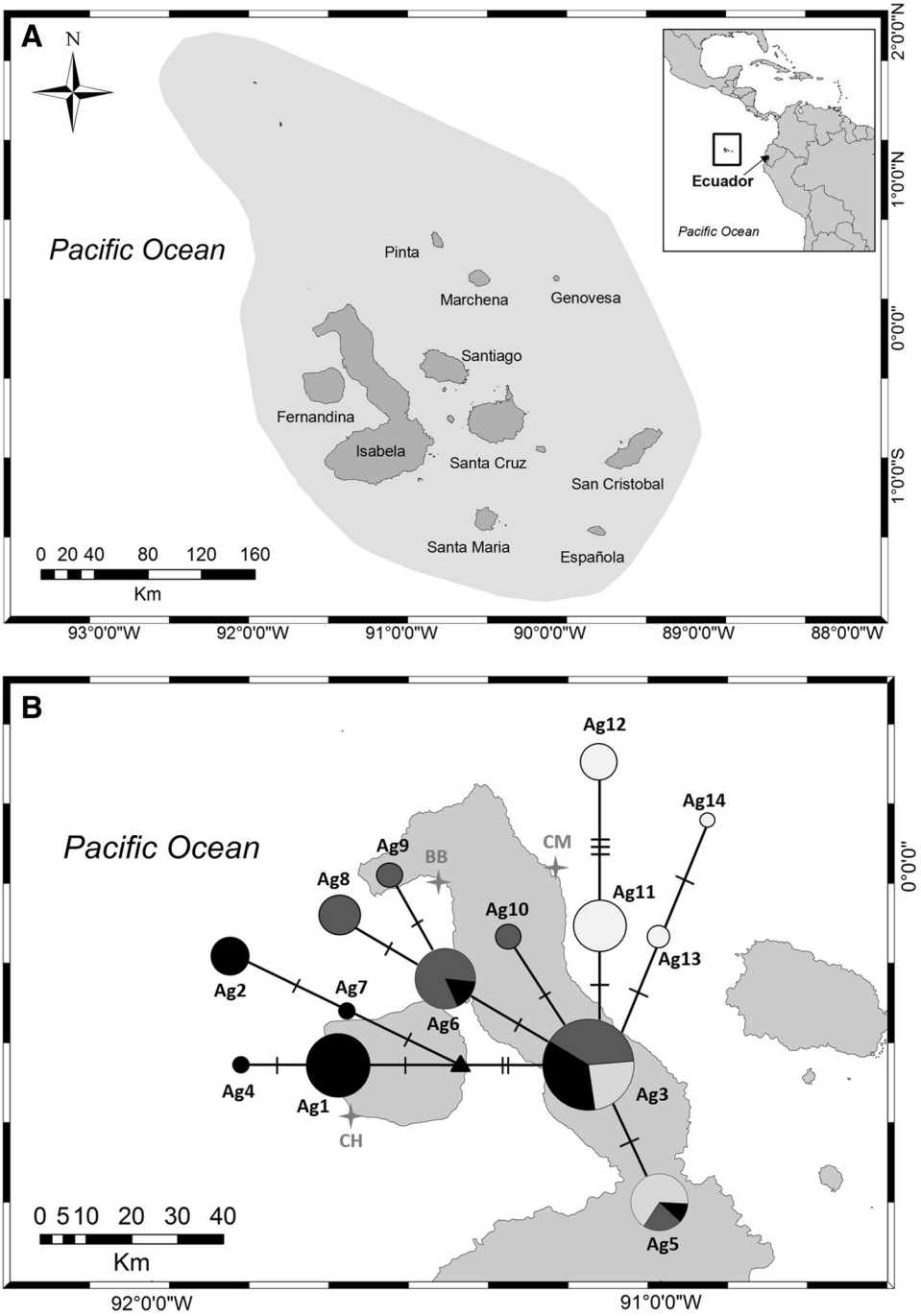

Fig. 1 **a** Map of the study area (Galapagos Islands) and the location of the three major *Arctocephalus galapagoensis* colonies sampled for this study. *BB* Banks Bay (Isabela Island), *CH* Cape Hammond (Fernandina Island), *CM* Cape Marshall (Isabela Island). The *grey shaded area* represents the species' distribution range. **b** *Median joining network* of mtDNA sequences representing distinct haplotypes as *circles*. Circle size is proportional to the haplotype frequency across all 87 sampled individuals. *Pie charts* indicate relative frequencies by sampling location CH (*black*), BB (*dark gray*) and CM (*light gray*). *Edges* connect haplotypes that differ by one base pair substitution. *Triangles* indicate potential intermediate haplotypes that were not sampled

Limberger 1985). These events take their toll on the GFS because they reduce local marine productivity in the Galapagos Islands, thereby affecting the entire food chain including top predators such as fur seals and sea lions (Wyrtki 1982; Philander 1983; Trillmich and Limberger 1985). ENSO events significantly decrease survival rates (Trillmich and Wolf 2008) and have been responsible for the death by starvation of significant proportions of the GFS population (Trillmich and Dellinger 1991), with the strongest events of the century (1982–1983 and 1996–1998) being associated with crashes of up to 50 % (Trillmich and Dellinger 1991; Trillmich and Limberger 1985; Alava and Salazar 2006; Bastida et al. 2007). Locally reduced food availability in particularly harsh years could potentially explain why the GFS has been observed foraging as far afield as the coastlines of Ecuador, Costa

Rica, Colombia, Mexico and Peru (Félix et al. 2001; Capella et al. 2002; Aurioles-Gamboa et al. 2004; Montero-Cordero et al. 2010).

Previous genetic studies of the GFS have either included small numbers of individuals to elucidate wider species relationships (Wynen et al. 2001; Wolf et al. 2007; Dasmahapatra et al. 2009) or focused on identifying vagrant animals using a handful of mtDNA sequences (e.g., Félix et al. 2001; Capella et al. 2002; Aurioles-Gamboa et al. 2004; Yonezawa et al. 2009; Montero-Cordero et al. 2010). Here, we carried out a study on the genetic structure, genetic diversity and bottleneck histories of all of the main breeding colonies of this species, using mtDNA and microsatellites to provide female and male mediated perspectives, respectively. We discuss the genetic consequences of natal philopatry and their implications for conservation and management.

Materials and methods

Sample collection and DNA extraction

A total of 90 *A. galapagoensis* tissue samples were collected from pups (49 males and 41 females) during 2004, comprising 30 samples each from the three main GFS breeding colonies at Cape Hammond, Fernandina Island (designated CH, 0°18′ S, 91°39′ W), Banks Bay, Isabela Island (designated BB, °02′ S, 91°24′ W) and Cape Marshall, Isabela Island (designated CM, 0°00′ S, 91°12′ W), respectively (Fig. 1). Tissue samples of $\sim$0.5 cm^3 were collected using piglet ear notch pliers (Majluf and Goebel 1992) from the interdigital membrane of the hind flipper of pups. All animals from each colony were sampled in the same local area and within a couple of hours of each other. Every sampled pup was individually identified using shave marks to ensure that no animal was sampled twice. In order to rule out the possibility of inclusion of closely related individuals, we calculated pairwise genetic relatedness (Lynch and Ritland 1999) using GenAlEx 6.5 (Peakall and Smouse 2006, 2012). Only one pair of specimens was estimated to be closely related ($r > 0.25$) and we therefore removed one of these animals from subsequent analyses.

Authorities of the Galapagos National Park (*Servicio Parque Nacional Galápagos*) approved sample collection and exportation under license number 099/04—SPNG of Project Social Structure in Sea Lion colonies—PC-01-03. The samples were cryo-preserved in 70 % ethanol at the Department of Animal Behaviour in Bielefeld, where genomic DNA was extracted following a standard phenol–chloroform protocol (Sambrook et al. 1989).

Mitochondrial DNA amplification and analyses

The following primers were used to amplify a 425 bp region of the mtDNA control region: R3 (L15926) THR 5′-TCA AAG CTT ACA CCA GTC TTG TAA ACC-3′ (Kocher et al. 1989), TDKD (H16498) 5′-CCT GAA GTA GGA ACC AGA TG-3′ (Slade et al. 1994). Each PCR was conducted in a 10 µl reaction volume containing 100 ng of template DNA, 20 mM Tris–HCl (pH 8.3), 100 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.25 mM dNTPs, 0.25 µM of each primer and 0.5 units of 5PrimeTaq polymerase (VWR). The following PCR conditions were used: 1 cycle of 5 min at 94 °C, 35 cycles of 30 s at 94 °C, 60 s at 65 °C and 60 s at 72 °C; and 1 final cycle of 7 min at 72 °C. The resulting PCR products were purified using shrimp alkaline phosphatase and exonuclease I (New England Biolabs) following the manufacturer's recommended protocol. All fragments were then sequenced from both ends on an ABI 3730xl capillary sequencer using the Applied Biosystems BigDye® Terminator v3.1 cycle sequencing kit. Sequence quality was checked within ChromasPro 1.7.4 (http://technelysium.com.au). Sequences were then aligned automatically within ClustalW 2.1 (Thompson et al. 1997) and one by one manually edited using Bioedit 7.1.3 (http://www.mbio.ncsu.edu/). Poor quality sequences at the beginning and end of the fragments were removed to yield a 220 bp stretch of high-quality sequence that was obtained for all individuals. Three individuals from CM did not recover high quality sequence data and were therefore removed from further analyses.

Haplotype (H_d) and nucleotide diversities (π) were quantified for the whole sample set and for each colony separately using Arlequin 3.5.1.2 (Excoffier and Lischer 2010) and DnaSP 5.10.1 (Rozas et al. 2003; Librado and Rozas 2009). Analysis of molecular variance (AMOVA) was conducted within Arlequin 3.5.1.2 (Excoffier and Lischer 2010) to quantify the amount of variation between and within colonies. AMOVA was conducted separately using F_{ST} (Weir and Cockerham 1984) and Φ_{ST} (Tajima 1993). Haplotype networks were constructed using the median-joining approach (Bandelt et al. 1999) implemented in Network 4.6.11 (http://www.fluxus-engineering.com).

Additionally, we calculated Fu's F_S (Fu 1997) and Tajima's D (Tajima 1989). Bayesian skyline reconstructions were implemented using BEAUTi 1.7.4 and BEAST 1.7.4 (Drummond et al. 2012) for (i) all data pooled, and (ii) each of the three populations. We used a HKY substitution model gamma site heterogeneity (generated by likelihood with PAUP 4.0b10 Swofford 2002) with eight categories and a strict molecular clock prior with Dickerson et al. (2010) mutation rate of 5.74×10^{-7} s/s/gen derived for *Callorhinus ursinus* (Hoffman et al. 2011). Following implementation of 30,000,000 Markov Chain Monte Carlo

(MCMC) iterations, a Bayesian skyline plot was generated using Tracer 1.5 (Drummond et al. 2012).

Microsatellite DNA amplification and analysis

We amplified 18 polymorphic loci previously developed for pinnipeds: ZcwB07, ZcwE04, ZcwE12, ZcwF07, ZcwB09, ZcwD02, ZcwE03, ZcwE05 designed for *Zalophus wollebaeki* (Hoffman et al. 2007; Wolf et al. 2006), ZcCgDh5.8 and ZcCgDh7tg designed for *Zalophus californianus* (Hernández-Velazquez et al. 2005), Hg1.3, Hg6.1, Hg6.3, Hg8.1 and Pv9 designed for *Halichoerus grypus* (Allen et al. 1995; Gemmell et al. 1997), PvcA and PvcE, designed for *Phoca vitulina* (Coltman et al. 1996) and Agaz2, designed for *Arctocephalus gazella* (Hoffman 2009). Forward primers were fluorescently labelled and PCRs were carried out in two separate multiplexed reactions using a Type It Kit (Qiagen) (for details see Table 6) following the manufacturer's recommended protocols. The following PCR profile was used: 1 cycle of 5 min at 94 °C, 8 cycles of 30 s at 94 °C, 90 s at 60 °C and 60 s at 72 °C, 20 cycles of 30 s at 94 °C, 90 s at 56 °C, 60 s at 72 °C; and 1 final cycle of 15 min at 72 °C. PCR products were resolved by electrophoresis on an ABI 3730xl capillary sequencer and allele sizes were scored automatically using the program GeneMarker v1.95 and subsequently manually inspected and adjusted when necessary.

Deviations from HWE and linkage disequilibrium (LD) were assessed using Arlequin with 1,000,000 MCMC steps, 100,000 dememorization steps and 10,000 permutations. Significance levels ($\alpha = 0.05$) for departure from HWE and for LD were corrected for multiple comparisons with the sequential Bonferroni test (Rice 1989). A single locus deviated significantly from HWE in BB, but no significant departures were found in the other colonies or after pooling all of the samples (Table 6). Significant LD was observed between ZcwE04 and ZcwE12, Pv9, PvcE and Hg1.3. We therefore removed locus ZcwE04 from subsequent analyses.

Arlequin was also used to estimate expected heterozygosity (H_e) and observed heterozygosity (H_o), to conduct AMOVA analysis of the microsatellite dataset, and to calculate *F*-statistics (F_{ST}). We then compared the genetic diversity of this species to previously reported values for several other pinniped species (Table 7).

To test for population structure without prior knowledge of sampling locations, we estimated the posterior probability of the data fitting the hypothesis of *K* clusters [Pr(X|K)], where *K* is the number of putative populations, using the program Structure 2.3.4 (Pritchard et al. 2000). We performed 10 independent runs for each *K* from $K = 1$ to 3 using 1,000,000 MCMC iterations and a burn-in period of 1,000,000. We checked for consistency among replicate runs for the same *K* value and then computed the arithmetic mean of the 10 runs. We also carried out a similar analysis using the program Structurama 2 (Hulesenbeck and Andolfatto 2007). This program uses a particularly efficient variant of MCMC called Gibbs sampling, where each MCMC cycle involves a Gibbs scan of all of the individuals. Hence the total number of MCMC cycles for the analysis is the product of the reported number of MCMC cycles and the number of individuals in the analysis. We set the number of populations as a random variable, a parameter that uses a Dirichlet process prior (Pella and Masuda 2006). We ran 1,000,000 cycles for the random variable prior of the number of populations. The first 100,000 cycles were discarded as burn-in.

To test the hypothesis that the GFS recently experienced one or more genetic bottlenecks, we compared the expected heterozygosity (H_e) at each of the microsatellite loci to values expected under neutrality and equilibrium conditions (H_{eq}). As shown by Cornuet and Luikart (1996), samples from populations that recently experienced bottlenecks tend to have $H_e > H_{eq}$. In order to generate the expected heterozygosities under neutrality–equilibrium, we used the program Bottleneck (Piry et al. 1999). This analysis was based on the general two-phase model (TPM), because most loci probably evolve according to a model intermediate between infinite allele model (Kimura and Crow 1964) and one-step stepwise mutation model (Otha and Kimura 1973; Di Rienzo et al. 1994). The TPM includes both stepwise mutations and mutations larger than single steps and appears to provide a reasonable fit to empirical evidence about the mutation process (Di Rienzo et al. 1994; Garza and Williamson 2001). We recorded the number of loci for which sample heterozygosity exceeded neutrality–equilibrium expectations and whether the overall set of deviations was significant (based on a one-tailed Wilcoxon test, with the alternative hypothesis of heterozygosity excess). The Arlequin software was also used to compute the Garza–Williamson modified index to verify the putative occurrence of a bottleneck and its influence on genetic variability, denoted as *M*, which refers to the mean ratio of the number of alleles to the range in allele size (Garza and Williamson 2001; Excoffier et al. 2005).

Results

Mitochondrial DNA

A 220 base pair fragment of the mtDNA control region was analysed for sequence variation in 87 GFS individuals sampled from BB, CH and CM (Table 1). Fourteen

Table 1 Genetic diversity of *Arctocephalus galapagoensis* based on control region mtDNA analysis

Control region mtDNA							
Populations	N	S	H	H_d	H_dSD	π (%)	πSD (%)
Banks Bay	30	5	6	0.77	0.04	0.5	0.3
Cape Hammond	30	8	7	0.76	0.04	1.1	0.4
Cape Marshall	27	6	6	0.81	0.03	0.9	0.3
Total	87	14	14	0.86	0.02	1.2	0.4

N number of samples, *S* segregating sites, *H* number of haplotypes, H_d haplotype diversity, H_dSD standard deviation of haplotype diversity, π nucleotide diversity, *πSD* standard deviation of nucleotide diversity

segregating sites were found, all transition substitutions and two insertion–deletion sites (found in BB and CH). A total of 14 haplotypes were identified, of which only two were shared among all three populations (Ag3 and Ag5) and one was shared between BB and CH (Ag6) (Fig. 1b; Table 2). The 11 remaining haplotypes were private to each colony (see Table 2; Fig. 1b for details). Haplotype and nucleotide diversity were moderately high: $H_d = 0.86 \pm 0.02$ and $\pi = 0.012 \pm 0.0009$.

Mitochondrial AMOVA analysis revealed evidence for strong population differentiation, with 33.9 % ($P < 0.001$) of the genetic variability being partitioned among the colo-

Table 2 List of individuals *Arctocephalus galapagoensis* that bear each mitochondrial DNA control region haplotype. Absolute frequency in the sample and geographic distribution of haplotypes

Haplotypes	Individuals	GenBank accession numbers	Frequencies	Localities	Islands
Ag1	FH01, FH06, FH07, FH08, FH15, FH17, FH24, FH25, FH27, FH28, FH30	KM030335	11	Cape Hammond	Fernandina
Ag2	FH02, FH09, FH18, FH23, FH29	KM030336	5	Cape Hammond	Fernandina
Ag3	FH03, FH05, FH11, FH14, FH16, FH20, FH21, FH22, FH26, IB07, IB12, IB13, IB16, IB17, IB22, IB23, IB26, IB27, IB29, IM01, IM19, IM23, IM24, IM25, IM28	KM030337	25	Cape Hammond Banks Bay Cape Marshall	Fernandina Isabela
Ag4	FH04	KM030338	1	Cape Hammond	Fernandina
Ag5	FH10, IB05, IB19, IM03, IM08, IM16, IM17, IM18, IM20	KM030344	9	Cape Hammond Banks Bay Cape Marshall	Fernandina Isabela
Ag6	FH12, FH19, IB01, IB03, IB04, IB06, IB11, IB15, IB18, IB20, IB24, IB25	KM030346	12	Cape Hammond Banks Bay	Fernandina Isabela
Ag7	FH13	KM030347	1	Cape Hammond	Fernandina
Ag8	IB02, IB08, IB09, IB28	KM030366	4	Banks Bay	Isabela
Ag9	IB10, IB21	KM030374	2	Banks Bay	Isabela
Ag10	IB14 IB30	KM030394	2	Banks Bay	Isabela
Ag11	IM02, IM04, IM09, IM12, IM13, IM14, IM21, IM22	KM030396	8	Cape Marshall	Isabela
Ag12	IM05, IM06	KM030400	2	Cape Marshall	Isabela
Ag13	IM07, IM10, IM11, IM15	KM030401	4	Cape Marshall	Isabela
Ag14	IM27	KM030421	1	Cape Marshall	Isabela

Table 3 Analysis of molecular variance (AMOVA) based on fixation indices (F_{ST} and Φ_{ST}) from mtDNA control region and 18 microsatellite loci for the population of *Arctocephalus galapagoensis* as a whole

Genetic differentiation Source of variation	mtDNA		Microsatellites	
	F_{ST}	Φ_{ST}	F_{ST}	R_{ST}
Among populations	0.132	0.339	0.015	0.035
Within populations	0.868	0.661	0.985	0.965

All values are significant at $P < 0.01$

nies (see Table 3). Pairwise Φ_{ST} estimates were of comparable magnitude between colonies: CM–BB ($\Phi_{ST} = 0.27$), CM–CH ($\Phi_{ST} = 0.31$), CH–BB ($\Phi_{ST} = 0.40$) (all significant at $P < 0.001$, Table 4). This pattern was also evident in the haplotype network, which showed marked differences in haplotype frequencies between geographically adjacent colonies (Fig. 1b). Tajima's D and Fu's F_S tests of selective neutrality yielded positive, but non-significant values (Tajima's D: 0.37 ± 0.66, Fu's F_S: 0.36 ± 0.60; Table 5) indicating no evidence for recent population expansion. Bayesian skyline plots constructed for the entire dataset and also for each population showed no discernible oscillations in N_{ef} over the last 8000 years (based on the mutation rate of Dickerson et al. 2010 as described in the "Materials and methods" section), other than a slight recent dip for which there is only tentative support (Fig. 2).

Microsatellites

All of the microsatellite loci were moderately polymorphic, with an average expected heterozygosity of 0.69 (SD = 0.17) and an average number alleles per locus of 7.44 (SD = 3.05) see Table 6 and supplementary data). AMOVA of the microsatellite data uncovered weak but significant ($F_{ST} = 0.015/R_{ST} = 0.035$, $P < 0.001$). Weak but significant genetic differentiation was also observed in pairwise population comparisons (Table 4). In Bayesian cluster analysis within the program Structure, the mean likelihood value for ten independent runs peaked at $K = 1$ (Fig. 3), consistent with a lack of population structure.

Table 5 Pairwise neutrality tests for populations of *Arctocephalus galapagoensis*

	Tajima's D	Tajima's D (P value)	Fu's F_s	Fu's F_s (P value)
Banks Bay	−0.26	0.43	−0.26	0.47
Cape Hammond	0.81	0.81	0.86	0.69
Cape Marshall	0.58	0.74	0.47	0.62
Total	0.37	0.66	0.36	0.60

Structurama generated similar results, with the Pr(X/K) being 99.12 % for $K = 1$.

The Bottleneck test did not provide support for a recent reduction in effective population size (normal L-shape distribution). However, results for Garza–Williamson's modified index, M, which assumes that $M < 0.68$, are suggestive of a reduction in population size ($M = 0.38 \pm 0.12$).

Discussion

This is the first investigation on population genetics of one of the world's most endangered pinniped species, the GFS. We provide data on the population genetic structure and genetic diversity of this species, interpret our findings with respect to what is known about the species' biology and discuss the implications of our results for conservation practice.

Genetic diversity and contrasting patterns of population structure

Moderate levels of genetic variability were found at both mtDNA sequences and 18 microsatellite loci. Overall levels of variability were comparable to those found in a variety of other pinniped species, although direct comparisons are made difficult by the fact that many of the loci screened and the sequence length in the different studies are not the same (Table 7).

Population structure was pronounced for mtDNA, but weak for nuclear markers. Over a third of the mtDNA variation was partitioned among the three main breeding

Table 4 Pairwise F-statistics among sampling localities of *Arctocephalus galapagoensis*: in the left for mtDNA control region and in the right for microsatellites

		mtDNA control region			Microsatellites		
		Banks Bay Φ_{ST}	Cape Hammond	Cape Marshall	Banks Bay R_{ST}	Cape Hammond	Cape Marshall
Banks Bay	F_{ST}	–	0.401*	0.271*	–	0.015	0.055*
Cape Hammond		0.129*	–	0.315*	0.010*	–	0.041*
Cape Marshall		0.146*	0.121*	–	0.025*	0.023*	–

* Significant values $P < 0.001$

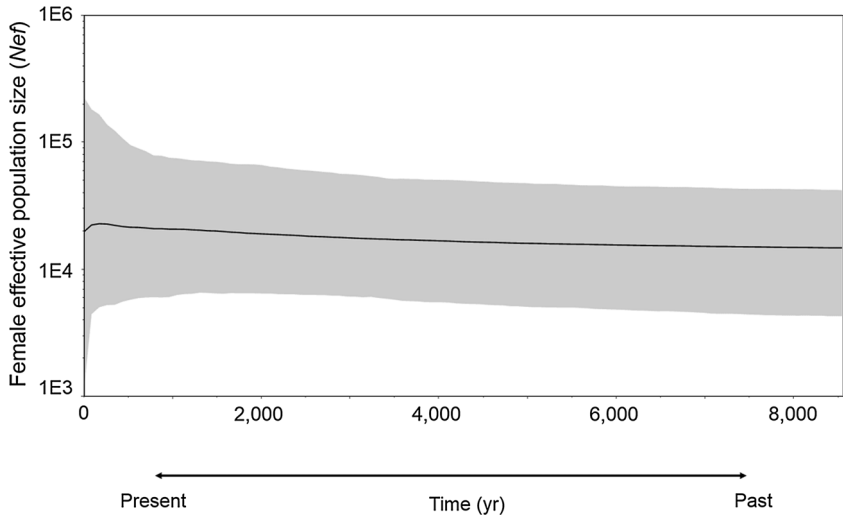

Fig. 2 Bayesian skyline plot of historical female effective population size of *Arctocephalus galapagoensis* (*straight line*) and the corresponding 95 % posterior probability interval (*grey area*)

colonies, despite these being separated by as little as 70 km, a distance that can easily be bridged during daily foraging trips (Jeglinski et al. 2013). In contrast, nuclear population structure was weak. This result is coherent with previous studies of pinnipeds showing strong mitochondrial structuring, but low differentiation for nuclear genes (Stanley et al. 1996; Andersen et al. 1998; Hoffman et al. 2006b; Davis et al. 2008; Campbell et al. 2008; Hoffman et al. 2009, including the Galapagos sea lion Wolf et al. 2008), a pattern that is consistent with the expectation that in pinnipeds females show strong natal philopatry and males are the dispersing sex (e.g., Fabiani et al. 2003; Dickerson et al. 2010, including the Galapagos sea lion Wolf and Trillmich 2007). However, in the case of GFS, strong matrilineal structure occurs between adjacent colonies that are separated by as little as 70 km. This pattern is consistent with mark-recapture studies showing that females of many pinniped species are capable of returning to within meters of their birth locations (Pomeroy et al. 2000; Wolf and Trillmich 2007; Hoffman and Forcada 2012).

The low differentiation of nuclear markers suggest sufficient gene flow to counteract inbreeding effects and maintain adaptive potential for the species as a whole. It follows that treating populations as ESUs and managing them separately is not warranted. Nevertheless, the strong matrilineal site fidelity evidenced by the clear structure of mitochondrial genetic variation is of concern. Crashes in abundance of individual populations put the whole species at risk because the strong site fidelity that is characteristic of otariid species compromises recolonization potential. In the GFS this is of particular relevance because the three major breeding populations investigated here comprise a substantial part of the total population. Overall, this justifies that conservation management considers each single population as a vital component of the entire species.

Historical variation in effective population size

The bottleneck test did not provide support for a recent reduction in effective population size. However, results for Garza–Williamson's modified index, M, which assumes that $M < 0.68$, are suggestive of a reduction in population size. These results suggest that anthropogenic exploitation and ENSO events may have had relatively little impact on overall levels of genetic diversity, despite the restricted geographic range of the species. This is consistent with the results of several analyses, all of which suggest that the population has not been subject to significant population size changes in the recent past. For instance, large values of both haplotype and nucleotide diversity at mtDNA could indicate that the original effective population size (N_e) of this species was large (Frankham et al. 2002) and are also an indication of a stable demographic history (Grant and Bowen 1998) (see Table 7). We also recovered non-significant values of Tajima's D and Fu's F_S (see Table 5), lending no support to a scenario of recent population expansion. Moreover, it is important to recognize that populations suffering a reduction in census size may not suffer a severe reduction of N_e (genetic bottleneck), due to metapopulation structure involving local extinctions and recolonizations (Pimm et al. 1989). This could be another explanation for the conserved levels of genetic diversity in this species.

It is also important to mention the potential contribution to nuclear diversity through male movements between sampled and unsampled colonies (e.g., Campagna et al. 1988; Hoelzel et al. 1999). Adult and sub-adult peripheral males, which are usually excluded from central breeding areas, could contribute towards genetic diversity if they are able to obtain copulations outside these colonies (e.g., Bartholomew 1970; Campagna et al. 1988; Boness 1991; Hoelzel et al. 1999). These males may disperse to other

Table 6 Measures of genetic diversity at 18 microsatellite loci in the *Arctocephalus galapagoensis* populations studied

Locus	Allele ranges	Banks Bay (n = 29)					Cape Hammond (n = 27)				
		A	AR	E	H_o	H_e	A	AR	E	H_o	H_e
ZcwE05[b]	189–195	4	3.99	0	0.44	0.47	3	3.80	0	0.38	0.39
ZcwD02[b]	198–250	12	11.60	1	0.93	0.91	13	12.60	2	0.88	0.89
ZcwB09[b]	191–207	6	5.90	1	0.83	0.77	5	4.00	0	0.77	0.80
ZcCgDh5[b]	319–349	8	7.80	1	0.76	0.73	7	6.50	1	0.69	0.78
Hg8.1[a]	178–186	4	3.80	0	0.41	0.40	4	4.90	0	0.46	0.50
ZcCgDh7t[b]	282–290	5	4.80	1	0.38	0.39	3	3.90	0	0.54	0.50
Hg6.1[b]	140–158	4	3.90	0	0.38	0.39	5	2.90	0	0.56	0.48
ZcwF07[b]	146–162	6	5.60	1	0.52	0.46	4	3.90	0	0.48	0.48
ZcwE03[b]	217–231	8	7.90	1	0.83	0.86	7	6.50	0	0.84	0.85
ZcwE12[b]	173–187	4	4.00	0	0.82[c]	0.73	7	5.90	1	0.81	0.82
ZcwE04[b]	120–144	8	7.90	0	0.97	0.87	9	7.80	0	0.88	0.85
ZcwB07[a]	182–198	7	6.80	0	0.82	0.73	8	6.80	0	0.84	0.82
Pv9[a]	172–182	6	5.80	0	0.79	0.68	6	5.00	0	0.69	0.73
Hg6.3[a]	225–239	5	4.80	1	0.48	0.49	4	3.80	0	0.58	0.54
PvcE[a]	120–136	6	5.80	0	0.66	0.72	5	4.60	0	0.73	0.69
Hg1.3[a]	230–260	7	6.90	0	0.85	0.83	10	8.50	1	0.96	0.87
PvcA[a]	151–163	7	6.80	1	0.68	0.79	6	4.90	0	0.76	0.80
Agaz2[a]	230–240	4	3.80	0	0.68	0.64	6	5.80	0	0.52	0.69
	Mean	6.17	5.99	0.44	0.68	0.66	6.18	5.67	0.28	0.69	0.69

Locus	Cape Marshall (n = 29)					Global population[d] (n = 84)				
	A	AR	E	H_o	H_e	A	AR[e]	E[f]	H_o	H_e
ZcwE05[b]	4	3.00	0	0.43[c]	0.44	4	3.65	0	0.43	0.44
ZcwD02[b]	13	12.60	2	0.89	0.89	17	12.99	1.66	0.91	0.90
ZcwB09[b]	4	5.00	0	0.79	0.66	6	5.71	0.33	0.80	0.77
ZcCgDh5[b]	7	6.90	0	0.75	0.67	9	7.26	0.66	0.73	0.73
Hg8.1[a]	5	4.00	1	0.52	0.61	5	4.57	0.33	0.48	0.54
ZcCgDh7t[b]	4	3.00	0	0.45	0.39	5	4.15	0.33	0.48	0.44
Hg6.1[b]	3	4.90	0	0.24	0.25	5	4.70	0	0.38	0.37
ZcwF07[b]	4	4.00	0	0.72	0.61	6	4.84	0.33	0.60	0.54
ZcwE03[b]	7	7.00	0	0.86	0.79	8	7.39	0.33	0.85	0.84
ZcwE12[b]	6	6.90	0	0.66	0.77	7	6.10	0.33	0.78	0.78
ZcwE04[b]	8	8.90	0	0.96	0.85	11	8.62	0	0.94	0.86
ZcwB07[a]	7	7.90	1	0.93	0.81	9	7.89	0.33	0.86	0.80
Pv9[a]	5	5.90	0	0.68	0.68	6	5.87	0	0.72	0.70
Hg6.3[a]	4	4.00	1	0.54	0.61	6	4.46	0.66	0.53	0.55
PvcE[a]	5	4.90	0	0.62	0.62	7	5.30	0	0.67	0.68
Hg1.3[a]	9	9.90	0	0.89	0.80	10	9.14	0.33	0.90	0.86
PvcA[a]	5	6.00	0	0.67	0.64	7	6.22	0.33	0.70	0.76
Agaz2[a]	6	5.90	0	0.86	0.74	6	5.31	0	0.66	0.69
	5.69	6.15	0.28	0.69	0.66	7.44	6.34	0.33	0.69	0.68

A number of alleles, *AR* allelic richness, *E* number of exclusive alleles, H_o observed heterozygosity, H_e expected heterozygosity

[a,b] Pooled markers in multiplex PCR

[c] Loci that deviated from H–W equilibrium after Bonferroni correction

[d] Samples for all populations pooled

[e] Allelic richness based on a sample size of 84 diploid individuals

[f] Arithmetic mean of exclusive alleles

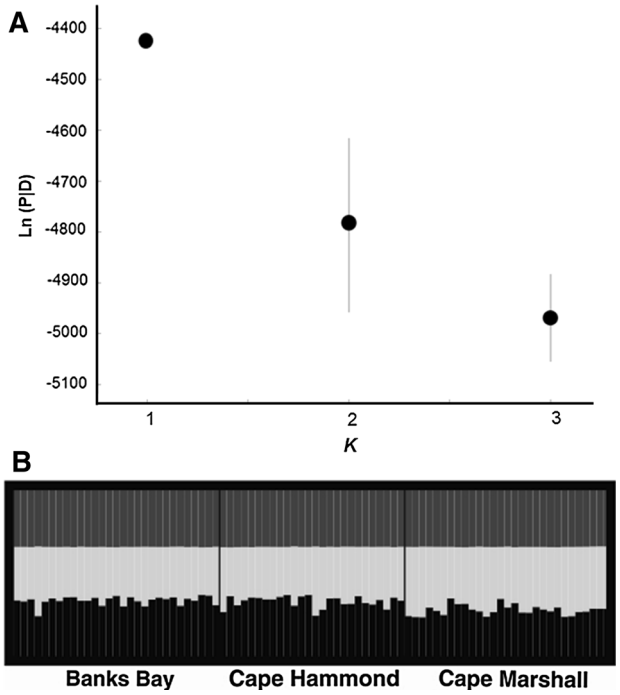

Fig. 3 **a** Log likelihood values as a function of the number genetically differentiated populations inferred from Bayesian STRUCTURE analysis of 18 microsatellite loci. **b** Proportional membership (q) of each *Arctocephalus galapagoensis* in the genetic clusters inferred by STRUCTURE with $K = 3$, without use of prior population (USEPOPINFO = 0). Each individual is denoted by a *vertical bar*, and the length of each bar shows the probability of membership in each cluster. In this case, all individuals have roughly the same probability of belonging to each sampling locality, suggesting that there is no population structure

colonies if they cannot establish in their original colonies, thereby breeding and leaving offspring in colonies other than those in which they were born, and establishing effective gene flow among colonies (Campagna et al. 1988; Boness 1991; Hoelzel et al. 1999). However, it is unclear if this occurs in the GFS, because according to Trillmich and Trillmich (1984) there is a marginal male effect, whereby females prefer areas defended by a strong territorial male which protects them from copulation attempts by marginal males.

The Bayesian skyline plot based on mtDNA and bottleneck tests of microsatellite data further provided no evidence of recent oscillations in effective population size, although a slight and weakly supported decrease in female N_e of around 6.2 % was observed in the recent past and the M value (Garza–Williamson's modified index) was lower than the threshold of 0.68. This suggests that the population size of GFS may have been historically rather similar to the current day estimate of 10,000–15,000 individuals (IUCN 2014). mtDNA has one quarter the N_e of nDNA (e.g., microsatellites), it traces only one independent coalescent event, and for microsatellites, a larger number of loci (20–100) may be required to detect genetic signatures of past population processes (Cornuet and Luikart 1996; Hoban et al. 2013). It also suggests that the catastrophic demographic changes recently documented in GFS (Trillmich and Limberger 1985; Trillmich and Dellinger 1991; Dekinger and Salazar 2010; IUCN 2014) apparently did not have a marked influence on the genetic diversity of the species that would be detectable with the number of loci used in this study (n = 18).

Several other pinniped species are thought to have been severely bottlenecked, primarily due to anthropogenic exploitation. In a few cases, these events are readily detectable using bottleneck tests (e.g., Oliveira et al. 2009; Hoffman et al. 2011) but many other species reveal no such signals (e.g., Klimova et al. 2014). The reason for this discrepancy is unclear but probably relates to variation in bottleneck timing and intensity. Antarctic fur seals, for example, were driven to the brink of extinction by extreme exploitation over the space of just a few decades. In contrast, GFSs probably did not experience such a dramatic reduction. Instead, the population may have been reduced many times over the course of the past few centuries, a pattern that may be more difficult to detect using classical bottleneck tests and will be difficult even with refined methodology and tens of thousands of markers (Shafer et al. 2015).

ENSO events can severely impact pinniped population sizes (e.g., Oliveira et al. 2006, 2009; Oliveira 2011) and have been responsible for significant but temporary reductions in GFS census sizes on at least two occasions, 1982–1983 and 1996–1998 (Trillmich and Limberger 1985; Trillmich and Dellinger 1991; Bastida et al. 2007). Some authors argue that ENSO events are both recurrent and ancient, going back to as far as 2 million years ago (De Vries 1987; Sandweiss et al. 1996), and that many animal species occupying marine environments affected by ENSOs may have adapted by developing flexible life history traits, which allow them to adjust to ever-changing environmental conditions (Majluf 1987). They might achieve this, for example, through extended female lactation periods, which may help to optimize offspring survival (Majluf 1987; Trillmich and Kooyman 2001). Flexible life history strategies can therefore help to buffer environmental stress which, in small populations, can be a decisive factor contributing to persistence and population recovery from demographic reduction. The flexibility is achieved by the mother's potential to adjust the duration of the lactation period thereby buffering offspring against times of low marine productivity during ENSO years (Trillmich and Wolf 2008; Trillmich 1990). A fuller understanding of the demographic response of the GFS to ENSO events only will be achieved with long-term individual-based data on key vital rates, including survival

Table 7 The mtDNA control region (left) genetic diversity of *Arctocephalus galapagoensis* based on haplotype (H_d) and nucleotide (π) diversities and microsatellite (right) genetic diversity in pinnipeds, based on expected heterozygosity (H_e) (or observed heterozygosity when only this information was available) and average number of alleles per locus (A)

Species	mtDNA control region				Microsatellites			
	No. of individuals	Fragment (bp)	H_d	π	No. of individuals	No. of loci	H_e	A
Galapagos fur seal (present paper)	87	220	0.86	0.012	84	17	0.68	7.44
Galapagos sea lion[a,n,r]	336	285	–	0.005	20	10/367	0.72/ 0.62	7.9/ 5.2
South American fur seal[b]	–	–	–	–	226	8	0.77	8.4
New Zealand fur seal[c]	–	–	–	–	383	11	0.75	11.8
Antarctic fur seal[d,o]	192	304	–	0.038	2106/20	9/15	0.8/0.74	12.4/–
Australian fur seal[e]	–	–	–	–	183	5	0.58	8.0
Subantarctic fur seal[f]	103	457	–	0.048	76	8	0.60	11.1
Northern fur seal[g]	578	381	0.99	0.024	578	7	0.79	11.7
Juan Fernandez fur seal[h]	31	298	0.90	0.030	–	–	–	–
Cape fur seal[i]	106	361	0.97	0.011	–	–	–	–
Guadalupe fur seals[j]	32	181	0.79	0.025	–	–	–	–
Australian sea lion[k,l]	208	360	0.90	0.016	217	5	0.54	4.5
New Zealand sea lion[n]	–	–	–	–	40	22	0.72	5.9
California sea lion[o,p]	52	283	0.60	0.020	58	12/16	0.61/ 0.72	6.8/ 6.4
Steller sea lion[q]	2599	196	–	0.011	668	13	0.66	7.9
Grey seal[r,s]	103	435	0.95	0.017	1183	9	0.74	–
Northern elephant seals[t,u]	100/40	–	0.41/ 0.40	0.0067/ 0.0066	–	–	–	–
Southern elephant seal[q,u,v]	48	300	–	0.021	263	2	0.59	–

[a] Hoffman et al. (2007)

[b] Oliveira et al. (2008)

[c] B. Robertson, A. Kalinin, H. Best, N. Gemmell (unpublished data, apud Robertson and Chilvers 2011)

[d] Hoffman and Amos (2005)

[e] Lancaster et al. (2010) (H_o only)

[f] L. Wynen, S. Goldsworthy, R. White, R. Slade (unpublished data, apud Robertson and Chilvers 2011) (H_o only)

[g] Dickerson et al. (2010)

[h] Goldsworthy et al. (2000)

[i] Mathee et al. (2006)

[j] Weber et al. (2004)

[k] Campbell R (in Robertson and Chilvers 2011)

[l] Campbell (2003)

[m] Wolf and Trillmich (2007)

[n] Acevedo-Whitehouse et al. (2009) (H_o only)

[o] Hernández-Velazquez et al. (2005)

[p] Maldonado et al. (1995)

[q] Hoffman et al. (2006a, b, 2009)

[r] Worthington-Wilmer et al. (1999)

[s] Fietz et al. (2013)

[t] Weber et al. (2000)

[u] Hoelzel et al. (1999)

[v] Hoelzel et al. (1993)

and fecundity, as well as data on ENSO frequency and intensity.

Conservation implications

Our study has important implications for the conservation management of the GFS. Historical demographic analyses indicate little sensitivity of the long-term effective population size to either historical exploitation or ongoing environmental fluctuations. However, despite the geographic range of this species being within the spatial scale of daily foraging trips (Jeglinski et al. 2013), strong matrilineal structuring is present. The lack of reciprocal monophyly for mtDNA and low differentiation estimated from microsatellite markers suggests, on the one hand, that the three major breeding populations of the species should be considered as a single ESU. On the other hand, conservation efforts should be directed towards all three populations due to strong mtDNA structure and the fact that philopatry is known to negatively affect the speed of recolonization (see above, Matthiopoulos et al. 2005), which is an important property for population recovery after ENSO events. Finally, it is important to emphasize that despite the GFS current population size and its moderate levels of genetic diversity in a single ESU, these fur seals will always be vulnerable to a variety of threats (e.g., feral dogs, infectious diseases, oil spills, entanglement in local net fisheries and ENSO events) due to their restricted distribution to a relatively small archipelago (IUCN 2014).

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