PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

FACULDADE DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

DIVERSIDADE GENÉTICA E ESTRUTURAÇÃO POPULACIONAL DO LOBO-MARINHO-DE-GALÁPAGOS, *Arctocephalus galapagoensis*

Fernando Ricardo Vieira Lopes

DISSERTAÇÃO DE MESTRADO

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Fernando Ricardo Vieira Lopes
Orientador: Sandro Luis Bonatto

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SUMÁRIO

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RESUMO

O lobo-marinho-de-Galápagos, *Arctocephalus galapagoensis*, apresenta uma das mais restritas distribuições geográficas dentro da família Otariidae, distribuindo-se especialmente à região noroeste das Ilhas Galápagos. Entre as principais ameaças à conservação da espécie está a caça, responsável pela quase extinção da espécie no início do século XIX, devido ao alto valor econômico e de subsistência da pele e gordura destes animais; e também os recorrentes eventos do fenômeno *El Niño*, o qual afeta toda a base da cadeia trófica do Pacífico equatorial e, por consequência, as espécies predadoras e de topo de cadeia trófica como o lobo-marinho-de-Galápagos. Tanto a caça, quanto os recorrentes eventos de *El Niño*, levaram a espécie à Lista Vermelha de Espécies Ameaçadas de Extinção da União Internacional para a Conservação da Natureza (do inglês *IUCN*), indicando que houve ≥50% de redução populacional observada nos últimos 30 anos. No entanto, até o momento, não há estudos que verificaram possíveis efeitos dos problemas mencionados sobre a variabilidade genética da espécie, nem como esta variabilidade está representada espacialmente ao longo da área de distribuição do lobo-marinho-de-Galápagos. Para obter as informações de diversidade genética, estruturação populacional e para verificar possíveis oscilações demográficas, bem como verificar como a variabilidade está distribuída no espaço nós utilizamos de técnicas moleculares, aplicando o uso de dois tipos de marcadores de DNA: um mitocondrial (mtDNA - de herança exclusivamente materna), através da aplicação de parte da região controladora, e outro nuclear (herança bi-parental), através da aplicação de 18 loci de microssatélites de DNA em amostras coletadas nas três maiores colônias da espécie: Cabo Hammond (Ilha Fernandina), Baía Banks (Ilha Isabela) e Cabo Marshall (Ilha Isabela). Verificamos com nossos resultados que mesmo as colônias analisadas estando distante cerca de 70 Km há uma forte fidelidade das fêmeas ao sítio de nascimento, com 33,9% da variação no mtDNA estando particionada entre colônias. Por outro lado, a estrutura populacional inferida através dos loci nucleares foi fraca. Nossos resultados além de mostrar uma forte fidelidade das fêmeas ao sítio de nascimento, mostrou que os machos são os principais responsáveis pelo fluxo gênico e que a fidelidade ao sítio de nascimento das fêmeas pode ser convertida em estruturação genética espacial em fina escala, mesmo em espécies que apresentam alta capacidade de deslocamento como é o caso de diversas espécies de pinípedes e, em especial, o lobo-marinho-de-Galápagos. Neste sentido, discutimos também neste estudo a importância da filopatria natal e consequente estruturação genética em fina escala para o manejo e conservação do lobo-marinho-de-Galápagos, neste que um dos maiores e mais representativos santuários da vida silvestre, o arquipélago de Galápagos.

ABSTRACT

The Galapagos fur seal, *Arctocephalus galapagoensis*, shows one of the most restricted species distribution range under the Otariidae family, with its distribution restricted to northwest of Galapagos Islands. Among the major threats to the conservation of Galapagos fur seals was the exploitation, that almost drove the species to extinction in the early nineteenth century due to the high economic and subsistence value of its skin and blubber. Currently, the species faces frequents events of *El Niño* that affect the base of the food chain in the equatorial Pacific Ocean, consequently the predatory and top predatory species like the fur seals. Both hunting and *El Niño* events led the species to Red List of Endangered Species of International Union for Conservation of Nature, indicating a ≥50% of population in the last 30 years. However, until now, there are no studies related to conservation problems and genetic variability and how genetic variability is represented in the space along the distribution range of Galapagos fur seal. To access the information about genetic diversity, population structure and demographic oscillation as well as how genetic variability is represented in the space we used molecular techniques applying two kinds of markers: a mitochondrial marker (control region, maternal inheritance) and nuclear marker (18 microsatellites loci, bi parental inheritance). The fur seals were sampled in the three major Galapagos fur seals colonies: Cape Hammond (Fernandina Island), Banks Bay (Isabela Island) and Cape Marshall (Isabela Islands). Our results showed that there is a strong female natal fidelity with 33.9% of mtDNA occurring among partitioned colonies. In this sense, this natal philopatry, was converted in fine-scale matrilineal population structure. In the other hand, the population structure inferred by nuclear loci was week. This suggests that males are the main responsible by gene flow among sampled localities, even in a highly mobile species like Galapagos fur seal. Here we discuss the importance of natal philopatry and fine-scale matrilineal population structure of Galapagos fur seal species and their implications for management and conservation in the one of the most representative wildlife sanctuaries, the Galapagos archipelago.

APRESENTAÇÃO GERAL

Esta dissertação de mestrado é apresentada sob forma de artigo científico e está configurada de acordo com as normas do periódico *Conservation Genetics*. Todas as legendas de figuras, figuras, tabelas e material suplementar estão incluídos ao final do artigo e estão conforme o permitido pelo periódico. Abaixo uma breve descrição sobre a biologia, ecologia e *status* de conservação da espécie.

O lobo-marinho-de-Galápagos, *Arctocephalus galapagoensis*, é endêmico do arquipélago de Galápagos (IUCN 2014) (00°35' S, 91°00' O) (Fig. 1A) e juntamente com o leão-marinho-de-Galápagos, *Zalophus wollebaeki*, são as únicas espécies de pinípedes que se reproduzem no Equador (Alava e Salazar 2006; IUCN 2014). O lobo-marinho-de-Galápagos ocorre principalmente na porção noroeste do arquipélago (nas ilhas Fernandina e Isabela), em locais onde há ressurgência de águas frias, ricas em nutrientes e alimentos (Alava e Salazar 2006). O lobo-marinho-de-Galápagos é considerado o menor lobo-marinho existente e apresenta pouco dimorfismo sexual quando comparada as demais espécies da família Otariidae. Os machos possuem de 1,5-1,6 m de comprimento e 60-68 kg de peso, enquanto as fêmeas possuem cerca de 1,1-1,3 m e pesam entre 21,5 e 33 kg (Jefferson et al. 2008; IUCN 2014).

O lobo-marinho-de-Galápagos foi intensamente caçado no início do século XIX, por causa do alto valor da sua pele e gordura. Devido a isso, entre 1816 e 1933 foi registrada a captura de cerca de 22.500 animais (Bastida et al. 2007; Reeves et al. 1992). A espécie foi primeiramente caçada por tripulantes de navios baleeiros que capturavam estes animais ao adentrar em terra firme na busca por alimento e água potável e, posteriormente, por caçadores profissionais de pinípedes. Há registros de que em apenas uma campanha de caça, conduzida pelo Capitão Fanning em 1816, cerca de 8.000 animais foram abatidos (Townsend 1934; Trillmich 1987). Neste sentido, a caça foi o maior problema de conservação enfrentado não só por esta espécie, bem como por todas as demais espécies de lobos e leões-marinhos. Desta maneira, a caça exploratória quase levou à extinção na natureza do lobo-marinho-de-Galápagos (Weber et al. 2004).

No final do século XIX os efeitos da caça haviam sido tão intensos que não se encontravam colônias reprodutivas efetivamente formadas, apenas pequenos grupos de lobos-marinhos dispersos pelo arquipélago (Trillmich 1987). No entanto, em 1957, uma pequena colônia reprodutiva de ~100 espécimes foi encontrada na Ilha Santiago por Eibl-Eibesfeldt

(1959). Entre os anos 1960 e 1970 novas colônias reprodutivas foram descobertas e novos censos estimaram a existência de 1.000-4.000 animais, de acordo com Leveque (1962) e Orr (1972). Entre 1977 e 1978, foram contabilizados 9.785 lobos-marinhos (Trillmich e Mohren 1981) nas principais ilhas de Galápagos e a partir deste valor 30.000 indivíduos foram estimados para o arquipélago como um todo. Atualmente, a IUCN estima a existência de 10.000 a 15.000 lobos-marinhos-de-Galápagos (IUCN 2014)

Durante os eventos de *El Niño*-Oscilação-Sul (ENSO, do inglês *El Niño-Southern Oscillation*), com aquecimento das águas superficiais do oceano Pacífico as correntes oceânicas que levam águas de alta produtividade primária ao arquipélago de Galápagos são afastadas daquela região e com isso toda a cadeia trófica dependente destes recursos é afetada. Estes eventos foram e continuam sendo responsáveis pela morte por inanição de uma parcela significativa da população de *A. galapagoensis* (Trillmich e Dellinger 1991). Acredita-se que os eventos de *El Niño* ocorridos em 1982-83 e 1996-98 foram responsáveis pela diminuição em até 50% da população em cada um dos períodos mencionados (Bastida et al. 2007; IUCN 2014; Trillmich e Dellinger 1991; Trillmich e Limberger 1985), acarretando, inclusive, em dispersões erráticas. Durante os eventos de ENSO vários espécimes já foram registrados na região continental do Equador, Costa Rica, Colômbia, México e possivelmente no Peru (Aurioles-Gamboa te al. 2004; Capella et al. 2002; Félix et al. 2001; Montero-Cordero et al. 2010), ou seja, de 1.000 a 3.000 km distante da sua área de distribuição original. *Arctocephalus galapagoensis* não possui movimentos migratórios fora das Ilhas Galápagos.

O lobo-marinho-de-Galápagos está protegido pela legislação equatoriana desde 1930. No entanto, sua proteção tornou-se efetiva somente após 1959, quando grande parte das Ilhas Galápagos foi declarada Parque Nacional (Seal Conservation Society 2010). As águas ao redor do arquipélago também são protegidas e incluem uma zona de proibição de pesca de 40 milhas náuticas (Heylings et al. 2002). Atualmente, o lobo-marinho-de-Galápagos encontra-se listado como "Em Perigo" na Lista Vermelha de Espécies Ameaçadas de Extinção da União Internacional para a Conservação da Natureza (IUCN), estando incluído na categoria *A2a*. Isto indica que houve ≥50% de redução populacional observada da espécie nos últimos 30 anos, sendo esta redução atribuída principalmente aos intensos eventos de *El Niño* de 1982-83 e 1996-98 (Trillmich e Limberger 1985; IUCN 2014). A espécie também está incluída no Apêndice II da Convenção sobre o Comércio Internacional de Espécies Ameaçadas da Fauna e Flora Silvestres (CITES 2014). Neste apêndice estão incluídas as espécies que não estão

necessariamente ameaçadas de extinção, mas que poderiam chegar a estar a menos que o comércio não seja rigidamente controlado e também aquelas consideradas espécies semelhantes às incluídas na lista CITES por motivos de conservação (CITES 2014).

Apesar da restrita distribuição geográfica, das grandes pressões exercidas em decorrência dos eventos de *El Niño* e da caça indiscriminada sofrida pela espécie, nenhum estudo avaliou a variabilidade genética, a existência de gargalos populacionais, a estruturação populacional nas Ilhas Galápagos e as suas consequências para o manejo e conservação da espécie. Os únicos estudos moleculares sobre *A. galapagoensis* estão basicamente relacionados às análises filogenéticas da família Otariidae e à identificação de indivíduos erráticos através da análise de poucas sequências de DNA mitocondrial (mtDNA) (*e.g.* Aurioles-Gamboa et al. 2004; Capella et al. 2002; Felix et al. 2001; Montero-Cordero et al. 2010; Wynen et al. 2001; Yonezawa et al. 2009). Neste sentido, este estudo abordou as questões mencionadas acima através da análise de marcadores moleculares extra-nucleares (DNA mitocondrial ou mtDNA) e nucleares (microssatélites) da população do lobo-marinho-de-Galápagos.

É importante mencionar que no grupo dos pinípedes (lobos-marinhos, leões-marinhos, focas e morsas), as fêmeas normalmente são filopátricas ao sítio de nascimento, sendo os machos os principais responsáveis pelo estabelecimento do fluxo gênico entre as populações (Riedman 1990; Fabiani et al. 2003). Desta maneira, a utilização apenas do marcador mitocondrial oferece resultados parciais, por ser insensível ao fluxo gênico mediado pelos machos. Assim, uma avaliação conjunta do mtDNA e de diversos locos de microssatélites, os quais refletem herança bi-parental nas populações (Hancock 1999), é essencial para um estimativa mais ampla sobre os processos atuantes sobre o genoma da espécie.

Os marcadores nucleares de microssatélites tem sido amplamente utilizados em pesquisas voltadas para determinação dos níveis de diversidade genética, para identificação de subdivisões geográficas entre populações de mamíferos aquáticos e recentemente na análise de demografia populacional histórica (Allen et al.1995; Gemmel et al. 1997; Luikart e Cornuet 1998; Oliveira et al. 2009). Tais marcadores são frequentemente utilizados em estudos de populações naturais por serem extremamente polimórficos, possuindo uma alta taxa de mutação, na ordem de 10^{-5} a 10^{-2} por geração (Jarne e Lagoda 1996), além de apresentarem alelos co-dominantes e de normalmente serem seletivamente neutros.

Além do conhecimento científico, a avaliação dos resultados gerados a partir da análise do mtDNA e dos loci de microssatélites de DNA das populações do lobos-marinhos-de-

Galápagos permitiram testar a hipótese dos efeitos de caça/ENSO sobre a variabilidade genética da espécie. Ou seja, se as reduções populacionais resultantes de caça ou ENSO foram suficientemente fortes para que fosse retirada variabilidade genética da espécie, bem como resultasse em oscilações negativas no tamanho efetivo populacional. Além disso, é fundamental que se saiba o quanto as espécies formalmente descritas apresentam subdivisões geográficas, pois tais diferenças resultam em diferentes estratégias de conservação e manejo ao longo da distribuição da espécie (Eizirik 1996; Frankham et al., 2002).

Referências

Alava JJ, Salazar S (2006) Status and Conservation of Otariids in Ecuador and the Galápagos Islands. In: Trites AW, Atkinson SK, DeMaster DP, Fritz LW, Gelatt TS, Rea LD, Wynne KM (org) Sea Lions of the world - 22nd Lowell Wakefield Fisheries Symposium. Alaska Sea Grant College Program, Anchorage, pp 495-519

Allen PJ, Amos W, Pomery PP, Twiss SD (1995) Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. Mol Ecol 4:653-662

Aurioles-Gamboa D, Schramm Y, Mesnick S (2004) Galapagos fur seals, *Arctocephalus galapagoensis*, in Mexico. LAJAM 3:77-80

Bastida R, Rodríguez D, Secchi E, Da Silva V (2007) Mamíferos Acuáticos de Sudamérica y Antártica, 2nd edn. Vázquez Manzini Editores, Buenos Aires

Capella JJ, Florez-Gonzáles L, Falk-Fernández P, Palácios DM (2002) Regular appearance of otariid pinnipeds along the Colombian Pacific coast. Aquat Mamm 28:67-72. doi:10.1098/rsbl.2007.0487

CITES: Convention on International Trade in Endangered Species of wild of fauna and flora (2013) http://www.cites.org. Accessed 01 September 2014

Eibl-Eibesfeldt I (1959) Survey on the Galapagos Islands. UNESCO Mission Rep.8, Paris

Eizirik E (1996) Ecologia molecular, genética da conservação, e o conceito de Unidades Evolutivamente Significativas. Braz J Genet 19 Suppl.: 23-29

Fabiani A, Hoelzel R, Galimberti F, Muelbert MMC (2003) Long-range paternal gene flow in the Southern elephant seal. Science 229:676. doi:10.1126/science.299.5607.676

Félix F, Lento G, Davis J, Chiluizal D (2001) El lobo fino de Galápagos *Arctocephalus galapagoensis* (Pinnipedia, Otariidae) en la costa continental de Ecuador, primeros registros confirmados mediante análises morfológicos y genéticos. Estud Ocean 20:61-66

Frankham R, Balou JD and Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge

Gemmell NJ, Allen PJ, Goodman SJ, Reed JZ (1997) Interespecific microsatellite markers for the study of pinniped popualtions. Mol Ecol 6:661-666

Hancock JM (1999) Microsatellites and other simple sequences: genomic contex and mutational mechanisms In: Goldstein DB, Schlotterer C (Eds). Microsatellites, Evolution and Applications.

Heylings P, Bensted-Smith R, Altamirano M (2002) Zonificación e historia de la Reserva Marina de Galápagos. In: Danulat E, Edgar GJ (eds.) Reserva Marina de Galápagos. Línea Base de la Biodiversidad. Fundación Charles Darwin/Servicio Parque Nacional Galápagos, Santa Cruz, Galápagos. 484 pp

IUCN: IUCN Red List of Threatened species (2014) http://www.iucnredlist.org/apps/redlist/details/2057/0. Accessed 01 October 2014

Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. Trends Ecol Evol 11-424-429

Jefferson TA, Webber MA and Pitman RL (2008) Marine mammals of the world 1ª Ed. Academic Press, 573 p

Leveque R (1963) Le statut actuel des vertebres rares et menaces de'larchipel des Galapagos. *Terre Vie* 110:397-430

Luikart G, Cornuet JM (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conserv Biol 12, 228-237

Montero-Cordero A, Fernández DM, Hernández-Mora G (2010) Mammalia, Carnivora, Otariidae, *Arctocephalus galapagoensis* Heller, 1904: First continental record for Costa Rica. Check List 6:630-632

Oliveira LR, Meyer D, Hoffman JI, Majluf P, Morgante JS (2009) Evidence of a genetic bottleneck in an *El Niño* affected population of South American fur seals, *Arctocephalus australis.* J Mar Bio Assoc U.K. 89:1717-1725. doi: 10.1017/S0025315409000162

Orr RT (1972) Galapagos fur seal (*Arctocephalus galapagoensis*). In Seals Proceedings of a working meeting of seal specialists on threatened and depleted seals of the world. IUCN Morges, 124-128

Reeves RR, Stewart BS and Leatherwood S (1992) The Sierra Club Handbook of seals and sirenians. Sierra Club Books, San Francisco

Riedman ML (1990) The Pinnipeds. Seals, sea lions and walruses. University of California Press, Berkeley

Seal Conservation Society (2010) Galapagos Fur Seal. http://www.pinnipeds.org/species/galfursl.htm Accessed 30 September 2010

Townsend CH (1934) The fur seal of the Galapagos islands. Zoologica 18:43-56

Trillmich F (1987) Galápagos Fur Seal: *Arctocephalus galapagoensis*. In: Croxal PL and Gentry RL (eds) Status, Biology and Ecology of Fur Seals. Proceedings of an International Symposium and Workshop. Cambrige, pp 23-27

Trillmich F and Dellinger T (1991) The effects of *El Niño* on Galápagos pinnipeds. In: Trillmich F, Ono KA (eds) Pinnipeds and El Niño: Responses to environmental stress. Springer-Verlag, Berlin, pp 66-74

Trillmich F, Limberger D (1985) Drastic effects of *El Niño* on Galapagos pinnipeds. Oecologia 67:19-22

Trillmich F and Mohren W (1981) Effects of the lunar cycle on the Galapagos fur seal, Arctocephalus galapagoensis. Oecologia (Berl.) 48:85-92

Weber DS, Stwart BS and Lehman N (2004) Genetic consequences of a severe Population Bottleneck in the Guadalupe fur seal (*Arctocephalus townsendi*). J Hered 95:144-153

Wynen LP, Goldsworthy SD, Insley SJ, Adams M, Bickham JW, Francis J, Gallo JP, Hoelzel AR, Majluf P, White RWG, Slade R (2001) Phylogenetic relationships within the Eared Seals (Otariidae: Carnivora): Implications for the historical biogeography of the family. Mol Phylogenet Evol 21:270-284

Yonezawa T, Kohno N, Hasegawa M (2009) The monophyletic origin of sea lion and fur seals (Carnivora; Otariidae) in the southern hemisphere. Gene 441:89-99

Artigo Científico

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

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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 mediated by sex-specific gene flow, 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 (ENSO) 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.

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 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) what is consistent with the expectation of male-biased dispersal (Perrin and Mazalov 2000).

65 Uniparental nonrecombining mtDNA has shorter coalescent times than nuclear DNA and is thus well
66 suited to delineate young evolutionarily significant units – ESUs (Corl and Ellegren 2012), that should ideally be
67 reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies of nuclear loci
68 (*e.g.* microsatellites) (*sensu* Mortiz 1994). ESUs objectively define units below the level of species that should be
69 prioritized for protection when they are under threat (Ryder 1986; Moritz 1994; Chan et al. 2006; Hedrick et al.
70 2006; Robalo et al. 2007; Bottin et al. 2007) in face of limited resources (Avise 1989). Characterization of
71 population structure and identification of ESUs allow the adoption of more effective conservation management
72 strategies, mainly related to the translocation and reintroduction of endangered species, in order to maximize the
73 overall viability of a metapopulation (McCarthy et al. 2004; Akçakaya et al. 2007).
74 In addition to the implications of female philopatry for ESU delineation, extreme philopatry can be of
75 concern where it is associated with a polygynous mating system, as is characteristic for otariid seals (Boness 1991).
76 In this case, declines in female numbers will strongly affect local operational sex ratios, impact local genetic
77 diversity and make local populations vulnerable to genetic (e.g. inbreeding, drift, bottlenecks), environmental (e.g.
78 *El Niño*, global climate change) or anthropogenic (e.g. hunting) impacts that may increase extinction risk
79 (Frankham et al. 2002). According to Cornuet and Luikart (1996), many populations around the world are suffering
80 demographic bottlenecks (reduction of census size) and genetic bottlenecks (reduction of effective population size,
81 N_e) as a result of increasing habitat fragmentation and isolation. The analysis of genetic diversity can be used to
82 test the hypothesis of whether a population that may have experienced recently a genetic bottleneck by comparing
83 its empirical heterozygosity (H_e) of a sampled population with the heterozygosity expected in an equilibrium
84 population (H_{eq}). In nonbottlenecked populations near mutation-drift equilibrium, H_{eq} will be equivalent to the
85 observed heterozygosity under Hardy-Weinberg equilibrium (H_e). However, if a population has suffered a recent
86 bottleneck, the mutation-drift equilibrium is transiently disrupted and H_e will exceed H_{eq} computed from the
87 number of alleles in the sample (Luikart and Cornuet 1998).

88 The Galapagos fur seal (GFS), *Arctocephalus galapagoensis,* is a non-migratory species that is endemic
89 and resident of the Galapagos Islands, Ecuador (00°35'S, 91°00'W, Fig. 1A), where it is mainly distributed across
90 the northern and western parts of the archipelago (mainly Fernandina and Isabela islands). The species' geographic
91 range is unusually small for a pinniped species, covering an area smaller than the Galapagos Marine Reserve (less
92 than 140,000 km^2). This limited distribution reflects the highly localized influence of an upwelling of cold,
93 nutrient-rich waters from the Humboldt and Cromwell currents, which provide sufficient food resources (Alava
94 and Salazar 2006; IUCN 2014). The species' small range and its mobility set the stage to study the potential impact
95 of philopatry at a high spatial resolution.
96 The GFS is also an important species from a conservation perspective. It was driven to the brink of
97 extinction by human hunting and no well-defined GFS breeding colonies remained in the archipelago towards the
98 end of the 19th century (Heller 1904 *apud* Trillmich 1987). Protective measures were put in place in 1930, but the
99 population only began to recover after 1959, when the Galapagos archipelago was declared a National Park (Seal
100 Conservation Society 2010). Nowadays, the population size is believed to range between 10,000 and 15,000
101 individuals (IUCN 2014). Currently, the GFS is listed in the appendix II of CITES, delineating species that are not
102 necessarily threatened by extinction but may become so unless trade is closely controlled (CITES 2014). The GFS
103 is also categorized as Endangered by IUCN Red List and falls into the A2a category, which applies to species that
104 have suffered a 50% or greater population decline over the last 10 years or three generations (IUCN 2014).
105 The GFS population is also subject to natural fluctuations due to *El Niño Southern Oscillation* (ENSO)
106 events (Wyrtki 1982; Philander 1983; Trillmich and Limberger 1985). These events take their toll on the GFS
107 because they reduce local marine productivity in the Galapagos Islands, thereby affecting the entire food chain
108 including top predators such as fur seals and sea lions (Wyrtki 1982; Philander 1983; Trillmich and Limberger
109 1985). ENSO events significantly decrease survival rates (Trillmich and Wolf 2008) and have been responsible
110 for the death by starvation of significant proportions of the GFS population (Trillmich and Dellinger 1991), with
111 the strongest events of the century (1982-83 and 1996-98) 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 mitochondrial DNA (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 management.

Material 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 ~0.5 cm^3 were collected using piglet ear notch pliers (Majluf and Goebel 1992) from the interdigital membrane of the hind flipper. 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 have 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 MgCl2, 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: one cycle of five min at 94 °C; 35 cycles of 30 s at 94 °C, 60 s at 65 °C and 60 s at 72 °C; and one final cycle of seven minutes 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 220bp stretch of high-quality sequence that was obtained for all individuals. Three individuals from Cape Marshall did not recover high quality sequence data and were therefore removed from further analyses.

158 Haplotype (Hd) and nucleotide diversities (π) were quantified for the whole sample set and for each
159 colony separately using Arlequin 3.5.1.2 (Excoffier 2010) and DnaSP 5.10.1 (Rozas et al. 2003; Librado and Rozas
160 2009). Analysis of Molecular Variance (AMOVA) was conducted within Arlequin 3.5.1.2 (Excoffier 2010) to
161 quantify the amount of variation between and within colonies. AMOVA was conducted separately using F_{ST} (Weir
162 and Cockerham 1984) and Φ_{ST} (Tajima 1993). Haplotype networks were constructed using the median-joining
163 approach (Bandelt et al. 1999) implemented in Network 4.6.11 (http://www.fluxus-engineering.com).
164 Additionally, we calculated Fu's F_S (Fu 1997) and Tajima's D (Tajima 1989). Bayesian skyline
165 reconstructions were implemented using BEAUTi 1.7.4 and BEAST 1.7.4 (Drummond et al. 2012) for (i) all data
166 pooled, and (ii) each of the three populations. We used a HKY substitution model gamma site heterogeneity
167 (generated by likelihood with PAUP 4.0b10 (Swofford 2002)) with eight categories and a strict molecular clock
168 prior with Dickerson et al. (2010) mutation rate of 5.74 $x10^{-7}$ s/s/gen derived for *Callorhinus ursinus* (Hoffman et
169 al. 2011). Following implementation of 30,000,000 Markov Chain Monte Carlo (MCMC) iterations, a Bayesian
170 Skyline Plot was generated using Tracer 1.5 (Drummond et al. 2012).
171
172 **Microsatellite DNA amplification and analysis**
173 We amplified 18 polymorphic loci previously developed for pinnipeds: ZcwB07, ZcwE04, ZcwE12,
174 ZcwF07, ZcwB09, ZcwD02, ZcwE03, ZcwE05 designed for *Zalophus wollebaeki* (Hoffman et al. 2007; Wolf et
175 al. 2006), ZcCgDh5.8 and ZcCgDh7tg designed for *Zalophus californianus* (Hernandez-Velasquez et al. 2005),
176 Hg1.3, Hg6.1, Hg6.3, Hg8.1 and Pv9 designed for *Halichoerus grypus* (Allen et al. 1995; Gemmel et al. 1997),
177 PvcA and PvcE, designed for *Phoca vitulina* (Coltman et al. 1996) and Agaz2, designed for *Arctocephalus gazella*
178 (Hoffman 2009). Forward primers were fluorescently labelled and PCRs were carried out in two separate
179 multiplexed reactions using a Type It Kit (Qiagen) (for details see Table 6) following the manufacturer's
180 recommended protocols. The following PCR profile was used: one cycle of five min at 94 °C; eight cycles of 30
181 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 one final

182 cycle of 15 min at 72 °C. PCR products were resolved by electrophoresis on an ABI 3730xl capillary sequencer
183 and allele sizes were scored automatically using the program GeneMarker v1.95 and subsequently manually
184 inspected and adjusted when necessary.

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

192 Arlequin was also used to estimate expected heterozygosity (He) and observed heterozygosity (Ho), to
193 conduct AMOVA analysis of the microsatellite dataset, and to calculate *F*-statistics (F_{ST}). We then compared the
194 genetic diversity of this species to previously reported values for several other pinniped species (Table 7). To take
195 potential sex-bias in dispersal into account, all analyses of population structure were conducted using the overall
196 dataset and separately for male and female pups.

197 population structure was tested without prior knowledge of sampling locations, we estimated the posterior
198 probability of the data fitting the hypothesis of *K* clusters [Pr(X|K)], where *K* is the number of putative populations,
199 using the program Structure 2.3.4 (Pritchard et al. 2000). We performed 10 independent runs for each *K* from *K*
200 = 1 to *K* = 3 using 1,000,000 MCMC iterations and a burn-in period of 1,000,000. We checked for consistency
201 among replicate runs for the same *K* value and then computed the arithmetic mean of the 10 runs. We also carried
202 out a similar analysis using the program Structurama 2 (Huelsenbeck and Andolfatto 2007). This program uses a
203 particularly efficient variant of MCMC called Gibbs sampling, where each MCMC cycle involves a Gibbs scan of
204 all of the individuals. Hence the total number of MCMC cycles for the analysis is the product of the reported
205 number of MCMC cycles and the number of individuals in the analysis. We set the number of populations as a

206 random variable, a parameter that uses a Dirichlet process prior (Pella and Masuda 2006). We ran 1,000,000 cycles
207 for the random variable prior of the number of populations. The first 100,000 cycles were discarded as burn-in.
208 The hypothesis that GFS recently experienced one or more genetic bottlenecks was tested comparing the
209 expected heterozygosity (H_e) at each of the microsatellite loci to values expected under neutrality and equilibrium
210 conditions (H_{eq}). As shown by Cornuet and Luikart (1996), samples from populations that recently experienced
211 bottlenecks tend to have $H_e > H_{eq}$. In order to generate the expected heterozygosities under neutrality-equilibrium,
212 we used the program Bottleneck (Piry et al. 1999). This analysis was based on the general two-phase model (TPM),
213 because most loci probably evolve according to a model intermediate between infinite allele model (IAM, Kimura
214 and Crow 1964) and one-step stepwise mutation model (SMM, Otha and Kimura 1973; Di Rienzo et al. 1994).
215 The two-phase model includes both stepwise mutations and mutations larger than single steps and appears to
216 provide a reasonable fit to empirical evidence about the mutation process (Di Rienzo et al. 1994; Garza and
217 Williamson 2001). We recorded the number of loci for which sample heterozygosity exceeded neutrality-
218 equilibrium expectations and whether the overall set of deviations was significant (based on an one-tailed
219 Wilcoxon test, with the alternative hypothesis of heterozygosity excess). The Arlequin software was also used to
220 compute the Garza-Williamson modified index to verify the putative occurrence of a bottleneck and its influence
221 on genetic variability, denoted as *M*, which refers to the mean ratio of the number of alleles to the range in allele
222 size (Garza and Williamson 2001; Excoffier et al. 2005).
223

224 ## Results

225 **Mitochondrial DNA**

226 A 220 base pair fragment of the mtDNA control region was analysed for sequence variation in 87 GFS
227 individuals sampled from Banks Bay (BB), Cape Hammond (CH) and Cape Marshall (CM) (Table 1). Fourteen
228 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 and Table 2). The 11 remaining haplotypes were specific to each colony (see Table 2 and Fig. 1B for details). Haplotype and nucleotide diversity were moderately high: Hd = 0.86 ± 0.02 and π = 0.012 ± 0.0009.

Mitochondrial Analysis of Molecular Variance (AMOVA) revealed evidence for strong population differentiation, with 33.9% ($P < 0.001$) of the genetic variability being partitioned among the colonies (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 8,000 years (based on the mutation rate of Dickerson et al. 2010 as described in the materials and methods), 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 ($P < 0.05$) population differentiation, both when male and female pups were analysed together (F_{ST} = 0.015 / R_{ST} = 0.035, $P < 0.001$) and separately (males F_{ST} = 0.019 / R_{ST} = 0.029 and females F_{ST} = 0.010 / R_{ST} = 0.029). Weak but significant genetic differentiaion 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. Structurama generated similar results, with the Pr(X/*K*) being 99.12% for $K = 1$.

253 The Bottleneck test did not provide support for a recent reduction in effective population size (normal L-
254 shape distribution). However, results for Garza-Williamson's modified index, *M*, which assumes that $M<0.68$, are
255 suggestive of a reduction in population size ($M = 0.38 \pm 0.12$).
256

257 ## Discussion

258 This is the first investigation on population genetics of one of the World's most endangered pinniped
259 species, the Galapagos fur seal. We provide data on the population genetic structure and genetic diversity of this
260 species, interpret our findings with respect to what is known about the species' biology and discuss the implications
261 of our results for conservation practice.
262

263 **Genetic diversity and contrasting patterns of population structure**

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

268 Population structure was pronounced for mitochondrial DNA, but weak for nuclear markers. Over a third
269 of the mtDNA variation was partitioned among the three main breeding colonies, despite these being separated by
270 as little as 70 km, a distance that can easily be bridged during daily foraging trips (Jeglinski et al. 2013). In contrast,
271 nuclear population structure was weak. This result is coherent with previous studies of pinnipeds showing strong
272 mitochondrial structuring, but weak population structure at nuclear level (Stanley et al. 1996; Andersen et al. 1998;
273 Hoffman et al. 2006b; Davis et al. 2008; Campbell et al. 2008; Hoffman et al. 2009, including the Galapagos sea
274 lion Wolf et al. 2008), a pattern that is consistent with the expectation that in pinnipeds females show strong natal
275 philopatry and males are the dispersing sex (*e.g.* Fabiani et al. 2003; Dickerson et al. 2010, including the Galapagos

276 sea lion Wolf and Trillmich 2007). Nevertheless, in the case of GFS, strong matrilineal structure occurs between
277 adjacent colonies that are separated by as little as 70 Km. This pattern is consistent with mark-recapture studies
278 showing that females of many pinniped species are capable of returning to within meters of their birth locations
279 (Pomeroy et al. 2000; Wolf and Trillmich 2007; Hoffman and Forcada 2012).

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

288

289 **Historical variation in effective population size**

290 The Bottleneck test did not provide support for a recent reduction in effective population size. However,
291 results for Garza-Williamson's modified index, *M,* which assumes that $M<0.68$, are suggestive of a reduction in
292 population size. These results suggest that anthropogenic exploitation and El Niño Southern Oscillation (ENSO)
293 events may have had relatively little impact on overall levels of genetic diversity, despite the restricted geographic
294 range of the species. This is consistent with the results of several analyses, all of which suggest that the population
295 has not been subject to significant population size changes in the recent past. For instance, large values of both
296 haplotype and nucleotide diversity at mtDNA could indicate that the original effective population size (N_e) of this
297 species was large (Frankham et al. 2002) and are also an indication of a stable demographic history (Grant and
298 Bowen 1998). We also recovered non-significant values of Tajima's *D* and Fu's F_S (see Table 5), lending no

299 support to a scenario of recent population expansion. Moreover, it is important to recognize that populations
300 suffering a reduction in census size may not suffer a severe reduction of N_e (genetic bottleneck), due to
301 metapopulation structure involving local extinctions and recolonizations (Pimm et al., 1989). This could be another
302 explanation for the conserved levels of genetic diversity in this species.

303 It is also important to mention the potential contribution to nuclear diversity through male movements
304 between sampled and unsampled colonies (e.g. Campagna et al. 1988; Hoelzel et al. 1999). Adult and sub-adult
305 peripheral males, which are usually excluded from central breeding areas, could contribute towards genetic
306 diversity if they are able to obtain copulations outside these colonies (e.g. Bartholomew 1970; Campagna et al.
307 1988; Boness 1991; Hoelzel et al. 1999). These males may disperse to other colonies if they cannot establish in
308 their original colonies, thereby breeding and leaving offspring in colonies other than those in which they were
309 born, and establishing effective gene flow among colonies (Campagna et al. 1988; Boness 1991; Hoelzel et al.
310 1999). However, it is unclear if this occurs in the GFS, because according to Trillmich and Trillmich (1984) there
311 is a marginal male effect, whereby females prefer areas defended by a strong territorial male which protects them
312 from copulation attempts by marginal males.

313 The Bayesian skyline plot based on mtDNA and bottleneck tests of microsatellite data further provided
314 no evidence of recent oscillations in effective population size, although a slight and weakly supported decrease in
315 female *Ne* of around 6.2% was observed in the recent past and the *M* value (Garza-Williamson's modified index)
316 was lower than the threshold of 0.68. This suggests that the population size of GFS may have been historically
317 rather similar to the current day estimate of 10,000-15,000 individuals (IUCN 2014). Mitochondrial DNA
318 (mtDNA) has one quarter the *Ne* of nDNA (*e. g* microsatellites), it traces only one independent coalescent event,
319 and for microsatellites, a larger number of loci (20-100) may be required to detect genetic signatures of past
320 population processes (Cornuet and Luikart, 1996; Hoban et al. 2013). It also suggests that the catastrophic
321 demographic changes recently documented in GFS (Trillmich and Limberger 1985; Trillmich and Dellinger 1991;

322 Denkinger and Salazar 2010; IUCN 2013) do not appear to have had a marked influence on the genetic diversity
323 of the species that would be detectable with the number of loci used in this study.

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

333 ENSO events can severely impact pinniped population sizes (e.g. Majluf 1991; Trillmich and Dellinger
334 1991; Trillmich et al. 1991; Oliveira et al. 2006, 2009, 2011) and have been responsible for significant but
335 temporary reductions in GFS census sizes on at least two occasions, 1982-83 and 1996-98 (Trillmich and
336 Limberger 1985; Trillmich and Dellinger 1991; Bastida et al. 2007). Some authors argue that ENSO events are
337 both recurrent and ancient, going back to as far as 2 million years ago (DeVries 1987; Sandweiss et al. 1996), and
338 that many animal species occupying marine environments affected by ENSOs may have adapted by developing
339 flexible life history traits, which allow them to adjust to ever-changing environmental conditions (Majluf 1987).
340 Fur seals, for instance, might achieve this, for example, through extended female lactation periods, which may
341 help to optimize offspring survival (Majluf 1987; Trillmich and Kooyman 2001). Flexible life history strategies
342 can therefore help to buffer environmental stress which, in small populations, can be a decisive factor contributing
343 to persistence and population recovery from demographic reduction. The flexibility is achieved by the mother's
344 potential to adjust the duration of the lactation period thereby buffering offspring against times of low marine
345 productivity during ENSO years (Trillmich and Wolf 2008; Trillmich 1990). A proper 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 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, Matthiopolous 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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374

375 **References**

376 Akçakaya HR, Mills G, Doncaster, CP (2007) The role of metapopulations in conservation. In: Macdonald, DW,
377 Service, K (eds) Key Topics in Conservation Biology. Blackwell Publishing, Oxford, pp 64-84

378 Alava JJ, Salazar S (2006) Status and Conservation of Otariids in Ecuador and the Galápagos Islands. In: Trites
379 AW, Atkinson SK, DeMaster DP, Fritz LW, Gelatt TS, Rea LD, Wynne KM (org) Sea Lions of the world -
380 22nd Lowell Wakefield Fisheries Symposium. Alaska Sea Grant College Program, Anchorage, pp 495-519

381 Allen PJ, Amos W, Pomery PP, Twiss SD (1995) Microsatellite variation in grey seals (*Halichoerus grypus*) shows
382 evidence of genetic differentiation between two British breeding colonies. Mol Ecol 4:653-662

383 Andersen L, Born E, Gjertz I, Wiig O, Holm LE, Bendixen C (1998) Population structure and gene flow of the
384 Atlantic walrus (*Odobenus rosmarus rosmarus*) in the eastern Atlantic Arctic based on mitochondrial DNA
385 and microsatellite variation. Mol Ecol 7:1323-1336.

386 Aurioles-Gamboa D, Schramm Y, Mesnick S (2004) Galapagos fur seals, *Arctocephalus galapagoensis*, in
387 Mexico. LAJAM 3:77-80

388 Avise JC (1989) A role for molecular geneticists in the recognition and conservation of endangered species. Trends
389 Ecol Evol 4:279-281

390 Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol
391 Evol 16:37-48

392 Bartholomew GA (1970) A model for the evolution of pinniped polygyny. Evolution 24:546-559

393 Bastida R, Rodríguez D, Secchi E, Da Silva V (2007) Mamíferos Acuáticos de Sudamérica y Antártica, 2nd edn.
394 Vázquez Manzini Editores, Buenos Aires

395 Boness DJ (1991) Determinants of mating systems in the Otariidae (Pinnipedia). In: Renouf D (ed) The Behaviour
396 of Pinnipeds. Chapman & Hall, London, pp 1–44

Bottin L, Tassin J, Nasi R, Bouvet J (2007) Molecular, quantitative and abiotic variables for the delineation of evolutionary significant units: case of sandalwood (*Santalum austrocaledonicum* Vieillard) in New Caledonia. Conserv Genet 8:99-109

Campagna C, Le Boeuf BJ, Capozzo HL (1988) Group raids: a mating strategy of male southern sea lions. Behaviour 105:224-249

Campbell RA, Gales NJ, Lento GM, Baker CS (2008) Islands in the sea: extreme female natal site fidelity in the Australian sea lion, *Neophoca cinerea*. Biol Lett 4:139-142

Capella JJ, Florez-Gonzáles L, Falk-Fernández P, Palácios DM (2002) Regular appearance of otariid pinnipeds along the Colombian Pacific coast. Aquat Mamm 28:67-72.

Chan C, Ballantyne KN, Aikman H, Fastier D, Daugherty CH, Chambers GK (2006) Genetic analysis of interspecific hybridisation in the world's only Forbes' parakeet (*Cyanoramphus forbesi*) natural population. Conserv Genet 7:493-506

CITES: Convention on International Trade in Endangered Species of wild of fauna and flora (2013) http://www.cites.org. Accessed 01 September 2014

Coltman DW, Bowen WD, Wright JM (1996) PCR primers for harbour seal (*Phoca vitulina concolour*) microsatellites amplify polymorphic loci in other species. Mol Ecol 5:161-163

Corl A, Ellegren H (2012) The genomic signature of sexual selection in the genetic diversity of the sex chromosomes and autosomes. Evolution 66:2138-2149

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001-2014

Dasmahapatra KK, Hoffman JI, Amos W (2009) Pinniped phylogenetic relationships inferred using AFLP markers. Heredity 103:168-177. doi: 10.1038/hdy.2009.25

Davis CS, Stirling I, Strobeck C, Coltman DW (2008) Population structure of ice-breeding seals. Mol Ecol 17:3078-3094

Dekinger J, Salazar S (2010) Possible effects of climate change in the populations of Galápagos pinnipeds. Notícias de Galápagos 67:45-49

De Vries TJ (1987) A review of geological evidence for ancient *El Niño* activity in Peru. J Geophys Res 92:14471-14479

Dickerson BR, Ream RR, Vignieri SN, Bentzen P (2010) Population structure as revealed by mtDNA and microsatellites in Northern fur seals, *Callorhinus ursinus*, throughout their range. PLoS ONE 5:1-9. e10671. doi: 10.1371/journal.pone.0010671

428 Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994) Mutational processes of simple-
429 sequence repeat loci in human populations. PNAS 1:3166–3170.

430 Drummond A, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7.
431 Mol Biol and Evol 29:1969-1973

432 Excoffier L, Laval G, Schneider (2005) Arlequin (version 3.0): An integrated software package for population
433 genetics data analysis. Evol Bioinform Online 1:47-50

434 Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics
435 analyses under Linux and Windows. Mol Ecol Res 10:564-567

436 Fabiani A, Hoelzel R, Galimberti F, Muelbert MMC (2003) Long-range paternal gene flow in the Southern
437 elephant seal. Science 229:676. doi:10.1126/science.299.5607.676

438 Félix F, Lento G, Davis J, Chiluizal D (2001) El lobo fino de Galápagos *Arctocephalus galapagoensis* (Pinnipedia,
439 Otariidae) en la costa continental de Ecuador, primeros registros confirmados mediante análises morfológicos
440 y genéticos. Estud Oceanol 20:61-66

441 Forcada J, Hoffman JI (2014) Climate change selects for heterozygosity in a declining fur seal population. Nature.
442 511: 462–465

443 Frankham R, Balou JD and Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press,
444 Cambridge

445 Fu YX (1997) Statistical Tests of neutrality of mutations against population growth, hitchhiking and background
446 selection. Genetics 147:915-925

447 Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci.
448 Mol Ecol 10:305-318

449 Gemmell NJ, Allen PJ, Goodman SJ, Reed JZ (1997) Interespecific microsatellite markers for the study of pinniped
450 popualtions. Mol Ecol 6:661-666

451 Goldsworthy SD, Page BC (2007) A Risk-Assessment Approach to Evaluating the Significance of Seal Bycatch
452 in two Australian Fisheries. Biol Conserv 139:269-285

453 Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: insights
454 from sardines and anchovies and lessons for conservation. J Herd 89:415-426

455 Greenwood PJ (1980) Mating Systems, philopatry and dispersal in birds and mammals. Anim Behav 28:1140-
456 1162

457 Hedrick PW, Lee RN, Hurt CR (2006) The endangered Sonoran top minnow: examination of species and ESUs
458 using three mtDNA genes. Conserv Genet 7:483-492

459 Hernández-Velazquez FD, Galindo-Sanchez E, Taylor MI, De la Rosa-Velez J, Cote IM, Schramm Y, Aurioles-
460 Gamboa D, Rico C (2005) New polymorphic microsatellite markers for California sea lions (*Zalophus*
461 *californianus*), Mol Ecol Notes 5:140-142

462 Hoban SM, Gaggiotti OE, Bertorelle G (2013) The number of markers and samples needed for detecting
463 bottlenecks under realistic scenarios, with and without recovery: a simulation-based study. Mol Ecol
464 22:3444-3450

465 Hoelzel RA, Le Boeuf BJ, Reiter J, Campagna C (1999) Alpha-male paternity in elephant seals. Behav Ecol
466 Sociobiol 46:298-306

467 Hoffman JI, Trathan PN, Amos W (2006a) Genetic tagging reveals extreme site fidelity in territorial male Antarctic
468 fur seals *Arctocephalus gazella*. Mol Ecol 15:3841–3847. doi: 10.1111/j.1365-294X.2006.03053.x

469 Hoffman JI, Matson C, Amos W, Loughlin TR, Bickham JW (2006b). Deep genetic subdivision within a
470 continuously distributed and highly vagile marine mammal, the Steller's sea lion *Eumetopias jubatus*. Mol
471 Ecol, 15:2821-2832

472 Hoffman IJ, Steinfartz S, Wolf JBW (2007) Ten novel dinucleotide microsatellite loci cloned from the Galápagos
473 sea lion (*Zalophus californianus wollebaeki*) are polymorphic in other pinniped species. Mol Ecol Notes
474 7:103-105

475 Hoffman IJ (2009) A panel of new microsatellite loci for genetic studies of Antarctic fur seals and other otariids.
476 Conserv Genet 10:989-992

477 Hoffman JI, Grant SM, Forcada J, Phillips CD (2011) Bayesian inference of a historical genetic bottleneck in a
478 heavily exploited marine mammal. Mol Ecol 20:3989-4008. doi: 10.1111/j.1365-294X.2011.05248

479 Hoffman JI, Forcada J (2012) Extreme natal philopatry in female Antarctic fur seals (*Arctocephalus gazella*).
480 Mamm Biol 77:71-73. doi: 10.1016/j.mambio.2011.09.002

481 Hulesenbeck JP and Andolfatto, P (2007) Inference of population structure under a Dirichlet process model.
482 Genetics 175:1787-1802

483 IUCN: IUCN Red List of Threatened species (2014) http://www.iucnredlist.org/apps/redlist/details/2057/0.
484 Accessed 01 October 2014

485 Jeglinski J, Goetz KT, Werner C, Costa DP, Trillmich F (2013) Same size–same niche? Foraging niche separation
486 between sympatric juvenile Galapagos sea lions and adult Galapagos fur seals. J Anim Ecol 82:694-706. doi:
487 10.1111/1365-2656.12019

488 Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. Genetics 49:725–
489 738

490 Klimova A, Fietz K, Olsen MT, Harwood J, Amos W, Hoffman JI (2014) Global population structure and
491 demographic history of the grey seal. Mol Ecol, 23:3999–4017

492 Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC (1989) Dynamics of
493 mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. PNAS
494 86:6196-6200

495 Librado P, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data.
496 Bioinformatics 25:1451-1452

497 Luikart G, Cornuet JM (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from
498 allele frequency data. Conserv Biol 12, 228-237

499 Lynch M, Ritland K (1999) Estimation pairwise relatedness with molecular markers. Genetics 152:1753-1766

500 Majluf P (1987) Reproductive ecology of female South American fur seals at Punta San Juan. Ph.D. thesis,
501 University of Cambridge

502 Majluf, P. 1991. El Niño effects on Pinnipeds in Peru. In: Trillmich F (Ed) Ecological studies. Springer, Berlin,
503 pp. 55-65

504 Majluf P, Goebel ME (1992) The capture and handling of female South American fur seals and their pups. Mar
505 Mamm Sci 8:187-190

506 Matthiopoulos J, Harwood J, Thomas L (2005) Metapopulation consequences of site fidelity for colonially
507 breeding mammals and birds. J Anim Ecol 74:716–727

508 McCarthy MA, Menkhorst PW, Quin BR, Smales IJ, Burgman MA (2004) Helmeted Honeyeater (*Lichenostomus*
509 *melanops cassidix*) in Southern Australia: assessing options for establishing a new wild population. In:
510 Akçakaya HR, Burgman MA, Kindvall O, Wood CC, Sjögren-Gulve P, Hatfield JS, McCarthy MA (eds)
511 Species Conservation and Management: Case Studies. Oxford University Press, Oxford, pp 410-420

512 Montero-Cordero A, Fernández DM, Hernández-Mora G (2010) Mammalia, Carnivora, Otariidae, *Arctocephalus*
513 *galapagoensis* Heller, 1904: First continental record for Costa Rica. Check List J 6:630-632

514 Moritz C (1994) Defining "Evolutionarily Significant Units" for conservation. Trends Ecol Evol 9:373-375

515 Oliveira LR, Arias-Schreiber M, Meyer D, Morgante, JS (2006) Effective population size in a bottlenecked fur
516 seal population. Biol Conserv 131:505-509

517 Oliveira LR, Meyer D, Hoffman JI, Majluf P, Morgante JS (2009) Evidence of a genetic bottleneck in an *El Niño*
518 affected population of South American fur seals, *Arctocephalus australis.* J Mar Bio Assoc U.K. 89:1717-
519 1725. doi: 10.1017/S0025315409000162

520 Oliveira LR (2011) Vulnerability of South American pinnipeds under *El Niño* Southern Oscillation events: 14:
521 237-252. In: Casalengo S (ed) Global warming impacts – Case studies on the economy, human health, and
522 on urban and natural environments. InTech, pp 1-17. doi:10.5772/25204

523 Otha T, Kimura M (1973) A model of mutation appropriate to estimate the number of electrophoretically
524 detectable alleles in a finite population. Genet Res 22:201–204

525 Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching
526 and research. Mol Ecol Notes 6:288-295

527 Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching
528 and research-an update. Bioinformatics 28:2537-2539

529 Pella J, Masuda M (2006) The Gibbs and split-merge sampler for population mixture analysis from genetic data
530 with incomplete baselines. Can J Fish Aquat Sci 63:576-596

531 Perrin N, Mazalov V (2000) Local Competition, inbreeding, and the evolution of sex-biased dispersal. Am Nat
532 155:116-127

533 Philander SFH (1983) El Niño Southern Oscillation phenomena. Nature 302:295-301

534 Pimm SL, Gittleman JL, MaCracken GF, Gilpin M (1989) Plausible alternatives to bottlenecks to explain reduced
535 genetic diversity. Trends Ecol Evol 4:176-178

536 Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the
537 effective population size using allele frequency data. J Hered 90:502-503

538 Pomeroy P, Twiss S, Redman P (2000). Philopatry, site fidelity and local kin associations within grey seal breeding
539 colonies. Ethology 106:899-919. doi:10.1046/j.1439–0310.2000.00610.x

540 Pritchard JK, Stephens M, Donnely PJ (2000) Inference of population structure using multilocus genotype data.
541 Genetics 155:945-959

542 Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223-225

543 Robalo JI, Doadrio I, Valente A, Almada VC (2007) Identification of ESUs in the critically endangered Portuguese
544 minnow *Chondrostoma lusitanicum* Collares-Pereira 1980, based on a phylogeographical analysis. Conserv
545 Genet 8:1225-1229

546 Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphims analyses by the
547 coalescent and other methods. Bioinformatics 19:2496-2497

548 Ryder OA (1986) Species Conservation and systematics: the dilemma of subspecies. Trends Ecol Evol 1:9-10

549 Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: A laboratory manual. Cold Spring Harbor
550 Laboratory, New York

551 Sandweiss DH, Richardson JB III, Reitz EJ, Rollins HB, Maasch KA (1996) Geoarchaeological evidence from
552 Peru for a 5000 years BC onset of *El Niño*. Science 273:1531-1533

553 Seal Conservation Society (2010) Galapagos Fur Seal. http://www.pinnipeds.org/species/galfursl.htm Accessed
554 30 September 2010

555 Shafer ABA, Gattepaille LM, Stewart REA, Wolf JBW (in revision) Demographic inferences using short-read
556 genomic data in an Approximate Bayesian Computation framework: in silico evaluation of power, biases,
557 and a proof of concept in Atlantic walrus

558 Shields WM (1982) Philopatry, inbreeding and the evolution of sex. State University of New York Press, New
559 York

560 Slade RW, Moritz C, Heidman A (1994) Multiple Nuclear-Gene Phylogenies: Application to Pinnipeds and
561 Comparsion with a Mitochondrial DNA Gene Phylogeny. Mol Biol Evol 11:341-356

562 Stanley H, Casey S, Carnahan J, Goodman S, Harwood J, Wayne RK (1996) Worldwide patterns of mitochondrial
563 DNA differentiation in the harbor seal (*Phoca vitulina*). Mol Biol Evol 13:368-382

564 Swofford DL (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer
565 Associates, Sunderland

566 Tajima F (1989) Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism. Genetics
567 123:585-595

568 Tajima F (1993) Simple methods for testing molecular clock hypothesis. Genetics 135:599-607

569 Thompson J, Gibson TJ, Plewniak F, Jeanmouguin F, Higgins DG (1997) The ClustalX windows interface:
570 flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res
571 24:4876-82

572 Trillmich F (1987) Galápagos Fur Seal: *Arctocephalus galapagoensis*. In: Croxal PL and Gentry RL (eds) Status,
573 Biology and Ecology of Fur Seals. Proceedings of an International Symposium and Workshop. Cambrige,
574 pp 23-27

575 Trillmich F (1990) The behavioral ecology of maternal effort in fur seals and sea lions. Behaviour 114: 1-20

576 Trillmich F and Dellinger T (1991) The effects of *El Niño* on Galápagos pinnipeds. In: Trillmich F, Ono KA (eds)
577 Pinnipeds and El Niño: Responses to environmental stress. Springer-Verlag, Berlin, pp 66-74

578 Trillmich F, Ono KA, Cost DP, DeLong RL, Francis JM, Gentry RL, Heath CB, LeBoeuf BJ, Majluf P, York AE.
579 1991. The effects of El Niño on pinniped populations in the eastern Pacific. In: Trillmich F (Ed) Pinnipeds
580 and El Niño.Springer, Berlin, pp. 247-270

581 Trillmich F, Kooyman GL (2001) Field metabolic rate of lactating female Galápagos fur seal (*Arctocephalus*
582 *galapagoensis*): the influence of offspring age and environment. Comp Biochem Phys 129:741-749

583 Trillmich F, Limberger D (1985) Drastic effects of *El Niño* on Galapagos pinnipeds. Oecologia 67:19-22

584 Trillmich F, Trillmich KGK (1984) The mating systems of pinnipeds and marine iguanas: convergent evolutionof
585 polygyny. Biol J Linn Soc 21:209-216

586 Trillmich F, Wolf JBW (2008) Parent-offspring and sibling conflict in the Galápagos fur seals and sea lions. Behav
587 Ecol Sociobiol 62:363-375

588 Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution
589 38:1358-1370

590 Wolf JBW, Tautz D, Caccone A, Steinfartz S (2006) Development of new microsatellite loci and evaluation of
591 loci from other pinnipeds species for the Galápagos Sea Lion (*Zallophus californianus wollebaeki*). Conserv
592 Genet 7:461-465

593 Wolf JBW and Trillmich F (2007) Beyond habitat requirements: individual fine-scale site fidelity in a colony of
594 the Galapagos sea lion (*Zalophus wollebaeki*) creates conditions for social structuring. Oecologia 152:553-
595 567

596 Wolf JBW, Tautz D, Trillmich F (2007) Galápagos and Californian sea lions are separate species: genetic analysis
597 of the genus *Zalophus* and its implications for conservation management. Front Zool 4:20

598 Wolf JBW, Harrod C, Brunner S, Salazar S, Trillmich F, Tautz D (2008) Tracing early stages of species
599 differentiation: Ecological, morphological and genetic divergence of Galápagos sea lion populations. BMC
600 Evol Biol 8:1-14

601 Wolf JBW, Trillmich F (2008) Kin in space. Social viscosity in a spatially and genetically sub-structured network.
602 Proc R Soc Lond B 275:2063-2069

603 Wynen LP, Goldsworthy SD, Insley SJ, Adams M, Bickham JW, Francis J, Gallo JP, Hoelzel AR, Majluf P, White
604 RWG, Slade R (2001) Phylogenetic relationships within the Eared Seals (Otariidae: Carnivora): Implications
605 for the historical biogeography of the family. Mol Phylogenet Evol 21:270-284

606 Wyrtki K (1982) The Southern Oscillation, ocean-atmosphere interaction and *El Niño*. Mar Technol Soc J 16:3-
607 10

608 Yonezawa T, Kohno N, Hasegawa M (2009) The monophyletic origin of sea lion and fur seals (Carnivora;
609 Otariidae) in the southern hemisphere. Gene 441:89-99

610

Figure legends

Fig. 1 a. Map of the study area (Galapagos Islands) and the location of the three major Galápagos fur seal colonies sampled for this study : BB: Banks Bay (Isabela Island), CH: Cape Hammond (Fernandina Island) and 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.

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

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 Galapagos fur seal 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.

FIGURES

Figure 1.

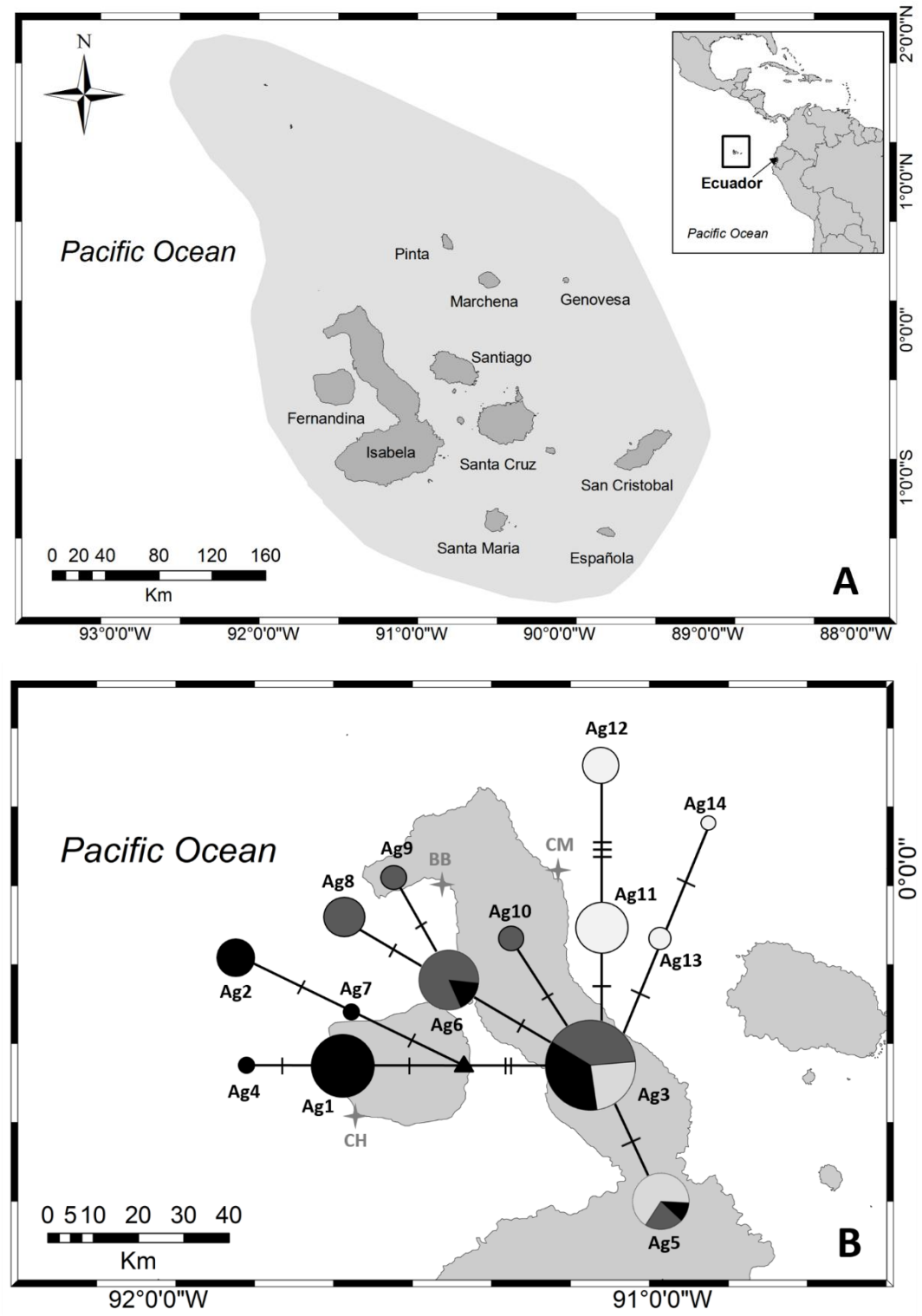


Figure 2.

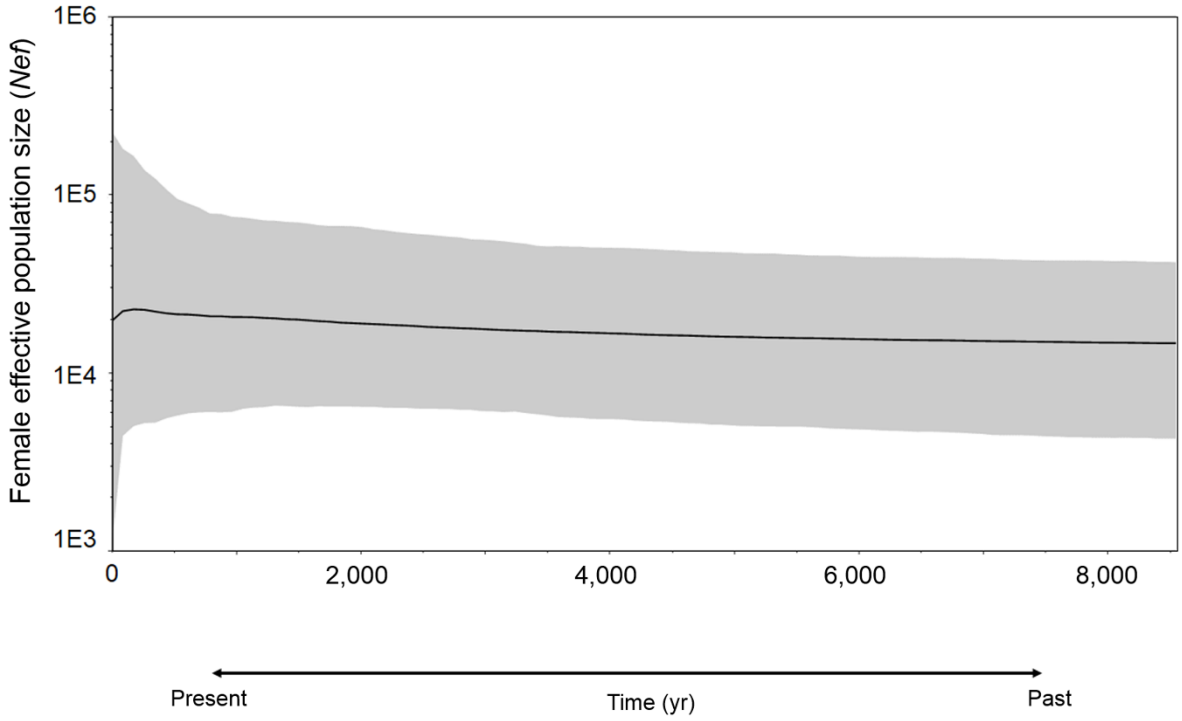
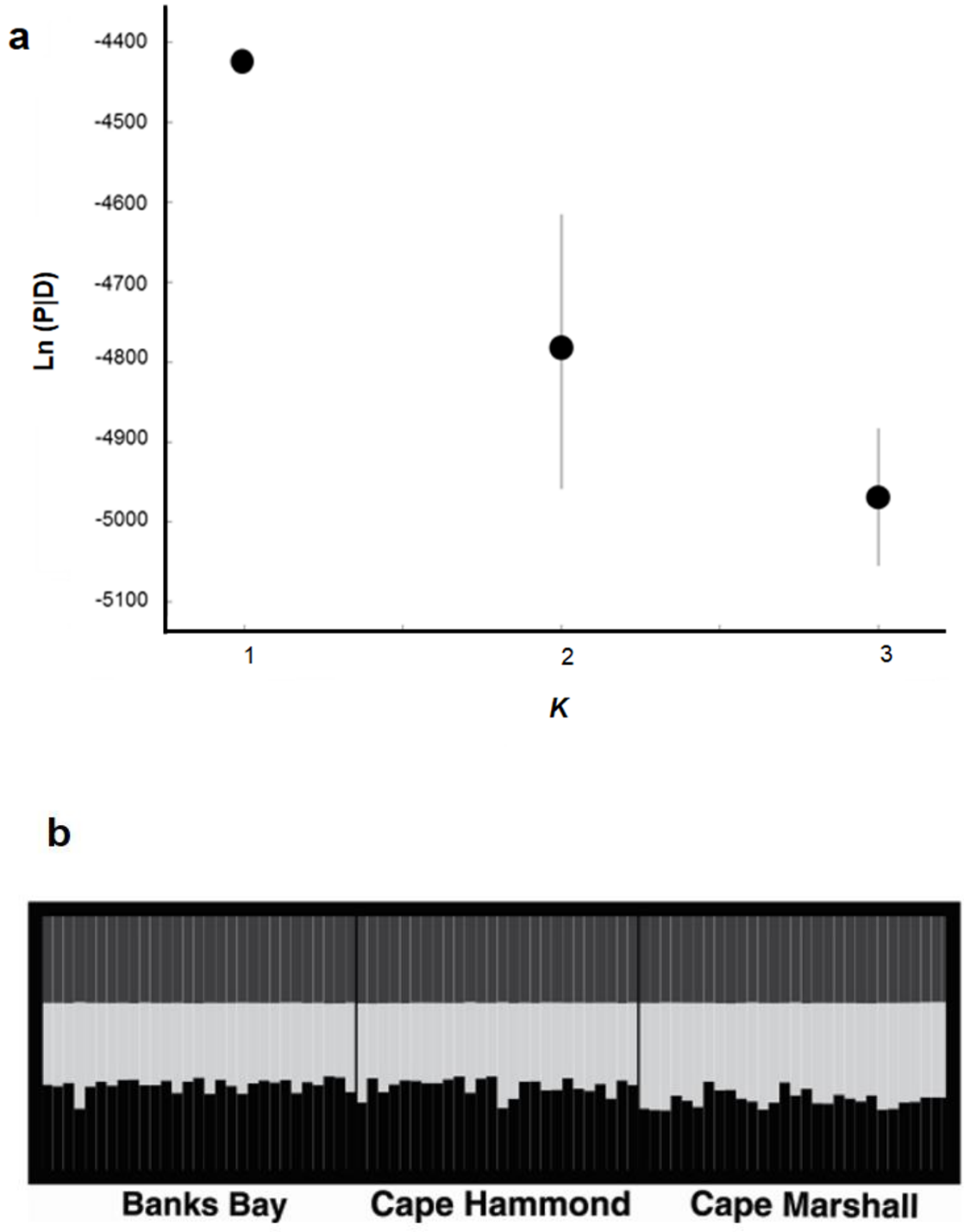

1E6
1E5
1E4
1E3
Female effective population size (Nef)
0
2,000
4,000
6,000
8,000
Present
Time (yr)
Past

Figure 3.


a
-4400
-4500
-4600
-4700
-4800
-4900
-5000
-5100
Ln (P|D)
1
2
3
K
b
Banks Bay
Cape Hammond
Cape Marshall

TABLES

Table 1. Genetic diversity of *Arctocephalus galapagoensis* based on control region mtDNA analysis. N = number of samples, S = segregating sites, H = number of haplotypes, Hd = haplotype diversity, HdSD = standard deviation of haplotype diversity, π = nucleotide diversity and πSD = standard deviation of nucleotide diversity.

	Control region mtDNA						
Population	**N**	**S**	**H**	**Hd**	**HdSD**	**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

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

Haplotype	Individuals	GenBank Accession Number	Frequency	Locality	Island
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. All values are significant at $P < 0.01$.

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

Table 4. Pairwise *F*-statistics among sampling localities for mtDNA control region and microsatellites.

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

* Significant values $P < 0.001$

Table 5. Pairwise neutrality tests.

	Tajima's D	Tajima's D (*p*-value)	Fu's Fs	Fu's Fs (*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

Table 6. Measures of genetic diversity at 18 microsatellite loci in the Galápagos fur seal populations studied.

		Banks Bay (n = 29)					Cape Hammond (n = 27)					Cape Marshall (n = 29)					Global population [a] (n = 84)				
Locus	**Allele range**	**A**	**AR**	**E**	**Ho**	**He**	**A**	**AR**	**E**	**Ho**	**He**	**A**	**AR**	**E**	**Ho**	**He**	**A**	**AR** [b]	**E** [c]	**Ho**	**He**
ZcwE05[2]	189-195	4	3.99	0	0.44	0.47	3	3.80	0	0.38	0.39	4	3.00	0	0.43 [d]	0.44	4	3.65	0	0.43	0.44
ZcwD02 [2]	198-250	12	11.60	1	0.93	0.91	13	12.60	2	0.88	0.89	13	12.60	2	0.89	0.89	17	12.99	1.66	0.91	0.90
ZcwB09 [2]	191-207	6	5.90	1	0.83	0.77	5	4.00	0	0.77	0.80	4	5.00	0	0.79	0.66	6	5.71	0.33	0.80	0.77
ZcCgDh5[2]	319-349	8	7.80	1	0.76	0.73	7	6.50	1	0.69	0.78	7	6.90	0	0.75	0.67	9	7.26	0.66	0.73	0.73
Hg8.1[1]	178-186	4	3.80	0	0.41	0.40	4	4.90	0	0.46	0.50	5	4.00	1	0.52	0.61	5	4.57	0.33	0.48	0.54
ZcCgDh7t[2]	282-290	5	4.80	1	0.38	0.39	3	3.90	0	0.54	0.50	4	3.00	0	0.45	0.39	5	4.15	0.33	0.48	0.44
Hg6.1[2]	140-158	4	3.90	0	0.38	0.39	5	2.90	0	0.56	0.48	3	4.90	0	0.24	0.25	5	4.70	0	0.38	0.37
ZcwF07[2]	146-162	6	5.60	1	0.52	0.46	4	3.90	0	0.48	0.48	4	4.00	0	0.72	0.61	6	4.84	0.33	0.60	0.54
ZcwE03[2]	217-231	8	7.90	1	0.83	0.86	7	6.50	0	0.84	0.85	7	7.00	0	0.86	0.79	8	7.39	0.33	0.85	0.84
ZcwE12[2]	173-187	4	4.00	0	0.82[d]	0.73	7	5.90	1	0.81	0.82	6	6.90	0	0.66	0.77	7	6.10	0.33	0.78	0.78
ZcwE04[2]	120-144	8	7.90	0	0.97	0.87	9	7.80	0	0.88	0.85	8	8.90	0	0.96	0.85	11	8.62	0	0.94	0.86
ZcwB07[1]	182-198	7	6.80	0	0.82	0.73	8	6.80	0	0.84	0.82	7	7.90	1	0.93	0.81	9	7.89	0.33	0.86	0.80
Pv9[1]	172-182	6	5.80	0	0.79	0.68	6	5.00	0	0.69	0.73	5	5.90	0	0.68	0.68	6	5.87	0	0.72	0.70
Hg6.3[1]	225-239	5	4.80	1	0.48	0.49	4	3.80	0	0.58	0.54	4	4.00	1	0.54	0.61	6	4.46	0.66	0.53	0.55
PvcE[1]	120-136	6	5.80	0	0.66	0.72	5	4.60	0	0.73	0.69	5	4.90	0	0.62	0.62	7	5.30	0	0.67	0.68
Hg1.3[1]	230-260	7	6.90	0	0.85	0.83	10	8.50	1	0.96	0.87	9	9.90	0	0.89	0.80	10	9.14	0.33	0.90	0.86
PvcA[1]	151-163	7	6.80	1	0.68	0.79	6	4.90	0	0.76	0.80	5	6.00	0	0.67	0.64	7	6.22	0.33	0.70	0.76
Agaz2[1]	230-240	4	3.80	0	0.68	0.64	6	5.80	0	0.52	0.69	6	5.90	0	0.86	0.74	6	5.31	0	0.66	0.69
	Mean	6.17	5.99	0.44	0.68	0.66	6.18	5.67	0.28	0.69	0.69	5.69	6.15	0.28	0.69	0.66	7.44	6.34	0.33	0.69	0.68

Number of alleles (A), allelic richness (AR), number of exclusive alleles (E), observed (Ho) and expected heterozygozity (He); [1,2] Pooled markers in multiplex PCR; [a] Samples for all populations pooled; [b] All richness based on a sample size of 84 diploid individuals; [c] Arithmetic mean of exclusive alleles; [d] loci that deviated from H-W equilibrium after Bonferroni correction

Table 7. Microsatellite genetic diversity in pinnipeds, based on expected heterozygosity (He) (or observed heterozygosity when only this information was available) and average number of alleles per locus (A).

Species	N° individuals	N° Loci	He	A
Galapagos fur seal (present paper)	84	17	0.68	7.44
Galapagos sea lion[1,13]	20	10/367	0.72/0.62	7.9/5.2
South American fur seal[2]	226	8	0.77	8.4
New Zealand fur seal[3]	383	11	0.75	11.8
Antarctic fur seal[4]	2106	9	0.8	12.4
Australian fur seal[5]	183	5	0.58	8.0
Subantarctic fur seal[6]	76	8	0.60	11.1
Northern fur seal[7]	462	8	0.80	17
Australian sea lion[8]	217	5	0.54	4.5
New Zealand sea lion[9]	40	22	0.72	5.9
California sea lion[10,13]	58	12/16	0.61/0.72	6.8/6.4
Steller sea lion[11]	668	13	0.66	7.9
Grey seal[12]	1883	9	0.74	-

[1] Hoffman et al. (2007)
[2] Oliveira et al. (2008)
[3] B. Robertson, A. Kalinin, H. Best, N. Gemmell (in Robertson and Chilvers 2011).
[4] Hoffman and Amos (2005).
[5] Lancaster et al. (2010) (Ho only).
[6] Wynen L, Goldsworthy S, White R, Slade R (in Robertson and Chilvers 2011) (Ho only).
[7] Dickerson et al. (2010).
[8] Campbell R (in Robertson and Chilvers 2011).
[9] Acevedo-Whitehouse et al. (2009) (Ho only)
[10] Hernandez-Velazquez et al. (2005)
[11] Hoffman et al. (2006)
[12] Worthington-Wilmer et al. (1999)
[13] Wolf et al. (2007)

Supplementary material

Galapagos Fur Seal microsatellite data

Tissue sample	*Location*	ZcwE05		ZcwD02		ZcwB09		ZcCgDh58		Hg8.1		ZcCgDh7t		Hg6.1		ZcwF07		ZcwE03		ZcwE12		ZcwE04		Pv9		Hg 6.3		PvcE		Hg 1.3		PvcA		Zcwb07		Agaz 2	
		a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
A01_IR1.fsa	Isabella (Banks Bay)	191	195	214	228	197	201	325	325	178	184	286	286	140	140	150	150	221	223	175	183	136	138	x	x	227	239	130	134	250	252	159	159	186	188	230	234
A02_IR2.fsa	Isabella (Banks Bay)	189	191	226	230	197	203	321	325	178	178	286	286	140	140	148	152	225	229	175	183	126	130	176	178	239	239	130	134	240	258	155	163	184	188	230	234
A03_IR3.fsa	Isabella (Banks Bay)	189	195	216	216	199	201	325	327	178	178	286	290	140	155	146	150	223	223	175	183	134	136	174	180	227	227	132	134	230	240	159	161	188	194	230	234
A04_IR4.fsa	Isabella (Banks Bay)	191	191	220	228	197	203	325	327	178	178	284	286	140	140	150	150	225	227	175	183	130	130	174	174	227	227	132	134	x	x	161	161	188	196	x	x
A05_IR5.fsa	Isabella (Banks Bay)	195	195	220	226	197	199	325	349	178	178	286	286	140	155	146	150	217	227	175	177	130	138	174	182	227	239	132	134	250	250	151	161	188	194	230	230
A07_IR7.fsa	Isabella (Banks Bay)	191	191	222	228	197	207	321	325	178	178	286	286	140	140	150	154	219	227	175	181	132	138	176	182	227	239	134	134	252	258	159	159	188	196	230	234
A08_IR8.fsa	Isabella (Banks Bay)	191	191	212	228	197	197	325	329	178	178	284	286	140	151	150	150	221	229	175	175	126	132	176	180	227	239	134	134	240	258	151	163	184	194	232	234
A09_IR9.fsa	Isabella (Banks Bay)	191	191	228	228	203	203	319	321	178	184	286	286	155	157	150	152	221	229	177	183	130	138	174	174	237	237	126	126	240	258	155	155	188	188	230	236
A10_IR10.fsa	Isabella (Banks Bay)	x	x	224	230	197	203	323	325	178	178	286	286	140	140	150	152	223	231	175	177	130	134	174	180	227	239	120	132	230	258	155	159	188	188	230	234
A11_IR11.fsa	Isabella (Banks Bay)	x	x	222	230	203	203	319	325	178	184	286	288	140	140	150	154	225	229	175	181	132	138	174	180	227	227	132	134	250	256	155	161	186	188	230	236
A12_IR12.fsa	Isabella (Banks Bay)	191	191	226	228	197	201	325	329	178	180	286	290	140	140	150	150	221	225	177	181	128	138	174	178	227	237	130	130	250	258	159	159	194	194	230	230
B01_IR13.fsa	Isabella (Banks Bay)	191	191	226	230	207	207	327	327	178	178	284	284	140	140	150	150	217	221	177	177	130	132	174	180	227	227	134	134	240	250	153	157	188	194	230	230
B02_IR14.fsa	Isabella (Banks Bay)	191	195	222	226	199	201	321	325	178	180	286	286	140	140	150	150	221	223	175	183	128	130	172	174	227	227	130	134	x	x	x	x	x	x	x	x
B03_IR15.fsa	Isabella (Banks Bay)	189	191	220	226	203	207	323	325	178	178	286	286	140	140	150	152	223	225	177	181	138	140	174	176	227	227	130	132	256	258	161	161	186	188	230	236
B04_IR16.fsa	Isabella (Banks Bay)	191	191	214	230	197	199	325	349	178	180	282	286	140	155	150	150	221	225	175	181	138	140	174	176	227	239	126	126	258	258	155	159	188	188	234	236
B05_IR17.fsa	Isabella (Banks Bay)	191	191	212	216	199	203	325	325	178	180	286	286	140	157	150	152	227	229	175	177	126	140	174	174	227	227	130	134	240	256	157	161	186	194	230	234
B06_IR18.fsa	Isabella (Banks Bay)	191	195	222	228	197	197	327	349	178	182	286	286	140	140	150	162	221	221	175	177	128	136	174	174	227	227	130	136	240	252	155	161	188	194	230	236
B07_IR19.fsa	Isabella (Banks Bay)	191	191	226	242	197	201	325	325	178	178	286	288	140	140	150	150	225	227	177	177	126	128	174	174	227	227	126	134	240	240	151	161	188	194	230	236
B08_IR20.fsa	Isabella (Banks Bay)	193	195	212	214	199	203	325	325	178	180	286	286	140	155	146	150	229	229	175	181	130	140	174	174	227	239	134	134	254	254	151	161	186	188	234	236
B09_IR21.fsa	Isabella (Banks Bay)	191	195	216	224	197	201	321	325	178	178	286	286	140	140	150	150	219	223	175	181	130	140	176	180	227	227	132	134	252	258	155	161	188	188	230	234
B10_IR22.fsa	Isabella (Banks Bay)	191	193	214	234	197	201	325	327	178	180	286	288	140	151	150	150	217	223	175	183	126	140	174	180	227	227	126	132	250	256	157	157	188	196	230	234
B11_IR23.fsa	Isabella (Banks Bay)	191	195	212	234	191	203	321	325	180	180	286	286	140	151	150	150	221	229	175	177	132	136	174	180	227	239	134	134	240	252	151	159	194	198	234	234
B12_IR24.fsa	Isabella (Banks Bay)	191	191	214	222	191	203	321	327	178	178	286	286	140	140	150	154	217	227	177	177	130	132	174	176	225	227	134	134	240	258	155	161	188	194	234	236
C01_IR25.fsa	Isabella (Banks Bay)	191	191	220	228	197	203	323	325	178	180	286	286	140	151	150	150	221	221	175	183	130	132	174	176	227	227	134	134	230	240	161	161	190	194	230	230
C02_IR26.fsa	Isabella (Banks Bay)	191	191	214	224	199	203	329	329	178	178	286	290	140	140	150	152	225	231	175	181	132	138	176	178	227	237	132	134	240	250	151	159	188	196	230	234
C03_IR27.fsa	Isabella (Banks Bay)	191	191	222	224	197	203	323	325	178	178	286	286	140	140	150	150	217	221	181	183	126	130	176	178	227	227	130	132	240	254	157	161	188	188	234	234
C04_IR28.fsa	Isabella (Banks Bay)	191	195	212	224	197	199	323	341	178	178	286	288	140	140	150	152	217	223	177	177	128	140	174	176	227	229	134	136	256	258	159	161	194	198	234	234
C05_IR29.fsa	Isabella (Banks Bay)	191	191	218	228	197	203	325	325	178	178	286	288	151	155	150	152	223	223	175	181	134	136	174	176	227	229	134	136	256	258	159	161	194	198	234	234
C06_IR30.fsa	Isabella (Banks Bay)	189	191	216	220	197	201	323	325	178	180	286	286	140	140	150	150	217	227	175	181	128	130	174	176	227	239	126	132	254	258	161	161	186	188	230	234
C07_IM1.fsa	Isabella (Cabo Marschall)	191	191	214	228	197	199	325	325	178	180	286	290	140	140	150	152	221	229	175	175	126	134	174	178	239	239	130	134	250	254	151	161	190	196	230	234
C08_IM2.fsa	Isabella (Cabo Marschall)	191	195	216	216	197	199	325	327	178	178	286	286	142	157	150	152	221	227	177	181	126	130	174	178	227	227	130	132	238	254	161	161	196	196	234	240
C09_IM3.fsa	Isabella (Cabo Marschall)	191	195	212	216	197	199	325	327	178	178	286	286	140	140	150	150	225	227	181	181	130	132	174	174	227	239	132	134	250	256	161	161	190	196	230	240
C10_IM4.fsa	Isabella (Cabo Marschall)	191	191	212	226	197	203	321	327	178	182	286	286	140	140	150	150	225	229	177	183	128	134	174	174	227	227	132	134	250	254	151	163	188	192	234	236
C11_IM5.fsa	Isabella (Cabo Marschall)	191	191	210	216	197	203	325	327	180	180	286	288	140	140	150	154	225	227	175	177	126	140	174	174	227	237	132	132	250	254	161	161	188	192	234	238
C12_IM6.fsa	Isabella (Cabo Marschall)	191	191	212	212	197	197	325	327	178	180	286	286	140	140	152	154	221	225	175	183	130	134	174	178	239	239	120	132	240	254	151	155	194	196	230	234
D01_IM7.fsa	Isabella (Cabo Marschall)	191	193	228	230	197	199	325	325	180	182	286	288	140	140	150	152	223	225	183	188	128	142	174	174	237	239	132	136	254	254	155	161	188	190	234	236
D02_IM8.fsa	Isabella (Cabo Marschall)	189	189	212	222	197	199	325	327	178	178	286	286	140	140	150	150	221	229	175	188	130	140	178	180	239	239	134	134	254	260	151	161	182	188	232	238
D03_IM9.fsa	Isabella (Cabo Marschall)	191	191	210	214	199	203	323	349	180	186	286	286	140	140	150	152	225	229	175	177	130	140	172	178	227	239	132	134	252	252	155	161	188	196	234	234
D04_IM10.fsa	Isabella (Cabo Marschall)	189	191	222	240	197	197	327	327	178	178	286	290	140	140	150	152	221	227	175	181	130	134	174	180	227	239	134	134	252	254	151	159	194	196	230	230
D05_IM11.fsa	Isabella (Cabo Marschall)	191	191	216	228	197	203	325	327	178	186	286	286	140	140	152	154	225	227	175	175	130	132	174	176	227	227	134	134	250	258	151	161	188	190	230	236
D06_IM12.fsa	Isabella (Cabo Marschall)	191	191	212	220	197	199	325	325	178	180	286	286	140	140	148	150	225	229	175	177	132	134	178	180	239	239	134	134	252	254	151	161	192	196	230	236
D07_IM13.fsa	Isabella (Cabo Marschall)	191	195	212	220	199	199	319	325	178	180	286	286	140	142	150	154	217	229	175	177	126	136	178	180	227	239	134	134	250	252	161	161	188	190	230	230
D08_IM14.fsa	Isabella (Cabo Marschall)	191	191	218	228	199	203	325	327	178	182	286	288	140	140	150	150	223	225	177	181	128	130	174	174	237	239	132	134	230	258	161	161	188	192	230	234
D09_IM15.fsa	Isabella (Cabo Marschall)	191	191	214	224	197	201	321	321	178	178	286	288	140	140	150	152	227	229	175	183	126	128	174	176	227	239	132	134	250	254	161	161	188	196	230	236
D10_IM16.fsa	Isabella (Cabo Marschall)	189	195	226	230	197	197	325	327	178	178	284	286	140	157	150	150	225	225	181	181	128	140	174	174	233	239	132	134	252	258	161	163	190	192	236	240
D12_IM18.fsa	Isabella (Cabo Marschall)	x	x	212	212	197	201	325	327	178	180	286	286	140	140	150	150	221	227	181	188	128	130	174	174	239	239	130	132	254	258	159	163	188	196	230	234
E01_IM19.fsa	Isabella (Cabo Marschall)	191	195	216	220	201	203	325	327	180	180	286	286	140	140	152	154	223	227	175	175	126	140	178	180	227	237	134	134	250	260	159	159	188	194	230	236
E02_IM20.fsa	Isabella (Cabo Marschall)	191	191	212	216	197	201	325	325	178	178	286	286	140	140	150	152	225	231	175	185	130	134	176	176	227	239	132	132	238	254	151	161	194	194	230	236
E03_IM21.fsa	Isabella (Cabo Marschall)	189	191	226	228	197	201	319	327	180	184	284	286	140	157	150	154	227	229	175	175	126	126	178	180	227	237	132	134	x	x	151	161	190	192	230	238
E04_IM22.fsa	Isabella (Cabo Marschall)	191	195	198	222	197	201	325	327	178	186	284	286	140	140	150	154	221	227	177	185	132	142	172	180	227	239	130	130	250	254	161	161	194	196	230	236
E05_IM23.fsa	Isabella (Cabo Marschall)	189	191	212	220	197	197	321	327	180	180	286	286	140	140	148	152	221	229	175	175	126	132	172	174	227	227	132	134	238	250	155	161	192	196	236	240
E06_IM24.fsa	Isabella (Cabo Marschall)	191	191	x	x	x	x	x	x	180	184	286	286	140	142	150	150	225	225	175	175	126	130	172	174	227	239	130	132	250	254	155	161	188	196	234	240
E07_IM25.fsa	Isabella (Cabo Marschall)	191	191	212	230	197	201	325	325	178	180	286	286	140	157	150	152	229	229	177	188	130	140	174	178	227	227	134	134	252	254	161	161	188	194	230	234
E08_IM26.fsa	Isabella (Cabo Marschall)	191	191	212	218	197	203	325	327	178	180	286	286	140	140	150	152	229	229	177	183	126	140	174	178	227	227	134	134	252	252	151	161	188	192	230	234
E09_IM27.fsa	Isabella (Cabo Marschall)	191	191	198	214	197	197	321	325	180	180	286	288	140	140	150	154	225	229	175	183	x	x	174	174	227	237	132	134	250	254	x	x	188	194	230	236
E10_IM28.fsa	Isabella (Cabo Marschall)	189	191	226	228	197	203	319	325	178	178	284	286	140	140	150	154	225	227	183	183	130	132	174	178	227	227	132	134	250	252	155	161	186	188	230	234
E11_IM29.fsa	Isabella (Cabo Marschall)	189	191	226	228	197	203	319	325	178	178	284	286	140	140	150	154	225	227	183	183	130	132	174	178	227	227	132	134	250	252	155	161	186	188	230	234
F02_FA1.fsa	Fernandina	189	195	212	220	197	201	325	327	178	178	284	286	140	151	150	152	227	227	177	185	130	132	172	172	227	227	134	134	238	250	151	163	194	194	230	230
F03_FA2.fsa	Fernandina	191	191	226	242	199	199	329	329	178	178	286	288	140	151	150	152	223	225	175	185	136	138	176	178	227	239	126	134	238	256	155	159	188	192	230	230
F04_FA3.fsa	Fernandina	191	195	212	216	199	203	325	327	178	178	286	286	140	155	150	150	223	227	175	177	130	136	178	178	227	239	126	134	240	240	151	161	188	196	230	230
F05_FA4.fsa	Fernandina	191	191	212	222	199	203	321	329	178	180	286	286	140	140	148	150	221	231	177	181	130	134	x	x	x	x	x	x	x	x	x	x	x	x	x	x
F06_FA5.fsa	Fernandina	189	191	212	222	199	203	325	325	178	178	286	286	140	140	150	152	223	227	175	181	130	138	174	174	x	x	126	132	230	252	151	159	186	186	230	234
F07_FA6.fsa	Fernandina	191	195	226	226	203	203	321	329	182	182	286	288	140	140	150	152	223	229	175	177	126	126	174	182	x	x	126	126	x	x	x	x	x	x	x	230
F08_FA7.fsa	Fernandina	189	191	212	250	203	203	325	327	178	180	284	286	140	140	146	150	223	227	175	181	134	138	174	174	227	237	126	130	250	252	151	161	188	198	230	232
F09_FA8.fsa	Fernandina	189	191	212	222	191	199	325	329	178	178	286	286	140	140	150	150	221	225	183	188	132	140	176	178	227	227	132	134	230	240	151	155	186	188	234	236
F10_FA9.fsa	Fernandina	191	191	220	230	191	203	323	329	178	184	284	286	140	151	150	150	229	229	177	183	130	134	176	182	227	227	134	134	252	254	151	155	188	194	x	x
F11_FA10.fsa	Fernandina	191	191	210	214	201	203	323	327	178	182	284	288	140	140	150	150	225	225	175	177	136	142	174	182	227	227	126	132	252	254	157	161	186	194	230	236
G01_FA12.fsa	Fernandina	191	191	212	222	197	201	321	325	178	182	288	288	140	155	150	152	221	231	173	175	134	138	174	176	237	239	126	134	242	250	159	159	186	192	234	240
G02_FA13.fsa	Fernandina	191	191	212	216	201	203	325	325	178	178	286	288	140	151	150	152	225	229	183	183	130	132	174	182	227	227	132	136	250	254	161	161	184	190	230	236
G03_FA14.fsa	Fernandina	191	191	216	218	191	199	327	329	178	178	286	288	140	151	150	150	217	227	183	185	128	138	174	176	227	227	132	134	252	260	151	161	184	194	230	234
G04_FA15.fsa	Fernandina	189	191	214	246	191	199	319	325	178	182	286	286	140	151	146	152	217	225	177	185	130	140	178	182	227	239	126	134	238	260	151	161	186	194	236	236
G05_FA16.fsa	Fernandina	191	191	212	226	201	201	319	325	178	180	286	286	140	140	150	150	221	225	183	183	132	134	174	178	227	227	134	134	238	250	151	151	188	196	230	234
G06_FA17.fsa	Fernandina	191	195	212	212	197	201	323	325	178	184	284	286	140	140	146	150	217	229	181	183	130	132	174	180	227	239	132	134	238	250	151	155	186	190	230	240
G07_FA18.fsa	Fernandina	191	191	228	230	199	201	319	329	178	182	286	286	x	x	x	x	x	x	181	183	132	134	178	182	227	227	126	134	240	252	161	161	194	194	234	234
G08_FA19.fsa	Fernandina	191	191	210	242	197	201	321	321	178	178	286	286	140	155	146	150	225	231	175	175	130	130	174	176	227	229	132	134	238	240	155	161	188	194	234	234
G09_FA20.fsa	Fernandina	191	191	214	222	201	203	325	325	178	182	286	286	151	155	150	150	225	227	175	188	126	132	174	174	227	239	130	130	252	256	161	161	188	196	230	236
G10_FA21.fsa	Fernandina	191	191	212	216	191	197	325	325	178	178	286	288	140	142	150	150	225	231	175	181	130	138	174	174	227	239	132	134	250	254	155	157	188	194	234	234
G11_FA22.fsa	Fernandina	191	191	212	230	191	191	323	331	178	178	284	286	140	157	150	150	221	229	183	183	136	136	174	176	227	239	132	134	240	250	155	157	184	190	234	234
H02_FA25.fsa	Fernandina	189	195	210	216	191	201	325	329	178	180	286	286	140	140	154	154	225	231	175	188	130	132	174	178	227	227	126	134	230	254	151	151	188	188	230	236
H03_FA26.fsa	Fernandina	191	191	214	216	199	203	321	327	184	184	284	286	140	140	150	152	221	225	181	183	130	134	174	178	227	229	134	134	238	254	151	159	186	194	236	236
H04_FA27.fsa	Fernandina	191	191	214	216	197	203	325	327	178	180	286	286	140	155	150	150	217	223	175	183	130	134	174	180	237	239	134	134	242	252	157	161	188	194	230	238
H05_FA28.fsa	Fernandina	191	191	216	216	201	201	327	327	178	178	286	286	140	151	150	150	221	221	181	181	130	136	174	174	227	229	132	134	252	254	159	161	194	196	230	230
H06_FA29.fsa	Fernandina	191	195	216	242	197	203	319	325	178	178	286	288	140	140	150	150	223	227	181	183	128	136	178	178	229	239	126	132	252	258	157	163	190	194	230	236
H07_FA30.fsa	Fernandina	191	191	214	226	199	201	325	325	178	180	286	288	140	140	150	154	225	229	177	185	132	138	174	182	227	227	126	134	250	252	161	163	184	188	230	230