RESEARCH ARTICLE

Genetic diversity and demographic history of the leopard seal: A Southern Ocean top predator

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Abstract

Leopard seals (*Hydrurga leptonyx*) are top predators that can exert substantial top-down control of their Antarctic prey species. However, population trends and genetic diversity of leopard seals remain understudied, limiting our understanding of their ecological role. We investigated the genetic diversity, effective population size and demographic history of leopard seals to provide fundamental data that contextualizes their predatory influence on Antarctic ecosystems. Ninety leopard seals were sampled from the northern Antarctic Peninsula during the austral summers of 2008–2019 and a 405bp segment of the mitochondrial control region was sequenced for each individual. We uncovered moderate levels of nucleotide ($\pi = 0.013$) and haplotype (Hd = 0.96) diversity, and the effective population size was estimated at around 24,000 individuals (NE = 24,376; 95% CI: 16,876–33,126). Consistent with findings from other ice-breeding pinnipeds, Bayesian skyline analysis also revealed evidence for population expansion during the last glacial maximum, suggesting that historical population growth may have been boosted by an increase in the abundance of sea ice. Although leopard seals can be found in warmer, sub-Antarctic locations, the species' core habitat is centered on the Antarctic, making it inherently vulnerable to the loss of sea ice habitat due to climate change. Therefore, detailed assessments of past and present leopard seal population trends are needed to inform policies for Antarctic ecosystems.

Introduction

Marine mammals are increasingly being impacted by human-induced climate change and many polar species may not be able to respond at the rate required for their long-term survival [1, 2]. However, species-specific responses to changing environments are far from uniform, even within the same ecosystem (e.g., Arctic [3]). Surprisingly, population trends remain

unknown for several conspicuous polar species, including top predators such as leopard seals, *Hydrurga leptonyx* [4]. Direct observations of this species in traditional surveys across the Antarctic are hindered by its broad and remote geographical distribution, low population density, and the adverse weather conditions that are commonly encountered in their main breeding habitats [5, 6].Therefore, indirect assessments, such as population genetic analyses, are invaluable for providing fundamental insights into the population dynamics and natural history of such species [7].

Contemporary neutral genetic variation retains valuable information that allows the inference of population sizes of past generations [8]. Specifically, the effective population size (N_E) scales with the magnitude of genetic drift in an ideal population at equilibrium [9]. Most importantly, fluctuations in this parameter can be used to detect changes in species abundance over time, which can reveal past responses to environmentally and anthropogenically induced changes in habitat availability. For several species of pinnipeds, population genetic studies have uncovered past demographic impacts of large-scale climate change events driven by the El Niño Southern Oscillation [10] and the potential effects of post-glacial ice retreat on population expansion, recolonization and population structure [11–13]. These studies are fundamental for predicting how species will respond to future climate perturbations and for identifying taxonomic units that deserve prioritized conservation status.

Explorations of large genetic datasets across pinniped lineages also highlight biological traits that influence genetic variation over time. For example, breeding habitat preference (land versus ice) is an important determinant of genetic variation in pinnipeds [14, 15], as substantial losses of genetic variation due to commercial sealing have been detected in gregarious pinniped species that breed on land [14]. Although this cumulative work represents a notable effort in terms of gathering samples and data for multiple pinniped species, some species have not yet been investigated. This reflects the difficulty of gathering samples from species breeding in remote, fluid and vast habitats such as the Antarctic pack ice.

The Lobodontini tribe (Antarctic ice seals) includes four species: the Ross seal (*Ommatophoca rossii*), the crabeater seal (*Lobodon carcinophagus*), the Weddell seal (*Leptonychotes weddelli*), and the leopard seal (*Hydrurga leptonyx*). Lobodontini underwent rapid radiation, possibly concurrent with their arrival in Antarctic waters [16], *c.* 5.49 MYA [17]. Morphological studies initially supported grouping Ross and Weddell seals, and crabeater and leopard seals, respectively as sister clades [18, 19] but a suite of molecular phylogenetic studies later found unequivocal support for a Weddell and leopard seal clade [16, 17, 20–23]. The mean divergence time between Weddell and leopard seals was estimated at 2.89 MYA (95% HPD range: 3.97–1.84 MYA) but the basal position within the group remains largely unresolved [17, 20].

The Weddell seal is one of the best-studied pinniped species [24] and is, therefore, typically included in foundational Antarctic habitat models (e.g., [25]). Conversely, Ross and leopard seals remain among the least studied pinnipeds, meaning that their impacts on Antarctic ecosystems are difficult to quantify [26]. Leopard seals have a broad circumpolar Antarctic and sub-Antarctic distribution, with individuals found very close to the Antarctic continent (e.g., Prydz Bay; [27]) to as far north as Sydney, Australia [28]. They are particularly challenging to study due to their solitary nature, pelagic lifestyle and scattered distribution [6]. Perhaps the best studied aspect of leopard seal biology is their diet, which generally consists of a variety of prey including krill, fish, penguins and other seal species [29]. Leopard seal diets vary geographically, being dominated by krill in some locations (Danco Coast; [30]) and focusing on the opportunistic consumption of endothermic prey in other regions (e.g., Adélie penguins, *Pygoscelis adeliae*, in Ross Island; [31]). Leopard seal diet composition also varies seasonally, with large females switching from smaller prey items to consume large numbers (21.3–37.6%) of Antarctic fur seal pups (*Arctocephalus gazella*) during the austral summers in the northern

Antarctic peninsula [29]. Due to their capacity for consuming endothermic prey and opportunistic / specialized feeding habits, leopard seals have a high potential to exert top-down pressure on coastal Antarctic ecosystems [32–34] with significant local consequences for the viability of prey populations [35]. For example, leopard seals have consumed an estimated 69.3% of all Antarctic fur seal pups born annually at Cape Shirreff in the north Antarctic Peninsula since 2010, contributing to the rapid collapse of this population [36].

Several visual surveys to estimate the abundance of leopard seals have been attempted [37–41], but most have yielded estimates with a high degree of uncertainty (Table 1). For example, the most recent global survey of leopard seals produced an estimate with a very large confidence interval (N = 35,500, 95% CI: 10,900–102,600 individuals) because of the difficulty of counting this elusive predator, which spends a significant portion of time underwater and rarely aggregates on ice [41]. Therefore, genetic approaches have great potential to aid in our basic understanding of leopard seal population dynamics. To date, published genetic analyses of leopard seals are limited [42, 43] and descriptions of key demographic parameters are lacking.

Descriptions of leopard seal demography are essential to understanding the dynamics of the Antarctic ecosystem. Importantly, in addition to exerting top-down pressure on their prey, leopard seals are also vulnerable to the indirect effects of fisheries, which reduce the availability of lower trophic level prey (e.g., krill, fishes) [32]. Therefore, knowledge of historical and current global leopard seal population abundance trends are critical for robust ecosystem-based models (e.g., [44]), which are key to ongoing conservation efforts in the Antarctic [45, 46]. Here, we utilize mitochondrial DNA (mtDNA) sequence data from 90 leopard seals to characterize neutral genetic diversity, estimate the effective population size, and reconstruct the recent demographic history of this species. These data not only provide fundamental knowledge relevant to understanding leopard seal biology, but also contribute important data for inclusion in expanded Antarctic ecosystem models that consider the potential for leopard seals to alter ecological processes and / or be threatened by ecological destabilization.

Materials and methods

Sampling

We sampled leopard seals at Cape Shirreff (62˚27'30"S; 60˚47'17"W), on the north coast of Livingston Island, South Shetland Islands (Fig 1). The Cape encompasses several seasonally, ice-free gravel and pebble beaches bordered by a permanent ice cap on its southern edge. We sampled a total of 90 leopard seals during identification-tagging efforts and captures during the austral summers (December to March) of 2008–09 through 2018–19. We obtained 2–4 mm^3 of skin from the hind flippers utilizing manual hole-punch pliers, or sterile biopsy punches. All tissue samples were preserved in 95% ethanol and stored at −20˚C. Sample collection efforts were undertaken by the U.S. Antarctic Marine Living Resources Program and information about sampled seals (e.g., Tag ID) is provided in the S1 File. This study adhered to the guidelines granted by the Office of Protected Resources, National Marine Fisheries Service, United States under the Marine Mammal Protection Permit Nos. 774–1874, 16472, and 20599.

DNA extraction and amplification

We extracted total genomic DNA using a commercial kit (DNeasy Tissue Kit, QIAGEN) according to the manufacturer's protocol and amplified a 465-base pair (bp) fragment of the mtDNA sequence of the control region (D-loop) using pinniped-conserved primers TDKD for forward (5′-CCTGAAGTAGGAACCAGATG- 3′) [48] and L15926 for reverse (5′-TCAAAGCTTACACCAGTCTTGTAAACC-3′) [49] annealing. We performed PCR

Table 1. Population sizes estimated for leopard seals, _Hydrurga leptonyx_, in Antarctica based on ship and aerial surveys. All estimates are rounded to the nearest 100.

Source	Spatial coverage	Study year(s)	Abundance
Eklund and Atwood (1962)	Ross Sea 68˚–70˚S; 166˚–177˚E Indian Ocean 64˚–65˚S; 105˚–112˚E	1956–1957	152,500
Erickson and Hanson (1990)	Most of the circumpolar pack-ice	1968/69–1982/83	296,500
Ainley (1985)	Ross Sea 62˚–78˚S; 106˚–170˚W	1976/77–1979/80	8,000
Southwell et al. (2012)	Most of the circumpolar pack-ice	1998–2000	35,500(10,900–102,600)

amplifications using 25˚l reactions containing 1˚l of each primer (10˚M), 2˚l of Bovine Serum Albumin (BSA; 20 mg/˚l), 12.5˚l of GO Taq green master mix (Promega) and 2˚l of template DNA (average concentration = 30ng/˚l). Reactions were cycled in a T-100 thermocycler (Bio-Rad) according to the following protocol: 1 min at 94˚, then 35 cycles of 94˚ for 1 min, 50˚ for

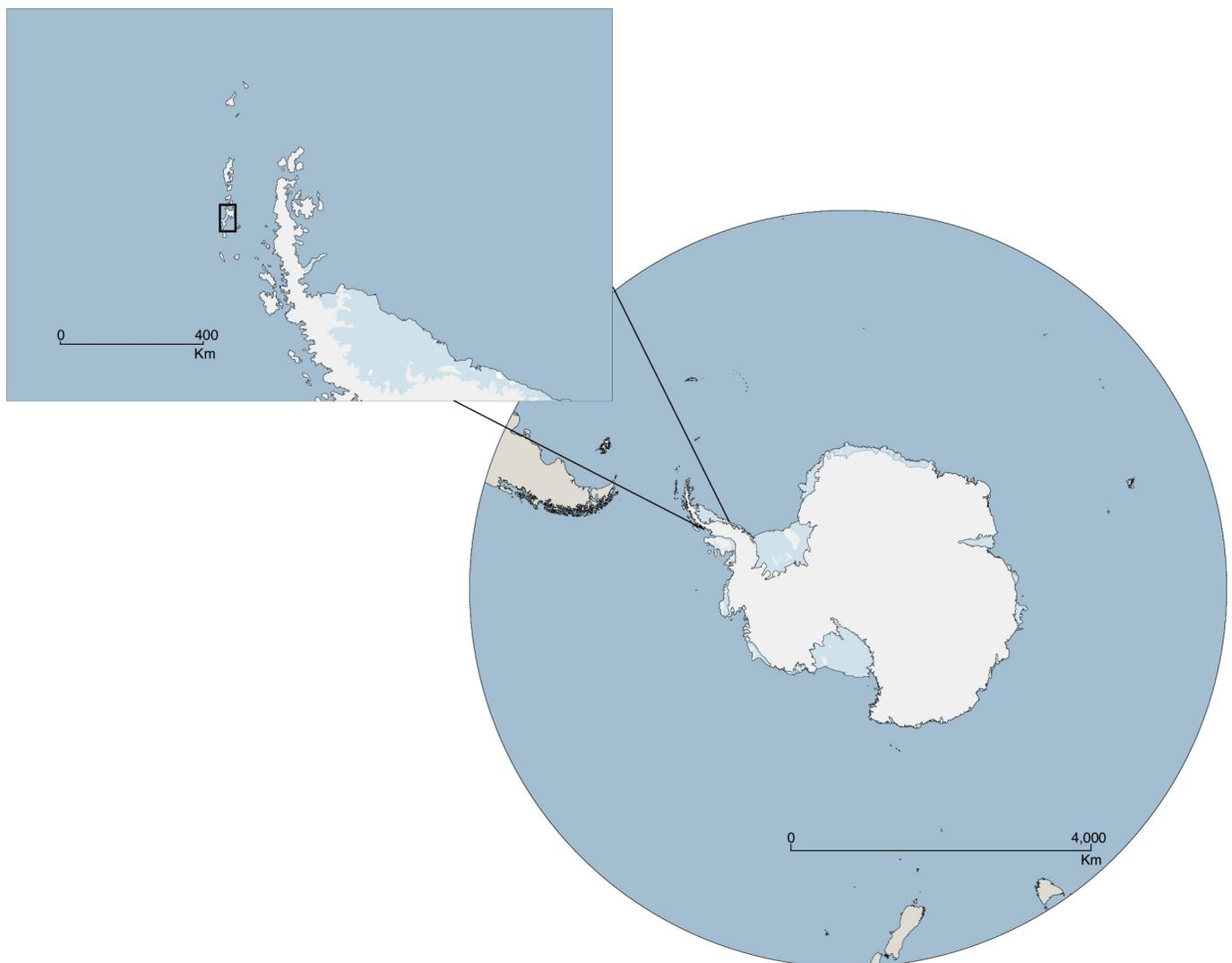**Fig 1. Livingston Island (indicated by the rectangle on insert) and its relative location to the Antarctic continent.** Leopard seals have been sampled for over a decade in Cape Shirreff, on the north coast of the island, by the U.S. Antarctic Marine Living Resources Program. Map designed utilizing Quantarctica detailed basemap [47] (Norwegian Polar Institute, https://www.npolar.no/quantarctica) within QGIS v. 3.30.

1 min and 72˚ for 1 min with a final extension at 72˚ for 7 min. PCR products were visualized on a 2% agarose gel with SYBR safe DNA stain (Invitrogen) to assess amplicon quality. Were enzymatically purified all successful PCR products, and sequenced most individuals in both directions using an Applied Biosystems 3730xl. We edited forward and reverse sequences manually using Geneious Prime v. 2021.1.2.2 [50], trimming ends to remove primer sequences and assuring that only high-quality calls (quality score > 85%) were included in downstream analyses. The post-trimmed sequence length was 405 bp and we aligned the resulting sequences using MUSCLE [51].

Genetic diversity

We selected DnaSP v.6.12.03 [52] for assessing the number of haplotypes (h), nucleotide diversity (π), and haplotype diversity (Hd). We also reconstructed a haplotype network using the Templeton, Crandall, and Sing method (TCS) [53, 54] within PopART (Population Analysis with Reticulate Trees) [55].

Effective population size

To calculate effective population size (N_E), we used a Bayesian Most Probable Estimate (MPE) of the theta (Θ) value generated by LAMARC v.2.1.9 [56]. This method uses a Markov chain Monte Carlo (MCMC) sampling technique to estimate population genetic parameters by sampling parameter values as well as genealogies. To estimate female effective population size (N_{EF}), we used the adjusted equation $N_{EF} = \Theta/2\mu$, where μ represents the mutation rate per site per generation. Assuming the population has a 1:1 sex ratio, the maternally inherited mitochondrial estimates were doubled to calculate the total N_E.

Neutrality test

We investigated deviations from neutrality (whether a population has had a significant history of expansion or contraction) via mismatch distribution analysis within Arlequin v. 3.5.2.2 [57]. The mismatch distribution depicts the observed number of pairwise nucleotide site differences between all of the sequences found in a group of samples [58, 59]. Mismatch distributions are typically unimodal and smooth for populations that have recently expanded, while multimodal and ragged distributions are typical of stable populations [59]. Arlequin tests for population expansion by fitting a model of sudden expansion and calculates Harpending's raggedness index (r) [60], which provides a measure of the smoothness of the empirical mismatch distribution. Expanding populations are expected to have lower values of r, whereas higher values are indicative of a stable population [60]. We conducted neutrality tests (Fu's Fs and Tajima's D) in Arlequin to infer demographic histories by determining whether the leopard seal sequences deviated from neutrality. Significant negative values indicate recent population expansion, while significant positive values indicate stable or bottlenecked populations [61–63].

Bayesian skyline plot analysis

To infer the timing and magnitude of past changes in population size, we implemented demographic reconstruction using a Bayesian skyline plot (BSP) analysis in BEAST v.1.10.4 [64]. BSP uses patterns of coalescence and assumes a single panmictic population to fit a demographic model to a sequence data set [65]. We analyzed the data set under the HKY substitution model [66] with a strict molecular clock, consistent with previous studies of other pinniped species based on the same mtDNA region (e.g., [67, 68]). We then used a coalescent Bayesian Skyline tree prior with six groups under a piecewise-constant model. Other priors

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used in this analysis were kept as default values. For the mutation rate, we opted for a value derived for the most closely related species, the Weddell seal (for the same mtDNA region: 1.14 x 10−7 substitutions per site per year (s/s/y)) [68]. We first converted the mutation rate units to s/s/gen (substitutions per site per generation) by multiplying Younger's et al.'s [68] rate by the generation time (GT) of leopard seals, which is estimated at 14 years [69]. The resulting mutation rate of 1.60×10^{-6} (s/s/gen) was then used in our analyses. We opted to run all BSP analyses for 75 million MCMC iterations with parameters logged every 10,000 steps, and the first 10% were discarded as burn-in. To visualize the skyline plot reconstruction over time, we used Tracer v. 1.7.2 [70].

Results

Genetic diversity

We identified 34 mtDNA haplotypes among 90 individuals (GenBank accession numbers OQ451774 — OQ451802; S1 File), nearly half of which (47.1%) were singletons. Among the haplotypes detected, five had been previously reported for the species (S1 File). The most common haplotype was detected in 10% of the samples and the most divergent haplotypes were separated by as many as 23 mutational steps (Fig 2). The sequences were characterized by moderate levels of both nucleotide and haplotype diversity (π = 0.013; Hd = 0.96; Table 2).
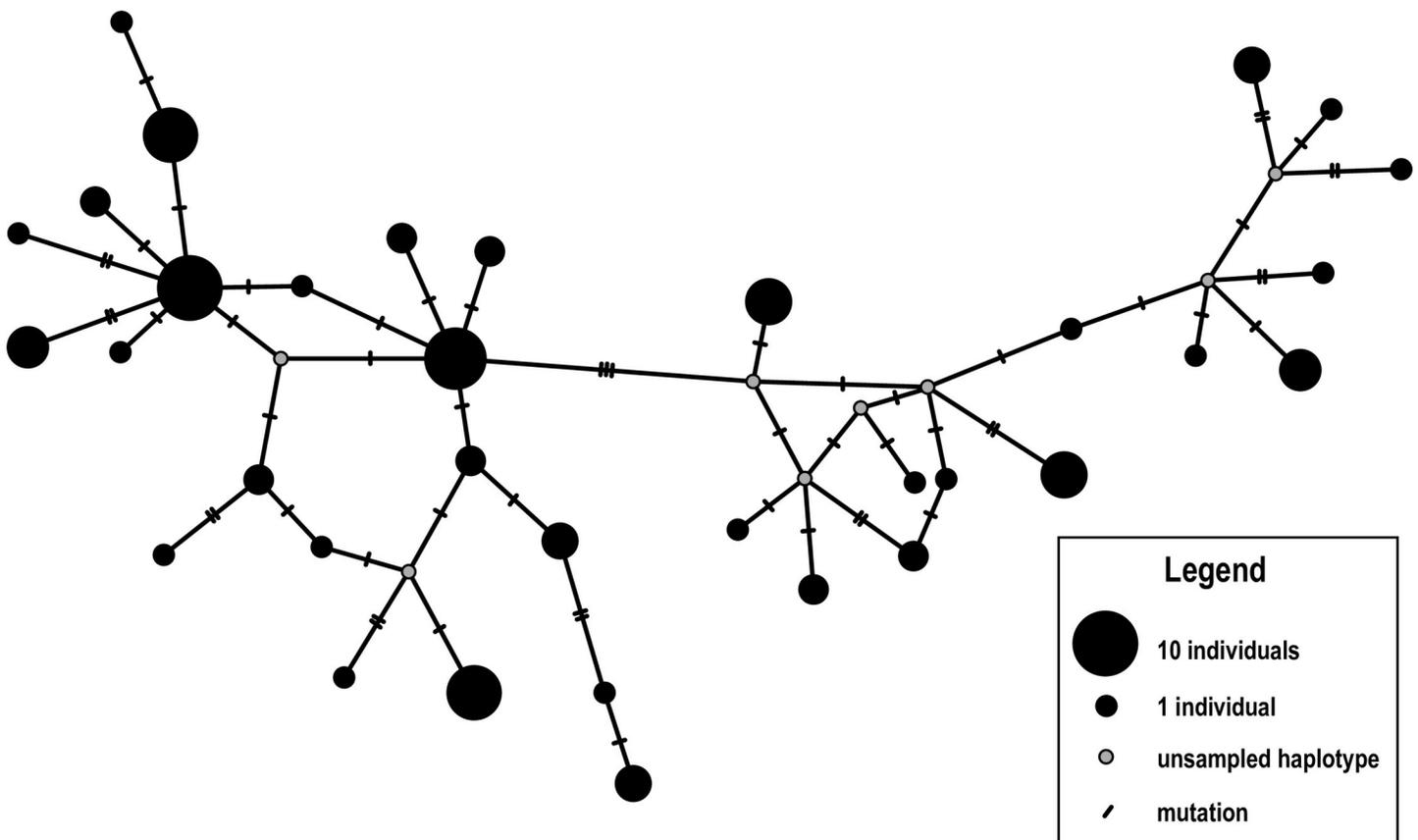

Fig 2. Haplotype network reconstruction of leopard seals, *Hydrurga leptonyx*, based on mtDNA sequencing data (n = 90; 405 bp mtDNA control region). This network was reconstructed via the TCS method [53, 54].

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Effective population size

We estimated Θ (theta) = 0.039 (0.027–0.053), which assuming a mutation rate (μ) for Weddell seals of 1.60×10^{-6} s/s/gen [68], yields an effective female population size estimate of 12,188 (95% CI = 8,438–16,563). Assuming a 1:1 sex ratio, the total effective population size for the species was estimated as 24,376 individuals (95% CI = 16,876–33,126; Table 2).

Demographic reconstruction

The observed mismatch distribution did not significantly depart from a unimodal shape (P > 0.05; Harpending's raggedness index = 0.008, P = 0.69; peak of distribution (τ) = 3.77; Fig 3A and Table 2). Additionally, neutrality tests yielded negative values (Fu's Fs = −14.76, P < 0.01; Tajima's D = − 0.297, P = 0.49; Table 2), indicative of an excess of rare polymorphisms. Together, these results are indicative of a past leopard seal population expansion.

To further investigate the timing of this population expansion, we reconstructed a historical timeline of female effective population size (N_{EF}). Our analyses indicated a population that experienced an accelerated period of expansion during the Pleistocene Epoch, starting around 35,000 years before present (KYA; Fig 3B). This accelerated rate of expansion coincided with the last glacial maximum (LGM) around 26–19 KYA; a time of increased glaciation [71]. Subsequently, N_{EF} reached and remained at its highest levels from 13,000–6,000 KYA. This period was followed by a post-glacial population decline that continues until the present. The N_{EF} estimate from the BSP analysis was similar to our independent N_{EF} estimate based on Θ (BSP N_{EF} = 10,000 vs. LAMARC Θ N_{EF} = 12,000).

Discussion

Due to the solitary nature of leopard seals and the vastness of their typical sea ice habitat, basic data on population trends are hampered by uncertainty. For this reason, leopard seals are not often incorporated into ecosystem models for the Southern Ocean, resulting in a knowledge gap regarding an important top predator. Here, we provide the first effective population size estimate for this species, based on a decadal sampling effort at Livingston Island, at the northern portion of the Antarctica peninsula. Our findings indicate that leopard seals have

Table 2. Comparison of genetic diversity indices and the results of neutrality analyses among Lobodontini seals.

Parameter	Leopard seal (this study)	Weddell seal (Curtis et al. 2009; 2011)	Crabeater seal (Curtis et al. 2009; 2011)	Ross seal (Curtis et al. 2009; 2011)
N	90	181	143	41
h	34	83	135	33
n singletons (%)	16 (47.1%)	49 (59.0%)	127 (94.1%)	26 (78.8%)
Hd	0.96	0.98	0.99	0.99
π	0.013	0.012	0.27	0.020
Θ (95 percentile)	0.039 (0.027–0.053)	0.075 (0.055–0.085)	0.576 (0.484–0.718)	0.088 (0.060–0.132)
Fu F$_S$ (P)	-14.76 (0.00)	-24.99 (0.00)	-24.09 (0.00)	-20.13 (0.00)
SSD (Mismatch P)	0.003 (0.58)	0.002 (0.56)	0.001 (0.82)	0.06 (0.04)
Raggedness (P)	0.008 (0.69)	0.007 (0.84)	0.002 (0.82)	0.017 (0.05)
τ	3.77	5.74	12.59	–
D	-0.297 (0.49)	–	–	–

N = sample size; **h** = number of haplotypes; **n singletons** = number of singleton haplotypes; **Hd** = haplotype diversity; π = nucleotide diversity; Θ = estimations of theta; **Fu Fs** = Fu's test of selective neutrality; **SSD** = sum of squared deviations, **Raggedness** = Harpending's raggedness index; τ = peak of nucleotide pairwise distribution, **D** = Tajima's D test.

A

B
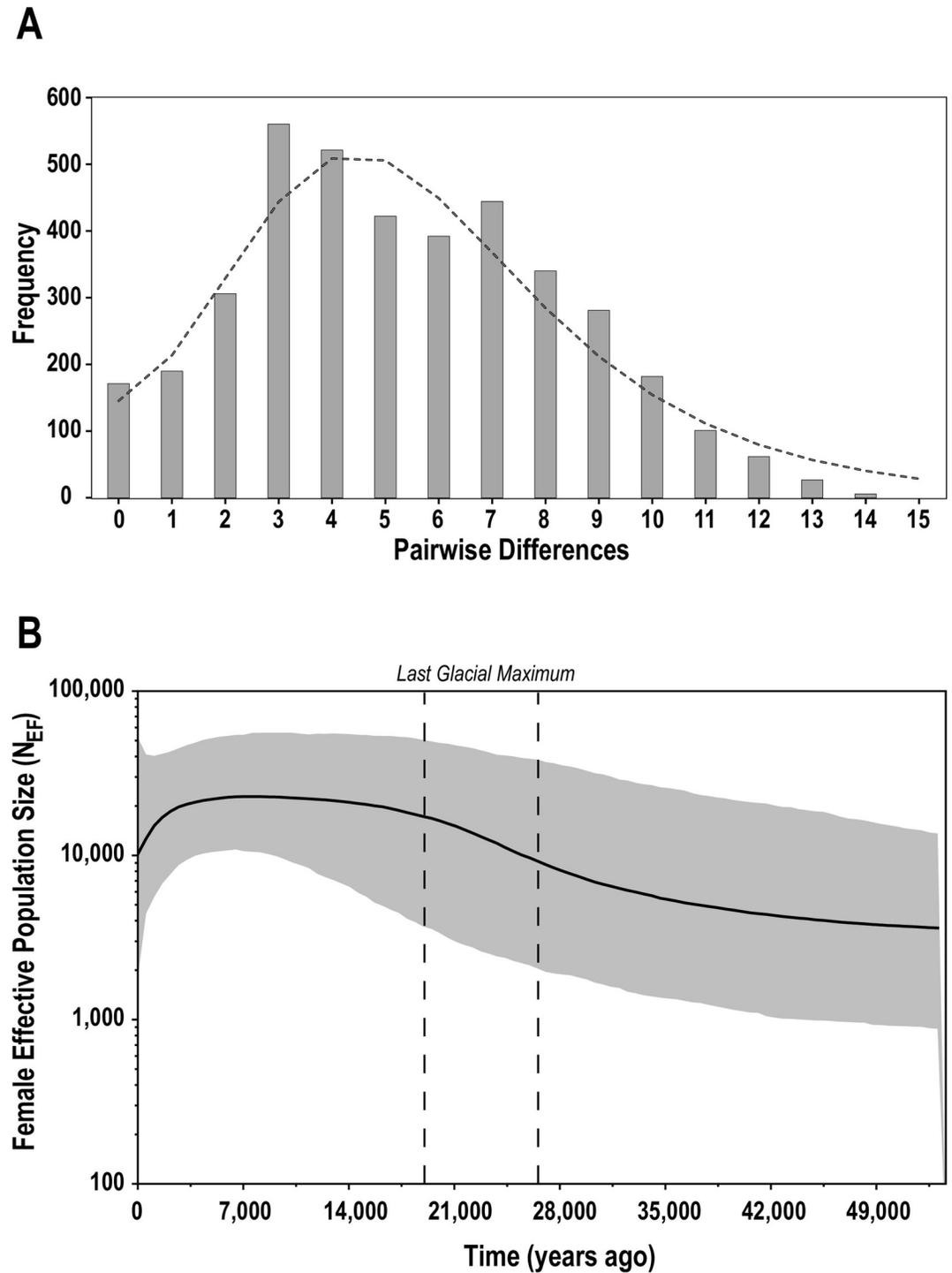
Fig 3. Demographic reconstruction of leopard seals, *Hydrurga leptonyx*, based on mtDNA sequencing data (n = 90; 405 bp). A. Mismatch distribution. Note that this distribution did not significantly depart from a unimodal shape (P> 0.05), rejecting constant population size; **B.** Bayesian Skyline Plot indicates timing of past expansion coincided with the Last Glacial Maximum (LGM; 26–19 KYA) [71].

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comparable levels of mitochondrial DNA diversity to their sister species, the Weddell seal. Furthermore, in line with other ice-breeding seal species [72, 73], historical population trends in this species appear to mirror the historical availability of sea ice, which was more extensive toward the end of the Pleistocene Epoch.

Genetic diversity

Our estimates of nucleotide and haplotype diversity for leopard seals were comparable to findings in other phocid species. Haplotype diversity (0.96) aligned with reports for southern elephant seals (*Mirounga leonina*, Hd = 0.96, N = 203) [74] and Weddell seals (Hd = 0.98, N = 181) [72], but was lower than a previous assessment for leopard seals based on a much smaller number of samples (Hd = 0.99; N = 13) [43]. Haplotype diversity was moderate compared to hooded seals (*Cystophora cristata*; Hd ≈ 1.0, N = 123) [75] but high compared to species that experienced strong bottlenecks such as northern elephant seals (*Mirounga angustirostris*; Hd = 0.41; N = 185) [76]. Similarly, our estimate of nucleotide diversity for the leopard seal (π = 0.013) was within the expected range for phocids, although it was nearly half the value reported for the crabeater seal (π = 0.27), which has a population size estimated in the order of millions [38, 40]. Leopard seal nucleotide diversity was somewhat lower than the Ross seal (π = 0.02, N = 41), but remarkably close to the reported nucleotide diversity of Weddell seals (π = 0.012) [72]. Previous molecular data clearly support *Hydrurga* and *Leptonychotes* as sister taxa [20–23] and a proposed divergence circa 2.89 MYA [17] is consistent with the observation of similar genetic diversity parameters between these species.

Effective population size vs. census size

The N_E estimate for leopard seals, 24,376 (95% CI: 16,876–33,126) is surprisingly high, considering the species' trophic level and generation time. Our BSP analyses also indicates that N_E was historically large. We argue that this may be related to a generalist foraging strategy that allows leopard seals to exploit a variety of prey. Despite being a top predator, recent studies have shown broad intra-specific variability with regard to diet, which is quite diverse and varies seasonally [29]. Variability is also observed in diving behavior [77], hunting strategies [78], the use of ice floes [79] and movements [34]. In fact, the traditional notion that leopard seals are exclusively found in the Antarctic has also recently been rejected [28]. Individuals are recorded year-round in the sub-Antarctic and more northerly locations [28, 79], but the contribution of the northernmost populations to the overall species population size is unknown, and the species' core breeding habitat is still presumably associated with circumpolar pack ice.

It would be helpful to extrapolate our N_E estimate for leopard seals to the census size (N_C), but this presents additional challenges. Recently, a comprehensive multi-species study of N_E in pinnipeds revealed a mean ratio of N_E/N_C of 31% [15], which is consistent with N_E/N_C ratios of between 20% and 30% reported for the brown bear (*Ursus arctos*): a terrestrial species that is better characterized than most marine mammals [80, 81]. Utilizing the mean N_E/N_C ratio of 31% [15], the contemporary leopard seal census population size (N_C) can be inferred at 78,632 (95% CI: 54,438–106,868). In this context, the most recent circumpolar survey of leopard seals estimated N_C at 35,500 [95% CI = 10,900–102,600; 41] appears slightly biased due to undercounting, but the 95% confidence interval nevertheless overlaps with our rough N_C estimate.

Our extrapolation based on the N_E/N_C ratio has a couple of key caveats. Although N_E and N_C are undeniably correlated, their ratio is species specific and can be as low as 10% [82]. Additionally, N_E/N_C is influenced by the choice of genetic marker, so its interpretation relies on precise knowledge regarding the distribution of genetic variation across the genome [83]; its impact on N_E is not fully understood. Therefore, an additional estimation of N_E for leopard

seals based on genome-wide data would be beneficial as has been demonstrated with Antarctic fur seals [84]. Despite this, our findings suggest that leopard seal visual surveys may under-count animals. Indeed, underwater passive acoustic surveys revealed a much higher mean density of leopard seals detected by passive acoustics compared to visual surveys in the Davis Sea: visual density = 0.006 leopard seals/km^2 vs. acoustics density = 0.31 seals/km^2 [6].

Demographic history

Demographic reconstruction revealed a population expansion during the late Pleistocene epoch. This expansion likely began approximately 35 KYA, which is somewhat more recent than proposed population expansions of Weddell seals in west Antarctica [72]; in fact, the peak of unimodal distribution (τ) for leopard seals is the lowest value among Lobodontini seals. Environmental conditions during the late Pleistocene varied considerably, but seasonal sea ice was perennial and extended to 45˚S [85, 86]. Curiously, although the expansion was underway during the LGM (26–19 KYA), it reached its peak after this time (6–13 KYA). This suggests that environmental conditions around that time increased the amount of available sea ice habitat for the leopard seals but their population expansion was also potentially related to ice-associated prey availability (e.g., increased numerical abundance and/or species diversity). For example, crabeater seals, considered part of the diet of leopard seals in Eastern Antarctica [e.g., 87], had a population expansion earlier in the Pleistocene [72, 73] consisting of an additional resource to leopard seals.

The post-glacial population decline of leopard seals detected in our analyses may be a consequence of more recent declines in the availability of breeding habitat, particularly in west Antarctica. This ice loss started during the Holocene (11.7 KYA) [88] and concurrently drove the population expansion of other pinniped species that benefit from ice-free conditions for breeding such as southern elephant seals [reviewed by 89] and Antarctic fur seals [13]. However, the recent decline that is evident in our skyline plot might alternatively be an artifact of the hidden effect of population structure on the BSP method [90]. For this reason, BSP analyses of significantly structured populations require a pooled sample approach (e.g., pooling samples from each sub-population) [68]. However, the only population structure study on leopard seals, based on the microsatellite genotypes of individuals sampled from six Antarctic and sub-Antarctic locations, revealed only very low levels of population differentiation (FST = 0.001 (– 0.002, 0.006)) [42], which appears negligible in this context. Additional historical demography analyses of leopard seals incorporating expanded geographic sampling should help clarify this finding.

Understanding the historical demography of a given species is indispensable to predicting the effects of global climate change, because ecological plasticity is inherently linked to intra-specific genetic variation [91], which in turn is tightly correlated with N_E. This is particularly true for the leopard seal, which unlike other Antarctic pinniped species (notably Antarctic fur seals and southern elephant seals), did not experience confounding direct anthropogenic impacts such as sealing on a large scale [72].

In conclusion, our study shows that despite variability in leopard seal behavior and their occurrence year-round in warmer sub-Antarctic locations, the expansion of ice habitat during the Pleistocene played a key role in the species past abundance, and sea ice availability is likely to continue shaping this species' demography into the future. In this context, the leopard seal emerges as a key indicator species of climate change in the Southern Ocean and should as such be regarded as an important component of future habitat modeling efforts in the region.

Supporting information

S1 File. Leopard seals, *Hydrurga leptonyx*, sequenced for mtDNA control region (405bp).
All individuals were tagged and sampled at Cape Shirreff, Livingston Island by the U.S. Antarctic Marine Living Resources (U.S. AMLR). GenBank accession numbers OQ451774 — OQ451802 correspond to new haplotypes detected in this study.
(DOCX)

Acknowledgments

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Writing – review & editing: Douglas J. Krause, Michael E. Goebel, Joseph I. Hoffman, Eric A. Lewallen.

References

1. Moore SE, Huntington HP. Arctic marine mammals and climate change: impacts and resilience. Ecological Applications. 2008; 18(2):157–65. https://doi.org/10.1890/06-0571.1 PMID: 18494369

2. Albouy C, Delattre V, Donati G, Frölicher TL, Albouy-Boyer S, Rufino M, et al. Global vulnerability of marine mammals to global warming. Scientific Reports. 2020; 10(1):1–12. https://doi.org/10.1038/s41598-019-57280-3 PMID: 31953496

3. Laidre KL, Stirling I, Lowry LF, Wiig Ø, Heide-Jørgensen MP, Ferguson SH. Quantifying the sensitivity of Arctic marine mammals to climate-induced habitat change. Ecological applications. 2008; 18(2):97–125. https://doi.org/10.1890/06-0546.1 PMID: 18494365

4. Hückstädt L. Hydrurga leptonyx. The IUCN Red List of Threatened Species 2015: e. T10340A45226422. 2015.

5. Southwell C, Paxton CGM, Borchers D, Boveng P, Rogers T, William K. Uncommon or cryptic? Challenges in estimating leopard seal abundance by conventional but state-of-the-art methods. Deep Sea Research Part I: Oceanographic Research Papers. 2008; 55(4):519–31. https://doi.org/10.1016/j.dsr.2008.01.005

6. Rogers TL, Ciaglia MB, Klinck H, Southwell C. Density can be misleading for low-density species: benefits of passive acoustic monitoring. PLoS One. 2013; 8(1):e52542. https://doi.org/10.1371/journal.pone.0052542 PMID: 23326339

7. Westbury MV, Thompson KF, Louis M, Cabrera AA, Skovrind M, Castruita JAS, et al. Ocean-wide genomic variation in Gray's beaked whales, *Mesoplodon grayi*. Royal Society open science. 2021; 8 (3):201788. https://doi.org/10.1098/rsos.201788 PMID: 33959341

8. Nadachowska-Brzyska K, Dutoit L, Smeds L, Kardos M, Gustafsson L, Ellegren H. Genomic inference of contemporary effective population size in a large island population of collared flycatchers (*Ficedula albicollis*). Molecular Ecology. 2021; 30(16):3965–73. https://doi.org/10.1111/mec.16025 PMID: 34145933

9. Wright S. Evolution in Mendelian populations. Genetics. 1931; 16(2):97–159. https://doi.org/10.1093/genetics/16.2.97 PMID: 17246615

10. De Oliveira LR, Meyer D, Hoffman J, Majluf P, Morgante JS. Evidence of a genetic bottleneck in an El Niño affected population of South American fur seals, *Arctocephalus australis*. Journal of the Marine Biological Association of the United Kingdom. 2009; 89(8):1717–25. https://doi.org/10.1017/S0025315409000162

11. De Bruyn M, Hall BL, Chauke LF, Baroni C, Koch PL, Hoelzel AR. Rapid response of a marine mammal species to Holocene climate and habitat change. PLoS genetics. 2009; 5(7):e1000554. https://doi.org/10.1371/journal.pgen.1000554 PMID: 19593366

12. Klimova A, Phillips CD, Fietz K, Olsen MT, Harwood J, Amos W, et al. Global population structure and demographic history of the grey seal. Molecular Ecology. 2014; 23(16):3999–4017. https://doi.org/10.1111/mec.12850 PMID: 25041117

13. Cleary AC, Hoffman JI, Forcada J, Lydersen C, Lowther AD, Kovacs KM. 50,000 years of ice and seals: Impacts of the Last Glacial Maximum on Antarctic fur seals. Ecology and evolution. 2021; 11 (20):14003–11. https://doi.org/10.1002/ece3.8104 PMID: 34707834

14. Stoffel MA, Humble E, Paijmans AJ, Acevedo-Whitehouse K, Chilvers BL, Dickerson B, et al. Demographic histories and genetic diversity across pinnipeds are shaped by human exploitation, ecology and life-history. Nature Communications. 2018; 9(1):1–12. https://doi.org/10.1038/s41467-018-06695-z PMID: 30446730

15. Peart CR, Tusso S, Pophaly SD, Botero-Castro F, Wu C-C, Aurioles-Gamboa D, et al. Determinants of genetic variation across eco-evolutionary scales in pinnipeds. Nature Ecology & Evolution. 2020; 4 (8):1095–104. https://doi.org/10.1038/s41559-020-1215-5 PMID: 32514167

16. Fulton TL, Strobeck C. Multiple markers and multiple individuals refine true seal phylogeny and bring molecules and morphology back in line. Proceedings of the Royal Society B: Biological Sciences. 2010; 277(1684):1065–70. https://doi.org/10.1098/rspb.2009.1783 PMID: 19939841

17. Fulton TL, Strobeck C. Multiple fossil calibrations, nuclear loci and mitochondrial genomes provide new insight into biogeography and divergence timing for true seals (Phocidae, Pinnipedia). Journal of Biogeography. 2010; 37(5):814–29. https://doi.org/10.1111/j.1365-2699.2010.02271.x

18. Hendey QB. The evolution and dispersal of the Monachinae (Mammalia: Pinnipedia): Annals of the South African Museum; 1972.

19. de Muizon C. Phocid phylogeny and dispersal. Annals of the South African Museum. 1982; 89:175–231.

20. Davis CS, Delisle I, Stirling I, Siniff DB, Strobeck C. A phylogeny of the extant Phocidae inferred from complete mitochondrial DNA coding regions. Molecular phylogenetics and evolution. 2004; 33(2):363–77. https://doi.org/10.1016/j.ympev.2004.06.006 PMID: 15336671

21. Fyler CA, Reeder TW, Berta A, Antonelis G, Aguilar A, Androukaki E. Historical biogeography and phylogeny of monachine seals (Pinnipedia: Phocidae) based on mitochondrial and nuclear DNA data. Journal of Biogeography. 2005; 32(7):1267–79. https://doi.org/10.1111/j.1365-2699.2005.01281.x

22. Arnason U, Gullberg A, Janke A, Kullberg M, Lehman N, Petrov EA, et al. Pinniped phylogeny and a new hypothesis for their origin and dispersal. Molecular phylogenetics and evolution. 2006; 41(2):345–54. https://doi.org/10.1016/j.ympev.2006.05.022 PMID: 16815048

23. Higdon JW, Bininda-Emonds ORP, Beck R, Ferguson SH. Phylogeny and divergence of the pinnipeds (Carnivora: Mammalia) assessed using a multigene dataset. BMC evolutionary biology. 2007; 7(1):1–19. https://doi.org/10.1186/1471-2148-7-216 PMID: 17996107

24. LaRue M, Salas L, Nur N, Ainley D, Stammerjohn S, Pennycook J, et al. Insights from the first global population estimate of Weddell seals in Antarctica. Science Advances. 2021; 7(39):eabh3674. https://doi.org/10.1126/sciadv.abh3674 PMID: 34559555

25. Oosthuizen WC, Reisinger RR, Bester MN, Steinhage D, Auel H, Flores H, et al. Habitat-based density models of pack-ice seal distribution in the southern Weddell Sea, Antarctica. Marine Ecology Progress Series. 2021; 673:211–27. https://doi.org/10.3354/meps13787

26. Southwell CJ, Paxton CGM, Borchers DL, Boveng PL, Nordøy ES, Blix AS, et al. Estimating population status under conditions of uncertainty: the Ross seal in East Antarctica. Antarctic Science. 2008; 20 (2):123–33. https://doi.org/10.1017/S0954102007000879

27. Rogers TL, Hogg CJ, Irvine A. Spatial movement of adult leopard seals (*Hydrurga leptonyx*) in Prydz Bay, Eastern Antarctica. Polar Biology. 2005; 28:456–63.

28. van der Linde K, Visser IN, Bout R, Krause DJ, Forcada J, Siniff D, et al. A review of leopard seal (*Hydrurga leptonyx*) births and pups using a standardised age-class classification system. Polar Biology. 2022; 45(7):1193–209. https://doi.org/10.1007/s00300-022-03053-0

29. Krause DJ, Goebel ME, Kurle CM. Leopard seal diets in a rapidly warming polar region vary by year, season, sex, and body size. BMC Ecology. 2020; 20:1–15. https://doi.org/10.1186/s12898-020-00300-y PMID: 32493329

30. Casaux R, Baroni A, Ramón A, Carlini A, Bertolin M, DiPrinzio CY. Diet of the leopard seal *Hydrurga leptonyx* at the Danco Coast, Antarctic Peninsula. Polar Biology. 2009; 32:307–10.

31. Ainley DG, Ballard G, Karl BJ, Dugger KM. Leopard seal predation rates at penguin colonies of different size. Antarctic Science. 2005; 17(3):335–40.

32. Forcada J, Malone D, Royle JA, Staniland IJ. Modelling predation by transient leopard seals for an ecosystem-based management of Southern Ocean fisheries. Ecological Modelling. 2009; 220(12):1513–21. https://doi.org/10.1016/j.ecolmodel.2009.03.020

33. Staniland IJ, Ratcliffe N, Trathan PN, Forcada J. Long term movements and activity patterns of an Antarctic marine apex predator: The leopard seal. PLoS One. 2018; 13(6):e0197767. https://doi.org/10.1371/journal.pone.0197767 PMID: 29870541

34. Kienle SS, Goebel ME, LaBrecque E, Borras-Chavez R, Trumble SJ, Kanatous SB, et al. Plasticity in the morphometrics and movements of an Antarctic apex predator, the leopard seal. Frontiers in Marine Science. 2022; 9. https://doi.org/10.3389/fmars.2022.976019

35. Boveng PL, Hiruki LM, Schwartz MK, Bengtson JL. Population growth of Antarctic fur seals: limitation by a top predator, the leopard seal? Ecology. 1998; 79(8):2863–77. https://doi.org/10.1890/0012-9658 (1998)079[2863:PGOAFS]2.0.CO;2.

36. Krause DJ, Bonin CA, Goebel ME, Reiss CS, Watters GM. The rapid population collapse of a key marine predator in the northern Antarctic Peninsula endangers genetic diversity and resilience to climate change. Frontiers in Marine Science. 2022; 8. https://doi.org/10.3389/fmars.2021.796488

37. Eklund CR, Atwood EL. A population study of Antarctic seals. Journal of Mammalogy. 1962; 43(2):229–38. https://doi.org/10.2307/1377094

38. Laws RM. Seals and whales of the Southern Ocean. Philosophical Transactions of the Royal Society of London B, Biological Sciences. 1977; 279(963):81–96. https://doi.org/10.1098/rstb.1977.0073

39. Ainley DG. Biomass of Birds and Mammals in the Ross Sea. Antarctic Nutrient Cycles and Food Webs. Berlin Heidelberg: Springer 1985. p. 498–515.

40. Erickson AW, Hanson MB. Continental estimates and population trends of Antarctic ice seals. Antarctic ecosystems: (Springer; 1990. p. 253–64.

41. Southwell C, Bengston J, Bester M, Blix AS, Bornemann H, Boveng P, et al. A review of data on abundance, trends in abundance, habitat use and diet of ice-breeding seals in the Southern Ocean. Ccamlr Science. 2012; 19:49–74.

42. Davis CS, Stirling IAN, Strobeck C, Coltman DW. Population structure of ice-breeding seals. Molecular Ecology. 2008; 17(13):3078–94. https://doi.org/10.1111/j.1365-294X.2008.03819.x PMID: 18494764

43. Hernández-Ardila LV, Barragán-Barrera DC, Negrete J, Poljak S, Riet-Sapriza FG, Caballero S. Insights into the genetic diversity of the leopard seal (*Hydrurga leptonyx*), inferred from mitochondrial DNA analysis, at Danco Coast, Antarctic Peninsula. Boletín de Investigaciones Marinas y Costeras-INVEMAR. 2021; 50:227–38. https://doi.org/10.25268/bimc.invemar.2021.50.SuplEsp.0951

44. Dahood A, Watters GM, de Mutsert K. Using sea-ice to calibrate a dynamic trophic model for the Western Antarctic Peninsula. PLoS One. 2019; 14(4):e0214814. https://doi.org/10.1371/journal.pone.0214814 PMID: 30939156

45. Watters GM, Hill SL, Hinke JT, Matthews J, Reid K. Decision-making for ecosystem-based management: evaluating options for a krill fishery with an ecosystem dynamics model. Ecological Applications. 2013; 23(4):710–25. https://doi.org/10.1890/12-1371.1 PMID: 23865224

46. Dahood A, Klein ES, Watters GM. Planning for success: leveraging two ecosystem models to support development of an Antarctic marine protected area. Marine Policy. 2020; 121:104109. https://doi.org/10.1016/j.marpol.2020.104109

47. Matsuoka K, Skoglund A, Roth G, de Pomereu J, Griffiths H, Headland R, et al. Quantarctica, an integrated mapping environment for Antarctica, the Southern Ocean, and sub-Antarctic islands. Environmental Modelling & Software. 2021; 140:105015. https://doi.org/10.1016/j.envsoft.2021.105015

48. Slade RW, Moritz C, Heideman A. Multiple nuclear-gene phylogenies: application to pinnipeds and comparison with a mitochondrial DNA gene phylogeny. Molecular Biology and Evolution. 1994; 11 (3):341–56. https://doi.org/10.1093/oxfordjournals.molbev.a040117 PMID: 8015430

49. Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, et al. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences. 1989; 86(16):6196–200. https://doi.org/10.1073/pnas.86.16.6196 PMID: 2762322

50. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012; 28(12):1647–9. https://doi.org/10.1093/bioinformatics/bts199 PMID: 22543367

51. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic acids research. 2004; 32(5):1792–7. https://doi.org/10.1093/nar/gkh340 PMID: 15034147

52. Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, et al. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular biology and evolution. 2017; 34(12):3299–302. https://doi.org/10.1093/molbev/msx248 PMID: 29029172

53. Templeton AR, Crandall KA, Sing CF. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics. 1992; 132(2):619–33. https://doi.org/10.1093/genetics/132.2.619 PMID: 1385266

54. Clement M, Snell Q, Walker P, Posada D, Crandall K. TCS: estimating gene genealogies. Proceedings 16th International Parallel and Distributed Processing Symposium; 2002; Ft. Lauderdale, FL, USA: IEEE Computer Society; 2002. p. 0184-.

55. Leigh JW, Bryant D. POPART: full-feature software for haplotype network construction. Methods in Ecology and Evolution. 2015; 6(9):1110–6. https://doi.org/10.1111/2041-210x.12410

56. Kuhner MK. LAMARC 2.0: maximum likelihood and Bayesian estimation of population parameters. Bioinformatics. 2006; 22(6):768–70. https://doi.org/10.1093/bioinformatics/btk051 PMID: 16410317

57. Excoffier L, Laval G, Schneider S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary bioinformatics. 2005;1:47–50. doi: 10.1177/117693430500100003.

58. Slatkin M, Hudson RR. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics. 1991; 129(2):555–62. https://doi.org/10.1093/genetics/129.2.555 PMID: 1743491

59. Rogers AR, Harpending H. Population growth makes waves in the distribution of pairwise genetic differences. Molecular biology and evolution. 1992; 9(3):552–69. https://doi.org/10.1093/oxfordjournals.molbev.a040727 PMID: 1316531

60. Harpending H. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Human biology. 1994; 66(4):591–600. PMID: 8088750

61. Tajima F. The effect of change in population size on DNA polymorphism. Genetics. 1989; 123(3):597–601. https://doi.org/10.1093/genetics/123.3.597 PMID: 2599369

62. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 1989; 123(3):585–95. https://doi.org/10.1093/genetics/123.3.585 PMID: 2513255

63. Fu YX. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 1997; 147(2):915–25. https://doi.org/10.1093/genetics/147.2.915 PMID: 9335623

64. Drummond AJ, Ho SY, Rawlence N, Rambaut A. A rough guide to BEAST 1.4. 2007.

65. Drummond AJ, Rambaut A, Shapiro B, Pybus OG. Bayesian coalescent inference of past population dynamics from molecular sequences. Molecular biology and evolution. 2005; 22(5):1185–92. https://doi.org/10.1093/molbev/msi103 PMID: 15703244

66. Hasegawa M, Kishino H, Yano T-a. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of molecular evolution. 1985; 22:160–74. https://doi.org/10.1007/BF02101694 PMID: 3934395

67. Hoffman JI, Grant SM, Forcada J, Phillips CD. Bayesian inference of a historical bottleneck in a heavily exploited marine mammal. Molecular Ecology. 2011; 20(19):3989–4008. https://doi.org/10.1111/j.1365-294X.2011.05248.x PMID: 21895820

68. Younger JL, Van Den Hoff J, Wienecke B, Hindell M, Miller KJ. Contrasting responses to a climate regime change by sympatric, ice-dependent predators. BMC Evolutionary Biology. 2016; 16(1):1–11. https://doi.org/10.1186/s12862-016-0630-3 PMID: 26975876

69. Pacifici M, Santini L, Di Marco M, Baisero D, Francucci L, Marasini GG, et al. Generation length for mammals. Nature Conservation. 2013; 5:87–94. https://doi.org/10.3897/natureconservation.5.5734

70. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic biology. 2018; 67(5):901–4. https://doi.org/10.1093/sysbio/syy032 PMID: 29718447

71. Clark PU, Dyke AS, Shakun JD, Carlson AE, Clark J, Wohlfarth B, et al. The last glacial maximum. science. 2009; 325(5941):710–4. https://doi.org/10.1126/science.1172873 PMID: 19661421

72. Curtis C, Stewart BS, Karl SA. Pleistocene population expansions of Antarctic seals. Molecular Ecology. 2009; 18(10):2112–21. https://doi.org/10.1111/j.1365-294X.2009.04166.x PMID: 19344354

73. Curtis C, Stewart BS, Karl SA. Genetically effective population sizes of Antarctic seals estimated from nuclear genes. Conservation Genetics. 2011; 12(6):1435–46. https://doi.org/10.1007/s10592-011-0241-x

74. Chua M, Ho SYW, McMahon CR, Jonsen ID, de Bruyn M. Movements of southern elephant seals (*Mirounga leonina*) from Davis Base, Antarctica: combining population genetics and tracking data. Polar Biology. 2022; 45(7):1163–74. https://doi.org/10.1007/s00300-022-03058-9

75. Coltman DW, Stenson G, Hammill MO, Haug T, Davis CS, Fulton TL. Panmictic population structure in the hooded seal (*Cystophora cristata*). Molecular Ecology. 2007; 16(8):1639–48. https://doi.org/10.1111/j.1365-294X.2007.03229.x PMID: 17402979

76. Hoelzel AR, Fleischer RC, Campagna C, Le Boeuf BJ, Alvord G. Impact of a population bottleneck on symmetry and genetic diversity in the northern elephant seal. Journal of Evolutionary Biology. 2002; 15(4):567–75. https://doi.org/10.1046/j.1420-9101.2002.00419.x

77. Krause DJ, Goebel ME, Marshall GJ, Abernathy K. Summer diving and haul-out behavior of leopard seals (*Hydrurga leptonyx*) near mesopredator breeding colonies at Livingston Island, Antarctic Peninsula. Marine Mammal Science. 2016; 32(3):839–67. https://doi.org/10.1111/mms.12309

78. Krause DJ, Goebel ME, Marshall GJ, Abernathy K. Novel foraging strategies observed in a growing leopard seal (*Hydrurga leptonyx*) population at Livingston Island, Antarctic Peninsula. Animal Biotelemetry. 2015; 3(1):1–14. https://doi.org/10.1186/s40317-015-0059-2

79. Siniff DB, Garrott RA, Rotella JJ, Fraser WR, Ainley DG. Opinion: Projecting the effects of environmental change on Antarctic seals. Antarctic Science. 2008; 20(5):425–35. https://doi.org/10.1017/S0954102008001351

80. Harris RB, Allendorf FW. Genetically effective population size of large mammals: an assessment of estimators. Conservation Biology. 1989; 3(2):181–91. https://doi.org/10.1111/j.1523-1739.1989.tb00070.x

81. Miller CR, Waits LP. The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): implications for conservation. Proceedings of the National Academy of Sciences. 2003; 100(7):4334–9. https://doi.org/10.1073/pnas.073553110

82. Frankham R. Effective population size/adult population size ratios in wildlife: a review. Genetics Research. 1995; 66(2):95–107. https://doi.org/10.1017/S0016672300034455

83. Charlesworth B. Effective population size and patterns of molecular evolution and variation. Nature Reviews Genetics. 2009; 10(3):195–205. https://doi.org/10.1038/nrg2526

84. Hoffman JI, Chen RS, Vendrami DLJ, Paijmans AJ, Dasmahapatra KK, Forcada J. Demographic reconstruction of Antarctic fur seals supports the krill surplus hypothesis. Genes. 2022; 13(3):541. https://doi.org/10.3390/genes13030541 PMID: 35328094

85. Gersonde R, Crosta X, Abelmann A, Armand L. Sea-surface temperature and sea ice distribution of the Southern Ocean at the EPILOG Last Glacial Maximum—a circum-Antarctic view based on siliceous microfossil records. Quaternary science reviews. 2005; 24(7–9):869–96.

86. Allcock AL, Strugnell JM. Southern Ocean diversity: new paradigms from molecular ecology. Trends in ecology & evolution. 2012; 27(9):520–8. https://doi.org/10.1016/j.tree.2012.05.009 PMID: 22727016

87. Hall-Aspland SA, Rogers TL. Summer diet of leopard seals (*Hydrurga leptonyx*) in Prydz Bay, Eastern Antarctica. Polar Biology. 2004; 27:729–34. https://doi.org/10.1007/s00300-004-0662-9

88. Cohen KM, Finney SC, Gibbard PL, Fan JX. The ICS international chronostratigraphic chart. Episodes Journal of International Geoscience. 2013; 36(3):199–204. https://doi.org/10.18814/epiiugs/2013/v36i3/002

89. Younger JL, Emmerson LM, Miller KJ. The influence of historical climate changes on Southern Ocean marine predator populations: a comparative analysis. Global Change Biology. 2016; 22(2):474–93. https://doi.org/10.1111/gcb.13104 PMID: 26391440

90. Heller R, Chikhi L, Siegismund HR. The confounding effect of population structure on Bayesian skyline plot inferences of demographic history. PloS one. 2013; 8(5):e62992. https://doi.org/10.1371/journal.pone.0062992 PMID: 23667558

91. Pauls SU, Nowak C, Bálint M, Pfenninger M. The impact of global climate change on genetic diversity within populations and species. Molecular ecology. 2013; 22(4):925–46. https://doi.org/10.1111/mec.12152 PMID: 23279006