

Low effective population size in the genetically bottlenecked Australian sea lion is insufficient to maintain genetic variation

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Abstract

Genetic bottlenecks can reduce effective population sizes (N_e), increase the rate at which genetic variation is lost via drift, increase the frequency of deleterious mutations and thereby accentuate inbreeding risk and lower evolutionary potential. Here, we tested for the presence of a genetic bottleneck in the endangered Australian sea lion (*Neophoca cinerea*), estimated N_e and predicted future losses of genetic variation under a range of scenarios. We used 2238 genome-wide neutral single-nucleotide polymorphisms (SNPs) from 72 individuals sampled from colonies off the southern (SA) and western (WA) coastline of Australia. Coalescent analyses using approximate Bayesian computation (ABC) methods indicated that both the SA and WA populations have experienced a historical genetic bottleneck. Using LD-based methods, we estimated contemporary N_e to be 160 (CI = 146–178) and 424 (CI = 397–458) for the WA and SA populations respectively. Modelled future population declines suggested that disease epidemics prompted the highest increases in inbreeding relative to fishery-related mortalities and other modelled threats. Small effective sizes and relatively low genetic variation leave this species vulnerable, and these risks may be compounded if current population declines are not reversed.

Introduction

Predicting the resilience of wildlife populations to current and future threats is key to designing effective conservation management (Angeler, Allen, Garmestani et al., 2018). Reductions in population size can lower genetic variation, increasing the frequency of deleterious alleles, the likelihood of inbreeding depression and the ability to adapt to novel challenges, ultimately leading to a greater risk of extinction (Frankham, 2005; Spielman, Brook and Frankham, 2004). The rate at which genetic variation is lost is primarily a function of the effective population size (N_e). Precise genetic-based estimates of N_e have only recently become possible due to the availability of large, high-resolution genomic datasets in non-model organisms (Waples et al., 2016). As a result, N_e is now an achievable measure that can be incorporated into conservation decision making (Frankham, 2003).

The N_e is the size of an ideal population where changes in allele frequencies and inbreeding (F) match the rate of

change observed in the population under investigation. An ideal population has a number of simplifying conditions, including being closed to migrants, possessing random mating, distinct generations and a mean number of one offspring per adult which varies according to a Poisson distribution (Frankham et al., 2010). Across generations, N_e influences the rate of loss of genetic variation due to drift, the extent of inbreeding and inbreeding depression, the adaptive potential and the extinction risks of populations (Charlesworth, 2009; Wright, 1931). In principle, the N_e provides a measure that is comparable across species with vastly different traits. This is valuable because the N_e is influenced by key demographic parameters that vary from that of an ideal population, such as the presence of non-breeding individuals, skewed sex ratios and variation in lifetime breeding success (Lee, Sæther and Engen, 2011; Palstra and Fraser, 2012). Consequently, N_e is often substantially less than census size (N_c) of the population (Lee et al., 2011; Waples, 2005) and predicting the magnitude of this difference requires detailed information on life history and/or genetic data (Frankham, 2010).

Genetic approaches to estimate contemporary N_e commonly use linkage disequilibrium methods, while past population size changes can be inferred from coalescence analyses (Luikart, Ryman, Tallmon *et al.*, 2010; Wang, Santiago and Caballero, 2016; Waples, Antao and Luikart, 2014). The linkage disequilibrium method allows for estimating contemporary N_e ($LD-N_e$) across pairs of loci that are unlinked and neutral, using genetic data from a single sampling event (Luikart *et al.*, 2010; Reid-Anderson, Bilgmann and Stow, 2019; Waples *et al.*, 2016). This approach is useful if a genetic bottleneck is suspected to have occurred very recently because the estimate is less likely to be moderated by pre-bottleneck characteristics of the population in question. Other single-sample methods include estimates based on the proportion of half siblings, though these tend to require a relatively large sample of the census size (Waples *et al.*, 2016).

The implications of a reduced N_e can be seen in species that have been subjected to substantial population size reductions, for example, the many pinniped species that were exploited during the sealing era (Stoffel, Humble, Pajmans *et al.*, 2018). For some pinnipeds that have experienced sustained reduction in population sizes, the loss of alleles has contributed to an ongoing decline in genetic variation and increase in homozygosity (e.g. the critically endangered Hawaiian monk seal, *Neomonachus schauinslandi*; Schultz, 2010). Reduced heterozygosity has been correlated with lower fitness in pinnipeds, in some cases resulting in poorer health (Acevedo-Whitehouse, Gulland, Greig *et al.*, 2003; Hoffman, Simpson, David *et al.*, 2014; Rijks, Hoffman, Kuiken *et al.*, 2008), poorer offspring survival (Coltman, Bowen and Wright, 1998), reduced mate attractiveness (Hoffman, Forcada, Trathan *et al.*, 2007) and less effective hunting (Hoffman, Forcada and Amos, 2010).

The Australian sea lion (*Neophoca cinerea*) has experienced a decline in both numbers and range due to historical commercial hunting pressures during the sealing era (~1800–1830) (Gales, Shaughnessy and Dennis, 1994). Being among the rarest otariids with a low total numbers of individuals (~10000) and declining pup production at most breeding sites across their relatively limited range (Goldsworthy, Mackay, Bilgmann *et al.*, 2017), they are listed globally as *Endangered* (IUCN) and are protected under Australian national and state legislations (Goldsworthy, 2015). Australian sea lion breeding colonies are currently only found in parts of southern and western Australian shelf waters (DSEWPC 2013). These breeding colonies are sparsely distributed along their range and breeding colony sizes tend to be relatively small for pinnipeds, typically consisting of tens of individuals. Females exhibit extreme philopatry and gene flow for males is restricted to a few 100 kilometers (Ahonen, Lowther, Harcourt *et al.*, 2016; Campbell, Gales, Lento *et al.*, 2008; Lowther, Harcourt, Goldsworthy *et al.*, 2012). Known threats are primarily bycatch-related mortality in commercial fisheries (mainly demersal gillnets) and marine debris entanglements (Goldsworthy and Page, 2007; Goldsworthy, Page, Shaughnessy *et al.*, 2010; Hamer, Goldsworthy, Costa *et al.*, 2013; Page, McKenzie, McIntosh

et al., 2004). Several secondary threats have also been identified (described in DSEWPaC, 2013) and the implications to demographic trends arising from the accumulative impact of these potential threats remain unknown (Mackay *et al.*, 2016). Overall, Australian sea lion numbers continue to decline despite the cessation of hunting in the last century and management interventions to mitigate contemporary known threats (Goldsworthy *et al.*, 2017; Goldsworthy *et al.*, 2015). It is possible that low genetic variation, compounded by drift, may be contributing to their continued decline as seen in other pinniped populations (e.g. Abadía-Cardoso, Freimer, Deiner *et al.*, 2017; Hoelzel, Fleischer, Campagna *et al.*, 2002).

Here, we tested whether the Australian sea lion has been subject to a genetic bottleneck, estimated current N_e and explored potential future losses of genetic variation resulting from different rates of population decline. We addressed this using coalescence analysis to test for the presence of genetic bottlenecks and estimated contemporary N_e using $LD-N_e$ and a sib-ship approach from genetic data collected at five Australian sea lion colonies. We then examined how contemporary genetic variation may be further reduced by key threats and their cumulative impacts. Given that genetic variation underlies resilience, this informs our understanding of the future capacity for this species to cope with anthropogenic impacts and other disturbances.

Materials and methods

Sample selection

We used samples from five Australian sea lion breeding colonies selected from an available collection of skin biopsy samples (see Ahonen *et al.*, 2016). The samples from three breeding colonies off South Australia (SA; Lilliput Island, Blefuscu Island and Olive Island) and two breeding colonies off the west coast of Western Australia (WA; North Fisherman Island and Beagle Island) were chosen on the basis of close geographic proximity to each other within a region and a large distance between the two regions (Fig. 1). We selected 15 individuals per colony with approximately equal ratios of males to females and included adults only to reduce biases for multiple generations.

DNA sequencing and genotyping

Genomic DNA was extracted from samples of Australian sea lions preserved in 100% ethanol. Next generation sequencing was performed by Diversity Technology Arrays (DArT; Canberra, Australia) using the DArTseq method (Jaccoud, Peng, Feinstein *et al.*, 2001). This double-digest restriction site-associated DNA (ddRAD) sequencing method uses a combination of *Pst*I and *Sph*I restriction enzymes to digest the DNA sample followed by Illumina sequencing (see Reid-Anderson *et al.*, 2019). The DArT-seq method also has its own pipeline to identify and call SNPs (DArTSoft14TM); however, this pipeline was not used here and the sequences were processed as followed.

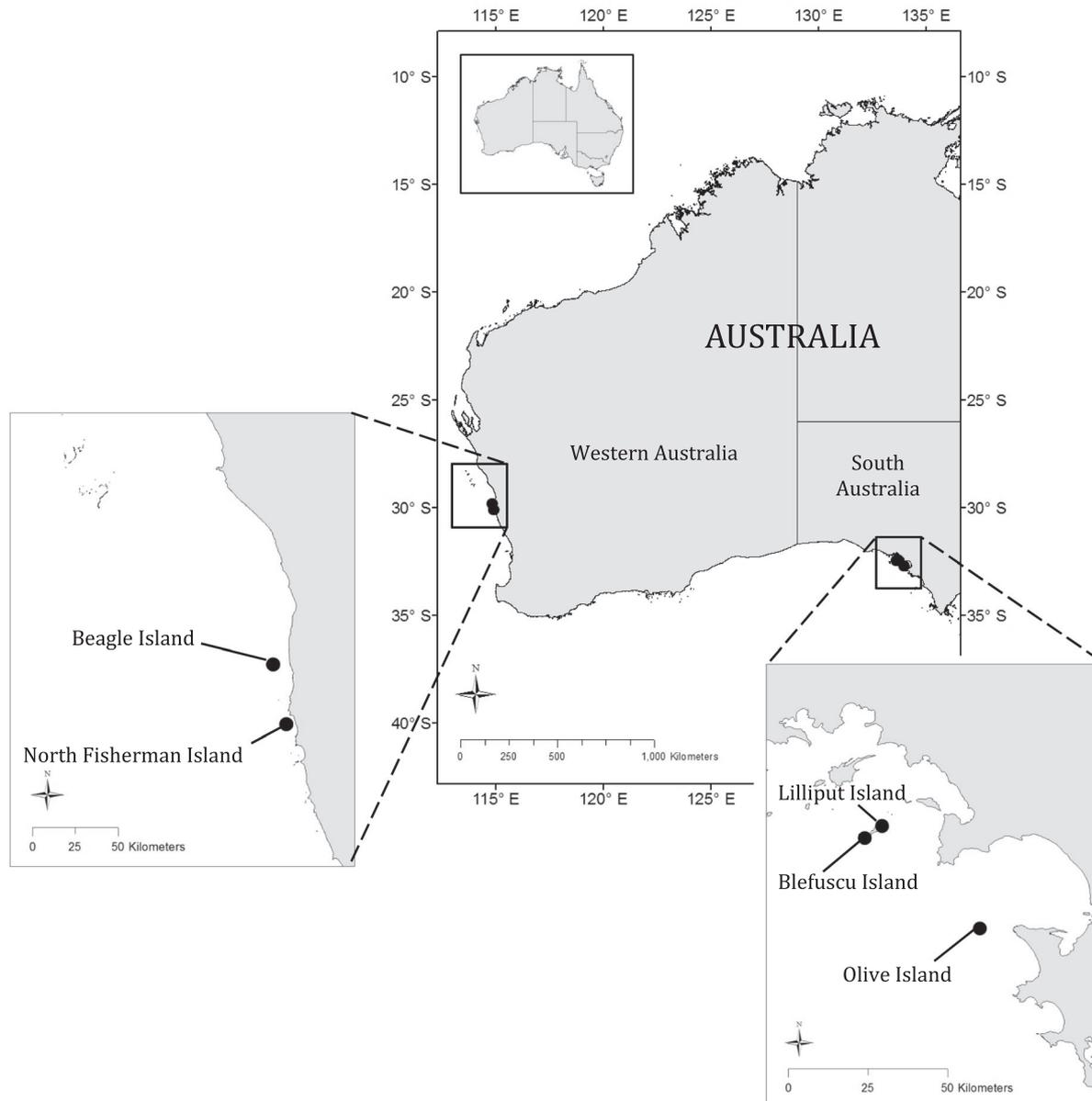


Figure 1 Geographic location of five breeding colonies of Australian sea lions (*Neophoca cinerea*) in Western Australia (North Fisherman Island, $n = 14$ and Beagle Island, $n = 16$) and South Australia (Lilliput Island, $n = 12$; Olive Island, $n = 15$ and Blefuscu Island, $n = 15$).

De-multiplexed raw sequences in FASTQ format obtained from DArT were processed with Cutadapt version 1.9.dev0 to remove adaptor sequences. Sequence quality was assessed using Fast QC 0.11.1 (Andrews, 2010). After removal of adaptors and restriction enzyme cutting sites, the trimmed sequences were 61bp in length. The STACKS pipeline version 1.44 was used to assemble sequences, discover loci and call SNPs (Catchen, Hohenlohe, Bassham *et al.*, 2013). *Ustacks* was used for the assembly of sequences *de novo* (parameters: $m = 4$, $M = 3$, $N = 5$, $-H$, $-R$, $-D$ – model type bounded 0–1 (not restricted) max locus 2), *Cstacks* (parameters: $n = 1$) to create the catalog,

Sstacks to stack sequence reads and the module *Populations* to call genotypes for loci having a minimum genotype likelihood of -10 . Filtering steps were then applied to retain SNPs that were biallelic, had a minimum read depth of four and were genotyped in at least 60% of individuals in the WA and SA populations each. Filtering also included retaining SNPs that had a global minor allele frequency (MAF) higher than 0.01 and a MAF separately for the WA and SA populations of 0.05. Detailed filtering parameters and SNPs retained at each step are listed in Table S1. The STACKS workflow is available at 'https://github.com/enormandeu/stacks_workflow'.

The raw sequences included around 15% duplicate runs for control of quality and reproducibility of results. Of the duplicate runs we retained only one of the duplicates by choosing the one with the lowest levels of missing data. After a further assessment of data quality, the level of missing data for the entire dataset was set to $\leq 1\%$. Sequences were then re-run through the filtering steps described above. Finally, where there were multiple SNPs per fragment, only a single SNP, the one with the highest MAF, was retained. For the demographic inference only, we used a dataset not filtered for MAF because minor alleles can be particularly informative for such simulations and filtering for MAF could bias the outcome of demographic inference.

F_{ST} outlier tests in BayeScan v2.1 (Foll and Gaggiotti, 2008) and OutFLANK (Whitlock and Lotterhos, 2015) were applied to identify and remove any SNPs putatively under selection in order to create an approximately neutral SNP dataset for subsequent analyses. BayeScan was run with the following parameters: number of threads 5000, thinning interval 10, number of pilot runs 20, length of pilot runs 5000, burn-in length 50000 and with prior odds 10 and 100 respectively. OutFLANK was run with parameters: LeftTrimFraction = 0.05, RightTrimFraction = 0.05, Hmin = 0.1 and qthreshold = 0.05.

Population genetic structure

In order to elucidate population genetic structure and identify populations for N_e estimations, we conducted an analysis of genetic structure using ADMIXTURE 1.3 (Alexander, Novembre and Lange, 2009; Alexander *et al.*, 2015). We used a range of population numbers ($K = 1-10$; a maximum of twice the number of sampled breeding colonies, to account for potential subdivision within colonies). We determined the value of K that best describes the data using ADMIXTURE's cross-validation (CV) procedure. The most likely number of K will exhibit the lowest CV error (Alexander *et al.*, 2015).

Test for a genetic bottleneck

We used the software DIYABC v2.1.0 (Cornuet, Pudlo, Veyssier *et al.*, 2014) that applies approximate Bayesian computation (ABC) to undertake coalescent simulations and thus infer the population history for the WA and SA Australian sea lion populations. DIYABC allows for the comparison of different historical scenarios involving population divergence, admixture and population size changes and the inference of demographic and historical parameters under the best-supported scenario. We tested whether the two Australian sea lion populations identified in our study had undergone a recent bottleneck, and if so what the magnitude of this bottleneck was. We, therefore, compared three scenarios of historical population size changes; (i) a bottleneck, (ii) an expansion through time and (iii) an equilibrium with no past variation in population sizes. For each scenario, N_e represents the contemporary population size, and for the scenarios

with past population size change, t is the time since population size has changed (in generations) and N_a and N_x correspond to ancestral population size for scenario (i) and (ii) respectively (see Fig. 2).

We used datasets of 42 samples from SA and 30 from WA with 1863 and 793 polymorphic SNPs, respectively, for SA and WA. We then simulated 5×10^6 genetic datasets under the three scenarios for the SA and WA populations respectively. Similarity between the simulated datasets and the real dataset is based on the default summary statistics proposed in DIYABC for a single population. Those summary statistics were used to select a scenario and to infer the parameter values under the best-supported scenario. As per default settings, the summary statistics represent mean values or variances over loci (single-sample; two-sample and three-sample statistics). For details about the computation of each statistic, see the DIYABC manual (<http://www1.montpellier.inra.fr/CBGP/diyabc/>).

After a few preliminary runs using the 'prior checking' option (see DIYABC manual for details), the prior distributions for all N_e and divergence time parameters were adjusted step by step, resulting in the parameters listed in Fig. 2. Uniform distributions were used for all parameters because the size of the sample and the limited number of loci used allow only rough estimation of all parameters (i.e. precision is only about an order of magnitude).

For all preliminary and final runs, we evaluated each analysis using a Bayesian equivalent of goodness of fit of the selected scenario, using the Bayesian 'model checking' option of DIYABC (Cornuet *et al.*, 2014; Cornuet, Ravigné and Estoup, 2010), see DIYABC manual section 2.10. In order for the model to be considered well fitted, the observed statistics had to fall within the distributions of simulated statistics. We simulated 16688 and 16658 datasets, respectively, for the WA and SA populations from the posterior distribution of parameters obtained under all scenarios to estimate these distributions. Principal component analysis applied on summary statistics was also used to visualize the fit between simulated and observed datasets. The three scenarios were compared using the logistic regression approach, and parameter estimation was performed for the scenario with the highest posterior probability.

Estimates of contemporary effective number of breeders (N_b) and effective population size (N_e)

Contemporary effective population sizes can also be estimated using linkage disequilibrium (LD) and sib-ship (Sib) methods. For the LD- N_e estimate, the effective number of breeders during a reproductive cycle, corrected for uneven contribution to the gene pool (N_b), was first estimated for both the SA and WA populations. We then conducted (i) a single-sample estimator approach applying the LD method (Waples and Do, 2008) of NeEstimator2 (Do, Waples, Peel *et al.*, 2014) for both populations. For the Sib- N_e estimate, we conducted (ii) a sib-ship reconstruction using genotype

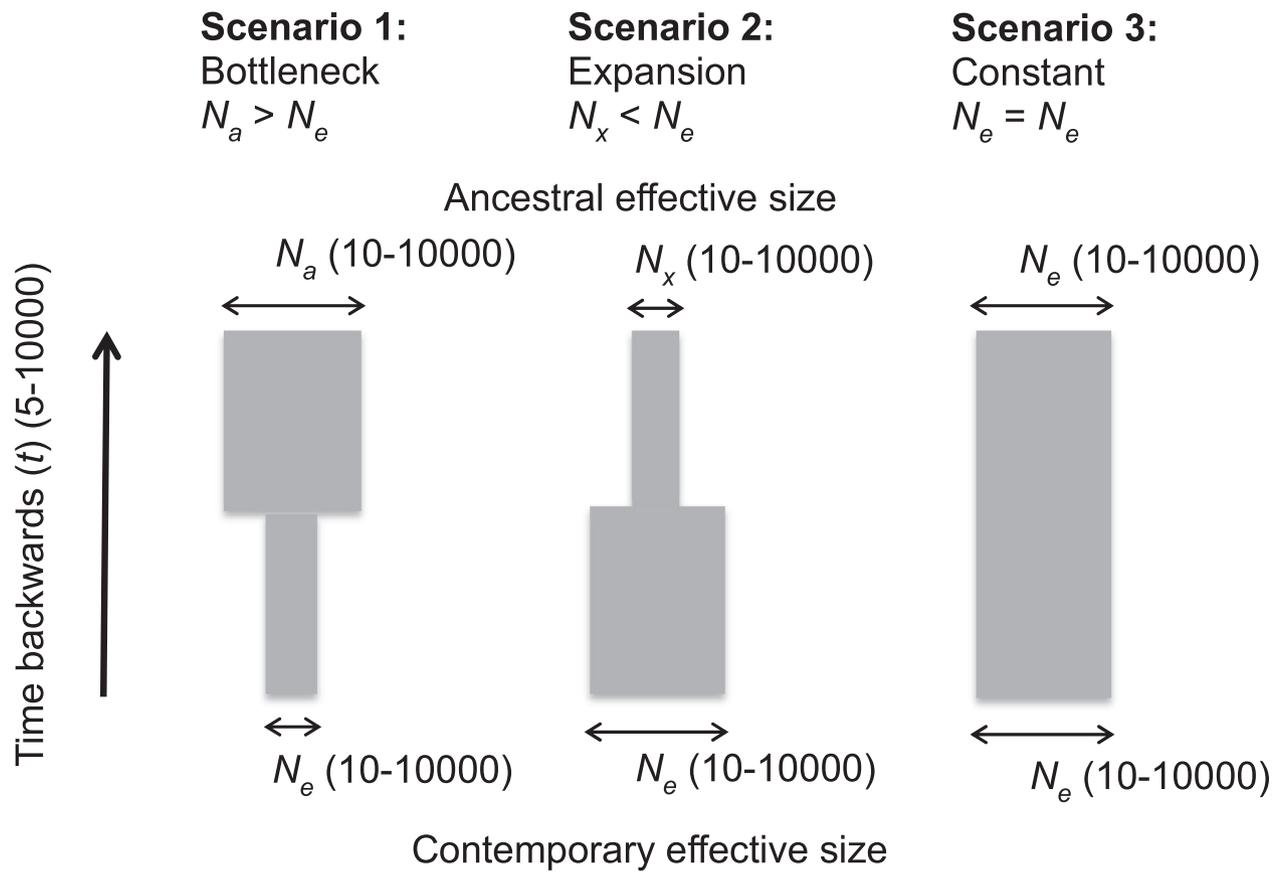


Figure 2 Demographic scenarios (bottleneck, expansion and constant effective size) tested with approximate Bayesian computation using DIYABC. Parameter values inferred in DIYABC: N_e = contemporary effective size, t = time of changed size (in generations), N_a and N_x = ancestral effective sizes, with $N_a > N_e$ (simulating a bottleneck model), $N_x < N_e$ (simulating an expansion model) and $N_e = N_e$ (simulating an equilibrium with no past variation in population sizes). The three scenarios are defined by relative changes in the effective size (N) at time (t) since present. Values of priors used are reported in parentheses.

data in COLONYv2 (Jones and Wang, 2010) for the SA and WA populations respectively. The high levels of female and male philopatry, and the isolation of Australian sea lion colonies, simplify the calculation of N_e , which can be biased by migration (Ryman, Laikre and Hössjer, 2019). Gene flow is more likely for the SA population given the close proximity of other colonies; however, of several single-sample methods used to estimate contemporary effective size, LD- N_e has been found to be the least influenced by migration (Ryman *et al.*, 2019).

NeEstimator2 includes an improved version of the LD- N_e algorithm of Waples and Do (2008) that accounts for missing data in the estimation of contemporary N_e (Peel, Waples, Macbeth *et al.*, 2013). Estimates were calculated assuming random mating and using lowest allele frequency cut-offs of 0, 0.1, 0.02 and 0.05. Waples and Do (2010) showed that thresholds of 0.05 for lowest allele frequencies generally lead to the least biased results.

A formula by Waples *et al.* (2014) was then used to correct for bias from overlapping generations, integrating two life-history parameters: adult life span (AL) and age at

maturity (α) (Table S2). A second formula by Waples *et al.* (2014) was used to derive the effective population size per generation ($N_{e(Adj2)}$) using $N_{b(Adj2)}$ and the same two life-history traits of AL and α . Finally, a third formula adopted from Waples *et al.* (2016) was applied that integrates the number of chromosomes for Otariids (for input parameters and formulas see Table S2). We adjusted for chromosome number because loci that are physically linked on a chromosome can result in a downwards bias of N_e and this adjustment reduces this bias (Waples *et al.*, 2016).

For the N_e estimates using sib-ship reconstruction in COLONYv2, we undertook three replicate runs in non-GUI mode using the full likelihood method and assuming random mating (for input parameters see Table S3).

To test whether the sample sizes and the number of SNPs used had sufficient statistical power to produce reliable estimates of N_e , we performed a power analysis in NeOGen (Blower, Riginos and Ovenden, 2019). NeOGen's overlapping generations model uses the underlying population simulator SIMUPOP (Peng and Kimmel, 2005). For detailed input parameters of the power analysis see Table S4.

Predictive modelling: loss of genetic variation over future generations

Future loss of genetic variation, over the next 260 years (18.4 generations; generation length of 14.1 years; Goldsworthy *et al.*, 2021), was simulated using BottleSim V2.6 (Kuo and Janzen, 2003) for the two genetic populations. We used the adjusted $N_{e(Adj3)}$ -LD estimates from WA and SA for the simulations. We also included $N_e = 50$ into the simulations for both populations because it has been regarded as a threshold number below which the effects of inbreeding depression are likely (Frankham, Bradshaw and Brook, 2014). To assess whether the current N_e would lead to a loss of genetic variation and an increase in inbreeding, we undertook the simulations with (i) keeping N_e stable throughout the simulation; and (ii) decreasing the N_e over generations to incorporate several decline scenarios. The latter included a range of plausible scenarios informed by current and future threats to Australian sea lions across their range. Rather than being absolute measures of decline in N_e , these scenarios were primarily designed to investigate the potential effect of different types of threats on the levels of future genetic variation.

The modelled decline scenarios included: (i) a 1.5% decline per year observed for Australian sea lion colonies on average across their distribution. The current IUCN listing of *Endangered* is based on a global assessment of the species (data were available for 30 subpopulations, accounting for ~75% of the species-wide pup production; IUCN; Goldsworthy, 2015, Goldsworthy *et al.*, 2021). The data showed that total pup production declined by 63% in three generations (Goldsworthy *et al.*, 2021), which is equivalent to a decline of 1.5%/year. We simulated an exponential decay by calculating a 1.5% reduction in N_e at each iteration (i.e. the decline becomes smaller as the N_e decreased). In pinnipeds, dynamics of N_e co-vary strongly with N_c (Peart, Tusso, Pophaly *et al.*, 2020) and we, therefore, expect to see a decline in N_e following the 1.5% decline in N_c that has occurred in SA over the past decades; (ii) four hypothetical scenarios resulting from disease outbreaks were based on declines seen with disease die-offs in other pinniped species (see Duignan *et al.*, 2014). In these scenarios, we simulated a one-off loss of individuals in year 1, losing 20%, 40%, 60% and 80% of the N_e followed by a 1.5%/year decline of the remaining N_e thereafter and (iii) a disturbance scenario based on ongoing chronic losses of individuals to the population (e.g. reaching bycatch trigger limits in the demersal gillnet fishery, or a combination of fisheries bycatch and other disturbances including entanglement in marine debris and currently unknown additional threats). For scenario iii, we simulated a decline of two individuals per year for the SA population. This decline was estimated from the currently implemented gillnet fishery management trigger limits and adding cryptic mortality from other sources at a low but constant rate. We assumed that mortality occurred for breeding adults (potentially reducing the N_e). Since most of the recent population decline was observed off southern Australia (Goldsworthy *et al.*, 2020, 2021), we did not simulate this scenario for the breeding colonies off the western coast.

Model parameters of all simulations were kept consistent to allow output comparisons: reproduction mode = dioecy with random mating; simulation module = diploid, multilocus, constant/decreasing population size; longevity of organism = 24; age at sexual maturity = 5; sex ratio = 1:1; generation overlap = maximum overlap; number of years = 260 (18.4 generations) and number of iterations = 1000.

Results

We retained 2238 genome-wide SNPs after filtering (see Table S1 for details on filtering steps and number of SNPs retained). The F_{ST} outlier tests identified one SNP putatively under selection using BayeScan and no SNPs using OutFLANK (data not shown). To produce a neutral dataset for subsequent analyses, we checked for differences in results including and excluding the identified SNP. Since results did not differ, we included the SNP (potentially a false positive) and treated the entire dataset of 2238 SNPs as neutral.

Clustering analysis

Clustering analyses in ADMIXTURE for the five sampling locations revealed two likely clusters with no further subdivisions: the WA population (North Fisherman Island and Beagle Island) and the SA population (Lilliput Island, Blefuscu Island and Olive Island) (Figure S1). The maximum distance among colonies in both populations was 50 km. When analyzing the SA and WA populations independently by removing the other population, the clustering analyses did not suggest any further subdivision (the most likely number of populations was $K = 1$ respectively). When plotting graphs for $K > 1$, some of the individuals showed different admixture proportions, although no clear pattern of subdivision into breeding colonies was observed (data not shown).

The results were in agreement with those from previous microsatellite and mtDNA control region sequences of Australian sea lions that covered a larger region off the WA and SA coasts and used larger sample sizes (Ahonen *et al.*, 2016; Campbell *et al.*, 2008; Lowther *et al.*, 2012).

Genetic bottleneck test

Analysis with DIYABC supported the presence of a genetic bottleneck for both the WA and SA populations. From our three competing scenarios, scenario (i) in which the population has experienced a bottleneck was strongly supported with a posterior probability of 1 (CI95 = [1, 1]), whereas scenarios (ii) of expansion and (iii) of equilibrium had no support (posterior probability of 0 with CI95 = [0,0], of 0 with CI95 = [0, 0] respectively). Principal component analysis applied on summary statistics revealed a good fit between simulated and observed datasets for the WA and SA population respectively (see Figure S2a,b). Given this result, we then inferred all parameter posterior distributions under the first scenario only, thus considering past reduction of population size. The estimated time of bottleneck was 65

Table 1 Effective number of breeders (N_b) for clusters of Australian sea lion colonies (*Neophoca cinerea*) from Western Australia ($n = 30$) and South Australia ($n = 42$)

Lowest allele frequency used	0.1	0.05	0.02	0.01	0+
Western Australia					
Beagle and North Fishermen Island					
Estimated N_b	96.1	<u>105.3</u>	124.5	138.8	138.8
Lower 95% CI	86.3	<u>95.9</u>	113.1	125.7	125.7
Higher 95% CI	108.3	<u>116.6</u>	138.2	154.8	154.8
South Australia					
Blefuscus, Lilliput and Olive Island					
Estimated N_b	245	<u>279.2</u>	281.3	298.7	298.7
Lower 95% CI	226.6	<u>260.5</u>	266.1	282.6	282.6
Higher 95% CI	266.5	<u>300.7</u>	298.1	316.7	316.7

N_b was estimated using the Linkage Disequilibrium method in NeEstimator including parametric 95% confidence intervals (CIs). Estimates that are underlined were used to derive N_e and adjusted values of N_e .

Table 2 Estimates of the effective number of breeders (N_b), effective population size (N_e) and adjusted N_e values for Australian sea lions (*Neophoca cinerea*) from Western Australia ($n = 30$) and South Australia ($n = 42$)

	Western Australia		South Australia		
	LCI	UCI	Blefuscus, Lilliput and Olive islands	LCI	UCI
N_b					
105	96	117	279	261	301
N_b (Adj2) -B					
110	100	122	292	273	315
N_e (Adj2) -B					
117	107	130	310	290	335
N_e (Adj3) -B					
160	146	178	424	397	458

LCI, lower 95% confidence interval (CI); UCI, upper 95% CI. Adjustments were undertaken with maximum age = 24 years and age at first maturity (females) = 5 years.

generations (23–409) ago for the WA population and 104 generations (31–357) ago for the SA population. The estimated strength of the bottleneck for both populations was a past population reduction of about 10-fold ($N_{SA} = 7530$ (2890–9920), $N_{SA} = 755$ (196–1430) and $N_{WA} = 7310$ (2640–9990), $N_{WA} = 673$ (146–1420)).

Contemporary effective population size

Estimates of contemporary effective numbers of breeders (N_b) using NeEstimator2 and using the minimum allele frequency of 0.05 resulted in estimates for the WA population of $N_b = 105$ (95% CI 96–117) and the SA population of $N_b = 279$ (95% CI 261–301) (see Tables 1 and 2). Adjustments of N_b and conversion to N_e with subsequent adjustments using 24 years for maximum age and 5 years for age

at first maturity for Australian sea lions (derived from Goldsworthy *et al.*, 2020, McIntosh, 2007) led to final values of $N_{e(Adj3)} = 160$ (95% CI 146–178) for the WA population and $N_{e(Adj3)} = 424$ (95% CI 397–458) for the SA population (see Table 2).

N_e estimates using the sib-ship method in COLONYv2 resulted in $N_e = 249$ (136–1018) for the WA population and $N_e = 298$ (177–856) for the SA population, when not assuming inbreeding. When assuming inbreeding, estimates were $N_e = 290$ (149–2757) for the WA population and $N_e = 410$ (228–1534) for the SA population. Estimates with NeEstimator (LD method), therefore, fell within the 95% CIs of the sib-ship estimates, but the sib-ship estimates resulted in substantially larger CIs than those of the LD estimates.

Power analyses with NeOGen revealed that sample sizes ($n_{WA} = 30$, $n_{SA} = 42$) combined with the number of SNP loci used ($n = 2238$) resulted in sufficient statistical power to reliably estimate LD- N_e . Furthermore, the N_e estimates from NeEstimator for the sample-loci combinations we used fell within the confidence intervals of the N_e estimates from NeOGen (see Figure S3).

Altogether, contemporary N_e was estimated with three approaches: ABC- N_e , LD- N_e and Sib- N_e . The estimated LD- N_e had the smallest confidence intervals of these three estimates and fell within the larger confidence intervals of the ABC- N_e and Sib- N_e estimates for WA and SA respectively. We, therefore, used contemporary LD- N_e only for subsequent modelling of genetic diversity and inbreeding over future generations. Ancestral effective population size estimates (ABC- N_a) were around 10-fold larger for WA and SA respectively.

Simulated losses of genetic variation and increase in inbreeding

Our forward simulations of losses of genetic variation and increase in inbreeding over the next 260 years, using the LD- $N_{e(Adj3)}$ estimates and simulating both constant (Fig. 3) and decreasing N_e scenarios, resulted in a loss of genetic variability and increase in inbreeding in each scenario (Fig. 4a,b). However, the magnitude of the loss of genetic variation and increase in inbreeding differed between the SA and WA populations and across the different scenarios. For simulations with constant N_e , a higher loss of genetic variation and increase in inbreeding over the next 260 years were seen for the WA population compared to the SA one (Table 3). The initial inbreeding coefficient (F) was higher for the WA (0.645 SD \pm 0.0101) than the SA (0.168 SD \pm 0.0079) population, but the magnitude of increase in inbreeding over the 260 years was lower for the WA (108.63%) compared to the SA (131.32%) population (Table 3, Fig. 3).

Overall, simulations with decreasing N_e estimates (different threat scenarios) lead to a considerably higher loss of genetic variation and increase in inbreeding over future generations compared to simulations with consistent N_e (Table 3, Fig. 4a,b). In the modelled disease outbreak scenarios, genetic variation was gradually lost and inbreeding

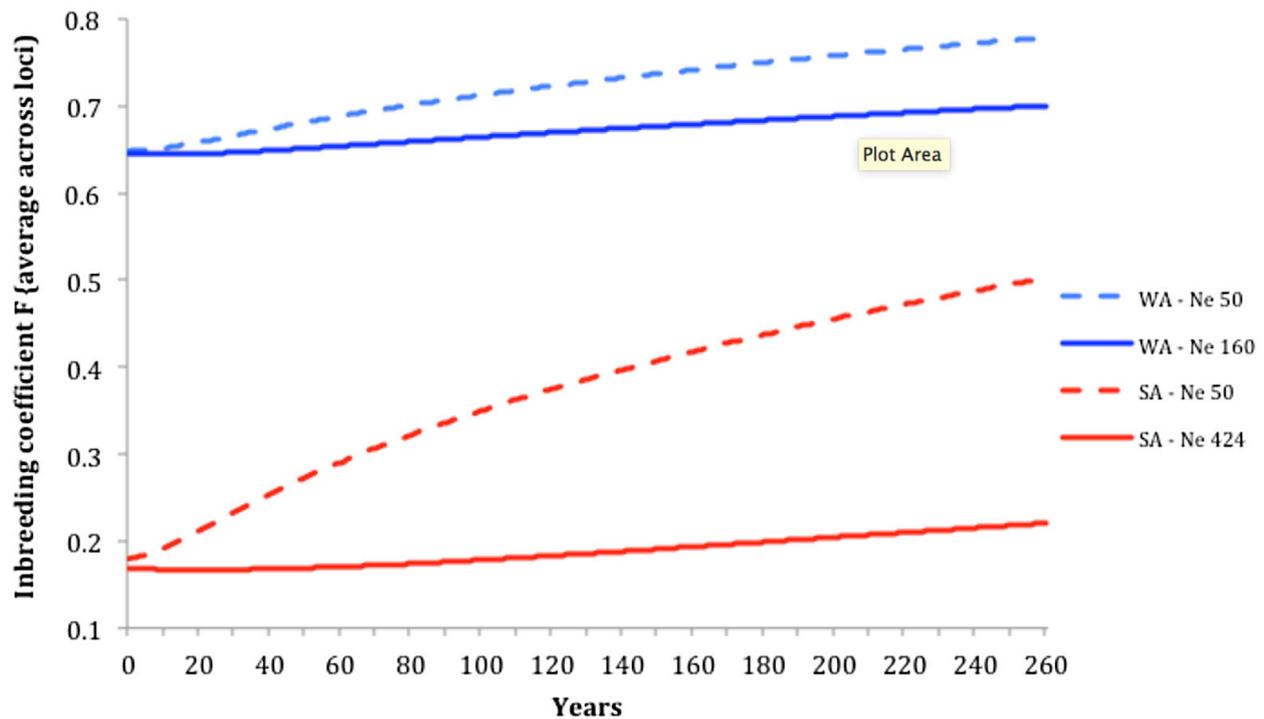


Figure 3 Projected increase in inbreeding (F) for Australian sea lions (*Neophoca cinerea*) in the South Australian ($N_e = 424$) and Western Australian ($N_e = 160$) population over the next 260 years (generation time of 14.1 years), including the hypothetical value of $N_e = 50$ (Frankham *et al.* 2014). N_e was kept constant over the 260 years.

increased over future generations in each scenario. For example, the magnitude of increase in inbreeding differed depending on the 20%, 40%, 60% or 80% disease scenarios and was higher for the WA compared to the SA population (Table 3). The model based on an 80% disease epidemic in the WA populations lead to a N_e with zero females (assuming equal sex ratios; $N_e < 2$) after 230 years (16.3 generations; generation length 14.1 years; Fig. 4b). Similarly, for the SA population, the cumulative scenario with two individuals lost per year lead to a decline of $N_e < 2$ after 211 years (15.0 generations; generation length 14.1 years; Table 3, Fig. 4a).

Overall, the starting level of inbreeding and predicted increase in inbreeding over 260 years was higher for the WA than the SA population, while the magnitude of increase was higher for the SA population (Fig. 3). When comparing all modelled declines scenarios, the disease epidemic scenarios resulted in the highest increases in inbreeding over future generations, in both populations, followed by the cumulative scenario of bycatch and other threats simulated for the SA population only (Fig. 4a,b).

Discussion

Our analysis of the endangered Australian sea lion from two populations off the west coast of WA and off SA revealed that both populations experienced a genetic bottleneck. The resulting contemporary effective population sizes are likely

too small to offset the erosion of genetic variation. Forward simulations showed that only modest declines in genetic variation are expected if the population sizes were to be maintained at their current sizes, yet they continue to decline (Goldsworthy *et al.*, 2017), increasing the rate at which genetic variation is lost. As expected, the greatest impacts on genetic variation and inbreeding were predicted when large, rapid initial population declines were simulated, as anticipated from a major disease outbreak. Ongoing cumulative losses from fisheries bycatch also had a considerable impact on genetic variation and inbreeding. Initial bycatch rates, however, were reduced from an estimated 16 adult females/year between 2006 and 2009 for the SA population (Olive, Lilliput and Blefuscu Islands combined; Goldsworthy *et al.* 2010) to a maximum allowed bycatch of two individuals/year post-2010 (Goldsworthy *et al.*, 2021). Modelled post-2010 maximum allowed bycatch rates show that even low chronic losses of individuals likely impact future genetic variation and inbreeding. The model also highlights the importance that this significant threat was managed because the loss of genetic variation and inbreeding would otherwise be exacerbated. There are concerns about the impacts of the WA gillnet fishery on Australian sea lion bycatch off the western coast of WA where currently less restrictive management measures and no verified onboard monitoring of bycatch take place.

The Australian sea lion has experienced a major decline in numbers as well as range contraction over the last few

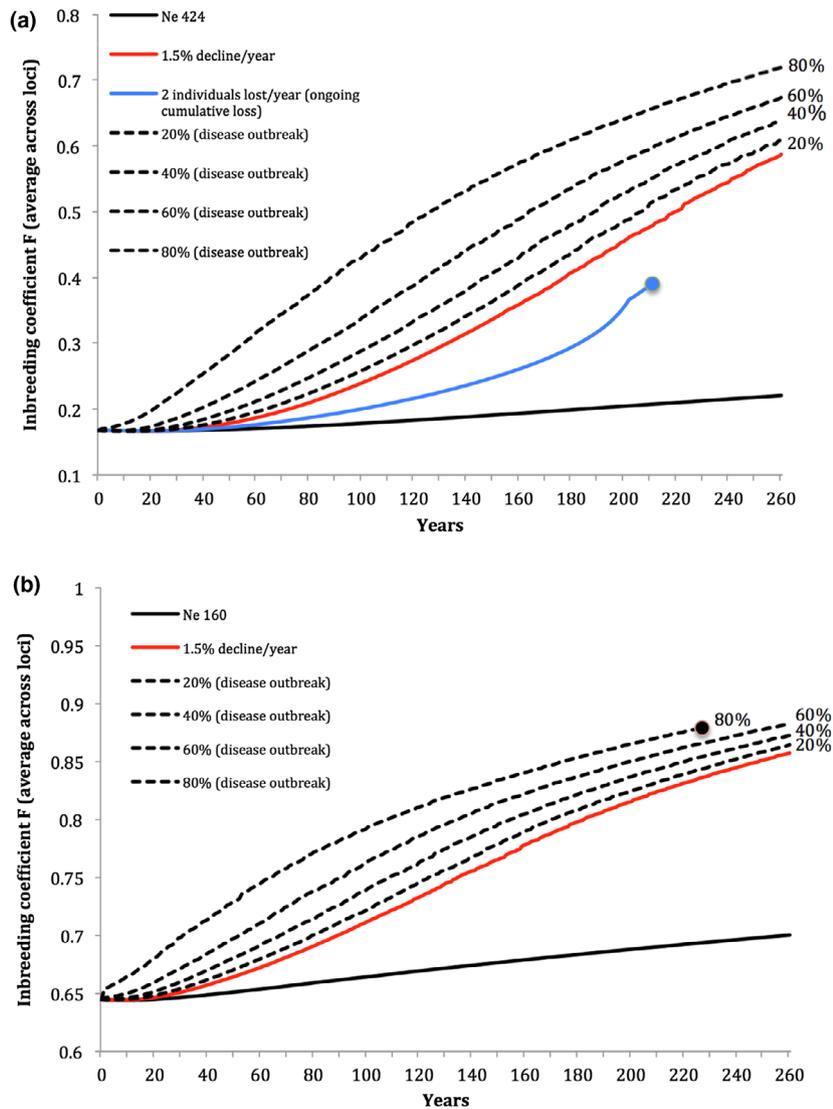


Figure 4 (a) Projected increase in inbreeding (F) for Australian sea lions (*Neophoca cinerea*) in the South Australian population ($N_e = 424$; Blefuscu, Lilliput and Olive Islands) over the next 260 years (generation time of 14.1 years) for several decline scenarios of N_e : 1.5% decline/year (IUCN listing); two individuals lost/year based on ongoing cumulative loss (e.g. fisheries bycatch, entanglement in marine debris and other unknown factors) and four hypothetical disease outbreak scenarios in which 20%, 40%, 60% and 80% of the N_e were lost in the first year with a decrease in N_e of 1.5% for each year thereafter. The round dot denotes the point of the projection at which the number of breeding females reaches 0, assuming equal sex ratios for the N_e . (b) Projected increase in inbreeding (F) for Australian sea lions (*Neophoca cinerea*) in the Western Australian population ($N_e = 160$; North Fisherman and Beagle Islands) over the next 260 years (generation time of 14.1 years) for several decline scenarios of N_e : 1.5% decline/year (IUCN listing); and four hypothetical disease outbreak scenarios in which 20%, 40%, 60% and 80% of the N_e were lost in the first year with a decrease in N_e of 1.5% for each year thereafter. The round dot denotes the point of the projection at which the number of breeding females reaches 0, assuming equal sex ratios for the N_e .

centuries (Gales *et al.*, 1994). Our estimates of N_e for the two populations that consisted of five breeding colonies are considerably lower than the expected N_e for these colonies (N_e -WA: 300–400 for Beagle/North Fisherman Islands; and N_e -SA: 600–800 for Olive/Lilliput and Blefuscu Islands, derived from pup counts with multipliers; Goldsworthy *et al.*, 2021). This is not unusual, especially given the reproductive skew inherent in the polygynous mating system of this species where some males contribute more offspring

than others (Ahonen *et al.*, 2016; Lee *et al.*, 2011; Nunney, 1993; Stoffel *et al.*, 2018). These life-history traits may increase susceptibility to the loss of genetic variation from a population bottleneck. The decline for the species is observed range wide but is generally higher for SA populations, but this may reflect a bias in sampling (Goldsworthy *et al.*, 2021). Although only a subset of colonies was included in this study, our conclusions are likely representative for the remaining colonies.

Table 3 Simulated loss of genetic diversity in Australian sea lions (*Neophoca cinerea*) in the Western Australian and South Australian populations over 260 years (18.4 generations), including standard error (SE), using constant values for effective population size (N_e) and scenarios with declining N_e

Location and scenario simulated	N_e	Year	H_0 Average (SE)	H_0 %	H_E Average (SD)	H_E %	OA Average (SD)	OA %	F Average	F %
Constant N_e										
South Australia (SA)	424	0	0.208 (0.0037)	100.00	0.208 (0.0036)	100.00	1.831 (0.0079)	100.00	0.168 (0.0079)	100.00
		260	0.202 (0.0035)	96.79	0.201 (0.0035)	96.59	1.777 (0.0078)	97.05	0.221 (0.0078)	131.32
Western Australia (WA)	160	0	0.093 (0.0033)	100.00	0.092 (0.0033)	100.00	1.354 (0.0101)	100.00	0.645 (0.0101)	100.00
		260	0.085 (0.0030)	91.80	0.084 (0.0030)	91.34	1.297 (0.0089)	95.80	0.700 (0.0090)	108.63
Hypothetical value (SA) (Frankham <i>et al.</i> 2014)	50	0	0.209 (0.0037)	100.00	0.206 (0.0036)	100.00	1.812 (0.0078)	100.00	0.180 (0.0079)	100.00
		260	0.159 (0.0028)	76.38	0.155 (0.0027)	75.16	1.485 (0.0074)	81.96	0.503 (0.0076)	279.77
Hypothetical value (WA) (Frankham <i>et al.</i> 2014)	50	0	0.093 (0.0033)	100.00	0.092 (0.0032)	100.00	1.812 (0.0078)	100.00	0.648 (0.0101)	100.00
		260	0.071 (0.0025)	76.46	0.069 (0.0024)	96.61	1.485 (0.0074)	81.96	0.778 (0.0073)	120.12
Declining N_e										
South Australia										
1.5% decline per year (IUCN listing)	424	0	0.208 (0.0037)	100.00	0.208 (0.0036)	100.00	1.831 (0.0079)	100.00	0.168 (0.0079)	100.00
		260	0.139 (0.0024)	66.67	0.129 (0.0023)	62.12	1.387 (0.0064)	75.72	0.587 (0.0069)	349.41
20% decline in year 1 + 1.5% IUCN (disease outbreak)	424	0	0.208 (0.0037)	100.00	0.208 (0.0036)	100.00	1.831 (0.0079)	100.00	0.168 (0.0079)	100.00
		260	0.132 (0.0023)	63.53	0.123 (0.0022)	59.29	1.367 (0.0062)	74.67	0.608 (0.0066)	362.07
40% decline in year 1 + 1.5% IUCN (disease outbreak)	424	0	0.208 (0.0037)	100.00	0.208 (0.0036)	100.00	1.831 (0.0079)	100.00	0.168 (0.0079)	100.00
		260	0.123 (0.0022)	58.94	0.114 (0.0020)	54.89	1.338 (0.0058)	73.09	0.638 (0.0062)	380.10
60% decline in year 1 + 1.5% IUCN (disease outbreak)	424	0	0.208 (0.0037)	100.00	0.208 (0.0036)	100.00	1.831 (0.0079)	100.00	0.168 (0.0079)	100.00
		260	0.111 (0.0020)	53.47	0.104 (0.0018)	49.80	1.305 (0.0053)	71.28	0.674 (0.0056)	401.18
80% decline in year 1 + 1.5% IUCN (disease outbreak)	424	0	0.208 (0.0037)	100.00	0.208 (0.0036)	100.00	1.831 (0.0079)	100.00	0.168 (0.0079)	100.00
		260	0.096 (0.0017)	46.27	0.090 (0.0016)	43.13	1.263 (0.0046)	68.97	0.719 (0.0049)	428.13
2 individuals lost per year (ongoing cumulative loss)	424	0	0.208 (0.0037)	100.00	0.208 (0.0036)	100.00	1.831 (0.0079)	100.00	0.168 (0.0079)	100.00
		211	0.188 (0.0033)	90.12	0.177 (0.0031)	84.89	1.576 (0.0078)	86.09	0.391 (0.0083)	232.53
Western Australia										
1.5% decline per year (IUCN listing)	160	0	0.093 (0.0033)	100.00	0.092 (0.0033)	100.00	1.354 (0.0101)	100.00	0.645 (0.0101)	100.00
		260	0.049 (0.0017)	52.71	0.045 (0.0016)	49.18	1.134 (0.0047)	83.71	0.857 (0.0050)	132.96
20% decline in year 1 + 1.5% IUCN (disease outbreak)	160	0	0.093 (0.0033)	100.00	0.092 (0.0033)	100.00	1.354 (0.0101)	100.00	0.645 (0.0101)	100.00
		260	0.047 (0.0016)	50.22	0.043 (0.0015)	46.89	1.127 (0.0045)	83.22	0.864 (0.0048)	134.04
40% decline in year 1 + 1.5% IUCN (disease outbreak)	160	0	0.093 (0.0033)	100.00	0.092 (0.0033)	100.00	1.354 (0.0101)	100.00	0.645 (0.0101)	100.00
		260	0.044 (0.0015)	47.17	0.041 (0.0014)	44.03	1.119 (0.0042)	82.65	0.872 (0.0045)	135.32
60% decline in year 1 + 1.5% IUCN (disease outbreak)	160	0	0.093 (0.0033)	100.00	0.092 (0.0033)	100.00	1.354 (0.0101)	100.00	0.645 (0.0101)	100.00
		260	0.041 (0.0014)	43.65	0.038 (0.0013)	40.75	1.110 (0.0039)	81.98	0.882 (0.0041)	136.85
80% decline in year 1 + 1.5% IUCN (disease outbreak)	160	0	0.093 (0.0033)	100.00	0.092 (0.0033)	100.00	1.354 (0.0101)	100.00	0.645 (0.0101)	100.00
		230	0.041 (0.0014)	44.28	0.038 (0.0013)	41.36	1.112 (0.0039)	82.10	0.880 (0.0037)	136.59

H_0 , observed heterozygosity; H_E , expected heterozygosity; OA, observed number of alleles; and F, inbreeding coefficient.

In cases where simulations were run over less than 260 years (18.4 generations), the last full year before the number of breeding females reached 0 was displayed in bold, assuming equal sex ratios (211 and 230 years respectively).

Our estimates of contemporary N_e using three methods (ABC- N_e , LD- N_e and Sib- N_e) for both the WA and the SA populations are below 1000, the N_e recommended to maintain evolutionary potential (Frankham, 2015). It has been suggested that maintaining $N_e > 100$ and retaining 80% or more of genetic variation will minimize inbreeding, and the IUCN Red List criteria derived from genetic considerations aim at maintaining a N_e that will allow retaining 90% of the initial heterozygosity for 100 years (Frankham, 2015; Gilpin and Soulé, 1986). We show that the Australian sea lion likely experienced a genetic bottleneck, and the relatively large confidence intervals around the parameter estimates are attributed to the relatively low number of polymorphic SNPs in the dataset of each population. The DIYABC simulations only use discrete generations; therefore, when overlapping generations apply, extrapolating genetic bottleneck timing in years is not reliable. However, the bottlenecks in the WA and SA populations are most likely linked to the colonial sealing era and the take of individuals by shipwrecked people. Between 1792 and 1849, an estimated minimum of 1 million seal (*Arctocephalus* spp.) and sea lions (*Neophoca cinerea* and *Phocarcos hookeri*) were harvested around Australia's southern coast, New Zealand and at the adjacent subantarctic islands, but the total harvest probably exceeded 1.5 million seals (Ling, 1999; Richards, 1994). Off Western Australia, Australian sea lions were also taken by shipwrecked sailors in the 17th and 18th centuries with only a few animals remaining in some colonies (Gales, Cheal, Pobar *et al.*, 1992).

In pinnipeds, human exploitation has resulted in genetic bottlenecks in approximately a third of species, with the first two of the three most heavily bottlenecked species (Mediterranean monk seal, *Monachus monachus*, Hawaiian monk seals, *Neomonachus schauinslandi* and northern elephant seal, *Mirounga angustirostris*) currently listed as endangered (Stoffel *et al.*, 2018). Although genetic variation was 21% lower in species of conservation concern, a clear relationship between conservation status and bottleneck strength was not evident (Stoffel *et al.*, 2018). While demographic recovery is possible with low levels of genetic variation, as seen with the northern elephant seal, this may simply reflect isolation and a fortuitous lack of exposure to contemporary threatening processes (Abadía-Cardoso *et al.*, 2017). The importance of maintaining genetic variation has also recently been exemplified by the Antarctic fur seal (*Arctocephalus gazella*) where females of low heterozygosity fail to breed against a background of three decades of declining heterozygosity attributed to climate change (Forcada and Hoffman, 2014). In this example, low homozygote pup survival leads to purging of strongly deleterious recessive mutations and increased heterozygosity over time (Forcada and Hoffman, 2014). The relevance of genetic variation to survival is further highlighted by recent calls for more genetic data to be integrated into the IUCN listing criteria (Frankham, 2015; Hunter, Hoban, Bruford *et al.*, 2018).

Reduced genetic variation is associated with increased risk of extinction in many taxa (Spielman *et al.*, 2004). The level of genetic variation observed in our dataset is difficult to

compare with datasets based on other genetic markers, such as microsatellites (e.g. Ahrens, Rymer, Stow *et al.*, 2018). However, the level of genetic variation measured in the Australian sea lion at microsatellite loci is comparable to microsatellite data collected for other highly bottlenecked and endangered pinnipeds. At colonies, including those evaluated here, the Australian sea lion exhibits allelic richness (Ar) and expected heterozygosity (He) ranging from 2.3 to 3.6 and 0.36 to 0.65 respectively (Ahonen *et al.*, 2016). These data compare with Ar and He data from the northern elephant seal, Mediterranean and Hawaiian monk seals, where collectively their Ar and He ranged 2.2–2.7 and 0.39–0.46 respectively (Stoffel *et al.*, 2018).

The Australian sea lion is listed as *Endangered* under the *IUCN Red List of Threatened Species* and is protected by Australian law. Mortality from fishery interactions was identified as a key threatening process and in South Australia this resulted in the successful implementation of management interventions between 2010 and 2012. Prior to this, the range-wide Australian sea lion pup production declined by approximately 63% over three generations, resulting in the Australian sea lion being classified as *Endangered* (Goldsworthy *et al.*, 2021). The estimated loss from fisheries interactions in South Australia was 374 individuals per breeding cycle (18 months; Gales *et al.*, 1994; Goldsworthy *et al.*, 2010), but new management interventions have reduced this bycatch (Australian Government, 2013). These interventions are likely to have minimized the rate at which genetic variation is lost. Our projections, using the population declines of 1.5%/year, markedly increased the loss of genetic variation and increased the level of inbreeding compared to simulations with a stable N_e . Although the introduction of trigger limits in gillnet fisheries off South Australia and sea lion exclusion devices in the Western and South Australian rock lobster fisheries have greatly reduced sea lion bycatch, our data show that even under current fisheries management practices the allowed levels of bycatch combined with other cumulative impacts will likely result in an erosion of genetic variation in the SA population (Lilliput, Blefuscu and Olive Islands). Such additional cumulative impacts include entanglements, climate change and other factors that are currently not well understood. These declines may, therefore, be underestimated because of the potential for under-reported bycatch (cryptic mortality) and currently unquantified threatening processes.

Infectious disease including morbillivirus, mycobacteriosis and internal parasite infections have been a major source of mortality in pinnipeds. The impact of infectious disease can be catastrophic, for example, morbillivirus can infect up to 95% of individuals and result in mortalities of 40–60% in seal colonies (Heide-Jorgensen and Harkonen, 1992; Kennedy, Kuiken, Jepson *et al.*, 2000; Klepac, Pomeroy, Bjørnstad *et al.*, 2009). Currently hookworm infestation is a major source of mortality in Australian sea lion pups (Marcus, Higgins and Gray, 2014). Additional disease outbreaks could be especially problematic for the Australian sea lion because isolation and limited exposure to pathogens have resulted in immunological naivety. Furthermore, the Major

Histocompatibility Complex (MHC), a section of the genome associated with immunity (Sommer, 2005), may be of low diversity in the Australian sea lion (MHC class II; Lau, Chow, Gray *et al.*, 2015). Lower MHC diversity and, more generally, low levels of genome-wide heterozygosity have been associated with greater risk of infection and disease load (e.g. Hoffman *et al.*, 2014).

Our simulations with a range of mortality rates suggest that infectious disease poses a major threat to genetic variation. Specifically, we simulated scenarios with 20–80% disease-related mortalities within the first year, values that are comparable to proportions of pinniped mass die-offs elsewhere (Duignan *et al.*, 2014). The genetic erosion detected after such population declines could potentially contribute to an extinction vortex (Gilpin and Soulé, 1986), especially for the high mortality scenarios. Reduced genetic variation in small populations can lead to an accumulation of deleterious alleles and reduced fitness (Frankham, 2005), and future research should test for evidence of inbreeding depression and the accumulation of deleterious alleles in Australian sea lion breeding colonies. In addition to maintaining genetic variation, managing disease risk includes strategies such as quarantine areas, feral pest management, disease management interventions and potentially genetic rescue (Frankham, 2015).

To manage the threatening processes impacting wildlife populations, forward simulations based on contemporary estimates of N_e can be used to evaluate the consequences of a range of scenarios. For example, the success of enhancing genetic variation by introducing individuals from other populations (genetic rescue) has been demonstrated in a variety of species (Frankham, 2015; Ralls, Sunnucks, Lacy *et al.*, 2020). The use of simulations showed that the fitness benefits of genetic rescue increase the probability of persistence, and monitoring the level of migrant ancestry and heterozygosity provide better indicators of the long-term benefits (Robinson, Bell, Dhendup *et al.*, 2020). However, the cost of long-term monitoring of genetic-based metrics precludes this management approach for most wildlife populations. Predictions based on well-informed simulations provide an efficient and cost-effective alternative (Ralls *et al.* 2020; Robinson *et al.* 2020).

In addition to estimating the benefits from genetic rescue, simulations can be applied to assess the relative impact of disturbances to genetic variation within wildlife populations. These include predicting direct anthropogenic impact, such as poaching or the implications of wild harvest to genetic variation and evolutionary change (Dunlop, Eikeset and Stenseth, 2015). This is especially relevant in marine environments where a meta-analysis of 140 marine fish species revealed lower genetic diversity in overfished populations (Pinsky and Palumbi, 2014). These approaches can also be applied to assess the outcome of low probability but high impact events, exemplified by infectious disease epidemics, for which social organisms are at heightened risk (Stow and Beattie, 2008). These predictions can then inform decisions on when interventions, such as prophylactic vaccination and quarantine programs should be used (e.g. Hawaiian monk

seal, *Neomonachus schauinslandi*, Baker, Harting, Barbieri *et al.*, 2017).

The rapid development of genomic techniques has coincided with the availability of increasingly refined modelling tools. In particular, individually based, spatially explicit eco-evolutionary models make use of data from a range of disciplines that traditionally worked in isolation, and broaden the scope of their application to conservation (Armansin, Stow, Cantor *et al.*, 2020; Schumaker and Brookes, 2018). These developments are timely given the limited resources available for conservation (Frankham *et al.*, 2010; Robinson *et al.*, 2020) and the poor performance of surrogates of genetic diversity (Hanson, Verissimo, Velo-Antón *et al.*, 2020).

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. (a) Cross-validation (CV) plot of values $K = 1-10$ for Australian sea lions (*Neophoca cinerea*) from five breeding colonies off Western and South Australia showing that the most likely number of populations for the data is $K = 2$; and (b) ADMIXTURE barplot showing the ancestry values for the most likely number of clusters of the dataset ($K = 2$).

Figure S2. (a) Western Australian (WA) sea lion population: plot of principal component analysis applied on summary statistics to visualize the fit between simulated (green) and observed (yellow) datasets for the in DIYABC inferred scenario 1 (bottleneck scenario). (b) South Australian (SA) sea lion population: plot of principal component analysis

applied on summary statistics to visualize the fit between simulated (green) and observed (yellow) datasets for the in DIYABC inferred scenario 1 (bottleneck scenario).

Figure S3. Power analysis sampling strategy plots with user specified sample locus combinations for Australian sea lions (*Neophoca cinerea*) of the (a) Western Australian population, and (b) South Australian population.

Table S1. Number of SNPs for Australian sea lions (*Neophoca cinerea*) before and after filtering, and number of SNPs that failed at each filtering step.

Table S2. Estimates of $N_{b(Adj2)}$, $N_{e(Adj2)}$ and $N_{e(Adj3)}$ using adjustments from Waples *et al.* (2014) and Waples *et al.* (2016).

Table S3. Input parameters for N_e estimates using the sibship reconstruction method in COLONYv2 (full likelihood method).

Table S4. Power analysis – NeOGen input parameters: life history – maximum possible age 26 years; maximum possible mating age 23 years (reduced likelihood of breeding as female approaches this age); minimum mating age 5 years (female age 6 at birth of first pup), litter size 1.