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**PNAS**

Molecular Ecology (6.2)

Title:

**xxx**

Short title:

xxx

K. Acevedo-Whitehouse 2

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L.W. Andersen 4

L. Chilvers ?

5

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N. Gemmell 7

S. Negro ?

H. Nichols X

A. Osborne ?

B. Robertson ?

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Keywords: Demographic history, bottleneck, Approximate Bayesian Computation, pinniped, conservation genetics.

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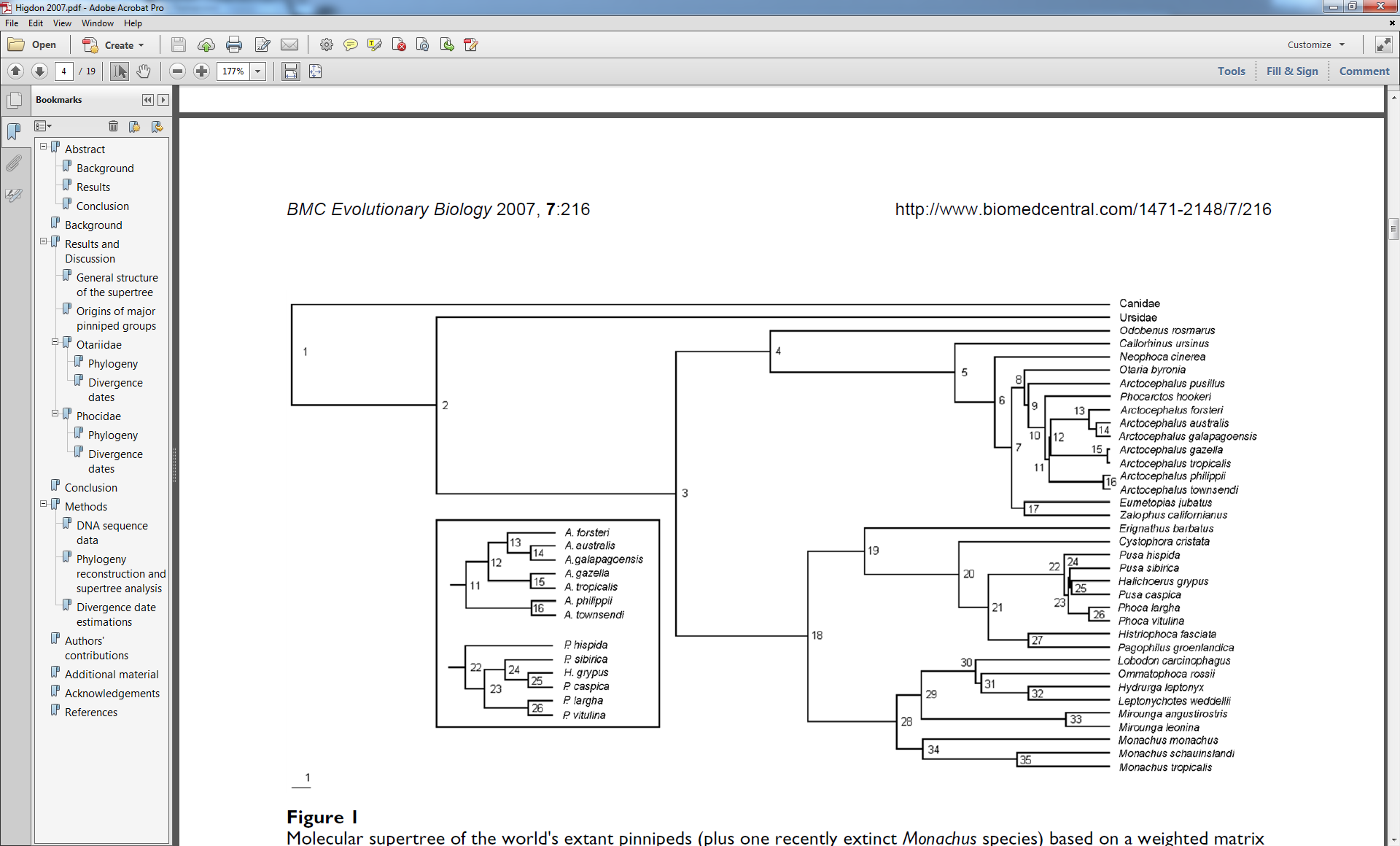
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|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Reference | Number of individuals | Number of loci | Number of loci analysed |
| Antarctic fur seal, *Arctocephalus gazella* | [[1](#_ENREF_1" \o "Hoffman, 2011 #3179)] | 246 | 21 | 21 |
| Galapagos fur seal, *Arctocephalus galapagoensis* | [[2](#_ENREF_2" \o "Lopes, in review #3835)] | 90 | 12 | 12 |
| Steller's sea lion, *Eumetopias jubatus* | [[3](#_ENREF_3" \o "Hoffman, 2006 #2558)] | 668 | 13 | 13 |
| Eastern Atlantic grey seal, *Halichoerus grypus* | [[4](#_ENREF_4" \o "Klimova, 2014 #3836)] | 1254 | 9 | 7 |
| Eastern Atlantic harbour seal, *Phoca vitulina* | [[5](#_ENREF_5" \o "Rijks, 2008 #2945)] | 204 | 27 | 27 |
| Galapagos sea lion, *Zalophus wollebaeki* | Wolf, J.B and Trillmich, F. (unpublished data) | 781 | 22 | 22 |
| South American fur seal, *Arctocephalus australis* | [[6](#_ENREF_6" \o "Rosa de Oliveira, 2008 #3214)] | 226 | 7 | 5 |
| Hooded Seal, *Cystophora cristata* | [[7](#_ENREF_7" \o "Coltman, 2007 #2894)] | 300 | 13 | 13 |
| Mediterranean monk seal, *Monachus monachus* | [[8](#_ENREF_8" \o "Pastor, 2004 #2191)] | 109 | 16 | 14 |
| Hawaiian monk seal, *Monachus schauinslandi* | [[9](#_ENREF_9" \o "Schultz, 2011 #3837)] and Schultz, J. (unpublished data) | 2386 | 18 | 17 |
| Bearded seal, *Erignathus barbatus* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 119 | 13 | 10 |
| Crabeater seal, *Lobodon carcinophagus* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 303 | 9 | 9 |
| Leopard seal, *Hydrurga leptonyx* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 150 | 14 | 12 |
| Arctic ringed seal, *Phoca hispida* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 303 | 10 | 10 |
| Ross Seal, *Ommatophoca rossi* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 90 | 9 | 9 |
| Weddell seal, *Leptonychotes weddelli* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 893 | 15 | 12 |
| Northern fur seal, *Callorhinus ursinus* | [[11](#_ENREF_11" \o "Dickerson, 2010 #3089)] | 492 | 8 | 7 |
| Saimaa ringed seal, *Phoca hispida saimensis* | [[12](#_ENREF_12" \o "Nyman, 2014 #3981)] | 172 | 17 | x |
| Lagoda ringed seal, *Phoca hispida ladogensis* | [[12](#_ENREF_12" \o "Nyman, 2014 #3981)] | 16 | 17 | x |
| New Zealand sea lion, *Phocarctos hookeri* | Osbourne, A. and Gemmell, N. (unpublished data) | 1205 | 14 | x |
| Atlantic walrus, *Odobenus rosmarus rosmarus* | Schafer, A.B.A. (unpublished data) | 623 | 10 | 10 |
| Atlantic walrus, *Odobenus rosmarus rosmarus* | Andersen, L.W.(unpublished data) | 555 | 15 | 15 |
| Northern elephant seal, *Mirounga angustrostrus* | Sanvito, S. and Galimberti, F (unpublished data) | 260 | 35 | 32 |
| Southern elephant seal, *Mirounga leonina* | Sanvito, S. and Galimberti, F (unpublished data) | 260 | 13 | 13 |
| California sea lion, *Zalophus californianus* | Acevedo-Whitehouse, K. (unpublished data) | 347 | 13 | 11 |
| South American sea lion, *Otaria flavescens* | Hoffman, J.I. (unpublished data) | 270 | 22 | 22 |



**Abstract**

Emerging Bayesian analytical approaches offer increasingly sophisticated means of reconstructing historical population dynamics from genetic data, but have been little applied to scenarios involving demographic bottlenecks. Consequently, we analysed a large mitochondrial and microsatellite dataset from the Antarctic fur seal *Arctocephalus gazella*, a species subjected to one of the most extreme examples of uncontrolled exploitation in history when it was reduced to the brink of extinction by the sealing industry during the late eighteenth and nineteenth centuries. Classical bottleneck tests, which exploit the fact that rare alleles are rapidly lost during demographic reduction, failed to provide convincing evidence for a bottleneck. In contrast, a strong signal of recent demographic decline was detected using both Bayesian skyline plots and approximate Bayesian computing, the latter also allowing derivation of posterior parameter estimates that were remarkably consistent with historical observations. This was achieved using only contemporary samples, further emphasizing the potential of these approaches to address important outstanding problems in conservation and evolutionary biology.

**Author summary**

Severe reductions in population size, termed bottlenecks, can deplete genetic variation and increase extinction risk. This has motivated the development of several genetic approaches for detecting bottlenecks within natural populations. Here, we applied state of the art Bayesian techniques to a well-documented case study, the Antarctic fur seal, which was hunted to the brink of extinction at South Georgia in the late eighteenth and nineteenth centuries. Using only contemporary DNA samples, we found strong evidence for a recent and dramatic reduction in population size, the extent and timing of which was in good agreement with the historical accounts of sealing captains. Our study suggests that Bayesian approaches could prove a powerful tool for conservation biologists interested in reconstructing the recent demographic history of threatened natural populations.

**Introduction**

Many natural populations have experienced severe demographic reductions, or population bottlenecks, due to over-exploitation or anthropogenically induced habitat destruction. This is a major cause of concern to conservation biologists because bottlenecks can lead to the loss of genetic variability, elevated levels of inbreeding and the fixation of mildly deleterious alleles, thereby increasing the risk of extinction and compromising adaptive evolutionary potential [[13](#_ENREF_13),[14](#_ENREF_14),[15](#_ENREF_15),[16](#_ENREF_16),[17](#_ENREF_17)]. Unfortunately however, detecting and measuring the impacts of such changes is not usually possible because patterns of historical abundance are seldom known. Consequently, there has been considerable interest in the development and application of methods for detecting bottleneck signatures using neutral genetic markers such as microsatellites.

One such approach exploits the fact that genetic drift is intensified in small populations, leading to concomitant changes in allele frequencies and in some cases the fixation or loss of particular alleles. This in principle allows variation in the effective population size to be measured when multiple, temporally spaced samples are available [[18](#_ENREF_18),[19](#_ENREF_19)]. However, this approach may underestimate the magnitude of severe bottlenecks because the loss of alleles constrains the extent to which allele frequencies are subsequently able to drift [[20](#_ENREF_20)]. Perhaps more importantly, most investigators are also unable to collect appropriately spaced temporal samples, particularly given that bottleneck testing is usually conducted *post hoc*. Approaches that attempt to elucidate demographic history from a single temporal genetic sample have therefore grown in popularity.

Three classical single-sample methods for detecting population bottlenecks are the heterozygosity excess [[21](#_ENREF_21)], mode-shift [[22](#_ENREF_22)] and *M*-ratio [[23](#_ENREF_23)] tests. The first and arguably most widely used of these is based on the premise that rare alleles are rapidly lost during a bottleneck but their loss only weakly influences heterozygosity. This generates a transient excess of heterozygosity (lasting up to 4 x *Ne* generations, where *Ne* is the bottleneck effective population size) relative to a population at equilibrium with an equivalent number of alleles. The second test [[22](#_ENREF_22)] measures the impact of the loss of rare alleles on the overall allele frequency distribution. The underlying rationale is that large, non-bottlenecked populations should have a high proportion of alleles at low frequency (<0.1), whereas alleles of intermediate frequency (e.g. 0.1-0.2) are expected to become more abundant after a severe bottleneck. Third, the *M*-ratio of Garza and Williamson [[23](#_ENREF_23)], defined as the ratio of the total number of alleles (*k*) to the allelic size range (*r*), may also be informative in respect of bottleneck history. This statistic exploits the fact that the loss of any allele during a bottleneck will reduce *k*, whereas only the loss of alleles at the extremes of the size range will reduce *r*. Consequently, *k* will tend to reduce more quickly than *r* in declining populations, leading to the expectation than *M* will be smaller in recently bottlenecked populations than in those at equilibrium.

Although the mode-shift test has not been extensively evaluated, heterozygosity excess and the *M*-ratio have both been shown to perform well at distinguishing bottlenecked from non-bottlenecked samples when applied to empirical datasets from species or populations with contrasting demographic histories [[21](#_ENREF_21),[23](#_ENREF_23),[24](#_ENREF_24),[25](#_ENREF_25)]. Recent simulations also suggest that these two measures may convey subtly different signals, the *M*-ratio for example being most likely to correctly identify a bottleneck when pre-bottleneck population size was large, the bottleneck lasted several generations or the population subsequently made a demographic recovery [[26](#_ENREF_26)]. However, a major drawback of both approaches is that they require simplifying assumptions to be made about the mutational mechanism of the genetic markers employed. These assumptions if incorrect have the potential to strongly influence equilibrium values of both heterozygosity conditional upon allele number and *M* [[26](#_ENREF_26),[27](#_ENREF_27),[28](#_ENREF_28)].

Fortunately, emerging analytical approaches drawing upon Bayesian methodologies provide a novel avenue for exploring demographic history independently of the bottleneck tests described above. For example, by implementing a flexible demographic model, Bayesian Skyline Plots (BSPs) allow changes in effective population size (*Ne*) over time to be described without the need for assumptions to be made about key demographic parameters [[29](#_ENREF_29)]. Potentially even more powerful is Approximate Bayesian Computation (ABC), an approach that allows for selection of the optimal demographic / evolutionary history and associated parameters among a set of hypothesized models [[30](#_ENREF_30)]. This is achieved by generating alternative simulated datasets based on assumptions about evolutionary and demographic parameters which are then compared to the observed data using summary statistics.

Surprisingly few studies have used ABC to infer the bottleneck histories of natural populations, and these have mostly employed a combination of ancient and contemporary samples to explore demographic events that occurred over time scales of thousands of years ago [[31](#_ENREF_31),[32](#_ENREF_32)]. However, most investigators do not have access to ancient DNA samples, while many are also more interested in relatively recent anthropogenically induced bottlenecks. At the same time, the use of ancient samples has largely restricted studies to using mitochondrial DNA (mtDNA) sequences, whereas the inclusion of multiple unlinked nuclear markers should substantially improve power [[31](#_ENREF_31)]. Finally, previous studies have lacked detailed historical observations with which to parameterize bottleneck scenarios for evaluation within the ABC framework.

The Antarctic fur seal (*Arctocephalus gazella*) provides an excellent opportunity to explore the relative abilities of established and emerging analytical approaches to detect a recent historical bottleneck. This species occurs predominantly to the south of the Antarctic Convergence, with 97% of the extant population breeding on the island of South Georgia (Figure 1)[[33](#_ENREF_33)]. Like most other members of the Arctocephalus genus, Antarctic fur seals were subject to uncontrolled exploitation for their fur and oil during the late eighteenth and nineteenth centuries. Uniquely however, the journals and logbooks of early explorers and sealing captains have been retained and scrutinized (see Supplementary Table 1) allowing reconstruction of the timing and extent of the demographic reduction (Figure 2). Detailed census data are similarly available with which to track the post-exploitation recovery of the population (see Supplementary Table 2). Together, these historical records allow the *a priori* parameterization of a plausible demographic model that can be tested using ABC.

The exploitation of Antarctic fur seals began shortly after the discovery of South Georgia by Captain James Cook in 1775. The most profitable strategy was to take as many seals as it was possible to kill in one season, as Mill [[34](#_ENREF_34)] comments: “Reckless extermination was the only method of seal-hunting resorted to on the islands of South Georgia and the coasts of South America so that the first in the field at a new sealing ground was sure of an immense booty, and late-comers as likely as not would go empty away”. Sealing at South Georgia reached its peak during the 1800-01 season (Figure 2) with thirty-one ships recorded as having been operating there, seventeen of which were responsible for a total catch of 112,000 seals [[35](#_ENREF_35)]. Such uncontrolled harvesting would have greatly depleted the population, probably explaining the brief abatement of sealing activity that followed until a resurgence in 1814. By 1822, Weddell [[36](#_ENREF_36)] estimated that up to 1.2 million seals had been taken at South Georgia, and the almost exterminated population was no longer able to sustain the industry.

After the last commercial catch of 170 fur seals in 1908, very few individuals were sighted until the 1950s. In 1915 a single juvenile male was found and immediately killed [[37](#_ENREF_37)] and in 1911 Larsen [[38](#_ENREF_38)] also reported sighting a group of 30 individuals. A dedicated fur seal survey found 38 animals at Bird Island in 1933 and deduced a total population of 60 [[37](#_ENREF_37)]. The Discovery expedition of 1936 subsequently reported 59 seals, including 12 pups, at the same location [[39](#_ENREF_39)], but the population is thought to have remained at around this level until the 1950s. Rapid population growth ensued in the 1960s and 1970s, and by 1990 fur seal numbers were estimated to have reached 1.5 million [[40](#_ENREF_40)]. The most recent estimate of 3 million was made at the XXIII Antarctic Treaty Consultative Meeting in 1999 (Specially Protected Species in Antarctica. XXIII ATCM / WP24. Agenda Item 7c; Meeting 1999).

Here, we genotyped 246 Antarctic fur seals from South Georgia at a 263-bp region of the hypervariable region 1 (HVR1) of the mitochondrial control region and 21 unlinked highly polymorphic microsatellites. Our aims were to explore the relative abilities of classical bottleneck tests and Bayesian approaches to recover a signal of historical exploitation and to estimate via ABC the distributions of key bottleneck parameters including timing and minimum population size. Our approach differs from previous studies using ABC in two main respects. First, our dataset comprises both mtDNA and microsatellite data, yielding greater genetic resolution as well as bi-parental perspectives. Second, population reduction resulting from harvesting and subsequent recovery are well documented in this species, providing a strong *a priori* demographic model.

**Results**

To test for a genetic signature of a historical population bottleneck, we genotyped 246 Antarctic fur seals from eight rookeries across South Georgia (Table 1) at 21 microsatellite loci and sequenced a 263-bp segment of the hypervariable region 1 (HVR1) of the mitochondrial control region. The microsatellite loci were highly informative, possessing on average 11.3 alleles and with a mean observed heterozygosity of 0.81 (Table 2). Following sequential Bonferroni correction to compensate for multiple statistical tests, none of the loci were found to deviate significantly from Hardy–Weinberg equilibrium and no pairs of loci exhibited significant linkage disequilibrium. A total of 26 mitochondrial haplotypes were found, eleven of which (Genbank accession numbers JF304904-JF304914‏) had not previously been described by Wynen *et al*. [[41](#_ENREF_41)].

Classical bottleneck tests

We first interrogated our microsatellite dataset for evidence of a genetic bottleneck using the heterozygosity excess approach of Luikart et al. [[22](#_ENREF_22)]. The results were highly dependent on the mutational model upon which the predicted relationship between heterozygosity and the number of alleles was based (Table 3). Thus, a significant excess of heterozygosity relative to expectations was detected under the IAM but not the SMM and *P*-values obtained for TPM models scaled positively with the proportion of multi-step mutations specified. This pattern probably reflects the greater power of the IAM to detect a bottleneck [[21](#_ENREF_21)], despite this model being unrealistic for most 'real' microsatellites [[42](#_ENREF_42)]. A mode shift in the allele frequency distribution was not detected.

Recent simulations [[26](#_ENREF_26)] suggest that when pre-bottleneck population size was large or the population made a demographic recovery, the ratio of the number of alleles to allelic size range may be more informative about bottleneck history than heterozygosity excess. Consequently, we also calculated the *M*-ratio of Garza and Williamson [[23](#_ENREF_23)]. The resulting value of 0.798 lies above the 0.7 threshold proposed by Garza and Williamson [[23](#_ENREF_23)], implying a lack of support for a bottleneck. Comparing this value against a null distribution derived from 10,000 theoretical populations in mutation-drift equilibrium, a bottleneck signature was only inferred below *θ* = 1.63, a value that corresponds to an unrealistically low pre-bottleneck *Ne* of 812 assuming a default microsatellite mutation rate of 5x10-4 [[43](#_ENREF_43)].

Approximate Bayesian Computing (ABC) analysis

Evaluation of the two proposed historic models indicated that the one incorporating a population bottleneck based on historical observations most accurately described the genetic data. This model received a posterior probability of between 0.99 and 1, while the model describing a constant population size through time received a posterior probability of between 0 and 0.01. In addition, Type I and Type II error rates for the selection of the bottleneck model were 0.18 and 0.12 respectively. The posterior distribution of selected parameters using values drawn from the 10,000 datasets closest to the observed are shown in Figure 5 and Table 4. These depict a genetic bottleneck that ended approximately 11 generations ago (mean = 12, median = 11, mode = 6 generations ago, 95% CI = 2–27) with an *Ne* at this time of approximately 139 (mean = 164, median = 139, mode = 122, 95% CI = 46–371). No clear posterior estimate of the time parameter associated with *Ne-historical* was recovered (data not shown). The statistical descriptors for contemporary *Ne* were much larger (mean = 744,000, mean = 742,000, mode = 178,000, 95% CI = 66,900–1,420,000) but exhibited a flat posterior distribution. Estimates of historical *Ne* were also comparatively large (mean = 777,000, median = 763,000, mode = 396,000, 95% CI = 155,000–1,430,000), but the lower bound of the CI for historical *Ne* was over twice as large as that estimated for contemporary *Ne*. Moreover, the posterior distribution of historical *Ne* dipped towards lower *Ne* values indicating resolution for estimating the posterior lower bound of this parameter.

With Bayesian analytical approaches, choices of prior distributions can have a large impact on posterior parameter estimates [[31](#_ENREF_31)]. Consequently, we explored the sensitivity of our analysis to a variety of different prior assumptions (see Materials and methods and Table 4 for details). Simulations involving prior parameter adjustments on sex ratio and mitochondrial mutation rate from the initial bottleneck model yielded largely unaltered estimates (Figure 5, Table 4). The main deviation observed was in the estimation of bottleneck and historic *Ne* values from simulations invoking a 1:5 sex ratio. These analyses described a larger bottleneck *Ne* (about double the size) and a historical *Ne* peaking at around a quarter of a million.

For our initial simulations, we chose prior distributions tightly bounded around values based on the available historical data. However, to explore sensitivity to these assumptions we replicated all of the above simulations with wider priors on *Ne* and time (Figure 6, Table 4). The bottleneck model was again highly supported over the constant population size model (posterior probabilities were 1 and 0 respectively), with type I and II error rate estimates being lower than those obtained from the initial simulations (0.08 and 0 respectively). Parameter estimates were almost identical to those obtained in our initial simulations with the exception of bottleneck *Ne*, which was consistently larger at around 700 and 1400 for analyses based on a sex ratio of 1 and 1:5 respectively.

Finally, we tested for any potential differences in the strength of the bottleneck signal contained within the mtDNA and microsatellite datasets by conducting additional simulations separately for each class of marker. For microsatellites, the bottleneck model was supported over the constant population size model regardless of *Ne* and time priors (Figures 5 and 6, Table 4). Posterior support values were also closely comparable to those reported above (e.g. bottleneck model = 0.94–0.96 versus constant population size model = 0.04–0.05 for simulations using the initial priors on *Ne* and time). In contrast, the mtDNA dataset was marginally better supported by the constant population size model than the bottleneck model (bottleneck model = 0.24–0.27; constant population size model = 0.72–0.75).

**Discussion**

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Conclusions

Although detailed historical records document a reduction in population size in the Antarctic fur seal down to 30 individuals or fewer, it seems likely that larger numbers may have escaped sealing, either by breeding at remote or inaccessible locations such as the Willis Islands [[41](#_ENREF_41)] or perhaps by remaining out at sea. Our results suggest that a dramatic reduction in population size did indeed take place, but that this may not have been substantial or long-lasting enough to have appreciably reduced levels of genetic diversity. Similar insights from other bottlenecked species offer to substantially improve our understanding of how historical demographic reductions influence contemporary genetic diversity, with important implications for the conservation and management of threatened natural populations.

**Materials and methods**

##### Tissue sample collection

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Tests for population substructure

To check for the presence of cryptic population structure within South Georgia, we calculated pairwise *F*st values among all of the sampling locations for both classes of marker. *F*st was calculated for microsatellites using Fstat version 2.9.3 [[44](#_ENREF_44)] and for mitochondrial DNA using Arlequin version 2.0 [[45](#_ENREF_45)]. Only three of the 72 resulting values were individually significant at *P* < 0.05, none of which remained so following sequential Bonferroni correction for multiple tests. Moreover, the results of subsequent data analyses were qualitatively very similar regardless of whether the full dataset was used or analyses were restricted to the study colony at Bird Island (data not shown).

Microsatellite-based bottleneck tests

To test for evidence of a genetic bottleneck, we first used the heterozygosity excess method of Luikart et al. [[22](#_ENREF_22)] implemented within the program Bottleneck v 1.2.02 [[46](#_ENREF_46)]. One potential drawback of this approach is that, although microsatellites evolve mainly by gaining or losing a single repeat unit (the Stepwise Mutation Model, SMM [[47](#_ENREF_47)]), occasional larger ‘jump’ mutations of several repeat units are also common [[42](#_ENREF_42),[48](#_ENREF_48)]. Consequently, Bottleneck allows the user to specify a range of mutation models, from the strict SMM through two-phase models (TPMs) with varying proportions of multi-step mutations to the infinite alleles model (IAM) where every new mutation is novel. For our analysis, four TPM models were evaluated with 1%, 5%, 10% and 30% multi-step mutations respectively and a default variance of 30. For each of the mutational models, the heterozygosity of each locus expected under mutation-drift equilibrium given the observed number of alleles (Hexp) was determined using 10, 000 simulations and then compared against observed heterozygosity (Ho). We then recorded the number of loci for which Ho was greater than Hexp and determined whether the overall set of deviations was statistically significant using sign, standardized differences and Wilcoxon signed ranks tests. Bottlenecked populations are also expected to exhibit a characteristic ‘mode shift’ in the frequency distribution of alleles away from the L-shaped distribution expected under mutation-drift equilibrium [[22](#_ENREF_22)]. Consequently, Bottleneck was also used to generate a qualitative descriptor of whether the observed allele frequencies at each locus deviate from such a distribution.

As an alternative test for a population bottleneck, we also calculated Garza and Williamson's *M*-ratio for our dataset using the program M\_P\_Val [[23](#_ENREF_23)]. The significance of the resulting value was determined by comparison against a distribution of *M* values calculated from 10,000 theoretical populations in mutation-drift equilibrium. Using conventional criteria, a significant reduction in population size is inferred if fewer than 5% of the replicates fall below the observed value of *M*. The program allows the user to modify three parameters that approximate the mutation process in natural populations: the proportion of mutations that are larger than a single step (*pg*), the average size of non-single-step mutations (Δ*g*) and *θ* = 4 *Neµ* (where *Ne* is the effective pre-bottleneck population size at equilibrium and *µ* is the mutation rate). We used the default settings of *pg* = 0.1 and Δ*g* = 3.5, and varied *θ* between 1 and 1000, the latter corresponding to an effective pre-bottleneck population size of 500,000 assuming a commonly used estimate of the dinucleotide microsatellite mutation rate of 5x10-4 mutants per gamete per generation [[43](#_ENREF_43)] as suggested by Garza and Williamson [[23](#_ENREF_23)].

Approximate Bayesian Computing (ABC) analysis

Statistical support for alternative historical scenarios that either included or excluded a bottleneck was tested within an ABC framework. This allowed us not only to determine whether or not a bottleneck is likely to have occurred, but also to estimate values of key parameters of interest. We initially simulated two primary historical models. The first incorporated a recent population bottleneck, with prior distributions broadly surrounding values derived from historical records (Figure 2). The model described an ancestral *Ne* uniformly distributed between 1 and 1.5 x 106, the upper bound encompassing Weddell's [[36](#_ENREF_36)] estimate of 1.2 million seals having been taken during the initial bout of harvesting. The occurrence of the bottleneck was described by two uniform distributions representing the time parameter associated with *Ne-historical* (15-100 generations ago) and the time associated with the end of the bottleneck (i.e. with the lowest value of *Ne-bottleneck*, 1-30 generations ago). The bottleneck model was constrained such that changes in effective population size occur sumultaneously. The prior on *Ne-bottleneck* was bounded between 1 and 1000, values that generously surround the 1911 census population of 30 individuals [[38](#_ENREF_38)]. *Ne-contemporary* was uniformly distributed between 1 and 1.5 x 106. For comparison, a null model of no population bottleneck was also defined, in which *Ne* was uniformly distributed through time (1-100 generations) and bounded between 1 and 1.5 x 106 individuals. For both models, the HVR1 mutation rate was defined by a uniform distribution with lower and upper bounds of 5.74 × 10-7 [[11](#_ENREF_11)] and 2.71 × 10-6 [[49](#_ENREF_49)] substitutions per site per generation respectively. The HKY + I + G (I = 0.57, G = 0.50) mutation model defined for HVR1 was determined using the hierarchical likelihood ratio test and Akaike information criterion, as implemented in ModelTest v 3.7 [[50](#_ENREF_50)]. For microsatellites, the Generalized Stepwise Mutation model [[51](#_ENREF_51)] was implemented with a mean rate uniformly distributed between 1.00 × 10-4 and 1.00 × 10-3 substitutions/generation. These simulations were performed assuming a 1:1 sex ratio.

To explore the influence of prior assumptions on posterior conclusions using ABC we next initiated a series of simulations incorporating specific prior parameter adjustments. Specifically, we examined the influence of assumptions on the prior distributions of the mitochondrial mutation rate and sex ratio on the posterior distributions of parameters of interest. This was important because the assumed value of *µmitochondrial* describes the source of genetic diversity for simulated populations while sex ratio influences the diversity of bi-parentally inherited markers. The effect of HRV1 mutation rate prior was explored by evoking a liberal prior bounded by 5.74 × 10-7 and 3.65 × 10-5. The effect of assuming a 1:1 sex ratio was assessed through additional simulations based on a sex ratio of 1 male to each 5 females. This sex ratio was drawn from field observations and it is unclear how accurately this value reflects the true genomic contributions of the two sexes.

In addition to the above simulations, all models were simulated a second time, but with broadened uniform priors on all parameters relating to *Ne* and time. Specifically, contemporary and historic *Ne* were distributed between 1 and 6 x 106, bottleneck *Ne* was distributed between 1-2000, timing of the start of the bottleneck was distributed between 1-500, and timing of the end of the bottleneck distributed between 1-50. Prior distributions of parameters for all analyses are described in Table 4. Finally, to assess whether the strength of the bottleneck signal differs between the mtDNA and microsatellite datasets, additional simulations were performed on each data type independently. These were carried out twice, once with the initially defined priors on *Ne* and time, and also following the broadened priors on these parameters.

For each model, one million genetic datasets were simulated with the defined demographic and marker parameters. Four summary statistics were then generated for the observed and simulated datasets: mean pairwise difference and Tajima’s *D* [[52](#_ENREF_52)] for HVR1, and mean heterozygosity and the mean number of alleles for microsatellites. Normalized Euclidean distances were calculated between the observed dataset and each of the simulated datasets using the local linear regression method of Beaumont et al. [[30](#_ENREF_30)]. The ten thousand datasets with the smallest Euclidean distances were then retained to build posterior parameter distributions, which were smooth weighted using the Locfit function within R version 2.9.1 [[53](#_ENREF_53)]. The posterior probabilities of each scenario were estimated using a logistic regression approach, providing both point estimates and 95% confidence intervals [[54](#_ENREF_54),[55](#_ENREF_55)]. Statistical measures of performance and Type I and Type II error rates were also calculated as a means of model checking [[56](#_ENREF_56)]. All of the above analyses were implemented within the DiyAbc v1 software package [[54](#_ENREF_54),[57](#_ENREF_57)].

Ethics statement

Tissue samples were collected and retained under permits issued by the Department for Environment, Food and Rural Affairs (License number AHZ/2024A/2005/1) and in accordance with the Convention on International Trade in Endangered Species of Wild Fauna and Flora. All field procedures were approved by the British Antarctic Survey (reference PEA 6).

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**Author contributions**

Conceived and designed the study: JH. Collated and analyzed the data: JH CP JF. Wrote the paper: JH CP

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**Figure Legends**

Figure 1 xx.

Figure 2 xx.

Figure 3 xx.

Figure 4 xx.

**Figure 5** Posterior density curves of model parameters based on 10,000 accepted values from 1x106 iterations of the initial bottleneck model (see Materials and methods for details). Continuous, large-dashed and short-dashed lines represent posterior density curves obtained for simulations with a sex ratio of 1, a sex ratio of 1:5 and with expanded priors on mitochondrial mutation rate respectively. Dotted lines represent results obtained for the microsatellite dataset only. Data from simulations using only mtDNA are not included due to the bottleneck model not being supported.

**Figure 6** Posterior density curves of model parameters based on 10,000 accepted values from 1x106 iterations of the bottleneck model with expanded priors on *Ne* and time (see Materials and methods for details). Continuous, large-dashed and short-dashed lines represent posterior density curves obtained for simulations with a sex ratio of 1, a sex ratio of 1:5 and with expanded priors on mitochondrial mutation rate respectively. Dotted lines represent results obtained for the microsatellite dataset only. Data from simulations using only mtDNA are not included due to the bottleneck model not being supported.

**Tables**

**Table 1** Numbers of Antarctic fur seals genotyped at the mitochondrial HVR1 and 21 microsatellite loci. For a map of the sampling locations within South Georgia, see Figure S1.

|  |  |  |
| --- | --- | --- |
| Location | Sampling site | Number of samples genotyped |
| Willis Islands | Main Island | 16 |
| Bird Island | Study colony | 142 |
| Freshwater beach | 25 |
| South Georgia | Prince Olav Harbour | 12 |
| Leith Harbour | 1 |
| Husvik | 12 |
| Cooper Bay | 14 |
| Annenkov Island | 15 |
| Wilson Harbour | 9 |
|  |  | 246 |



**Table 2** The number of loci with heterozygosity excess and test probabilities obtained using a range of mutational models (see methods for details) within the program Bottleneck [[46](#_ENREF_46)].The mode test revealed normal L-shaped distributions under all of the scenarios tested. *P*-values significant at α < 0.05 without correction for multiple statistical tests are highlighted in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Mutational model | No. of loci with heterozygosity excess | Sign test  *P*-value | Standardized differences test  *P*-value | Wilcoxon test  *P*-value |
| IAM | 21 | **<0.0001** | **<0.0001** | **<0.0001** |
| TPM70 | 20 | **<0.001** | **<0.0001** | **<0.0001** |
| TPM90 | 17 | **0.008** | **0.002** | **<0.001** |
| TPM95 | 16 | 0.075 | **0.018** | **0.008** |
| TPM99 | 16 | 0.076 | 0.326 | 0.320 |
| SMM | 12 | 0.531 | 0.373 | 0.919 |

Table 3 Prior uniform distributions, mean, median, mode, quantiles, and estimates of bias and precision (MRB = mean relative bias, RMSE = root mean square error) for the posteriors of parameters calculated from simulations of historic models that differed in prior bounds of specific parameters.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Model | Prior | Mean | Median | Mode | 5% | 95% | MRB | RMSE |
| *Ne-contemporary* | **Initial** | 1–1.5×106 | 7.44×105 | 7.42×105 | 1.78×105 | 6.69×104 | 1.42×106 | 6.50 | 98.68 |
| Sex ratio = 1:5 |  | 7.44×105 | 7.45×105 | 2.24×105 | 7.53×104 | 1.42×106 | 2.36 | 18.19 |
| Broadened *µ*seq |  | 7.38×105 | 7.33×105 | 4.32×105 | 7.00×104 | 1.43×106 | 5.76 | 58.36 |
| Microsatellites only |  | 7.45×105 | 7.44×105 | 1.94e×105 | 7.28×104 | 1.42×106 | 4.49 | 40.78 |
| **Broadened** | 1–6×106 | 2.98×106 | 2.97×106 | 6.98×105 | 2.79×105 | 5.72×106 | 2.13 | 11.90 |
| Sex ratio = 1:5 |  | 2.95×106 | 2.92×106 | 2.74×105 | 2.73×105 | 5.67×106 | 6.10 | 60.50 |
| Broadened *µ*seq |  | 3.01×106 | 3.04×106 | 5.17×106 | 2.85×105 | 5.71×106 | 7.80 | 105.60 |
| Microsatellites only |  | 3.00×106 | 2.98×106 | 2.72×106 | 2.93×105 | 5.70×106 | 2.83 | 19.90 |
| *Ne-bottleneck* | **Initial** | 1–1000 | 164 | 139 | 122 | 46 | 371 | 0.62 | 1.85 |
| Sex ratio = 1:5 |  | 356 | 313 | 215 | 92 | 821 | 0.77 | 2.70 |
| Broadened *µ*seq |  | 169 | 140 | 133 | 48 | 429 | 0.60 | 2.69 |
| Microsatellites only |  | 188 | 153 | 153 | 53 | 563 | 0.68 | 2.97 |
| **Broadened** | 1–2000 | 658 | 662 | 688 | 217 | 1070 | 0.43 | 1.49 |
| Sex ratio = 1:5 |  | 1200 | 1250 | 1360 | 469 | 1810 | 0.48 | 3.64 |
| Broadened *µ*seq |  | 660 | 667 | 678 | 228 | 1090 | 0.42 | 1.30 |
| Microsatellites only |  | 665 | 655 | 593 | 216 | 1100 | 0.33 | 0.98 |
| *Ne-historical* | **Initial** | 1–1.5×106 | 7.77×105 | 7.63×105 | 3.96×105 | 1.55×105 | 1.43×106 | 1.56 | 22.52 |
| Sex ratio = 1:5 |  | 3.60×105 | 2.42×105 | 1.17×105 | 6.39×104 | 1.10×106 | 3.29 | 33.13 |
| Broadened *µ*seq |  | 7.48×105 | 7.20×105 | 3.59×105 | 1.44×105 | 1.42×106 | 1.07 | 8.64 |
| Microsatellites only |  | 6.31×105 | 5.94×105 | 9.56×103 | 1.19×104 | 1.41×106 | 2.90 | 35.65 |
| **Broadened** | 1–6×106 | 2.41×106 | 2.05×106 | 7.40×105 | 3.20×105 | 5.49×106 | 0.83 | 3.52 |
| Sex ratio = 1:5 |  | 9.07×105 | 4.35×105 | 9.88×104 | 6.79×104 | 3.67×106 | 2.03 | 20.00 |
| Broadened *µ*seq |  | 2.29×106 | 1.92×106 | 5.74×105 | 2.87×105 | 5.42×106 | 1.31 | 6.57 |
| Microsatellites only |  | 2.79×106 | 2.76×106 | 1.13×104 | 1.23×105 | 5.67×106 | 9.04 | 133.37 |

**Table 3 continued**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Model | Prior | Mean | Median | Mode | 5% | 95% | MRB | RMSE |
| *τbottleneck-end* | **Initial** | 1–30 | 12 | 11 | 6 | 2 | 27 | 0.77 | 2.49 |
|  | Sex ratio = 1:5 |  | 13 | 12 | 6 | 2 | 27 | 0.75 | 2.31 |
|  | Broadened *µ*seq |  | 12 | 11 | 5 | 2 | 27 | 0.81 | 2.49 |
|  | Microsatellites only |  | 13 | 12 | 7 | 3 | 28 | 1.05 | 2.867 |
|  | **Broadened** | 1–50 | 17 | 13 | 5 | 2 | 42 | 0.97 | 3.08 |
|  | Sex ratio = 1:5 |  | 17 | 13 | 4 | 2 | 43 | 0.67 | 2.38 |
|  | Broadened *µ*seq |  | 17 | 13 | 7 | 2 | 43 | 1.00 | 3.56 |
|  | Microsatellites only |  | 18 | 14 | 1 | 2 | 44 | 0.95 | 2.93 |
| *τbottleneck-start* | **Initial** | 15–100 | 70 | 74 | 100 | 32 | 98 | 0.18 | 0.59 |
|  | Sex ratio = 1:5 |  | 69 | 72 | 96 | 29 | 98 | 0.16 | 0.57 |
|  | Broadened *µ*seq |  | 71 | 74 | 99 | 32 | 98 | 0.14 | 0.56 |
|  | Microsatellites only |  | 71 | 74 | 94 | 31 | 98 | 0.19 | 0.59 |
|  | **Broadened** | 1–2000 | 316 | 329 | 490 | 104 | 485 | 2.5 | 17.88 |
|  | Sex ratio = 1:5 |  | 284 | 287 | 279 | 77.0 | 475 | 1.60 | 14.18 |
|  | Broadened *µ*seq |  | 316 | 327 | 466 | 105 | 484 | 3.62 | 3.56 |
|  | Microsatellites only |  | 320 | 333 | 497 | 108 | 486 | 2.83 | 23.33 |
| *µmitochondrial* | **Initial** | 5.78×10-7–2.74×10-6 | 1.67×10-6 | 1.67×10-6 | 6.27×10-7 | 6.80×10-7 | 2.64×10-6 | 0.20 | 0.61 |
|  | Sex ratio = 1:5 |  | 1.64×10-6 | 1.65×10-6 | 2.70×10-6 | 6.43×10-7 | 2.62×10-6 | 0.15 | 0.58 |
|  | Broadened *µ*seq |  | 1.84×10-5 | 1.84×10-5 | 7.52×10-6 | 2.37×10-6 | 3.47×10-5 | 0.98 | 3.14 |
|  | **Broadened** |  | 1.65×10-6 | 1.65×10-6 | 5.78×10-7 | 6.67×10-7 | 2.65×10-6 | 0.21 | 0.65 |
|  | Sex ratio = 1:5 |  | 1.57×10-6 | 1.52×10-6 | 5.78×10-7 | 6.31×10-7 | 2.65×10-6 | 0.22 | 0.68 |
|  | Broadened *µ*seq | 5.78×10-7–3.65×10-5 | 1.85×10-5 | 1.85×10-5 | 1.21×10-6 | 2.22×10-6 | 3.47×10-5 | 1.29 | 4.18 |
| *µmicrosatellite* | **Initial** | 1.00×10-4–1.00×10-3 | 4.69×10-4 | 4.31×10-4 | 2.66×10-4 | 1.34×10-4 | 9.12×10-4 | 0.29 | 0.92 |
|  | Sex ratio = 1:5 |  | 4.85×10-4 | 4.53×10-4 | 2.49×10-4 | 1.35×10-4 | 9.27×10-4 | 0.26 | 0.84 |
|  | Broadened *µ*seq |  | 4.75×10-4 | 4.35×10-4 | 2.98×10-4 | 1.35×10-4 | 9.23×10-4 | 0.33 | 0.93 |
|  | Microsatellites only |  | 5.24×10-4 | 5.00×10-4 | 4.12×10-4 | 1.56×10-4 | 9.38×10-4 | 0.32 | 0.91 |
|  | **Broadened** |  | 3.16×10-4 | 2.46×10-4 | 1.44×10-4 | 1.18×104 | 7.68×10-4 | 0.25 | 0.78 |
|  | Sex ratio = 1:5 |  | 3.90×10-4 | 3.26×10-4 | 1.78×10-4 | 1.37×104 | 8.61×10-4 | 0.24 | 0.68 |
|  | Broadened *µ*seq |  | 3.12×10-4 | 2.45×10-4 | 1.38×10-4 | 1.17×104 | 7.59×10-4 | 0.18 | 0.73 |
|  | Microsatellites only |  | 3.53×10-4 | 2.85×10-4 | 1.83×10-4 | 1.18×104 | 8.17×10-4 | 0.28 | 0.79 |

**Supplementary table 1** Details of the pinniped microsatellite datasets used for this study. All

Shown are the number of individuals and microsatellite loci genotyped for each species. The number of loci analysed refers to the number of microsatellite loci that did not deviate significantly from HWE after FDR correction and hence which were retained for analysis (See Materials and methods for details).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Reference | Number of individuals | Number of loci | Number of loci analysed |
| Antarctic fur seal, *Arctocephalus gazella* | [[1](#_ENREF_1" \o "Hoffman, 2011 #3179)] | 246 | 21 | 21 |
| Galapagos fur seal, *Arctocephalus galapagoensis* | [[2](#_ENREF_2" \o "Lopes, in review #3835)] | 90 | 12 | 12 |
| Steller's sea lion, *Eumetopias jubatus* | [[3](#_ENREF_3" \o "Hoffman, 2006 #2558)] | 668 | 13 | 13 |
| Eastern Atlantic grey seal, *Halichoerus grypus* | [[4](#_ENREF_4" \o "Klimova, 2014 #3836)] | 1254 | 9 | 7 |
| Eastern Atlantic harbour seal, *Phoca vitulina* | [[5](#_ENREF_5" \o "Rijks, 2008 #2945)] | 204 | 27 | 27 |
| Galapagos sea lion, *Zalophus wollebaeki* | Wolf, J.B and Trillmich, F. (unpublished data) | 781 | 22 | 22 |
| South American fur seal, *Arctocephalus australis* | [[6](#_ENREF_6" \o "Rosa de Oliveira, 2008 #3214)] | 226 | 7 | 5 |
| Hooded Seal, *Cystophora cristata* | [[7](#_ENREF_7" \o "Coltman, 2007 #2894)] | 300 | 13 | 13 |
| Mediterranean monk seal, *Monachus monachus* | [[8](#_ENREF_8" \o "Pastor, 2004 #2191)] | 109 | 16 | 14 |
| Hawaiian monk seal, *Monachus schauinslandi* | [[9](#_ENREF_9" \o "Schultz, 2011 #3837)] and Schultz, J. (unpublished data) | 2386 | 18 | 17 |
| Bearded seal, *Erignathus barbatus* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 119 | 13 | 10 |
| Crabeater seal, *Lobodon carcinophagus* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 303 | 9 | 9 |
| Leopard seal, *Hydrurga leptonyx* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 150 | 14 | 12 |
| Arctic ringed seal, *Phoca hispida* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 303 | 10 | 10 |
| Ross Seal, *Ommatophoca rossi* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 90 | 9 | 9 |
| Weddell seal, *Leptonychotes weddelli* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 893 | 15 | 12 |
| Northern fur seal, *Callorhinus ursinus* | [[11](#_ENREF_11" \o "Dickerson, 2010 #3089)] | 492 | 8 | 7 |
| Atlantic walrus, *Odobenus rosmarus rosmarus* | Schafer, A. (unpublished data) | 623 | 10 | 10 |
| Atlantic walrus, *Odobenus rosmarus rosmarus* | Andersen dataset | 555 | 15 | 15 |
| Northern elephant seal, *Mirounga angustrostrus* | Sanvito, S. and Galimberti, F (unpublished data) | 260 | 35 | 32 |
| Southern elephant seal, *Mirounga leonina* | Sanvito, S. and Galimberti, F (unpublished data) | 260 | 13 | 13 |
| California sea lion, *Zalophus californianus* | Acevedo-Whitehouse, K. (unpublished data) | 347 | 13 | 11 |
| South American sea lion, *Otaria flavescens* | Hoffman J.I. (unpublished data) |  |  |  |