Genetic Evidence of a Population Bottleneck and Inbreeding in the Endangered New Zealand Sea Lion, *Phocarctos hookeri*

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Abstract

The New Zealand sea lion (NZSL) is of high conservation concern due to its limited distribution and its declining population size. Historically, it occupied most of coastal New Zealand, but is now restricted to a few coastal sites in southern mainland New Zealand and the sub-Antarctic Islands. NZSLs have experienced a recent reduction in population size due to sealing in the 1900s, which is expected to have resulted in increased inbreeding and a loss of genetic variation, potentially reducing the evolutionary capacity of the species and negatively impacting on its long-term prospects for survival. We used 17 microsatellite loci, previously shown to have cross-species applications in pinnipeds, to determine locus- and population-specific statistics for 1205 NZSLs from 7 consecutive breeding seasons. We show that the NZSL population has a moderate level of genetic diversity in comparison to other pinnipeds, and provide genetic evidence for a population reduction, likely caused by historical sealing, and a measure of allele sharing/parental relatedness (internal relatedness) that is suggestive of increased inbreeding in pups that died during recent epizootic episodes. We hypothesize that population bottlenecks and nonrandom mating have impacted on the population genetic architecture of NZSLs, affecting its population recovery.

Subject areas: Population structure and phylogeography; Conservation genetics and biodiversity

Key words: bottleneck, diversity, microsatellites, structure

A species that undergoes a reduction in population size often experiences increased inbreeding and an associated loss of genetic variation (Frankham et al. 2002). This can be detrimental because loss of genetic variation through inbreeding can increase identity-by-descent, leading to increased homozygosity and the expression of deleterious recessive alleles throughout the genome (Frankham 2005), including...
at loci influencing species fitness (Charlesworth and Charlesworth 1987). These genetic hallmarks of inbreeding depression (reduced heterozygosity and allelic variation) are often detectable via neutral loci, such as microsatellites. These markers have been used as surrogates for full genome information to determine the extent of inbreeding and species fitness in many vertebrates (Colman and Slate 2003; Chapman et al. 2009) and can provide a good estimation of evolutionary potential (Crnokrak and Roff 1999, Frankham et al. 1999, 2002; Willi et al. 2006). Microsatellites are often considered the ideal marker for studies of population and conservation genetics because mutations within microsatellites are generally nonfunctional (Jarne and Lagoda 1996; Ellegren 2000; Buschiazio and Gemmell 2006). However, variation at microsatellite loci has also been associated with disease resistance and/or susceptibility in numerous species (Acevedo-Whitehouse et al. 2003; Cohas et al. 2009; Mainguy et al. 2009), and studies in pinnipeds have shown that variation at microsatellite loci is associated with traits relative to fitness and disease status (Colman et al. 1998a, 1998b; Bean et al. 2004; Pastor 2004; Acevedo 2006; Hoffman 2006; Kretzman 2006; Rijks 2008).

For a population to survive and grow, it requires both genetic variation and reproductive success. These traits are lacking in most threatened species, which often display decreased reproductive fitness and disease resistance (Reed and Frankham 2003). The New Zealand sea lion (NZSL, Phocarctos hookeri), of the order Carnivora, suborder Pinnipedia, is the largest native and only seal endemic to New Zealand (Bryden et al. 1998). It is one of the rarest sea lions in the world, with recent population estimates ranging between 8600 and 11300 individuals (Geschke and Chilvers 2009). Threats to its ongoing survival remain acute, with pup production over the last decade having declined 40% (Chilvers et al., 2007, 2009). The species is considered Endangered by IUCN and Nationally Critical under the New Zealand Threat Listing (Baker et al. 2010).

Prior to the arrival of humans in New Zealand around AD1300, the NZSL ranged from the far north of the North Island, through Stewart Island and down to the sub-Antarctic, with colonies also present on the Chatham Islands (Childerhouse and Gales 1998). Sea lions were hunted for food by indigenous New Zealand populations and this subsistence hunting saw the depletion of the sea lion population from the northern North Island by c.AD1500 and the replacement of a mainland haplotype with one that had previously been restricted to the sub-Antarctic (Collins et al. 2014). Following European settlement of New Zealand, the NZSL was subsequently extirpated from the New Zealand mainland through commercial sealing activities by AD1826 (Childerhouse and Gales 1998). In 1896, the NZSL was declared a protected species.

The NZSL range is now restricted mainly to the remote Auckland Islands (71% of total pup production, Robertson and Chilvers 2011) and Campbell Island (27% of total pup production (Gales and Fletcher 1999; Childerhouse et al. 2005; Chilvers et al. 2007; Maloney et al. 2012). Within the Auckland Islands group there are 4 main breeding areas; Sandy Bay (Enderby Island), Figure of Eight Island, South East Point, and Dundas Island. Threats to the stability and growth of the population now include incidental by-catch from, and resource competition with, trawler fisheries in the immediate area (Chilvers 2008; Meynier et al. 2010) and episodic disease events which result in high levels of mortality of pups especially, but also adults (Baker 1999). Such disease events have occurred 3 times since the first known event was observed in the 1997/1998 breeding season (Baker 1999; Wilkinson et al. 2006). The 1997/1998 episode resulted in mortality of 52.6% of pups at 2 months of age (5 times higher than in nonepizootic years where the average is 10.2%, Castinel et al. 2007), along with at least 74 adult females, and was possibly caused by a Campylobacter bacterium (Baker 1999). In 2001/2002 and 2002/2003, further seasons of unusually high mortality took place, with the death of 31.3% and 22.1% of pups at 2 months of age (also significantly higher than in nonepizootic years) born in these years, respectively, from infection with the opportunistic bacterium Klebsiella pneumoniae (Wilkinson et al. 2006). Based on this recent history, it is likely that the population will remain static (Chilvers 2012) if the current trend of periodic epizootic events continues within the NZSL population continues.

The breeding season of the NZSL is from mid-December to mid-January and the mean date for pup birth is late December (Chilvers et al. 2006). Female NZSLs usually give birth for the first time at 4–6 years old, with males achieving dominant male status at 10–12 years old (Perrin et al. 2008). The epizootics of recent years are now impacting on the number of adults available for breeding, as shown by estimates of the reduction in the number of adult females being recruited to the breeding population (Wilkinson et al. 2006). It was predicted that the mortalities of 2001/2002 and 2002/2003 would result in between 2.3% and 4.6% fewer adult females contributing to the breeding population by the 2007/2008 breeding season (Wilkinson et al. 2006).

Here, we explore a panel of 17 trans-species microsatellites, derived from other pinniped species, that successfully cross-amplify between pinniped species (Colman et al. 1996; Gemmell et al. 1997; Davis et al. 2002; Hoffman et al. 2007) and that are also polymorphic in the NZSL. (Osborne et al. 2011, 2013) to examine genetic variability in this species. We assembled a large, multi-year, and multi-location collection of samples from living males, females, and pups, as well as pups that succumbed to disease. Due to the documented demographic history, and the recent and ongoing decline in population size, we question whether genetic variation may be impacting on survivorship through epizootic episodes and limiting population growth. In addition, we are interested to know if the NZSL genome has retained genetic hallmarks indicative of the historic bottleneck; if detected, such patterns of variation would again be detrimental to population persistence. We use these data to address whether: 1) Auckland Islands breeding colonies represent one or multiple subpopulations; 2) the population retain a genetic signal indicative of the bottleneck/reduction due to 19th century hunting; and 3) pups which succumbed to disease exhibited lower heterozygosity and higher internal relatedness (IR; i.e., measures of inbreeding) compared to those that survived. We hypothesize that patterns of genetic variation detected in this study may indicate that population size reduction, and associated genetic changes, may be impacting on the NZSLs population recovery and contributing to what appears to be a susceptibility to epizootic disease (Baker 1999; Wilkinson et al. 2006; Osborne et al. 2013).

**Methods**

**Sampling**

The NZSL sample used here includes 1205 individual animals collected between the 2000/2001 and 2006/2007 Austral summer breeding seasons (Table 1). All NZSL samples in this study were collected from sites within the Auckland Islands group (Figure 1, 50°42’S 166°5’E). The majority of NZSL samples were collected from Sandy Bay on Enderby Island, which is the most intensively monitored site. A proportion of pup samples were also collected during the 2003/2004 breeding season from Dundas Island (n = 42), South East Point on Enderby Island (n = 32), and Figure of Eight Island (n = 24) in the Auckland Islands group (Figure 1). Pups were
Table 1. Sources of NZSL samples used in this study

<table>
<thead>
<tr>
<th>Year/location</th>
<th>Dead pups</th>
<th>Live pups</th>
<th>Adult females</th>
<th>Adult males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sandy Bay</td>
<td>Sandy Bay</td>
<td>Dundas Island</td>
<td>South East Point</td>
</tr>
<tr>
<td>2000/2001</td>
<td>31</td>
<td>103</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001/2002</td>
<td>18</td>
<td>84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002/2003</td>
<td>6</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003/2004</td>
<td>30</td>
<td>77</td>
<td>42</td>
<td>32</td>
</tr>
<tr>
<td>2004/2005</td>
<td>59</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005/2006</td>
<td>73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006/2007</td>
<td>49</td>
<td>34</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Years represent Austral summer breeding seasons where samples were collected, and each cell shows the number of animals sampled per year. The 2003/2004 season includes samples from 4 breeding colonies: Sandy Bay, Enderby Island; Dundas Island; South East Point, Enderby Island; Figure of Eight Island. All adult NZSLs were alive at the time of sampling.

*Territorial adult males.

Figure 1. Breeding distribution of the NZSL. There are 3 recognized breeding areas: 2 in the Auckland Islands, 1 on Campbell Island Motu Ihupuku and also a colony on the Otago Peninsula (Chilvers and Wilkinson 2008). Sampling locations used in this study are Sandy Bay on Enderby Island, Figure of Eight Island, South East Point, and Dundas Island. Figure from Robertson and Chilvers (2011).
predominantly sampled from marked females in each year of the study. All samples of live sea lions were collected under approved animal ethics protocols and appropriate permits (Department of Conservation Animal Ethics Permit Approval AEC86 [1 July 1999]), and dead pups were sampled at necropsy.

DNA Extraction

DNA was extracted from NZSL skin biopsies (live pups) or other internal tissue (dead pups) using an adaptation of the Chelex 100 (Bio-Rad, Hercules, CA, USA) extraction protocol (Walsh et al. 1991). Briefly, approximately 1 mm$^3$ of tissue was suspended in a digesting solution consisting of 5% Chelex 100, 100 mM NaCl, 50 mM Tris (pH 8.0) 1% SDS, and 10 mM EDTA. About 10 mg/ml each of proteinase K and RNase were added and samples were digested overnight at 55 °C. Samples were centrifuged at 12 000 rpm for 1 min to precipitate the debris and transferred to a new tube containing 5% Chelex in TE (10 mM Tris pH 8.0, 1 mM EDTA). Finally, samples were again centrifuged at 12 000 rpm and stored at −20 °C until used.

Microsatellite Amplification and Genotyping

Polymerase chain reaction (PCR) experiments were carried out as previously described (Osborne et al. 2013) with primers referenced in Table 2. PCR products were genotyped using an ABI 3730x DNA Analyser (Genomic Analysis Service, University of Otago) and analyzed using the program GeneMapper (both Applied Biosystems, Carlsbad, CA, USA).

Analyses of Population Statistics, Diversity and Bottleneck

Raw microsatellite allele lengths were converted into allele bins using the program FlexiBin (Amos et al. 2007). This program was chosen due its ability to help to prevent miscalling of microsatellite alleles. Allele frequencies, observed and expected heterozygosities, null allele frequencies (Pemberton et al. 1995) and conformation to Hardy–Weinberg equilibrium (HWE) were determined using the program Cervus (Kalinowski et al. 2007) Estimates for HWE at each locus were subject to Bonferroni correction to account for testing across multiple loci at 0.05/n (n = the number of loci being tested). The same program was used to check for repeat genotypes/duplicate sampling of individuals. IR for each of the 1205 individual NZSL was calculated using the program GENHET (Coulon 2010), implemented in the statistics package R (R Core Development Team 2010). Heterozygosity–heterozygosity correlations (Balloux et al. 2004) were calculated using the R extension package Rhh (Alho et al. 2010). Analyses of variance (ANOVA), Tukey's post hoc tests of significance, Pearson’s correlation coefficient, and one- and two-sample t-tests were also calculated using R. A random sample of 10% of PCR amplifications, and subsequent genotyping, were repeated for error checking to ensure reliability of data.

Population substructure among the different breeding beaches (Sandy Bay, Dundas Island, Figure of Eight Island, and South East Point) was examined using a conventional test of differentiation using microsatellite data; pairwise $F_{ST}$ values (Weir and Cockerham 1984) were calculated between live pups sampled from the different breeding grounds in 2003/2004 using FSTAT version 2.93 (Goudet 2001). Structure v2.3.3 (Pritchard et al. 2000; Falush et al. 2003, 2007) using pups solely sampled during the 2003/2004 breeding season was also used to test for any “cryptic” structuring, and visualized using STRUCTURE HARVESTER v0.6.94 (Earl and Vonholdt 2012).

We tested for a change in effective population size (bottleneck) from allele frequencies using 2 approaches: Bottleneck v1.2.02 (Cornuet and Luikart 1996) and Approximate Bayesian Computation using Do It Yourself ABC (DIYABC) v1.0.4.39 (Cornuet et al. 2010). We used Bottleneck to test for recent effective population size reductions, based on patterns of heterozygosity excess or deficit (Cornuet and Luikart 1996). In contrast, DIYABC was employed to infer past population history through comparison to a variety of models informed by our knowledge of the population’s demographic history. In this instance,

### Table 2. Microsatellite variation using samples spanning 7 breeding seasons (2000/2001–2006/2007)

<table>
<thead>
<tr>
<th>Microsatellite</th>
<th>Accession</th>
<th>Reference</th>
<th>Fragment size (bp)</th>
<th>N</th>
<th>k</th>
<th>$H_o$</th>
<th>$H_e$</th>
<th>Null allele frequency</th>
<th>Genotyping error rate per locus (%)</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg8.10</td>
<td>G02096</td>
<td>1</td>
<td>184–193</td>
<td>115</td>
<td>6</td>
<td>0.451</td>
<td>0.43</td>
<td>−0.025</td>
<td></td>
<td>0.1341</td>
</tr>
<tr>
<td>Lc5</td>
<td>AF417694</td>
<td>3</td>
<td>156–186</td>
<td>1203</td>
<td>5</td>
<td>0.445</td>
<td>0.444</td>
<td>0.0002</td>
<td>1.06</td>
<td>0.9937</td>
</tr>
<tr>
<td>Pvc</td>
<td>G02096</td>
<td>1</td>
<td>171–179</td>
<td>1201</td>
<td>5</td>
<td>0.457</td>
<td>0.484</td>
<td>0.0297</td>
<td>0</td>
<td>0.0375</td>
</tr>
<tr>
<td>Zcwe03</td>
<td>AM039821</td>
<td>9</td>
<td>210–216</td>
<td>1191</td>
<td>4</td>
<td>0.532</td>
<td>0.537</td>
<td>0.0014</td>
<td>0.61</td>
<td>0.6313</td>
</tr>
<tr>
<td>G1A</td>
<td>—</td>
<td>—</td>
<td>180–195</td>
<td>1195</td>
<td>8</td>
<td>0.584</td>
<td>0.611</td>
<td>0.0216</td>
<td>0</td>
<td>0.2764</td>
</tr>
<tr>
<td>M11A</td>
<td>—</td>
<td>4</td>
<td>142–177</td>
<td>1199</td>
<td>6</td>
<td>0.61</td>
<td>0.634</td>
<td>0.0196</td>
<td>0</td>
<td>0.0045</td>
</tr>
<tr>
<td>Hg6.1</td>
<td>G02091</td>
<td>1</td>
<td>150–166</td>
<td>1199</td>
<td>8</td>
<td>0.654</td>
<td>0.646</td>
<td>−0.0069</td>
<td>1.09</td>
<td>0.675</td>
</tr>
<tr>
<td>Lc28</td>
<td>AF140584</td>
<td>2</td>
<td>134–150</td>
<td>1203</td>
<td>5</td>
<td>0.658</td>
<td>0.679</td>
<td>0.0161</td>
<td>0</td>
<td>0.0824</td>
</tr>
<tr>
<td>Pvc</td>
<td>—</td>
<td>—</td>
<td>131–164</td>
<td>1200</td>
<td>9</td>
<td>0.735</td>
<td>0.727</td>
<td>−0.005</td>
<td>2.15</td>
<td>0.6794</td>
</tr>
<tr>
<td>Pvc</td>
<td>U65444</td>
<td>6</td>
<td>156–179</td>
<td>1195</td>
<td>8</td>
<td>0.773</td>
<td>0.735</td>
<td>−0.0271</td>
<td>1.08</td>
<td>0.011</td>
</tr>
<tr>
<td>Hg6.3</td>
<td>G02092</td>
<td>1</td>
<td>210–250</td>
<td>1147</td>
<td>7</td>
<td>0.707</td>
<td>0.745</td>
<td>0.0268</td>
<td>0</td>
<td>0.0774</td>
</tr>
<tr>
<td>OrrFCB1</td>
<td>G34933</td>
<td>5</td>
<td>178–210</td>
<td>1180</td>
<td>12</td>
<td>0.739</td>
<td>0.769</td>
<td>0.0199</td>
<td>0.69</td>
<td>0.009</td>
</tr>
<tr>
<td>H116</td>
<td>AF140582</td>
<td>2</td>
<td>139–167</td>
<td>1201</td>
<td>14</td>
<td>0.711</td>
<td>0.783</td>
<td>0.0438</td>
<td>1.08</td>
<td>0.01</td>
</tr>
<tr>
<td>OrrFCB7</td>
<td>G34928</td>
<td>5</td>
<td>200–212</td>
<td>1191</td>
<td>7</td>
<td>0.776</td>
<td>0.795</td>
<td>0.0133</td>
<td>0.64</td>
<td>0.8127</td>
</tr>
<tr>
<td>Hg4.2</td>
<td>G02090</td>
<td>1</td>
<td>144–177</td>
<td>1195</td>
<td>14</td>
<td>0.818</td>
<td>0.819</td>
<td>−0.001</td>
<td>0</td>
<td>0.2246</td>
</tr>
<tr>
<td>ZcweC03</td>
<td>AM039819</td>
<td>9</td>
<td>240–269</td>
<td>1071</td>
<td>15</td>
<td>0.776</td>
<td>0.829</td>
<td>0.032</td>
<td>3.18</td>
<td>0.054</td>
</tr>
<tr>
<td>ZcGxDh5.16</td>
<td>YJ676477</td>
<td>8</td>
<td>208–246</td>
<td>1183</td>
<td>19</td>
<td>0.826</td>
<td>0.896</td>
<td>0.0397</td>
<td>0</td>
<td>0.024</td>
</tr>
</tbody>
</table>

we know from historic records that the NZSLs were subject to intense hunting during sealing in the mid 1800s, which resulted in significant population size reduction (Childerhouse and Gales 1998). Using DIYABC we tested the fit of our data to scenarios of historic population size reduction (Scenario 1, Supplementary Table 1) compared to a scenario in which the population size remains stable (Scenario 2, Supplementary Table 1). Prior distributions were chosen from recent research and historical evidence (Childerhouse and Gales 1998; Geschke and Chilvers 2009): N, effective size of current population; t0, time parameter associated with the historical effective population size; N0, pre-bottleneck effective population size; db, time parameter associated with the end of the bottleneck event (Table 3). We used the default values for microsatellite mutation models based on Cornuet et al. (2010). Scenario comparison was based on calculation of simulated versus observed summary statistics (mean number of alleles, mean gene diversity, mean allele size variance, and mean M index [the ratio of the number of alleles to the range in allele size; Garza and Williamson 2001] across loci).

Data Availability
We have deposited the primary data underlying these analyses as follows:
Sampling locations and microsatellite genotypes: Dryad (Baker 2013).

Results
Population Statistics
Of the 1205 individuals in this study, 1176 (98%) were genotyped at 15–17 loci, with 1001 (84%) genotyped at all 17 loci (Table 2). Where individuals were unable to be genotyped at every locus, they were subjected to repeat PCR 2 additional times before conceding lack of amplification of a locus in a particular individual. After error-checking of genotypes using a random 10% of the total sample set (10% of samples genotyped once more each), the error rate for our dataset is estimated as 0.67% across all 17 loci (per locus error rate reported in Table 2). No duplicate genotypes were detected in our data, confirming that no animal had been sampled and included in the cohort twice.

The 1205 individuals in this study, detailed in Table 4A, B, comprise 219 adult females, 213 adult males, and 773 pups (of which 580 are live and 193 are dead). Population-wide (Auckland Islands) statistics were calculated from 7 breeding seasons using all 17 loci (Figure 1). Observed heterozygosity (H0) averaged over all loci was 0.66 (range 0.445–0.826) while expected heterozygosity (He) was 0.68 (range 0.43–0.896). We identified between 4 and 19 alleles at microsatellite loci within the NZSL population, average 8.9 (Table 2). After Bonferroni correction for multiple tests none of the 17 loci deviated significantly from HWE (Bonferroni corrected \( \alpha = 0.002 \)). Null alleles were not detected at any of the 17 loci used.

To place the level of variation detected in the NZSL in a comparative context, we plotted overall expected heterozygosity (He) against the number of alleles detected for 11 pinniped species (Figure 2), using data for other pinnipeds sourced from Table 1 of Robertson and Chilvers (2011). We detected a linear relationship between the number of alleles and H0 (Pearson’s correlation coefficient of \( r = 0.61 \)). This linear relationship exists despite the varying number of individuals used in each of the studies incorporated in this analysis, and shows that the NZSL has a moderate level of genetic variation in comparison to other pinniped species.

Heterozygosity–heterozygosity correlations (Balloux et al. 2004) performed in R calculated across all loci in the NZSL population indicated a small but significant correlation among loci at 0.041 (95% CI = 0.009–0.071, Supplementary Figure 1).

Population Differentiation
Live pups from the 2003/2004 breeding season were used to test for population differentiation, from 4 different breeding beaches: Sandy Bay, Enderby Island \( n = 77 \); Dundas Island, \( n = 42 \); Figure of 8 Island, \( n = 24 \) and South East Point, Enderby Island \( n = 32 \). Pairwise comparisons of the fixation index \( F_{ST} \) (Weir and Cockerham 1984) found statistically significant structuring based on microsatellite genotype data between South East Point and Dundas Island, and South East Point and Enderby Island (Supplementary Table 2; Wright, 1978). However, the \( F_{ST} \) values themselves are very small, at levels which generally suggest no population structure (Hartl et al. 1997; Frankham et al. 2002); we suggest that the 2 positive \( P \)-values obtained here are reflective of the kinship contained within the sampled populations, and represent the spatial pattern of genetic differentiation that can appear when sampling species that exist in familial clusters (Knutsen et al. 2011).

To investigate the potential for cryptic population substructuring, the true K (number of populations) was inferred using the program STRUCTURE (Pritchard et al. 2000). The data was subjected to a burn-in period of 5000 and 50 000 Markov chain Monte Carlo (MCMC) repeats, and this was performed 4 times for each of 4 hypothesized K = 1 to K = 4. When K is approaching its true value, the log likelihood for each K (L(K)) plateaus, and has a high variance between runs. (The most likely K was inferred using STRUCTURE HARVESTER; Earl and Vonholdt 2012). Raw STRUCTURE outputs and the estimated log probability of the data indicated that the most likely K = 1. All other estimated values of K have high variance between runs and large standard deviations (Supplementary Table 3).

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Prior distribution</th>
<th>Conditions</th>
<th>Mean of the posterior distribution</th>
<th>Median of the posterior distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective population size of current population</td>
<td>( N )</td>
<td>6000–15000</td>
<td>8070</td>
<td>7490</td>
</tr>
<tr>
<td>Time assoc. with historical Ne</td>
<td>( t_0 )</td>
<td>1–10 000</td>
<td>6800</td>
<td>7100</td>
</tr>
<tr>
<td>Time (generations) assoc. with end of bottleneck</td>
<td>( db )</td>
<td>15–25</td>
<td>19.8</td>
<td>19.6</td>
</tr>
<tr>
<td>Pre-bottleneck effective population size</td>
<td>( N_0 )</td>
<td>15 000–100 000</td>
<td>28 400</td>
<td>22 900</td>
</tr>
</tbody>
</table>

Table 3. Parameters associated with demographic history scenarios, as used in DIYABC, along with calculations of posterior distributions of these parameters, as determined by DIYABC.
Table 4. Details on genetic diversity in the NZSL using 17 microsatellites

<table>
<thead>
<tr>
<th>Population</th>
<th>Year</th>
<th>Location</th>
<th>Sample size (n)</th>
<th>No. alleles (k)</th>
<th>Allelic richness</th>
<th>$H_S$</th>
<th>$H_E$</th>
<th>IR</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>—</td>
<td>—</td>
<td>1205</td>
<td>8.94</td>
<td>4.07</td>
<td>0.66</td>
<td>0.68</td>
<td>0.03</td>
<td></td>
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<tr>
<td>Adult females</td>
<td>2000/2001</td>
<td>SB</td>
<td>111</td>
<td>6.82</td>
<td>3.89</td>
<td>0.65</td>
<td>0.67</td>
<td>0.041</td>
<td>0.0052*</td>
</tr>
<tr>
<td>Pups</td>
<td>2000/2001</td>
<td>SB</td>
<td>134</td>
<td>7.12</td>
<td>6.43</td>
<td>0.67</td>
<td>0.67</td>
<td>0.004</td>
<td>0.7945</td>
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<tr>
<td>Territorial</td>
<td>2000/2001</td>
<td>SB</td>
<td>18</td>
<td>5.71</td>
<td>4.13</td>
<td>0.61</td>
<td>0.68</td>
<td>0.129</td>
<td>0.0085*</td>
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<tr>
<td>Adult females</td>
<td>2001/2002</td>
<td>SB</td>
<td>69</td>
<td>6.35</td>
<td>3.93</td>
<td>0.64</td>
<td>0.67</td>
<td>0.069</td>
<td>0.0022*</td>
</tr>
<tr>
<td>Adult males</td>
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<td>SB</td>
<td>85</td>
<td>6.59</td>
<td>3.94</td>
<td>0.62</td>
<td>0.67</td>
<td>0.091</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Pups</td>
<td>2001/2002</td>
<td>SB</td>
<td>102</td>
<td>6.59</td>
<td>6.19</td>
<td>0.68</td>
<td>0.68</td>
<td>0.006</td>
<td>0.7158</td>
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<tr>
<td>Adult females</td>
<td>2002/2003</td>
<td>SB</td>
<td>39</td>
<td>5.94</td>
<td>3.90</td>
<td>0.66</td>
<td>0.67</td>
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<td>0.3446</td>
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<tr>
<td>Adult males</td>
<td>2002/2003</td>
<td>SB</td>
<td>110</td>
<td>7.24</td>
<td>4.12</td>
<td>0.67</td>
<td>0.69</td>
<td>0.041</td>
<td>0.0035*</td>
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<td>Pups</td>
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<td>SB</td>
<td>71</td>
<td>6.63</td>
<td>6.20</td>
<td>0.67</td>
<td>0.68</td>
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<td>0.1805</td>
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<td>Pups</td>
<td>2003/2004</td>
<td>SB, DI, Fig 8, SEP</td>
<td>205</td>
<td>7.53</td>
<td>6.45</td>
<td>0.67</td>
<td>0.68</td>
<td>0.021</td>
<td>0.0495*</td>
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<tr>
<td>Pups</td>
<td>2004/2005</td>
<td>SB</td>
<td>105</td>
<td>6.88</td>
<td>6.31</td>
<td>0.67</td>
<td>0.68</td>
<td>0.023</td>
<td>0.1406</td>
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<tr>
<td>Pups</td>
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<td>SB</td>
<td>73</td>
<td>6.53</td>
<td>6.19</td>
<td>0.66</td>
<td>0.68</td>
<td>0.041</td>
<td>0.0043*</td>
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<td>SB</td>
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<td>6.47</td>
<td>6.09</td>
<td>0.67</td>
<td>0.66</td>
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<td>0.3712</td>
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</table>

Bottleneck analyses

BOTTLENECK (Cornuet and Luikart 1996) was chosen to model a population bottleneck based on the stepwise mutation model, SMM (Ohta and Kimura 1973), the two-phase model, TPM (Di Rienzo et al. 1994), and the infinite alleles model, IAM (Estoup et al. 1995). All 3 models were used because microsatellites only very rarely conform to strict SMM (Cornuet and Luikart 1996), and the program calculates the probability of heterozygote excess based on each of the assumed microsatellite mutation models. Live pups from 2003/2004 ($n = 205$) were chosen to provide a “genomic snapshot” of the level of variation in the NZSL population at this point in time. Using the program’s sign test, heterozygote excess was detected under the IAM ($P < 0.0001$). Also under IAM, we found no loci with heterozygote deficiency but 17 with heterozygote excess ($P < 0.0001$). Modeling under the SMM resulted in a similar output; the probability for heterozygote excess was $P < 0.0001$ with 16 loci predicted to be displaying heterozygote excess. The two-phase model TPM was implemented with $70\%$ of loci mutating under SMM and $30\%$ under IAM, with variance set at the default of 30. Under the TPM, the sign test gave the probability of heterozygote excess as $P = 0.003$. Thus, with all 3 models a significant excess of heterozygous individuals was detected, providing strong evidence of a bottleneck within the last 4Ne generations.
Approximate Bayesian Computation

To further investigate the bottleneck, we employed Approximate Bayesian Computation to test whether our data were better fitted to a population that had undergone a bottleneck, or maintained constant population size. We defined 2 models describing these differing demographic histories: Scenario 1) using priors dictating that a population size contraction had occurred between 25 and 15 generations ago (to coincide with the onset and end of commercial sealing activities); or Scenario 2) that no population size contraction had occurred (Supplementary Table 1). The posterior probabilities of each scenario were calculated by measuring the similarities between simulated and observed data. The scenario with the highest posterior probability, determined by logistic regression, was Scenario 1 \((P = 0.94, 95\% CI = 0.89–0.98)\), consistent with a history of a population bottleneck. According to this scenario, the population suffered a severe size contraction between 25 and 15 generations ago, timing that coincides with the end of 19th century commercial sealing activities in the NZSL habitat range. The adequacy of the model was assessed by comparing where the observed summary statistics lie among those generated by the simulated datasets. Three of the 4 summary statistics in each scenario all deviated significantly \((P < 0.05, \text{Supplementary Table 4})\) from the simulated data; however, Scenario 1 was a better fit to the simulated data than Scenario 2. The significant deviations likely result from the estimations of the prior distributions, because we do not know with precision the historical size of the population. Nevertheless, we can see from the principal component analysis (Supplementary Figure 2) and the posterior probabilities that these data are highly supportive of Scenario 1 (Scenario 1: \(P = 0.94, 95\% CI = 0.89–0.98\); Scenario 2: \(P = 0.06, 95\% CI = 0.0197–0.1004\)). Parameter estimation was based on 300 datasets from Scenario 1 and underwent a logit transformation (Table 3). The current effective population size \((N)\) was estimated as 7490 individuals, and the bottleneck (time associated with the lowest Ne) was calculated to have occurred 19.6 generations ago. N0, the pre-bottleneck effective population size, was estimated to be 22,900.

IR and Pup Mortality

IR (Amos et al. 2001) is an index of similarity (weighted by allele frequencies within the population) of the parental half-genotypes for a given individual. It therefore reflects the kinship of an individual’s parents relative to a random pairing of individuals within the population, and is often used as a proxy for measures of inbreeding, because a positive IR value indicates some kinship of the parents. IR values vary around zero, ranging from -1 (least inbred) to 1 (most inbred). We tested the mean IR scores for all dead pups and then all live pups against an hypothesized mean of zero using a one-sample t-test. We found that dead pups showed a level of IR that was significantly higher than zero, with \(P = 0.0001\), at a mean IR of 0.0195 (95% CI = 0.0188–0.0202, \(t = 4.9316, DF = 297, SD = 0.16\)). Mean IR of live pups was 0.015 (95% CI = 0.002–0.027, \(t = 2.3428, DF = 580, SD = 0.15\)), which was also significantly greater than zero at \(P = 0.0195\), albeit less so than dead pups. There was a highly significant difference in mean IR between dead pups and live pups, \(P = 0.004\) (mean = 0.032, 95% CI = 0.01–0.05, \(t = 2.8742, DF = 876\)). In adult NZSLs (Table 4A), the mean level of IR was significantly higher than zero in adult females 2000/2001; territorial males 2000/2001; adult females 2001/2002; and adult males 2001/2002.

To account for any potential biases in the above analyses that might result from combining data for pups from multiple years, and which may include a number of half-siblings, we calculated IR values for each population subset (Table 4A, B). An ANOVA was performed on the IR values for each population subset firstly based on Table 4A. Significant differences exist between the IR values of the subsets in Table 4A \((P = 0.0007, \text{Supplementary Table 5a})\). Tukey’s post hoc testing showed that the significance differences between the population subsets in Table 4A is driven by the significant differences between the high IR value of the adult males sampled in 2001/2002 and 1) pups 2000/2001, \(n = 134, P = 0.0039\); 2) pups from 2001/2002, \(n = 102, P = 0.0132\); 3) pups from 2003/2004, \(n = 205, P = 0.0294\). All other comparisons between population subsets were not significant \((P > 0.05)\). When we separated dead and live pups and recalculated the ANOVA without adult NZSLs (as in Table 4B) there was no significant difference in IR between any of the pup groups (Supplementary Table 5b). This indicates that our results for mean IR between live and dead pups reflect the true nature of IR differences between a large group of live pups and a large group of dead pups, and are not skewed by the inclusion of pups from multiple breeding years.

Discussion

Here, we have presented locus- and population-specific statistics for 17 microsatellite loci in the NZSL at the Auckland Islands. Across 7 breeding seasons, the average allelic diversity was 8.94 with an observed \((H_{obs})\) and expected \((H_{exp})\) of 0.66 and 0.68, respectively. Average error rate for microsatellite genotyping was low at 0.67%. Heterozygosity is correlated among loci in the NZSL population, indicating that our data display identity disequilibrium and that the loci are not hetero- or homozygous independently of each other. We provide a measure of microsatellite variation (IR) that is suggestive of a higher occurrence of potentially inbred individuals among pups that died during epizootic episodes. Consistent with a previous studies of NZSL population structure based on mitochondrial DNA (Ashton 1999; Collins et al. 2014) we found no evidence of population structure or differentiation between NZSL breeding beaches, suggesting that there is gene flow between the different breeding areas in the Auckland Islands. Analyses with the program Bottleneck provide statistical evidence for a recent population size reduction, which was significant regardless of the model of microsatellite mutation that is assumed.

Allele frequencies in large, randomly mating populations approach equilibrium when factors causing deviation of loci from HWE, such as inbreeding, population fragmentation, selection and migration, are limited. Equilibrium is expected in most outbreeding populations, however agreement with expectations does not mean that the loci are free from influences such as selection, since these effects are often small and difficult to detect. Thus HWE provides a null hypothesis against which to detect inbreeding, selection, migration, or population substructure/fragmentation (Frankham et al. 2002). The NZSL has a very restricted distribution, within which there is no evidence of population substructure (Robertson et al. 2006; Osborne et al. 2013, 2015; Collins et al. 2014). Hence, gene flow should occur among breeding beaches, evidenced by estimation here of the most likely number of populations \((K)\) as 1, the low values of pairwise \(F_{ST}\) values between breeding sites, and lack of detectable deviations from Hardy–Weinberg expectations when considering the dataset as a whole.

Average heterozygosity and allelic diversity over a panel of loci are commonly used to characterize genetic diversity in a species or population. A previous study of microsatellite diversity in 39 NZSLs described an unexpectedly high level of diversity (an observed heterozygosity of
microsatellite loci of 0.72) considering the recent and current population history of the NZSL (Acevedo-Whitehouse et al. 2009). With a larger sample set, we expand on this and show that mean observed heterozygosity in NZSLs is 0.66, with expected heterozygosity at 0.68. It is interesting to compare this value to that observed in the New Zealand fur seal (NZFS) Arctocephalus forsteri, which have higher estimates of observed heterozygosity (0.66–0.74) dependent on geographic location (Robertson and Gemmell 2003; Caudron et al. 2009). This is noteworthy because the NZSL and NZFS have similar recent population histories in that they were both heavily hunted up to and during the 19th century (Childerhouse and Gales 1998; Lalas and Bradshaw 2003), but their modern trajectories are very different; the NZFS is a thriving population experiencing annual increases in pup production at some sites during the early 2000s of more than 32% per annum (Boren et al. 2006). In stark contrast the NZSL has failed to recover to pre-hunting levels and is now in significant decline (Taylor 1971; Wilkinson et al. 2003, 2006) with pup production having declined 40% in the preceding decade (Chilvers 2009), which has been strongly driven by fisheries bycatch (Robertson and Chilvers 2011).

Dependent on population history, simple observed and expected heterozygosity at a panel of microsatellite loci may not always be wholly indicative of genome wide genetic diversity, because a population bottleneck has to be sustained and severe before any large effect on overall levels of heterozygosity is detectable. Population bottlenecks reduce both allele frequency and heterozygosity; Genetic drift can be associated with this reduction (Garza and Williamson 2001) and can lead to some alleles in the population being lost, or becoming very rare, and some going to fixation. Thus, the first evidence of a genetic bottleneck is the loss of rare alleles (Nei et al. 1975; Watterson 1984; Maruyama and Fuerst 1985; Cornuet and Luikart 1996). Therefore, in a population that has undergone contraction, there will be fewer rare alleles present than one would expect for a population at mutation-drift equilibrium (Luikart and Cornuet 1998). In the Auckland Islands population as a whole, the average allelic diversity of 8.94 is lower than the range of allelic diversity in the NZFS, which is 9.4–15.3 dependent on geographic location (Robertson and Gemmell 2005; Caudron et al. 2009), while observed heterozygosity remains at a moderate level compared to other pinnipeds with similar demographic histories (Table 1 in Robertson and Chilvers 2011 and Figure 2). This suggests that despite maintenance of a moderate level of observed heterozygosity, allelic diversity is low and provides some genetic evidence to support historical observations of a population bottleneck (Osborne et al. 2013; Collins et al. 2014).

Most outbred, randomly mating populations exist at mutation-drift equilibrium. In such populations, there is an equal probability that a locus will show heterozygote excess or heterozygote deficit, so that approximately 50% of loci will show a slight heterozygote excess and 50% will show a slight heterozygote deficit (Luikart and Cornuet 1998). This is in contrast to recently bottlenecked populations which are expected to show a majority of loci with heterozygote excess as a result of the loss of rare alleles. The sign test (Cornuet and Luikart 1996) was implemented to determine if a population possesses a significant number of loci with heterozygote excess. This test can determine if the population has been recently bottlenecked, where recent is defined as within 4N_e generations (Cornuet and Luikart 1996). The NZSL population shows significant heterozygote excess regardless of the model of microsatellite mutation employed. This evidence of heterozygote excess is consistent with the historical data available documenting the intensive exploitation of this species’ during the peak of sealing in the 18th and 19th centuries (Lalas and Bradshaw 2001), and is also likely influenced further by the longer term reduction in the species’ distribution post human settlement c. AD1300, and recent epizootic events and resource competition between NZSLs and fisheries, resulting in NZSL death (Chilvers 2008; Robertson and Chilvers 2011).

Evidence for heterozygote excess and genetic confirmation of historical observations of a bottleneck is further supported by the implementation of Approximate Bayesian Computation. We find that the scenario depicting a population size contraction between 25 and 15 generations ago fits our data, with a highly significant posterior probability, compared to a null scenario depicting a constant population size. This suggests that our data are indeed reflective of a population that has been through a population size bottleneck, roughly 19 generations ago, consistent with the known population history.

The bottleneck analyses performed here are based on simulations that assume uniform allele mutation rates and modes of microsatellite evolution (i.e., perfect repeat length intervals). It is important to note that microsatellites do not always conform to these assumptions, and non-conformation is more likely when using trans-species markers, as used in this, and many other studies of pinnipeds (e.g., Coltman et al. 1996; Gelatt et al. 2001; Davis et al. 2002; Wolf et al. 2006). Unusual allele repeat length distributions are a possible sign of a bottleneck, and because there is the potential for trans-species markers to also display this trait, we acknowledge that the bottleneck signature detected here may be influenced by use of trans-species markers. However, the 3 different modes of microsatellite evolution used in the Bottleneck program, and the well-established demographic history of this species, provides significant confidence that the signature detected in this species is genuine.

While inference of a bottleneck is valuable, it is also helpful to know if inbreeding is occurring in the population, which may be a consequence of reduced population size following a bottleneck. While it is well-known that estimates of multilocus heterozygosity have often been used as proxies of inbreeding, heterozygosity estimates are expected to only correlate weakly with inbreeding coefficients calculated through pedigrees (Balloux et al. 2004; Slate et al. 2004; Alho et al. 2010; Grueber et al. 2011). Calculation of heterozygosity–heterozygosity correlations (Balloux et al. 2004) can indicate whether the microsatellite data under study is reflective of genome-wide heterozygosity, under the premise that heterozygosity estimates calculated from 2 randomly chosen sets of markers should be similar (Alho et al. 2010). Repeated random division of this microsatellite dataset in half and recalculation of a measure of multilocus heterozygosity (in this case, IR) attains a mean correlation that is positive and significant. This means that heterozygosity is correlated among the loci used in this study, and that the multilocus heterozygosity estimates calculated here are able to identify an inbreeding signature, if present (Supplementary Figure 1).

To investigate whether any subsets of the NZSL population contained individuals that may have parents that are more related to one another than one would expect from a truly random pairing, we chose to use a measure of parental relatedness and microsatellite allele sharing, IR (Amos et al. 2001) that is based on a measure of genetic relatedness between individuals (Queller and Goodnight 1989). Using this metric, those individuals that are more homozygous have a higher IR score, and in this metric, the sharing of rare alleles is weighted more than the sharing of common alleles. While this measure can be problematic in species where there is admixture and immigration (because the sharing of new alleles would be accounted for as being “rare” (Aparicio et al. 2006), neither admixture nor immigration occur in the NZSL population.
Overall, dead pups as a group showed a level of IR that is significantly higher than live pups. This indicates that dead pups have parents that show a higher level of allele sharing and therefore are potentially more inbred than would be expected compared to pups that survived. It is possible that this pattern is reflective of the selection pressure that opportunistic pathogens exert in epizootic years, during which more homozygous pups may be more susceptible to disease (Coltman et al. 1999). This is an interesting possibility that warrants further investigation. All NZSL adults, both male and female, display mean IR scores that are significantly higher than zero. We hypothesize that this trend may be explained by recent epizootic events in the NZSL: the adult NZSLs in this study were sampled from 2001 onwards; since NZSLs reach breeding between 4–6 years (females) and 10–12 years (males, Perrin et al. 2008), it is likely that some of the females are the progeny of those that survived the 1997/1998 epizootic, or are those that survived through this disease outbreak (both males and females). The adult groups in the cohort may therefore provide a snapshot of the population at points where it was more inbred—the animals that have survived through recent epizootics may be more genetically similar to one another than could be expected in a random cohort. Increased IR of adults may lead to increased IR of pups, with further epizootics potentially increasing overall IR, however those with the highest IR values are still more likely to die during an epizootic episode than those with a lower IR. The high IR detected in this study suggests that genetic variation in the NZSL population needs to be fully considered in conservation management plans.

Conclusions

The Auckland Islands NZSL population has experienced a reduction in population size as a result of 19th century sealing activities, and exists in relative isolation. Our analyses identified signatures consistent with the presence of more inbred individuals following a bottleneck, which is likely a consequence of historic range reduction, intensive exploitation, recurrent epizootic events and ongoing commercial fishery pressures. If both human interactions (fishermen bycatch and resource competition) and negative environmental effects (e.g., further disease outbreaks) continue to occur, then these may accelerate the decline of the NZSL via further population reduction and loss of genetic diversity.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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References


