

Have Antarctic minke whales increased in abundance because of 20th century whaling?

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ABSTRACT

Severe declines in megafauna worldwide illuminate the role of top predators in ecosystem structure. In the Antarctic, the ‘Krill Surplus Hypothesis’ posits that the killing of more than two million large whales led to competitive release for smaller krill eating species like the Antarctic minke whale. If true, the current size of the Antarctic minke whale population may be unusually high as an indirect result of whaling. Here we estimate long-term population size of the Antarctic minke whale prior to whaling by sequencing eleven nuclear genetic markers from 52 modern samples purchased in Japanese meat markets. Based upon Bayesian estimates of mutation rate and coalescence times among loci, we calculate long-term population size to be 670,000 individuals (95% CI: 374,000-1,150,000). Our estimate of long-term abundance is similar to or greater than contemporary abundance estimates, suggesting that competitive release and the ‘Krill Surplus Hypothesis’ are not required to explain current Antarctic minke whale abundance.

INTRODUCTION

Ecologists have long debated the relative roles of top-down (consumer-driven) and bottom-up (resource-driven) forces in shaping natural communities (Menge, 1992; Strong, 1992; White, 1978; Hairston et al., 1960; Hunter & Price, 1992). Trophic cascades stemming from removal of top-predators provide compelling support for consumer-driven control of food webs in many environments (Estes & Palmisano, 1974; Pace et al., 1999; Laws, 1970; McLaren & Peterson, 1994), including large, complex marine ecosystems (Estes et al., 1998; Frank et al., 2005). The commercial hunting of nearly two million whales (Figure 1a & b) from the Southern Ocean in the early 1900’s (Clapham & Baker, 2002) provides an opportunity to investigate the ecological consequences of top-predator removal on an oceanic scale. The hunted whales would have consumed at least 150 million tons of krill annually (Laws, 1977), leading many authors to suggest that their removal led to competitive release for smaller krill-eating

organisms (Conroy, 1975; Sladen, 1964; Payne, 1977; Laws, 1977; Croxall & Prince, 1979) – a top-down hypothesis often referred to as the ‘Krill Surplus Hypothesis.’ However, krill populations in the Southern Ocean are currently thought to be regulated by recruitment which is positively correlated with sea ice cover (Loeb et al., 1997; Nicol et al., 2000; White & Peterson, 1996) – a bottom-up explanation that has profound implications for krill and krill-dependent species amidst rising global temperatures (Nicol et al., 2008; Croxall et al., 2002; Forcada et al., 2006; McMahon & Burton, 2005; Weimerskirch et al., 2003). Understanding the role of whale defaunation in Antarctic ecosystems is therefore an important goal for ecological research.

One species hypothesized to have benefited from a krill surplus is the Antarctic minke whale, *Balaenoptera bonaerensis* (Burmeister 1867) (Figure 1c). Population size increases in Antarctic minke whales during the latter half of the 20th century have been inferred by increases in recruitment and decreases in age at sexual maturity (Thomson & Butterworth, 1999; Kato, 1985; Kato, 1983; Kato & Sakuramoto, 1991; Masaki, 1979; Butterworth et al., 1999) (but see Cooke, 1985; de la Mare, 1985). Some suggest that minke whales in the Southern Ocean increased in population size by approximately 8-fold after the removal of the large baleen whales (blue, fin, sei, humpback) (Ohsumi, 1979), and, at their current level, may be inhibiting the recovery of over-exploited whale species and reducing human food resources (Morishita & Goodman, 2000; Ohsumi, 1979; IWC, 1994; ICR, 2006). While there is a lack of firm data on pre-whaling population sizes and competition among whale species (Gales et al., 2005), some agencies advocate culling minke whales as a way to reduce competition with fisheries and support recovery of endangered whale species (ICR, 2006).

Assessing whether or not removal of large baleen whales had a cascading effect on minke whale population size requires knowledge of the Antarctic marine ecosystem before the onset of 20th century whaling. Recently, scientists have employed genetic data to assay past population sizes of baleen whales and other species (Alter et al., 2007; Roman & Palumbi, 2003; Shapiro et al., 2004; Atkinson et al., 2008), based upon the relationship between genetic diversity (θ) and long-term effective population size (N_e) ($\theta = 4N_e\mu$, where μ is the average mutation rate). Initial reconstructions of historical population sizes of whales using genetic data were limited by reliance on single loci and incomplete oceanic sampling (Lubick, 2003; Holt & Mitchell, 2004; Roman & Palumbi, 2003), but recent efforts using multiple loci and simulations of “ghost” populations (Alter et al., 2007) have helped overcome some of the initial challenges.

To assess the likelihood of a post-whaling competitive release in the Antarctic minke whale, we estimate long-term effective population size using eleven unlinked, nuclear markers. We describe a general method for capturing uncertainty in estimates of effective size due to unresolved gametic phase in highly heterozygous sequences and a new way to incorporate mutation rate variation among loci. If Antarctic minke whales are, at present, unusually abundant due to whaling, we would expect our estimate of long-term population size to be significantly lower than contemporary estimates of abundance.

METHODS

Sample collection and sequencing - To obtain genetic samples, 52 whale meat samples were purchased from Japanese meat markets and copies of the whale genome were amplified using Whole Genome Amplification (Lasken & Egholm, 2003). The sampled individuals were originally killed in Antarctic management Areas III, IV, V and VI (Figure 1b) by the Japanese Whale Research Program under the Special Permit in the Antarctic (JARPA). Eleven nuclear introns (Table 1) were amplified from these samples and sequenced using standard PCR and sequencing protocols. Individuals were sequenced in both directions when possible and all variable sites were checked by eye using Sequencher ver. 4.8 (Gene Codes Corporation, Ann Arbor, MI). Despite multiple attempts, not all individuals sequenced successfully for every intron, resulting in variation in the final sample sizes (mean 40, range: 20 – 52). PHASE 2.1 (Stephens et al., 2001) was used to reconstruct gametic phase using a burn-in of 10,000 iterations and run length of 10,000 iterations. To ensure that each sample was unique, we confirmed that none of the samples had identical sequences at all loci. We found no significant linkage disequilibrium (LD) among loci after correcting for multiple comparisons, therefore we considered the loci independent.

Equilibrium, neutrality and sub-division - To determine if our sequences were evolving in a manner consistent with equilibrium and neutrality, Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) tests were performed using the software DnaSP (Rozas et al., 2003). Tajima's D uses an estimate of θ based on the average number of pairwise differences (θ_π) and θ based upon the number of segregating sites (θ_s), while Fu's Fs is based upon the haplotype frequency distribution and θ_π . In neutrally evolving sequences, both values will be approximately equal to zero, while balancing selection or population expansion will result in values that are significantly greater or less than zero, respectively. We also used DnaSP to calculate the minimum number of recombination events in the sample (Hudson & Kaplan, 1985) and found that 6 of the 11 introns showed evidence of recombination. Therefore, coalescent simulations ($n = 10,000$) incorporating the per gene recombination parameter (R) were used to generate 95% confidence intervals for both Tajima's D and Fu's Fs statistics.

Accounting for the potential influence of population sub-division and/or interspecific gene flow on estimates of genetic diversity is one of the primary challenges to successfully using genetic data to estimate long-term population size (Alter et al., 2007; Atkinson et al., 2008). Genetic and morphological research indicates that Antarctic minke whales are reproductively isolated from all other forms of minke whales (reviewed in Rice, 1998) and recent genetic data indicate that they likely diverged from their most recent common ancestor, the common minke whale, at a minimum of 4 to 5 million years ago (Pastene et al., 2007). This time frame is approaching $4N_e$ generations, the average time at which nuclear loci are expected to become reciprocally monophyletic, thus, our genetic estimate is unlikely to be an artifact of gene flow from other species.

Population sub-division can increase coalescence time between genes and inflate estimates of genetic diversity. While preliminary reports suggested there may be

population sub-structure within Antarctic minke whales ($\phi_{st} = 0.0090$, $p = 0.0025$) (Pastene et al., 1996), these distinctions are weak. To further investigate the possibility of population sub-structure, we estimated the most likely number of populations (K) within our data set using the program Structure 2.2 (Pritchard et al., 2000). To avoid the potentially confounding effect of background linkage disequilibrium (LD) between closely linked sites within an intron, the maximum a posteriori haplotypes from PHASE at each intron were recoded to represent a single locus with n alleles. We performed three independent runs at each K value ($K = 1, 2$, and 3) using a burn-in period of 100,000 iterations and a run length of 500,000 repetitions. The best K value was selected based upon the highest average log likelihood score.

Estimation of θ - LAMARC version 2.1.3 was used to simultaneously estimate θ while incorporating recombination in the model (Kuhner, 2006). In contrast to summary statistic estimates of θ (θ_s , θ_π , etc.), LAMARC accounts for uncertainty in the data by integrating over the space of possible genealogies using Markov chain Monte Carlo (MCMC). Initial runs with PHASE indicated that 8 of the 11 loci contained some sites where the gametic phase could not be resolved with high confidence (probability threshold $< 90\%$). While LAMARC has an option for entering data as “phase unknown,” initial tests indicated inputting samples from PHASE’s posterior distribution produced tighter convergence across runs. Therefore, to account for the uncertainty in the data resulting from unknown gametic phase, LAMARC was run on 10 realizations from PHASE’s posterior distribution for each of the 11 introns.

For 9 of the 11 loci we applied the best fitting mutation models according to the phylogenetic analysis by Jackson et al (*in review*); for the remaining 2 loci we used the mutation models inferred by Alter et al (2007) (Appendix 1) (Alter et al., 2007). To accommodate inter-locus variation in mutation rate, we implemented LAMARC’s gamma model for mutation rate variation. The gamma model option models individual locus mutation rates as being drawn from a gamma distribution with mean 1 and a shape parameter estimated from the data. This approach has the advantage of being generally applicable to species without individual locus mutation rates. Comparing the distribution of variation in estimates of the individual locus substitution rates with the gamma distribution estimated by LAMARC confirmed that the gamma model provides a good fit to the data (data not shown). For the recombination rate, we used a flat prior on a log scale from $1E-05$ to 10. For θ we used a flat prior from $1E-05$ to 0.4, achieving nearly identical results on both the log and the linear scale.

We used LAMARC’s Bayesian option and achieved excellent mixing and concordance between replicate runs. For the final analysis, each PHASE realization was run in LAMARC 3 times with different random number seeds using 150,000 iterations of burn-in and 600,000 iterations after burn-in, taking samples every 20 iterations. The final θ values were obtained by allowing LAMARC to combine information across the 10 alternate PHASE realizations and the three runs as 30 separate replicates for a total of 18 million MCMC iterations after burn in. Because the current implementation of LAMARC allows the gamma model only within the likelihood framework, we developed our own extension of LAMARC (called GUFUL-Gamma Updating For Bayesians

Using LAMARC) that allows the gamma model to be applied in a Bayesian framework. This program uses the estimated posterior densities of θ for each locus as input to a Markov chain Monte Carlo algorithm that provides samples from the posterior distributions of the overall θ , the locus-specific relative rate parameters, and the scale parameter of the gamma distribution. GUFBUL will be distributed as a small subcomponent of the LAMARC package (personal communication, Mary Kuhner, University of Washington, Dept. of Genome Sciences) along with full details of its implementation. Implementation details are also available directly from Eric Anderson at eric.anderson@noaa.gov.

Simulating effects of sub-structure and limited sampling on θ – The extent to which limited geographic sampling will influence estimates of whole-ocean genetic diversity depends upon the degree of population sub-structure. To investigate the relationship between population sub-structure and θ , we simulated seven populations in an Antarctic ring, joined by stepping stone migration with migration rates ranging between 1.25 and 50 migrants per population per generation. Using the program Makesamples (Hudson, 2002), we set $\theta = 4Nm$ to 3.75; this corresponds to a per nucleotide $\theta = 0.0071$ in a sequence of average length for our study. We simulated two scenarios: 1) Single sub-population sampling: 84 sequences sampled from a single subpopulation, and 2) Multi-population sampling: 12 sequences sampled from each of the 7 populations. We simulated 50,000 coalescent trees with the infinite sites mutation model at each migration rate and under each of the two sampling scenarios. From each replicate we estimated θ using the number of segregating sites, θ_s and θ based upon pairwise differences θ_π .

Calculating census population size from θ - The conversion of θ into effective population size (N_e) is based upon the relationship, $\theta = 4N_e\mu$, where μ is the average mutation rate. To calculate μ for the Southern Ocean minke whale, we sampled with replacement from among 11 previously published or in press individual locus mutation rates for the 11 loci in our study; 2 of the individual locus mutation rates were from Alter *et al* (2007), while 9 were taken from a Bayesian analysis of baleen whale phylogeny and fossil history (Jackson *et al in review*). For each re-sampled locus, a sample mutation rate was drawn from the posterior distribution of the estimated mutation rate or uniformly from the 95% confidence intervals on the mutation rate.

To convert μ from units of mutations per base pair per year into mutations per base pair per generation, we needed an estimate of the generation time for Southern Ocean minke whales. The average age of sexually mature individuals can be used as a proxy for generation time, assuming fecundity is constant with age (Roman & Palumbi, 2003). Most data suggest that minke whales reach sexual maturity at approximately 7 years old (Klinowska, 1991; Olsen, 1997). We calculated the average age of sexually mature individuals (from 7-53 yrs old) using commercial and JARPA catch records from 43,236 individuals as reported in Table 1 of Butterworth *et al* (1999). To more accurately reflect uncertainty in our estimate we sampled uniformly from between the lower and upper bounds of year-to-year and area-to-area estimates.

To convert N_e to census population size (N_c) requires knowledge of the ratio of mature adults to the effective number of adults (N_{mature}/N_e) as well as the proportion of juveniles in the population. Estimating N_{mature}/N_e requires information on variation in lifetime reproductive success for whales. In the absence of such information, we based our approximation of N_{mature}/N_e on equation (1) in Nunney and Elam (1994): $N_e = N/(2-T^{-1})$, where T = generation length (as calculated by the methods described above). To approximate juvenile abundance we estimated the ratio of total population size to total adults from corrected age structure information from the 1987/88 and 1988/89 Japanese research takes as reported in Table 2 of Kato *et al* (1991; 1990) (Kato et al., 1991; Kato et al., 1990). We chose to use the corrected age structure information over the combined commercial and JARPA catch records from Butterworth *et al* (1999) to avoid the potential for under-representing juvenile abundance (Butterworth et al., 1999). Again, we sampled with replacement 1 million times to generate confidence intervals around our estimate of the ratio of total population size to total adults.

RESULTS

Equilibrium, neutrality and sub-division - Among eleven nuclear introns, nucleotide diversity averaged 0.00387 (range: 0.00074-0.01352), with an average of 11.2 haplotypes per locus (range: 4-25) (Table 1). These values are higher than for other baleen whales: for example, nucleotide diversity is nearly 4 times higher than seen in gray whales (Alter et al., 2007). The results of Tajima's D and Fu's F_s tests (see *SI*) were consistent with neutrality and equilibrium (Table 1): no Tajima's D values and only one of eleven Fu's F_s tests was significantly different from zero. The STRUCTURE analysis did not reveal any hidden population structure in our data, suggesting the most likely number of populations (K) was $K=1$ (log likelihood = -1427) with $K=2$ (log likelihood = -1431) and $K=3$ (log likelihood = -1437) being less likely.

Estimation of θ - From locus to locus, estimates of θ varied from 0.0010 to 0.0174 (Table 2), presumably reflecting variation among loci in mutation rate and coalescence history. Incorporating information across all 11 loci and alternative phases, we estimated the posterior mean θ to be 0.0071 (95% CI: 0.0045 – 0.0112) (Table 2).

Simulating effects of sub-structure and limited sampling on θ - Simulations suggested that population structure would not significantly increase θ unless migration between sub-populations was so low that the expected $Phist > 0.10$ (an order of magnitude higher than the previously calculated $Phist$ for Antarctic minke whales (see above)) or $Nm < 2.5$ (Fig. 2). Furthermore, our simulated θ differed little regardless of whether samples were drawn from a single sub-population or drawn evenly from across all sub-populations, suggesting that even though our samples are from a limited geographic area, our θ estimate reflects ocean-wide genetic diversity.

Calculating census population size from θ - In order to convert from θ into effective population size we calculated an average mutation rate and generation time. Using individual locus mutation rates from Alter et al (2007) and a Bayesian analysis of baleen whale phylogeny and fossil history, the overall mutation rate was estimated to be

4.54×10^{-10} bp⁻¹ year⁻¹ (95% CI: 3.50×10^{-10} to 5.75×10^{-10}). Our estimate was reduced from the slightly higher average rate used by Alter et al (2007) (4.8×10^{-10} bp⁻¹ year⁻¹). Using the average age of sexually mature individuals as a proxy for generation time, we calculated the average generation length across the combined dataset to be 17.65 years. There was considerable variation in generation length across years, sample areas, and sample methods (commercial and JARPA catches from Area IV and Area V) (Fig. 3). Sampling uniformly from within lower and upper bounds of year-to-year and area-to-area estimates resulted in an age range between 14.60 – 21 years. Using our estimated θ from LAMARC and our multi-locus mutation rate in mutation per base pair per generation, we calculated the effective size of the Antarctic minke whale population to be 199,849 (95% CI: 140,519 - 349,736).

In order to convert from effective population size into census population size we incorporated juvenile abundance and variation in reproductive success. We estimated juvenile abundance or the ratio of total population size to total adults to be 100:67 or 1.48 (95% CI: 1.39 – 1.59). We approximated variation in reproductive success or the ratio N_{mature}/N_e to be ~2 based upon equation (1) in Nunney and Elam (1994). Our estimate of N_{mature}/N_e estimate was on the low end of estimates for low fecundity mammals and consistent with previous estimates used for whales (Frankham, 1995; Sheldon et al., 2001). Multiplying the product of the two above ratios by our estimate of effective population size gives an estimate of census population size of 671,000 individuals. Bootstrap re-sampling across the variation in mutation rate, generation time, the ratio of total population size to total adults and from the posterior distribution of effective size yielded 95% CI from 374,000 – 1,150,000, we (Fig. 4).

DISCUSSION

Current and long-term abundance estimates - Our estimate of long-term population size of 671,000 individuals (95% CI: 374,000 – 1,150,000) spans the range of estimates from three circumpolar surveys conducted under the supervision of the IWC: 608,000 (CV = 0.089) for the years 1978-1984; 766,000 (CV = 0.091) from 1985-1991 (Branch & Butterworth, 2001); and an unpublished report to the IWC that suggested 338,000 (CV=0.079) for the years 1991-2004 (Branch, 2006a). While differences between the first two and the last abundance estimates remain controversial (Branch & Butterworth, 2001; Clapham et al., 2007), our estimate is similar to or slightly higher than these ecological estimates. Similarity between genetic long-term and contemporary short-term population estimates for Antarctic minke whales stand in marked contrast to the strong differences seen in previous comparisons for humpback, fin and grey whales (Roman & Palumbi, 2003; Alter et al., 2007). One major factor that might explain the similarity between genetic long-term and contemporary short-term population estimates for Antarctic minke whales is that minke whales have not been hunted to near extinction like the other species. Our results suggest that demographic estimates can mirror ecological estimates in populations that have not been severely over-hunted and provide support to previous studies using similar genetic methods to estimate long-term abundance in over-hunted baleen whale species (Roman & Palumbi, 2003; Alter et al., 2007).

Confidence limits and possible refinements - The confidence intervals surrounding both current and long-term average estimates are large and thus the power to detect a small (<3-fold) increase in population size is low. However, even if minke whale abundance was at the low end of our confidence limits just prior to whaling (~374,000), our results are difficult to reconcile with previous suggestions of a recent 8-fold increase in Antarctic minke whale abundance (Ohsumi, 1979). Our results indicate that current abundance is within the range of abundance prior to whaling when all whale species co-existed at their pre-exploitation levels. Therefore, competitive release and the Krill Surplus Hypothesis are not required to explain present Antarctic minke whale abundance.

Refinements to both current and long-term estimates may improve our ability to detect changes in population size. For example, abundance estimates based upon the circumpolar surveys are considered minimum estimates because they do not include whales missed on the track-line, whales north of the survey region, and whales inside the pack ice (Branch & Butterworth, 2001). Statistical improvements to the analysis of circumpolar survey data for Antarctic minke whales are ongoing and it is thought that a consensus regarding present abundance will be reached at the 2009 meeting of the IWC (Justin Cook, *pers. comm.*). In regards to long-term abundance, additional life table information for minke whales would help refine our estimate of several necessary demographic parameters such as generation length, juvenile abundance, and N_{mature}/N_e . For example, at present we use an N_{mature}/N_e of ~2 based on equation (1) in Nunney and Elam (1994). Our estimate of N_{mature}/N_e is on the low end of estimates for low fecundity mammals and consistent with previous estimates used for whales (Frankham, 1995; Sheldon et al., 2001). However, a more refined estimate of this ratio could be attained with direct measurements of lifetime variation in reproductive success; higher variation in reproductive success would lead to a $N_{\text{mature}}/N_e > 2$ and a larger estimate of the long-term population size. Future research may also improve our estimate of the mutation rate (μ). Recently it has been suggested that μ evolves faster over shorter timescales than over longer timescales (Ho et al., 2005). Alter and Palumbi (2009) used inter-genic comparisons in mtDNA to suggest that previous phylogenetic rate estimates for the quickly evolving mtDNA control region are likely to be low by a factor of approximately 2.6 (Alter & Palumbi, 2009). Rates calibrated by comparison among individuals identical at cytochrome b sequences were 5-5.4% per million years, instead of the 2% derived from comparisons among species (Alter & Palumbi, 2009). Slowly evolving nuclear genes are less likely to suffer this problem, but if true, then our estimate of μ based on interspecific comparisons would be an underestimate, and a slower μ for Antarctic minke whales would reduce our estimate of long-term population size. Addressing the various sources of uncertainty surrounding both the long-term and the current abundance estimates provides an interesting area for future research.

Implications for the Antarctic Marine Ecosystem - Why would the removal of more than 2 million whales fail to result in competitive release for minke whales? One possibility that has been mentioned (Kawamura, 1978), but not thoroughly investigated is that minke whales are not resource limited because krill abundance exceeds the demands of krill-dependent predators in the Antarctic Marine Ecosystem. Another possibility is that, as the smallest baleen whale in the world, minke whales may not use krill in the

same way and at the same time as whales that are between 3 and 11 times heavier (Fig. 1a). Niche specialization would make competitive release less likely and recent evidence indicates minke and humpback whales partition food resources by depth within the water column, krill size, and aggregation area (Friedlaender et al., 2006; Friedlaender et al., 2008). Within the Antarctic marine ecosystem, other krill eating species also have failed to show the competitive release predicted under the 'Krill Surplus Hypothesis,' including Adelie penguins and crab-eating seals (reviewed by Ballance et al., 2006; Ainley et al., 2007). In these cases, other limits to population size besides krill levels have been implicated. For example, recent evidence indicates population levels in Adelie penguins may be set by the availability of sea ice rather than diet (Croxall et al., 2002; Fraser et al., 1992; Taylor & Wilson, 1990). For minke whales, mechanisms that limit population size are not well understood, though the role of food supplies outside the Antarctic summer, predators, and disease are possible areas for future focus (Branch, 2006b).

Trophic cascades stemming from removal of top-predators can have direct and indirect effects that ripple throughout ecosystems. Vertical effects of top-predator removal on lower trophic levels make up some of the most dramatic cases of trophic cascades (Estes & Palmisano, 1974; Pace et al., 1999; Laws, 1970; McLaren & Peterson, 1994), but horizontal effects in which predator declines cause competitive release for other predators within a trophic level are increasingly observed (Trewby et al., 2008). The 'Krill Surplus Hypothesis' predicted that the removal of approximately two million whales would lead to competitive release for smaller krill eating species. However, the similarity between our long-term estimate and current estimates based on ecological data suggest that competitive release and the 'Krill Surplus Hypothesis' are not required to explain current Antarctic minke whale abundance. Our results add to a growing body of literature suggesting that top-down forces alone may not fully explain population regulation within the Antarctic marine ecosystem (Ainley et al., 2007).

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Table 1. Summary statistics for 11 introns sequenced in Southern Ocean minke whales. N = number individuals, N_S = number of polymorphic sites, N_H = number of distinct haplotypes as determined by PHASE, ver. 2.1 (Stephens *et al.* 2001), π = nucleotide diversity (Tamura and Nei).

Intron	N	N_S	N_H	π	Tajima's D	Fu's Fs
ACTA	34	9	7	0.00255	-1.09	-1.27
BTN	32	6	6	0.00120	0.50	0.54
CAT	38	13	13	0.00685	0.78	-1.81
CHRNA	49	16	16	0.01352	0.02	-3.95
CP	20	24	19	0.00717	-0.04	-0.92
ESD	47	23	25	0.00572	0.01	-16.17
FGG	41	13	12	0.00084	-0.01	-0.35
GBA	44	4	5	0.00061	-1.58	-4.66
LAC	45	12	12	0.00198	-0.06	-1.22
PTH	42	4	4	0.00144	-1.06	-1.11
RHO	52	3	4	0.00074	-1.37	-3.59

*Number in bold refers to a significant deviation from zero as determined by 95% confidence intervals generated using coalescent simulations in DNAsp (Rozas *et al.* 2003).

Table 2. *Posterior mean theta (θ) estimated using LAMARC.*

Marker	θ	min	max
ACTA	0.0061	0.0023	0.0119
BTN	0.0013	0.0005	0.0026
CAT	0.0105	0.0051	0.0187
CHRNA	0.0201	0.0102	0.0350
CP	0.0081	0.0043	0.0145
ESD	0.0145	0.0081	0.0232
FGG	0.0044	0.0021	0.0076
GBA	0.0042	0.0013	0.0093
LAC	0.0065	0.0028	0.0120
PTH	0.0043	0.0012	0.0102
RHO	0.0051	0.0014	0.0118
OVERALL	0.0071	0.0045	0.0112

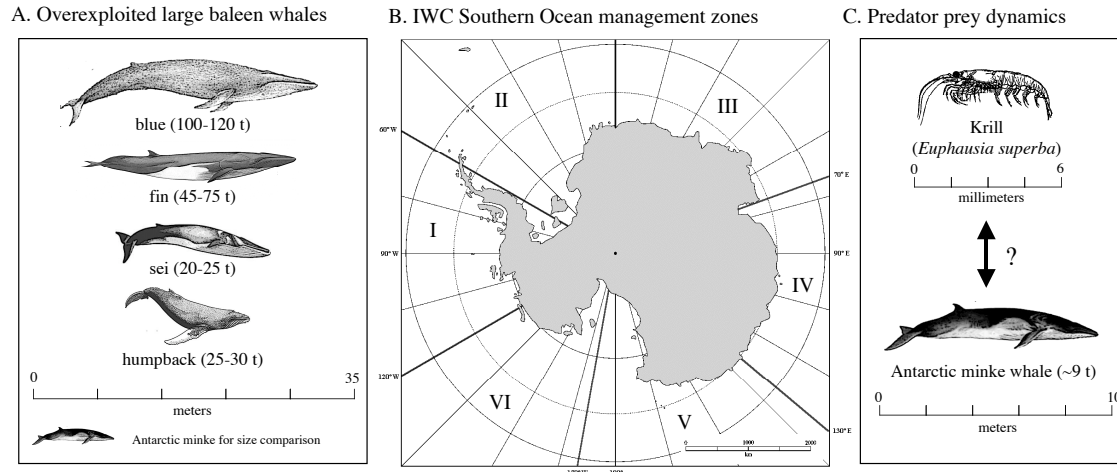


Figure 1. The Southern Ocean ecosystem: A) Four main species of baleen whale hunted in the early 1900's. The figure illustrates that a blue whale in the Antarctic measuring up to 29 m and weighing between 100-120 metric tonnes (t) is approximately 3 times longer and more than 11 times heavier than an Antarctic minke whale, measuring up to 9 m and weighing up to 9 t. B) Southern Ocean management zones as defined by the IWC. C) Krill, the main food source of baleen whales. The question mark refers to the unknown relationship between krill abundance and minke whale abundance in the Southern Ocean.

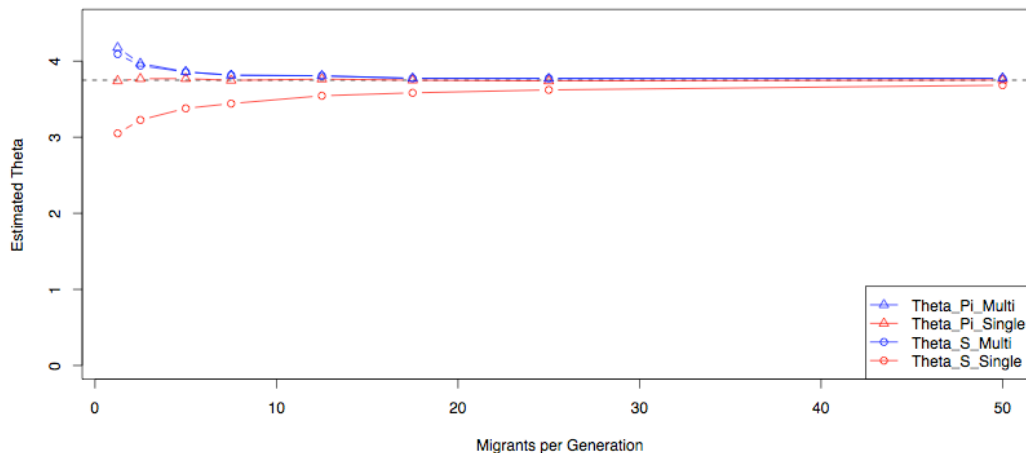


Figure 2. Population sub-structure simulation results demonstrating that θ estimated using segregating sites and pairwise differences differed little regardless of whether samples were drawn from a single sub-population or drawn evenly from across 7 sub-populations. Simulations are described in the supplemental information text. The horizontal dotted black line at 3.75 shows the expected value of q under panmixia (same number of individuals, but no population substructure). Blue lines show results for the multipopulation sampling scenario; red the single population sampling scenario. The results show that across a wide range of migration rates, estimates of θ are close to the value expected under panmixia.

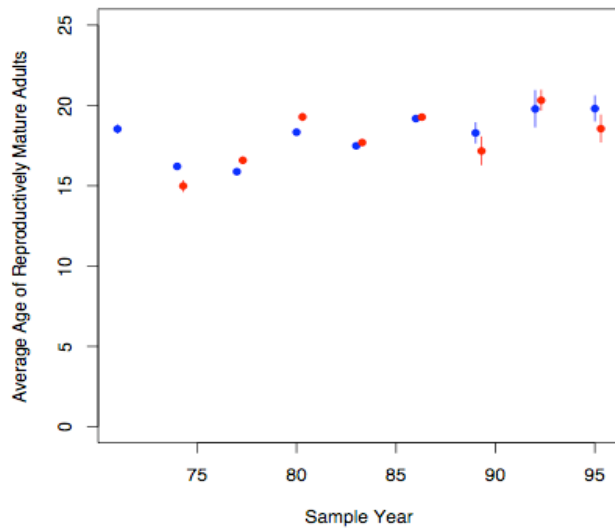


Figure 3. The figure illustrates considerable variation in generation length across years and areas in the Antarctic minke whale. Generation length was estimated as the average age of sexually mature individuals from commercial and JARPA catch-at-age matrices (see Table 1 in 17). Blue dots indicate catches in management Area IV and red dots indicate catches in Area V (SI Fig. 1).

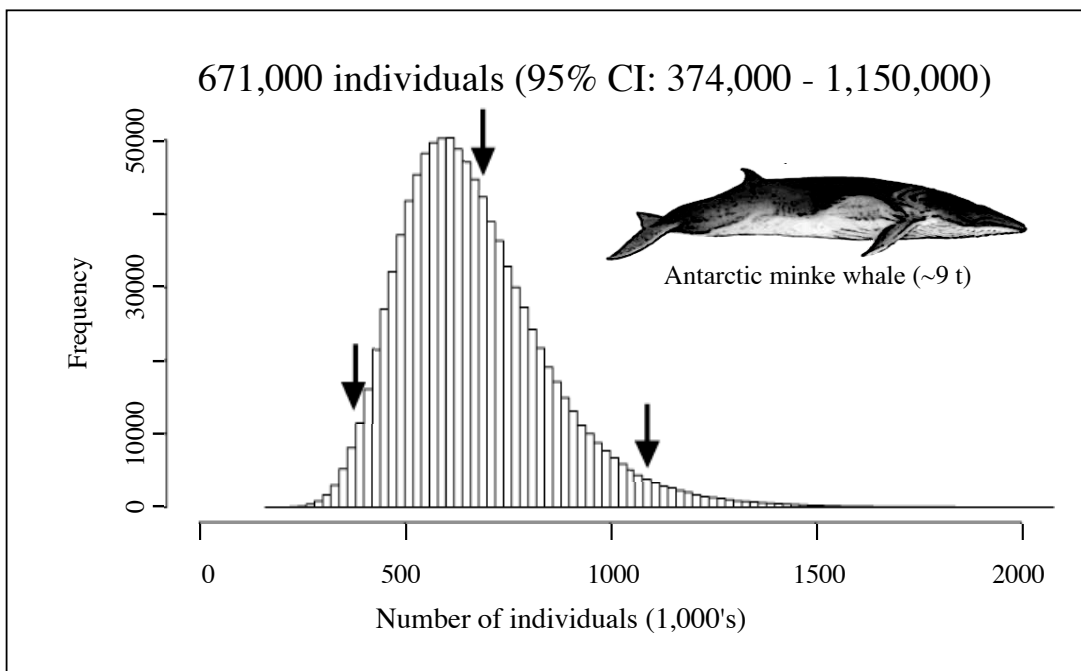


Figure 4. Distribution of long-term census population size estimates, taking account of uncertainty in θ , mutation rate, generation time, and the ratio of total population size to total adults. The arrows represent the mean value and the upper and lower 95% confidence intervals.

Appendix 1. Nuclear intron description

Gene Abbreviation		Associated Gene	Mutation Model	Approx. Length (BP)
ACTIN		Actin	GTR	440
BTN		Butyrophilin	F84	1280
CAT		Catalase	GTR	470
CHRNA	Cholinergic Receptor Nicotinic Alpha		F84	250
CP		Ceruloplasmin	F84	1000
ESD		Esterase D	GTR	670
FGG		Fibrinogen Gamma Chain	F84	1000
GBA		Glucosidase, Beta; Acid	F84	300
LAC (LALBA)		alpha Lactalbumin	GTR	530
PTH		Proteolipid protein	F84	270
RHO		Rhodopsin	F84	180