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RESEARCH ARTICLE

Temporal genetic samples indicate small effective population size of the endangered yellow-eyed penguin

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Abstract There is an increasing awareness that the long-term viability of endemic island populations is negatively affected by genetic factors associated with population bottlenecks and/or persistence at small population size. Here we use contemporary samples and historic museum specimens (collected 1888–1938) to estimate the effective population size (N_e) for the endangered yellow-eyed penguin (*Megadyptes antipodes*) in South Island, New Zealand, and evaluate the genetic concern for this iconic species. The South Island population of *M. antipodes*—constituting almost half of the species' census size—is thought to be descended from a small number of founders that reached New Zealand just a few hundred years ago. Despite intensive conservation measures, this population has shown dramatic fluctuations in size over recent decades. We compare estimates of the harmonic mean N_e for this population, obtained using one moment and three likelihood based-temporal methods, including one method that simultaneously estimates migration rate. Evaluation of the N_e estimates reveals a harmonic mean N_e in the low hundreds. Additionally, the inferred low immigration rates ($m = 0.003$) agree well with contemporary migration rate estimates between the South Island and subantarctic populations of *M. antipodes*. The low N_e of South Island *M. antipodes* is likely affected by strong fluctuations in population size, and high variance in reproductive success. These results show that genetic concerns for this population are valid and that the long-term viability of this species may be compromised by reduced adaptive potential.

Keywords Museum specimens · Temporal method · *Megadyptes antipodes* · New Zealand · Island · Microsatellites

Introduction

Untangling the relative roles of genetic and demographic factors that affect the persistence of endangered populations is a fundamental goal of conservation biologists and wildlife managers. In New Zealand, exotic mammalian predators have played a dramatic role in the decline and extinction of endemic fauna (Clout 2001; Duncan and Blackburn 2004), but intense conservation efforts have resulted in the eradication or control of these predators in localised mainland and offshore areas. Recent New Zealand conservation studies have also started to highlight the potential role of genetic factors in shaping the long-term viability of persisting endemic populations (Jamieson 2007; Jamieson et al. 2008). In particular, it is recognised that the loss of genetic diversity and increased levels of inbreeding—due to population bottlenecks and/or persistence at small population sizes—might have reduced mean population fitness and adaptive potential (Allendorf 1986; Lande and Shannon 1996; Frankham et al. 2002; Keller and Waller 2002).

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Effective population size (N_e), defined as the size of an ideal population experiencing the same rate of genetic drift as the actual population under consideration (Wright 1931; Frankham 1995; Willi et al. 2006; Palstra and Ruzzante 2008), is a key parameter in studies of genetic diversity. Historically, estimation of N_e has been notoriously difficult, but this situation has been much improved by recent statistical developments facilitating the estimation of N_e from temporal genetic samples (Nei and Tajima 1981; Pollak 1983; Waples 1989; Wang 2001; Berthier et al. 2002; Beaumont 2003; Wang and Whitlock 2003). These so-called temporal methods estimate the harmonic mean of a population's variance effective size based on the change in allele frequencies over the time interval separating the temporally spaced samples. The use of museum specimens is particularly promising in the estimation of N_e for species with long generation times (Wandeler et al. 2007). Here we use contemporary and historical samples to estimate N_e for the endangered yellow-eyed penguin (*Megadyptes antipodes*) in South Island, New Zealand, and evaluate the genetic concern for this iconic species.

Megadyptes antipodes is thought to have expanded its range from the subantarctic islands to South Island, around 500 years ago, after the arrival of Polynesians but before settlement by Europeans and their commensals (Fig. 1; Boessenkool et al. 2009a). Based on low contemporary migration rates (<2%) between South Island (including surrounding islands such as Stewart Island) and subantarctic yellow-eyed penguins, and the relatively low levels of genetic variation of the current South Island population, it is thought that the South Island population descended from a small number of founders (Boessenkool et al. 2009b). Nevertheless, around 40% (~800 nests, ~2,200 individuals) of *M. antipodes* globally are now found on and around South Island (McKinlay 2001).

By the 1980s, non-native predators—chiefly mustelids and cats, introduced by Europeans in the late 19th century—had caused major egg and chick predation (Darby and Seddon 1990) and prompted the implementation of intensive predator trapping around *M. antipodes* breeding areas. Despite these recent conservation measures, however, *M. antipodes* population sizes have remained highly unstable (McKinlay 2001; Moore 2001). This demographic instability has been attributed to changes in food supply (van Heezik and Davis 1990), climatic variations (Peacock et al. 2000) and disease epidemics (e.g. Gill and Darby 1993; Department of Conservation unpublished data). Regardless of their underlying causes, such fluctuations in population size are a primary factor leading to substantial reductions in N_e (Frankham 1995).

Based on the suggested recent founding of South Island *M. antipodes*, with subsequent fluctuations in population size, conservation biologists hold genuine concerns for this population. In particular, the ongoing emergence of novel diseases (for example, a diphtheria-like disease linked to infection by a strain of *Corynebacterium*; Department of Conservation, unpublished data) suggests that the adaptive potential of this population may be limited, a concern that may become increasingly important with predicted climate change. In this study we use microsatellite analyses of contemporary and historic South Island samples to test for temporal changes in genetic diversity over the last century, and to provide genetic estimates of N_e.

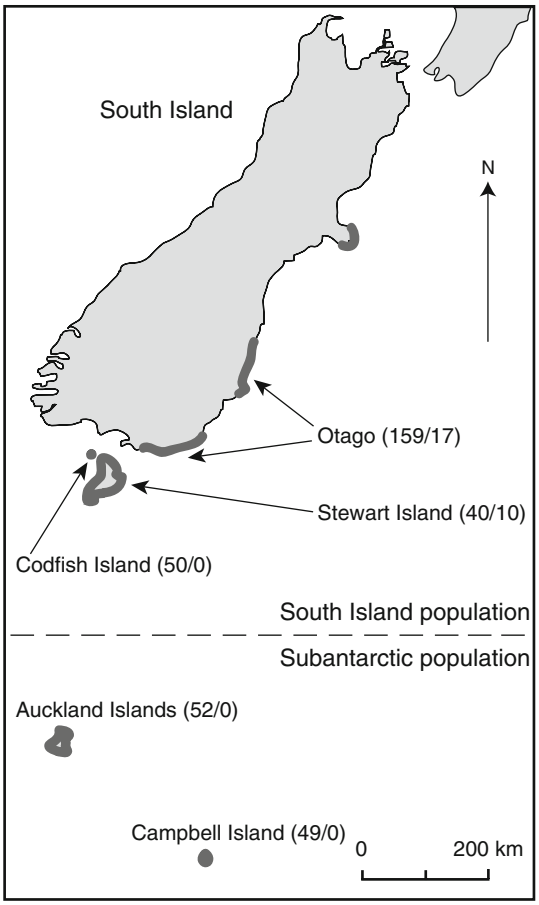

Fig. 1 Map of the South and subantarctic islands of New Zealand. The *dark grey line* represents the current breeding range of *Megadyptes antipodes*. *Arrows* point to the geographical locations where samples were collected. Sample sizes for contemporary/historic samples are given in *brackets*. The *dashed line* symbolically represents the genetic split between South Island and subantarctic populations

Materials and methods

Study area and sampling

Yellow-eyed penguin blood samples ($N = 249$) were collected between 2005 and 2008 at five breeding areas on and around South Island, including Stewart and Codfish Islands

(Fig. 1). Together, these areas form the South Island yellow-eyed penguin population (Boessenkool et al. 2009b). A total of 101 additional samples were collected from the subantarctic Auckland and Campbell Islands (genotypes of these samples are used for N_e estimates that allow for migration, see below). Details of blood sampling methods are described in Boessenkool et al. (2009b).

To facilitate sampling of historic yellow-eyed penguin specimens we contacted a total of 128 museums around the world. Toe pad samples were obtained from 35 specimens collected between 1888 and 1938 at several locations on the South Island and on Stewart Island (Fig. 1, for sample details see electronic supplementary material). These 35 samples included almost all yellow-eyed penguins specimens from the South Island with an explicit collection date (<1950) that are currently held in museum collections.

DNA extraction and genotyping

DNA from contemporary samples was extracted and purified using 40 μg proteinase K in 5% Chelex (Biorad: Walsh et al. 1991). All samples were genotyped at 12 microsatellite loci previously developed for yellow-eyed penguins (Man03, Man08, Man13, Man21, Man22, Man27, Man39, Man47, Man50, Man51, Man54, Man55; Boessenkool et al. 2008). Microsatellite primer sequences and polymerase chain reaction (PCR) conditions for contemporary samples are described in Boessenkool et al. (2008).

For DNA extraction of historic toe pad samples a ~1 × 2 mm piece was rehydrated by a 24 h wash in 1 ml 10 mM Tris–HCL (pH 8.0). Following rehydration, toepad samples were finely cut with a sterile scalpel blade and DNA was extracted using the Chargeswitch Forensic DNA Purification Kit (Invitrogen) or the DNeasy Tissue Kit (Qiagen) following manufacturers' instructions. No differences were observed in extraction or amplification success between these two kits. Historic samples were amplified at the same 12 loci described above, with the exception of Man22 and Man27 which did not amplify consistently for the historic samples. These two loci were therefore omitted from all further analyses. PCR reactions (10 μl) contained 2 μl DNA, 0.5 μM of each primer, 0.5 U *Taq* DNA polymerase (Mango *Taq*, Bioline), 1× *Taq* buffer, 0.8 μM dNTP and 1.5 μM $MgCl_2$, with the addition of betaine and DMSO (1.1 M and 2%, respectively) if necessary (see Boessenkool et al. 2008). The amplification profile was 2 min at 94°C, 35–50 cycles of 15 s at 96°C, 15 s at 45–50°C and 30 s at 72°C, followed by a 4 min final extension at 72°C.

To prevent contamination of historic DNA with exogenous DNA or PCR products, all DNA extractions and PCR set-up of historic samples were performed inside a UV hood in a laboratory where no contemporary yellow-eyed penguin DNA or vertebrate PCR products have ever been present. Standard precautions for the analysis of historic DNA were closely adhered to, including the use of filter tips, UV radiation and cleaning of materials with bleach and/or 70% ethanol before and after each laboratory session, and maintenance of a one-way flow from the historic DNA laboratory to the modern/post-PCR laboratory. Historic samples were extracted in small batches of nine samples and potential contamination was monitored by negative extraction and PCR controls. To minimise the risk of erroneous genotypes due to allelic dropout and the amplification of false alleles (Taberlet et al. 1996; Sefc et al. 2003), 2–7 successful amplifications were obtained for each historic sample before a genotype was scored, and genotypes were only scored when every allele was observed at least twice.

Disequilibrium and genetic diversity

Deviations from Hardy–Weinberg proportions and linkage equilibrium were tested using GENEPOP 4.0 (Rousset 2008) for contemporary South Island, contemporary subantarctic, and historic South Island samples separately. Markov chain parameters employed 10,000 dememorizations, 1,000 batches and 10,000 iterations. Significance levels were adjusted for multiple comparisons using Bonferroni corrections (Rice 1989). Genetic diversity was quantified for the ten loci that amplified consistently in contemporary and historic samples, using the total number of alleles and expected and observed heterozygosity calculated in GENETIX 4.05.2 (Belkhir et al. 1996–2004). Calculations of allelic richness were performed using FSTAT 2.9.3 (Goudet 2002) to adjust for sample size differences. Statistical significance of differences in genetic diversity between historic and contemporary South Island samples was tested with a Wilcoxon signed rank test in SPSS ($\alpha = 0.05$). The difference in genetic diversity between subantarctic and contemporary South Island is discussed extensively in Boessenkool et al. (2009b), and subantarctic diversity is included here for comparative purposes only.

Effective population size

The quantification of N_e using temporal methods requires an estimation of the number of generations (T) separating the temporally spaced sampling points. We calculated average generation time using the formula $\Sigma(x\ l_x\ b_x)/\Sigma(l_x\ b_x)$, where x is age, l_x is the proportion of individuals surviving to age x and b_x is the reproductive output at age x (Begon et al. 2006; see electronic supplementary material). Yearly adult survival of *M. antipodes* was set to 0.856 (Richdale 1957) and reproductive output set to 1.16

fledglings per pair (Darby and Seddon 1990). Maximum age was set to 20 years (Richdale 1975; Department of Conservation unpublished data) and variation in age at first breeding as estimated by Richdale (1957) was incorporated in the analysis. Using these estimates, average generation time of *M. antipodes* was calculated at 7.7 years (see electronic supplementary material). The time span between the collection year of contemporary samples (2006) and the weighted average collection year for historic samples (1901) was 105 years, resulting in $T = 14$. To account for uncertainty in T we also present estimates of N_e using $T = 12$ and $T = 16$.

We used one moment-based and three likelihood-based approaches of the temporal method to obtain estimates of N_e. These methods typically assume discrete generations, no selection, no mutation, and a closed panmictic population. Although our dataset violates the first of these assumptions, any bias due to overlapping generations can be minimised if samples are taken more than ten generations apart (Waples and Yokota 2007), which is the case in our study. The effects of migration are more complex (Wang and Whitlock 2003; Fraser et al. 2007a; Palstra and Ruzzante 2008), and we therefore included an estimator of N_e that relaxes the assumption of a closed population (this estimator is referred to as N_{eOPEN}, in contrast to the other estimators, which are referred to as $N_{eCLOSED}$). Finally, previous research has shown that significant, albeit relatively low, F_{ST} values within the South Island may indicate sub-structuring among several of the sampled breeding areas (Boessenkool et al. 2009b), violating the assumption of panmixia. This population genetic structure is very weak, however, and it has formerly been concluded that the South Island can be broadly regarded as a single population (Boessenkool et al. 2009b). This conclusion is further supported by the lack of any significant departure from Hardy–Weinberg proportions in the pooled South Island samples (see Results). We nevertheless evaluate the possible effect of substructuring on our estimates of N_e (see Discussion).

First, we calculated the moment-based estimator from Waples (1989; extended from Nei and Tajima 1981 and Pollak 1983) using the program NeEstimator (Peel et al. 2004). Second, we applied the coalescent-based likelihood method from Beaumont (2003) as implemented in the program TMVP (which is based on the program TM3 from Berthier et al. 2002). We assumed no change in N_e during the sampling interval and calculated N_e as the mode of the posterior distribution. The MCMC simulation was performed with 50,000 updates of which ten percent were discarded as burnin. The size of importance sampling was 100, the thinning interval was 10 and the size of the proposal distribution of parameter updates was 0.5. Third, we estimated N_e with the pseudo-likelihood based approach from Wang (2001) using the program MLNE. Finally, we applied the pseudo-likelihood method from Wang and Whitlock (2003; also implemented in MLNE) that relaxes the assumption of no migration by jointly estimating N_e (N_{eOPEN}) and the migration rate m. This method requires allelic data from the source population (the subantarctic population) and at least two samples from the focal population (the contemporary and historic South Island population). The method assumes migration is constant, that all sources are sampled and that the source population is sufficiently large that allele frequencies are temporally stable, although the method is relatively robust to violations of the latter assumption (Wang and Whitlock 2003). For all likelihood-based methods, maximum N_e (N_{eMAX}) was set to 1,000. Higher values of N_{eMAX} affected only the upper bound of the 95% confidence interval (CI) when this fell above 1,000 in MLNE, but never influenced point estimates or the lower bound of the CI. For TMVP analyses, increasing N_{eMAX} only lead to marginal increases of the upper bound of the CI (data not shown).

Results

All 350 contemporary samples amplified at all 12 microsatellite loci with the exception of six samples from South Island, which have missing genotypes for one (three samples), three (two samples) or five loci (one sample), respectively. Of the 35 historic samples, DNA was successfully extracted from 27 samples and a total of 249 genotypes were scored at ten loci (historic samples did not amplify at loci Man22 and Man27). Eight historic samples had missing genotypes at one (four samples), three (one sample), four (two samples) and six loci (one sample), respectively. Allelic dropout was encountered in 16 out of 224 PCR amplifications of confirmed heterozygous historic samples. These 16 cases of allelic dropout were restricted to four of the 27 historic samples, with most instances occurring multiple times in replicate amplifications of the same locus (e.g. for one sample, dropout was observed in five out of seven replicate amplifications of locus Man47). The amplification of a false allele was detected in just one out of a total of 634 successful PCRs.

Disequilibrium and genetic diversity

There was no evidence for linkage disequilibrium between any pairs of loci, and no loci showed significant departure from Hardy–Weinberg proportions. Eight out of ten loci were polymorphic in the contemporary South Island *M. antipodes* samples, and these same eight loci showed variation in the historic samples. In contrast, all ten loci were polymorphic in the subantarctic population. Genetic

diversity estimators were slightly lower historically compared to estimates from contemporary samples of the South Island population, but these differences were not significant (all P values > 0.05, Table 1).

Effective population size

Point estimates of the harmonic mean of N_e for the South Island population of *M. antipodes* varied between 128 and 656 ($T = 14$) for the different methods applied (Table 2). Wang's pseudo-likelihood method gave the highest estimate with a large CI of which the upper bound was limited by our setting of N_{eMAX} = 1,000. The moment-based estimator (Waples 1989) gave a slightly lower point estimate and, similar to Wang's estimator, the CI were large (note that an upper bound cannot be set for the moment-based estimator). N_e estimates from Beaumont's (2003) likelihood-based method and the joint estimator of N_{eOPEN} and m from Wang and Whitlock (2003) were similar with highly congruent CIs. The N_e estimates are relatively robust to the number of generations (T) between sampling periods, showing only slight increases in N_e with increasing T (Table 2). Estimates of m were low ($m = 0.003$, CI 0.002–0.007) and consistent for different values of T (Table 2).

Table 1 Genetic diversity at ten microsatellite loci in contemporary and historic *M. antipodes*

Location	N	L_{poly}	A/locus	$A_{richness}$	H_E	H_O
South Island						
Contemporary	249	8	3.0	2.5	0.38	0.37
Historic	27	8	2.2	2.2	0.36	0.33
Subantarctic	101	10	4.5	3.7	0.47	0.45

L_{poly} = number of polymorphic loci, A/locus = mean number of alleles per locus, $A_{richness}$ = allelic richness, H_E = expected heterozygosity, H_O = observed heterozygosity

Discussion

Effective population size estimates of South Island yellow-eyed penguins

Using microsatellite DNA analyses of historic (1888–1938) and contemporary samples we estimate the harmonic mean N_e of South Island *M. antipodes* between 124 and 656 with lower bounds of the CI varying between 67 and 228 and upper bounds between 431 and >1,000. The evaluation of CIs in addition to point estimates of N_e is essential, because CIs generated by different analytical methods are often more consistent than point estimates (Fraser et al. 2007a). Additionally, the lower bound of the CI gives important insight into the status of a population with respect to critical conservation thresholds (Hansen et al. 2002). In the current study, the four methods applied to estimate N_e varied in their point estimates and their CIs (Table 2), a finding which raises questions about the relative accuracy of the different techniques.

Moment-based estimators such as the estimator from Waples (1989) are known to overestimate N_e and have low precision (resulting in large CIs), particularly when populations experience rapid genetic drift and allele frequencies are skewed (Wang 2001; Berthier et al. 2002; Jorde and Ryman 2007; Palstra and Ruzzante 2008). Furthermore, the bias of this estimator seems to increase with increasing generations between samples (Tallmon et al. 2004). In contrast, simulations have shown that both Beaumont's (Beaumont 2003) and Wang's estimators (Wang 2001) show reduced bias when ten generations have passed between samples, with the former becoming very accurate and precise (Tallmon et al. 2004). Interestingly, our estimate of N_e from Beaumont's method, and in particular the associated CI ($N_e = 124$, CI 67–504), was very similar to the joint estimator ($N_{eOPEN} = 196$, CI 92–431) of Wang and Whitlock (2003), while Wang's N_e estimate (Wang 2001) was three times larger with an upper bound of the CI above 1,000. The N_{eOPEN} estimator from Wang and Whitlock (2003) is considered to be superior to the closed

Table 2 Effective population size estimates (N_e) and their confidence intervals (CI) for South Island *M. antipodes*, estimated using four different temporal methods

T	Estimated N_e (95% confidence interval)				
	Waples (1989)	Beaumont (2003)	Wang (2001)	Wang and Whitlock (2003)	
				N_e	m
12	237 (77–1,141)	97 (55–405)	576 (200 to >1,000)	184 (85–390)	0.003 (0.002–0.007)
14	277 (90–1,331)	124 (67–504)	656 (228 to >1,000)	196 (92–431)	0.003 (0.002–0.007)
16	317 (103–1,521)	144 (73–559)	737 (255 to >1,000)	200 (101–448)	0.003 (0.002–0.006)

T = number of generations passed, m = migration rate

population estimators ($N_{eCLOSED}$) and expected to give more realistic values of N_e, because it relieves the assumption of no migration.

Nevertheless, the effect of migration on N_e is complex and should be addressed cautiously (Wang and Whitlock 2003; Fraser et al. 2007a; Palstra and Ruzzante 2008). Ignoring immigration can lead to either upward or downward biases of N_e depending on 1) the extent of gene flow, 2) the sampling interval and 3) the genetic differentiation between focal versus source population(s) (Wang and Whitlock 2003; Fraser et al. 2007a). Many studies have found $N_{eOPEN} < N_{eCLOSED}$, particularly in cases where spatial genetic structuring is weak or moderate, and associated migration rates (sometimes unrealistically) high (Fraser et al. 2007a). In these scenarios it is thought that $N_{eCLOSED}$ estimates the N_e of the entire metapopulation, rather than the N_e of the population of interest (Wang and Whitlock 2003). With the exception of Beaumont's estimator, we also find $N_{eOPEN} < N_{eCLOSED}$ in the present study. This result may seem surprising given that *M. antipodes* has low migration rates (CI 0.002–0.007; see also Boessenkool et al. 2009b). Genetic differentiation between our two populations is strong, however, and our sampling interval was relatively long (14 generations). Consequently, migrants may have significantly altered genetic diversity on the South Island during the sampling interval, leading to an upward bias of estimated $N_{eCLOSED}$. The above reasoning, however, does not explain the strong overlap between our estimates of N_{eOPEN} and Beaumont's $N_{eCLOSED}$. Beaumont's estimator is the only estimate that is based upon coalescent theory, and perhaps this estimator is affected differently by such migration patterns. The above issues further emphasize the complex interaction between N_e and m, and reiterate that our understanding of the influence of m on N_e and their estimators is currently incomplete (Fraser et al. 2007b).

Similar to the complex relationship between N_e and m, the effect of population substructuring can bias estimates of N_e either upwards or downwards. Specifically, whereas genetic differentiation among subpopulations will lead to an increase in the population-wide N_e, estimate, variance in reproductive success or productivity among subpopulations will, conversely, reduce the estimate of N_e (Whitlock and Barton 1997; Nunney 1999). Whether either of these effects has biased the findings of the present study is difficult to establish, but we believe that any bias due to substructuring will be weak because F_{ST} values among South Island *M. antipodes* samples are low and only rarely significant (Boessenkool et al. 2009b). Furthermore, no departures from Hardy–Weinberg proportions were observed in either the modern or the historical samples. Although exploring the possible effects of substructuring would undoubtedly be interesting, there are too few historic samples to estimate N_e for separate breeding areas within the South Island.

Although historic samples provide a valuable means to estimate N_e using temporal genetic analyses, the inherent scarcity of such samples means that low sample sizes are unavoidable. Our sample size of 27 for the historic South Island population is substantially lower than the sample size of fifty that is typically recommended (Palstra and Ruzzante 2008), which may have compromised the precision of our estimates of N_e. Furthermore, the historic samples were collected over a 50-year period. The analytical consequences of pooling samples across multiple generations have not, to our knowledge, been evaluated, but it is conceivable that such pooling could distort allele frequency estimation and thereby bias estimates of N_e.

It is difficult to calculate the N_e/N_c (effective population size/census population size) ratio in *M. antipodes* because, in fluctuating populations, the harmonic mean N_e is weighted towards the smallest values of N_e during the sampling interval (Leberg 2005), and we cannot calculate the harmonic mean N_c over the time interval used to calculate the harmonic mean N_e. Dividing our point estimates of N_e (124, 196, 277, 656) by the current census size (2,200 for the total South Island population, including surrounding islands) gives ratios of 0.06, 0.09, 0.13 and 0.30, respectively, but this may be a slight underestimate of the actual ratio as the harmonic mean N_c over the time interval is likely to be less than the current census size. With the exception of 0.30, these estimates appear close to the average N_e/N_c ratios found in natural populations of vertebrate taxa ($N_e/N_c = 0.10$–0.11; Frankham 1995). Fluctuating population size is arguably the most important factor reducing this ratio (Frankham 1995). Indeed, close monitoring of yellow-eyed penguins on the South Island has revealed strong fluctuations in the total number of breeders during the last two decades, with the lowest population estimate recorded in the 1990/1991 season when as few as 140 pairs bred on the South Island (Gill and Darby 1993), versus approximately 500 breeding pairs on the South Island in more recent years (Department of Conservation, unpublished data). The second most important factor leading to low N_e/N_c ratios is variance in reproductive success (Frankham 1995). Such variance has been shown to exist in yellow-eyed penguins, and parental 'quality' is likely an important component determining this variation (Efford and Edge 1998; Bull 2005). Unfortunately, no comparable estimates of N_e/N_c exist for any other penguin species (Frankham 1995; Palstra and Ruzzante 2008). Long-term estimates of N_e for Galápagos and Magellanic penguins were calculated by Akst et al. (2002) but these analyses are not directly comparable to our estimates for *M. antipodes* because they involve much longer time-scales. Indeed, application of the method applied by

Akst et al. (2002) to South Island *M. antipodes* would yield non-credible N_e estimates (data not shown)—values as high as the current census size.

Conservation implications

The minimum N_e required to retain sufficient evolutionary potential is thought to approximate 500, although thresholds as high as 5,000 have been proposed (Franklin 1980; Franklin and Frankham 1998; Lynch and Lande 1998). Coping with certain environmental challenges, such as the introduction of disease and toxins, may require only an adaptive response at a few specific loci, and the population size needed to maintain sufficient genetic variation at such loci is more likely to lie in the thousands than in the hundreds (Willi et al. 2006). Not withstanding the limitations of our sampling, most of our N_e estimates (and especially the lower bounds of the CI) for South Island *M. antipodes* are well below such critical thresholds required to maintain adaptive potential. This finding is particularly notable in the context of the regular disease epidemics experienced by this population. Furthermore, South Island *M. antipodes* already have low genetic diversity compared to the subantarctic population at neutral loci and immigration rates are sufficiently low for the population to be considered demographically isolated (Boessenkool et al. 2009b). The low effective population size estimates presented in the current study imply that the South Island population will likely experience the loss off genetic variation due to random drift, potentially eroding adaptive potential. Given predicted increases in rates of environmental variations due to climate change (NIWA 2008), the maintenance of adaptive genetic diversity in *M. antipodes* may become increasingly important. These results suggest that the South Island population of yellow-eyed penguins will remain vulnerable and unstable in the near future, and ongoing monitoring of the population, in addition to continued predator trapping, is therefore essential.

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