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Evaluating Accuracy and Bias in Genetic Monitoring Under Scenarios of Population Decline

Susan Kasko Munster

A Thesis Submitted to the Graduate Faculty of GRAND VALLEY STATE UNIVERSITY<br>In<br>Partial Fulfillment of the Requirements<br>For the Degree of<br>Master of Science

## Biology

August 2015

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Susan Kasko Munster

2015

## DEDICATION

I would like to dedicate this work to my family, without whom I could not have made the step to return to graduate school or complete my degree. I need to thank first and foremost, my husband,

Frank, for his unending support and encouragement. I need to thank my children for their patience and both of my parents for their support and time to make all of this possible. I would also like to thank my sister for all the help she has provided.

I would also like to thank my highly supportive and ever-patient advisor Amy Russell for her willingness to take me on as a graduate student, her guidance and encouragement, as well as her sense of humor. I don't know how I could have done half of this without you.

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#### Abstract

Accurate and unbiased estimates of current effective population size are of primary importance in making informed decisions for conservation purposes. One species of concern, the eastern red bat (Lasiurus borealis), is experiencing ongoing population losses due to wind turbine collisions. The proportion of the population being affected is currently unknown, although recent estimates of total fatalities at wind turbines over the time from 2000-2011 are in the hundreds of thousands for this species alone. The roosting habits of eastern red bats make it difficult to monitor their census population size $\left(N_{c}\right)$; thus, genetic estimates of effective population size $\left(N_{e}\right)$ may provide an alternative monitoring tool. Because they mutate rapidly, microsatellite loci are one of the most promising classes of genetic data for monitoring recent changes in effective population size. To test the accuracy of microsatellite-based estimators for monitoring population declines in large populations, simulated microsatellite data sets were created based on eastern red bat population parameters under multiple scenarios of decline. Simulated data sets were then analyzed using coalescent-based msvar analyses, frequency-based $M$-ratio tests, and simple measures of genetic diversity. When parameters estimated using msvar were compared to the known parameters with which data sets were created, it was found that msvar produced precise and unbiased estimates of ancestral effective population size $\left(N_{A}\right)$, but routinely yielded imprecise estimates of current $N_{\mathrm{e}}$ that were typically biased upwards by an order of magnitude or more. $M$-ratios correctly indicated decline in $40.3 \%$ of data sets, mostly those simulated under demographic scenarios with a large $N_{A} . \theta\left(=4 N_{e} \mu\right)$ was calculated using the known parameters, coalescent point estimates, repeat number variance, homozygosity, and mean allele frequencies. Of these, the coalescent estimates of $\theta$ were the least accurate when compared to known $\theta$. These results indicate that caution is warranted when using genetic data to


estimate current $N_{\mathrm{e}}$, particularly for large $\left(N_{\mathrm{e}} \geq 1000\right)$ populations, and that coalescent-based estimates of $N_{\mathrm{e}}$ may be of little practical utility in monitoring large populations over short timescales.

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## INTRODUCTION

## Conservation genetics

A primary concern in any conservation effort is accurately estimating and monitoring changes in census population size. Census population size $\left(N_{c}\right)$ is the number of adults in a population within a given area (Luikart et al. 2010). Genetic estimates of $N_{c}$ are a useful alternative when field counts are impractical or unlikely to be accurate (Luikart et al. 2010). Methods for estimating $N_{c}$ from genetic data are similar to traditional capture-mark-recapture (CMR) methods, the difference being that genetic profiles are used as the identification "tag" for individuals. CMR methods of estimating $N_{c}$ can be performed on open or closed populations and can be designed to accommodate capture rate variability (Seber 1973). Many of these estimates presume that captures, and recaptures if applicable, are random, and produce more reliable estimates if a significant proportion of the population ( $20 \%$ or more) is captured and/or recaptured (Seber 1973, Luikart et al. 2010). If populations are large, widespread, or if capture/recapture rates are low, the use of CMR techniques may produce imprecise or inaccurate estimates of $N_{c}$.

It is not always possible to accurately estimate the census size for a species from genetic samples, but the effective population size can be estimated. Effective population size $\left(N_{e}\right)$ is the size of a Wright-Fisher population experiencing the same levels of genetic drift or inbreeding as the population being studied (Kimura \& Crow 1963). $N_{e}$ can be used to estimate $N_{c}$ using the ratio $N_{e} / N_{c}$, if this relationship is known. It is thought that the $N_{e} / N_{c}$ ratio is more likely to be predictable for species with reduced fecundity and temporally stable reproductive rates (Luikart et al. 2010). Frankham (1995) found that $N_{e}$ is typically $10-20 \%$ of the census size, although there are published $N_{e} / N_{c}$ ratios ranging from 0.04 (Bartley et al. 1992) to 1.07 (Driscoll 1999)
utilizing data collected in a single season. Additionally, studies have attempted to estimate $N_{e} / N_{c}$ ratios from field observations for a population over multiple decades to assess the stability of this ratio (Hauser et al. 2002, Ardren \& Kapuscinski 2003), and found annual variation due to reproductive success rates and fluctuations in population size (Ardren \& Kapuscinski 2003) and selection, selective sweeps, exploitation of populations, and/or genetic drift (Hauser et al. 2002).

Methods for estimating a population's current effective size make use of many different aspects of molecular data. Waples \& Do (2010) developed a method to estimate current $N_{e}$ based on linkage disequilibrium in single-sample genetic data, but the authors found this approach to be accurate only for $N_{e}<200$. Two other common methods of estimating current $N_{e}$ from genetic data are based on loss of heterozygosity and change in allele frequencies (Hill 1972, Waples 1989). Both of these methods detect population decline using one summary statistic, such as when $N_{e}$ is determined using only changes in allele frequency or by comparing the observed heterozygosity to the expected heterozygosity (Luikart et al. 2010). Because these methods assess population decline by examining a single aspect of the data, they may not have sufficient power to detect recent population declines (Girod et al. 2011). An alternate method of estimating current $N_{e}$ from genetic data is to use coalescent-based methods (Wakeley 2009, Wakeley \& Sargsyan 2009). Using coalescent theory, mutations are situated within genealogies to allow estimation of the time between coalescent events (Kingman 1982, Wakeley 2009). The shape of coalescent genealogies and distribution of coalescence times allows one to infer characteristics such as current effective population size from genetic samples (Hudson 1991, Nielsen \& Slatkin 2013).

Estimates of current effective population size can be pertinent to the conservation status of a species. Estimates of $N_{e}$ can reveal histories of population size change and shed light on the
relative importance of genetic drift or natural selection in a population (Hare et al. 2011). $N_{e}$ can also be used to assess or predict loss of heterozygosity in a population (Allendorf et al. 2013). Considering that $N_{e}$ may be a small proportion of $N_{c}$, the genetic variability of breeding individuals is of great importance to the long-term genetic health of a species (Frankham 2005).

## Genetics of species in decline

There are several concerns relating to the genetic diversity of a population experiencing a decrease in size. Over time, populations in decline tend to have less genetic diversity and lower heterozygosity (Peery et al. 2012). This effect was seen with the Mauritius kestrel, which showed a $57 \%$ decrease in heterozygosity after the population declined to fewer than 50 individuals (Groombridge et al. 2000). Declining populations also generally see an increase in inbreeding, which can lead to the loss of allelic diversity and the fixation of detrimental alleles (Amos \& Balmford 2001). For example, a significant negative correlation between inbreeding coefficient and sperm quality was found in the endangered Iberian lynx (Ruiz-Lopez et al. 2012). Both heterozygosity and inbreeding depression are of concern for species survival in conservation biology (Frankham 1995). For any declining population, genetic variation is expected to decrease over time (Nei et al. 1975). Therefore, evaluation and monitoring of the genetic variation found within a species could be used as a tool to determine if the population size is decreasing (Cornuet \& Luikart 1996, Luikart et al. 2010). If estimates of $N_{e}$ are found to be effective early indicators of population declines then they might be used to monitor species nearing threatened or endangered status before genetic diversity has decreased to dangerously low levels, potentially compromising the ability of a species to adapt to changing conditions (Frankham 2005).

The importance of genetic diversity to fitness and survival has been a matter of debate. Lande (1988) proposed that species generally went extinct due to environmental changes or Allee effects before genetic factors had a significant impact. This has been shown to apply in cases of extreme population declines (Halliday 1980, Stoner \& Ray-Culp 2000), but, in general, extrinsic factors are not alone in decreasing the average fitness of a population (Frankham 2005). Fundamentally, it is assumed that a loss of genetic variation within a species contributes to an increase in the risk of extinction (Frankham 1995). A loss of heterozygosity throughout the genome is typically seen in declining populations (Spielman et al. 2004b). Such decreases in heterozygosity may impair a species' ability to cope with novel diseases or other changes in their environment (Spielman et al. 2004a). Heterozygosity has also been correlated with other factors that affect fitness, such as reproductive success, mate selection, resistance to parasites, and adaptations to local conditions (Ujvari \& Belov 2011). Isle Royale wolves exhibited decreased heterozygosity during the decades since their establishment on the island in 1950 (Hedrick et al. 2014). A significant increase in the inbreeding coefficient was also observed as the population declined. The crash of this population may be due to inbreeding effects, loss of heterozygosity, extrinsic factors, or some combination of those forces (Hedrick et al. 2014).

One mitigating factor that was thought to counteract the effects of inbreeding in declining populations is the purging of deleterious alleles (Lande 1988). Inbreeding has been found to purge some deleterious alleles, but not enough of them are lost to fully compensate for the negative effects of inbreeding (Spielman et al. 2004a). Reed et al. (2003) documented that populations with higher levels of inbreeding had increased rates of extinction under experimental conditions. Sixty-one percent of inbred Drosophila populations went extinct by the sixtieth generation, and extant groups exhibited impaired fitness compared to controls. The negative
impacts of fixing some deleterious alleles were far greater than any benefits incurred from the purging of other deleterious alleles. Additionally, as population census size dwindles, selection acts less effectively against deleterious alleles, which instead may continue to accumulate in surviving generations. This increases the genetic load on the population and can cause an 'extinction vortex' (Amos and Balmford 2001), which is a negative genetic feedback loop that leads inexorably to extinction.

Genetic data have been used in a number of different ways to assess population trends. Using genetic samples with high mutation rates is desirable in detecting recent population decline, as these should be sensitive to more recent changes in population size. There are multiple types of genetic data from which to choose, including mitochondrial DNA sequences, genome-wide single nucleotide polymorphisms (SNPs), and autosomal microsatellite loci. Mitochondrial DNA is a maternally inherited circular chromosome found within mitochondria. Single nucleotide polymorphisms can be found in any type of DNA and refers to variations found at individual base pairs. Microsatellite loci are regions of DNA that consist of short repeating motifs (Pierce 2014). All of these options have been used in analyses of genetic demography (Moritz 1994, Pritchard et al. 1999, Schneider \& Excoffier 1999, Sunnucks 2000, Marth et al. 2004, Gutenkunst et al. 2009), but microsatellites have the highest rate of mutation (International SNP Map Working Group 2001, Kumar \& Subramanian 2002, Antao et al. 2011) and therefore hold the most promise for detecting recent population declines.

## Threats to eastern red bats

Currently, many migratory bat species are suffering population losses due to wind turbine collisions (Arnett et al. 2008), white nose syndrome (Blehert et al. 2009), mine closures (Ellison et al. 2003), and habitat fragmentation (Meyer et al. 2009). In North America, the three species
most affected by turbine-related mortality are eastern red bats (Lasiurus borealis), hoary bats (L. cinereus), and silver-haired bats (Lasionycteris noctivagans; Kunz et al. 2007). Eastern red bats are tree-roosting bats that migrate in the fall and spring (Harvey et al. 2011), roost singly or in small groups in trees, and are mainly active at night (Shump \& Shump 1982). This makes estimating population census size through direct counts very difficult because of their widespread, but sparse and unpredictable, distribution throughout the landscape. There have been multiple studies that have estimated the number of eastern red bats killed per year by wind turbines (Johnson et al. 2004, Barclay et al. 2007, Kunz et al. 2007, Cryan \& Barclay 2009, Cryan 2011), but it has not been possible to evaluate these losses as a proportion of the population because the total population census size is unknown.

Projections from the U. S. Department of Energy predict continued growth in installed wind capacity, with an observed increase of $8.2 \%$ in 2014 and expected increases of $12.9 \%$ and 12.0\% in 2015 and 2016, respectively (U.S. Energy Information Adminstration 2015). Losses due to wind turbines are also spatially variable, with disproportionately high rates at some locations (Barclay et al. 2007). Considering the expected growth in this energy sector, affected bat species are likely to continue experiencing decreases in population size (Cryan 2011). Unfortunately, legal regulations often present difficulties in monitoring affected populations and assessing population trends over large spatial scales. Utility companies operating wind turbines are not uniformly required to enact mitigation measures unless endangered species are being affected. Wildlife mortality data are often considered to be proprietary information of utility companies (Arnett \& Baerwald 2013), and reporting requirements for wildlife fatalities varies across states and provinces. For example, the state of Texas, which had the greatest operating
capacity of wind turbines in 2008, has no requirement for utility companies to report bat or bird fatalities (Arnett et al. 2008).

In light of these factors, eastern red bats are likely to be of conservation concern in the near future. Although this species is not legally protected, Arnett and Baerwald (2013) calculated that 143,023 to 287,403 eastern red bats were killed by wind turbines between 2000 and 2011 in the United States and Canada. Without an accurate population census size estimate, it is not possible to know what proportion of the total population is represented by these mortality data. A more circumstantial indicator of decline is the tenfold decrease in the number of eastern red bats submitted for rabies testing over a span of nearly four decades (Winhold et al. 2008). However, this study was limited to Michigan and does not necessarily reflect eastern red bat losses over their range in eastern North America (Arroyo-Cabrales et al. 2008). Overall, available evidence indicates that eastern red bats are experiencing population declines, which suggests that these populations may be subject to the associated genetic effects of decreasing heterozygosity, increasing rates of inbreeding, and lowered adaptability. Considering the difficulties in estimating census population size for this species, genetic monitoring presents an appealing alternative for estimating effective population size and monitoring population decline. Understanding the accuracy and precision of genetic monitoring techniques is essential before applying these estimates to wildlife management decision-making. Through evaluation of simulated datasets, where population parameters are known, it is possible to determine the accuracy and precision of parameter estimates from genetic samples. Because the true parameter values are not known for wild populations, the accuracy and precision of estimates produced from the genetic monitoring of real populations is difficult if not impossible to assess.

In order to test the potential for using microsatellite loci to monitor recent demographic declines in large populations, I simulated microsatellite data based on population parameters from eastern red bats. I then used coalescent (msvar) and frequency-based ( $M$-ratio, $\theta$ ) analyses of the simulated data to assess the accuracy and bias associated with these analytical methods.

## MATERIALS AND METHODS

## Simulated sample design

In this study, datasets were simulated using ms (Hudson 2002). Simulated data were created in three sampling scenarios. The first sampling scenario constituted 20 diploid individuals and 50 microsatellite loci, similar to what is commonly done in some analyses of genomic data (David et al. 2003). The second sampling scenario was created with 50 diploid individuals each genotyped at 10 microsatellite loci, which has been seen commonly in frequentist population genetic studies (Cornuet et al. 1999). A third sampling scenario, intended to emulate data that might be collected for phylogeographic analyses, was created with 20 diploid individuals and 10 microsatellite loci (Hunter et al. 2012).

For each sampling scenario, data were simulated under a model of exponential decline in a single panmictic population, as is indicated for eastern red bats (Vonhof \& Russell 2015). The population history involved a stable ancestral population of a given effective population size $\left(N_{A}\right)$, experiencing a rate of decline $(r)$ for a specified number of generations $(t)$ to a current effective population size $\left(N_{e}\right)$ at the time of sampling (Table 1, Figure 1). Demographic scenarios included ancestral effective population sizes $\left(N_{A}\right)$ of $1,000,10,000$, and 100,000 diploid individuals. For each of these ancestral effective sizes, populations were simulated under rates of decline $(r)$ of $-1 \%,-5 \%,-10 \%$, and $-50 \%$ per generation. The time $(t)$ at which the population decline began was specified as $1,5,10,50$, and 100 generations in the past. Current effective population sizes $\left(N_{e}\right)$ were calculated from the previous three parameters using the equation:

$$
N_{e}=N_{A}(1+r)^{t} ;
$$

where $N_{e}$ is the current effective population size, $N_{A}$ is the ancestral effective population size, $r$ is the rate of population decline, and $t$ is time in generations (Malthus 1798). Any simulations in
which $N_{e}$ was less than 10 were discarded and not used for further analyses. The coalescentscaled rate of decline $\alpha$ was determined as specified by Hudson (2002):

$$
\alpha=-\left(\frac{1}{t}\right) \ln \left(\frac{N_{e}}{N_{A}}\right)
$$

After the data sets were simulated in ms under the infinite sites model, they were converted to microsatellite data using the script ms2ms.pl (Pidugu \& Schlötterer 2006).

Mutation rates for microsatellite loci in bats are unknown. For this study, the mutation rate was estimated at $\mu=1 \times 10^{-3}$ mutations per locus per generation by taking the geometric mean of multiple mutation rates reported for other mammals (Dietrich et al. 1992, Edwards et al. 1992, Weissenbach et al. 1992, Mahtani \& Willard 1993, Weber \& Wong 1993, Ellegren 1995, Goldstein \& Pollock 1997, Brinkmann et al. 1998). This mutation rate was used to calculate $\theta$ (= $\left.4 N_{A} \mu\right)$ for the data simulations in ms. An average generation time of 5 years was used based on the life history of eastern red bats. The average generation time for eastern red bats is not known, but individuals become sexually mature during their first fall (Cryan et al. 2012) and may live as long as 12 years (Harrad \& Jackson 1961), so a mean reproductive age of 5 years seemed reasonable.

## Coalescent analyses using msvar

Msvar v.1.3 (Storz \& Beaumont 2002) was used to obtain coalescent-based estimates of population parameters for each data set. This program utilizes a closed population model in which a population, at some time in the past, had an ancestral effective population size $N_{A}$ that decreased by rate $r$ for $t$ generations to the current time at which point the effective population size was $N_{e}$. For this analysis, all parameters and settings are given on the $\log _{10}$ scale. Msvar also requires hyperpriors for each parameter $\left(N_{A}, N_{e}, \mu\right.$, and $\left.t\right)$. The hyperpriors are $\alpha$, the mean of the normal distribution for each variable, $\sigma$, the standard deviation of $\alpha, \beta$, the mean of the standard
deviation for each variable, and $\tau$, the standard deviation of the standard deviation. I specified hyperpriors for each parameter, $N_{A}, N_{e}, \mu$, and $t$, as follows. For $N_{A}$ and $N_{e}, \alpha$ was set to one order of magnitude higher than the known (i.e., simulated) value to allow for greater variation with each simulation and $\sigma$ was set to 2 , in accordance with Storz \& Beaumont (2002). For the mutation rate $\mu, \alpha$ was equal to the $\log$ of the mutation rate and $\sigma$ was set to 0.5 , also as demonstrated by Storz \& Beaumont (2002). For $t, \alpha$ was equal to the $\log$ of the number of generations since decline with $\sigma$ set to 2 (Storz \& Beaumont 2002), to allow for flexibility in estimating the parameter. For all parameters, $\beta$ was set to zero, indicating that there was no variation in model parameters amongst loci, and $\tau$ was set to 0.5 in accordance with the examples provided with msvar v.1.3 and Storz and Beaumont (2002). MCMC chains were run for a minimum of $2 \times 10^{9}$ steps (or more as needed to reach convergence) and parameter estimates were assessed every 10,000 steps, resulting in 20,000 estimates for each parameter. Each simulated data set was analyzed three times to allow comparisons of runs generated with different starting random numbers.

When each set of three runs were completed for a data set, the runs were checked for convergence with the Gelman-Rubin statistic in $R$ ( $R$ Core Team 2014) using the gelman.diag() function in the CODA package (Plummer et al. 2006). The first $50 \%$ of each chain was discarded as a burn-in, and the Gelman-Rubin statistic was calculated from the second half of the chains to determine whether convergence occurred within a run. Convergence was assessed from the potential scale reduction factor (PSRF), which compares the variability within each chain to the variability between chains. Any runs that did not have a PSRF $\leq 1.1$ (Beaumont 1999) were rerun with more steps, some up to $1 \times 10^{10}$, to determine if the run would converge within a reasonable time. Analyses with a PSRF $\leq 1.1$ were considered to have converged, and were used
to estimate $N_{A}, N_{e}, \mu$, and $t$ to compare to the known parameters used to create the data sets. Analyses with a PSRF $\geq 1.1$ were considered as not converging and were discarded. After discarding the first half of the data points collected, data from each of the triplicate runs were combined (Okello et al. 2008, Bourke \& Frantz 2010, Jordan et al. 2013) and modes, highest posterior density (HPD) $90 \%$ intervals, posterior probability plots, and boxplots for each parameter estimate were constructed using R. These results are referred to as pooled results. Medians were substituted for any variables that yielded more than one mode for the pooled results. Mode and median were both used because they are not as influenced by outliers as the mean would be. All pooled results were observed to have numerous outliers in boxplots. Frequentist analyses using M-ratio and $\theta$

As a population declines, it is initially expected to lose rare alleles and thus decrease the total number of alleles at a locus. Rare alleles tend to be lost at a higher rate than common alleles through genetic drift. Because drift is a random process, it may result in the loss of any allele in the entire range of allele sizes found at a locus. The $M$-ratio (Garza \& Williamson 2001) compares the number of alleles to the allelic range using the formula:

$$
M=k / r
$$

where $k$ is the total number of alleles and $r$ is the range of allele sizes. While $k$ is affected by the loss of any alleles, $r$ is only affected when the smallest or largest alleles are lost (Garza \& Williamson 2001). It is expected that a population that is in decline would lose rare alleles faster than it would decrease the range size of alleles; thus, $k$ would decrease at a faster rate than would $r$, and $M$ would be smaller in a declining population than in a population at equilibrium.
$M$-ratios were calculated for each simulated microsatellite data set using M_P_val.app software (Garza \& Williamson 2001). The $M_{C}$, or critical value, was also determined for each
data set from 10,000 replicate simulations of an equilibrium population based on the known $\theta\left(=4 N_{\mathrm{e}} \mu\right)$, number of loci, sample size, size of the largest mutation, and the proportion of multi-step mutations using the Critical_M software (Garza \& Williamson 2001). The $M_{C}$ given for each data set is the minimum of the $95 \%$ confidence interval from those simulations; calculated $M$-ratios below this threshold are indicative of populations in decline (Garza \& Williamson 2001).
$\theta$ is a basic measure of diversity that combines the current effective population size $\left(N_{e}\right)$ with the mutation rate $(\mu)$ for a population. So long as the mutation rate remains constant for a population, $\theta$ may be used to monitor for changes in the effective population size (Garza \& Williamson 2001, Spong et al. 2000). $\theta$ was calculated multiple ways to determine whether any particular measure showed potential as a proxy for monitoring changes in population size. $\theta$ was determined using the equation:

$$
\theta=4 N_{e} \mu \quad(\text { Kimura 1968) }
$$

This was done first using the known parameters for each data set and is shown as $\theta . \theta_{\text {col }}$ was calculated using the modes of $N_{e}$ and $\mu$ from the msvar output for each scenario for any run that demonstrated convergence. $\theta$ was also calculated directly from the simulated microsatellite data using three different methods in the Pegas package in R (Paradis 2010). The theta.msat( x ) function in this package calculated $\theta$ based on the repeat number variance $\left(\theta_{v} ;\right.$ Kimmel et al. 1998), expected homozygosity ( $\theta_{h} ;$ Kimmel et al. 1998), and mean allele frequencies ( $\theta_{x}$; Haasl \& Payseur 2010).

## RESULTS

## Msvar estimates

In nearly all analyses, the ancestral effective population size $\left(N_{A}\right)$ was estimated very accurately and precisely using msvar (Figures 2-11). Posterior distributions for this parameter were characterized by a single mode close to the known parameter value, with relatively little error; an example is shown in Figure 2. Across all pooled results in the 50 loci and 20 individuals sampling scenario, the same level of precision is observed across varying rates of decline and with varying time of decline. In demographic scenarios with $N_{A}=1000$, point estimates range between 3.01-3.29 compared to a known $\log \left(N_{A}\right)=3.00$ (Table 2, Figure 3). Similar levels of accuracy are achieved in estimating larger ancestral effective population sizes of 10,000 (Table 3, Figure 4) and 100,000 (Table 4, Figure 5). Point estimates of $N_{A}$ range between $4.01-4.27$ (known $\left.\log \left(N_{A}\right)=4.00\right)$ when $N_{A}$ is 10,000 and between 5.03-5.21 $\left(\mathrm{known} \log \left(N_{A}\right)=5.00\right)$ when $N_{A}$ is 100,000 .

Estimates of $N_{A}$ from other sampling scenarios yielded results that were similarly accurate and precise. Pooled results with a sampling scenario of 50 individuals and 10 loci provided a range of $N_{A}$ estimates of 2.96-3.29 for a known $\log \left(N_{A}\right)=3.00$ (Table 5, Figure 6). For demographic scenarios with larger $N_{A}$, parameter estimates produced in msvar ranged between 3.89-4.22 (known $\log \left(N_{A}\right)=4.00$; Table 6, Figure 7) and between 4.90-5.26 (known $\log \left(N_{A}\right)=5.00$; Table 7, Figure 8). For the sampling scenario with 20 individuals, 10 loci, and an ancestral effective population size of 1000 , similar results were seen for $N_{A}$ estimates (Table 8, Figure 9), with $\log \left(N_{A}\right)$ ranging from $2.91-3.46$ (known $\left.\log \left(N_{A}\right)=3.00\right)$. Simulations with $N_{A}=$ 10,000 produced estimates ranging from $3.92-4.41$ (known $\log \left(N_{A}\right)=4.00$; Table 9, Figure 10), and those with $N_{A}=100,000$ ranged from $4.89-5.19($ known $=5.00$; Table 10, Figure 11$)$.

Ancestral effective population size estimates are both accurate and precise when compared to the known value (Figure 3). This accuracy is consistent regardless of the time since decline, rate of decline, or initial effective population size ( $N_{A}$, Figures 3-5). Sampling scenarios with 20 individuals and 50 loci and those with 50 individuals and 10 loci also demonstrate similarly accurate estimates of $N_{A}$ (Figures 4 and 7). Less accuracy is seen with a less intense sampling effort (20 individuals and 10 loci, Figures 9-11).

This level of precision and accuracy was not seen in estimates of current effective population size $\left(N_{e}\right)$. Multiple examples demonstrate the range of patterns seen in $N_{e}$ estimates (Figure 12A-D). More than half of the simulated data sets yielded posterior probability plots for $N_{e}$ similar to Figure 12A, with imprecise and inaccurate estimation of the known. Some analyses showed considerable variation in estimates of $N_{e}$ among individual runs (e.g., Figure 12B). Some analyses (e.g., Figure 12C) showed improved precision over those exemplified in Figure 12A, but lacked accuracy in estimating $N_{e}$. A small number of simulations accurately and precisely estimated $N_{e}$, with little inter-run variation (e.g., Figure 12D). In simulations with 20 individuals, 50 loci, and $N_{A}=1000$, nine of the fourteen converging pooled results were $\pm 0.5$ orders of magnitude or more from the known (Table 2, Figure 13). Five of the pooled results produced point estimates that were more precise, deviating between 0.024 orders of magnitude less and 0.453 orders of magnitude more than the known. For demographic scenarios of $N_{A}=10,000$ (Table 3, Figure 14), $N_{e}$ was overestimated by half an order of magnitude or more in twelve of fifteen simulations, with point estimates deviating between 0.727 orders of magnitude and 2.056 orders of magnitude greater than the known. Three simulations produced point estimates that were more precise, deviating by only 0.229 orders of magnitude less to 0.311 orders of magnitude more than the known. Only three simulations converged from the demographic
scenario with $N_{A}=100,000$ (Table 4, Figure 15). All three overestimated the current effective population size, with point estimates of this parameter deviating between $0.704-1.537$ orders of magnitude greater than the known.

Results from the 50 individuals and 10 loci sampling scenario were similar. In the demographic scenario that had an $N_{A}=1000$, pooled results from seven of fourteen simulations overestimated the current effective population size by a half of an order of magnitude or more, with point estimates deviating between $0.503-1.732$ orders of magnitude greater than the known $N_{e}$ (Table 5, Figure 16). The remaining seven pooled results were more precise, with point estimates deviating by 0.084 orders of magnitude less to 0.374 orders of magnitude greater than the known. In the demographic scenario with an $N_{A}$ of 10,000 (Table 6, Figure 17), $N_{e}$ was overestimated by half of an order of magnitude or more in pooled results from fifteen of seventeen simulations; estimates deviated between $0.664-3.401$ orders of magnitude greater than the known value for $N_{e}$. Pooled results from the simulations modeling -1\% decline over 100 generations (estimate $=3.730$, known $\left.\log \left(N_{e}\right)=3.566\right)$ and $-10 \%$ decline over 1 generation (estimate $=4.090$, known $\left.\log \left(N_{e}\right)=3.957\right)$ were more precise than other simulations within this sampling scenario. Only nine simulations converged for the demographic scenario with $N_{A}=$ 100,000 (Table 7, Figure 18). The current effective population size was overestimated by half of an order of magnitude or more (deviation of $0.933-1.888$ orders of magnitude greater than the known) for seven of nine simulated data sets. Pooled results from two data sets ( $-1 \%$ decline over 50 generations and $-5 \%$ decline over 100 generations) yielded more precise estimates of $N_{e}$ (estimate $=5.270$, known $\log \left(N_{e}\right)=4.783$; estimate $=2.860$, known $\log \left(N_{e}\right)=2.829$; respectively) than other simulations in this sampling scenario.

Pooled results from the 20 individuals and 10 loci sampling scenario yielded levels of precision in estimating $N_{e}$ comparable to the previous two sampling scenarios. In nine of fourteen demographic scenarios with $N_{A}=1000$, point estimates of $N_{e}$ deviated from the known by half of an order of magnitude or more ( 0.644 less than to 1.686 orders of magnitude greater than the known, Table 8, Figure 19). Data sets from the five remaining scenarios produced estimates that deviated between 0.061 less than to 0.367 orders of magnitude greater than the known value of $N_{e}$. Point estimates of $N_{e}$ overestimated the known in fourteen of seventeen demographic scenarios with an ancestral effective population size of 10,000 , with estimates deviating between 0.582 and 2.056 orders of magnitude greater than the known value for $N_{e}$ (Table 9, Figure 20). Three pooled results yielded better precision in estimating $N_{e}$, with point estimates deviating between 0.459 less than to 0.389 orders of magnitude greater than the known. $N_{e}$ was overestimated in eleven of fifteen data sets simulated under a demographic scenario with $N_{A}=100,000$, with the point estimate deviating between $0.542-1.976$ orders of magnitude greater than the known $N_{e}$ (Table 10, Figure 21). Point estimates of $N_{e}$ from the remaining four data sets deviated from the known by 0.178 less than to 0.447 orders of magnitude greater than the known. Across all sampling scenarios and demographic scenarios, of the pooled results producing estimates within a half an order of magnitude of the known, most (28 of 32) data sets were simulated under conditions of population decline lasting for 50-100 generations and/or with a rate of decline of $-1 \%$ or $-5 \%$.

Although $N_{e}$ was not accurately estimated, there were some general trends that were demonstrated by the boxplots. For most analyses, the known value fell within the second quartile of the posterior distribution (e.g., Figure 13A), and the point estimate was typically greater than the known. Demographic scenarios with smaller $N_{A}(=1000$, Figure 16) yielded estimates that
were more accurate rather than those with a larger $N_{A}(=10,000$, Figure 17; $=100,000$, Figure 18), with the known typically falling within the second quartile for scenarios with $N_{A}=1000$. Results from sampling scenarios with 20 individuals and 50 loci were fairly similar to those with 50 individuals and 10 loci as shown in Figure 13B and 16B, where simulations at 1, 5, and 10 generations produce broad and imprecise estimates of $N_{e}$ and simulations at 50 generations actually underestimate the known $N_{e}$. Simulation results for the 20 individuals and 50 loci and the 50 individuals and 10 loci sampling scenarios each also had knowns that were narrowly outside of the second quartile (13A, C, D, 16B, D). Both of these sampling scenarios produced more accurate results than the 20 individuals and 10 loci sampling scenario (Figure 19) where the known is not within the second quartile in multiple simulations, and there were two estimates that have second quartiles far removed from the known (Figure 19A at 10 generations; 19D at 5 generations). The sampling scenario with 20 individuals and 50 loci did have two simulations (Figure 13A at 100 generations, 13B at 50 generations) that precisely estimated the known $N_{e}$, whereas the sampling scenario with 50 individuals and 10 loci only had one scenario precisely estimating $N_{e}$ (Figure 16B at 50 generations). The sampling scenario with 20 individuals and 10 loci (Figure 19) did not have any precise estimates of $N_{e}$, indicative of the effects of both the number of individuals and the number of loci sampled on resolution power.

Additionally, estimates of current effective population size tended to lose precision over time, with notable deviations between the point estimate and known at 50 or 100 generations of decline (e.g., Figure 17B, C, D). Of all data sets tested, only 32 of 119 (26.9\%) estimated the current effective population size within half an order of magnitude and 16 of those 32 occurred at the most extreme number of generations $(t)$ of decline: at $-1 \%$ decline for 50 or 100 generations, $-5 \%$ decline for 50 or 100 generations, or $-10 \%$ decline for 50 generations (Tables 2, 3, 5-10).

Nine data sets (Tables $2,3,5,8,9,10$ ) actually underestimated the known $N_{e}$. Seven of the nine underestimates were also seen at the most extreme number of generations $(t)$ of decline: at 50 or 100 generations since decline, and at $-1 \%,-5 \%$ or $-10 \%$ rate of decline. Most (7 of 9 ) of these data sets were characterized by few alleles per locus, as would be expected after many generations of strong genetic drift. One hundred and ten of 119 data sets ( $92.4 \%$ ) provided point estimates of $N_{e}$ that were greater than $N_{A}$ for the given scenario, yielding a false signal of population growth rather than decline. Additionally, posterior probability plots (Figure 12) and $90 \%$ HPD intervals are indicative of the lack of precision for $N_{e}$ estimates (Tables 2-10). For example, with a known $\log \left(N_{e}\right)=3.00$, the $90 \%$ HPD typically ranged between 1 to 7 ( $=10$ to 10 million, Table 2). In summary, the imprecision of $N_{e}$ point estimates indicates they should be viewed as an unreliable measure for short-term population monitoring. In addition, $N_{e}$ point estimates were consistently biased upward from the known across pooled results, regardless of sampling scenario or demographic scenario.

Msvar accurately and precisely estimated the known mutation rate, $\log \left(10^{-3}\right)=-3.00$. This result was observed in nearly all simulations performed. Posterior density curves illustrate unimodal distributions with modes very close to the known value and little deviation between runs (Figure 22). No changes in precision were observed from differing scenarios, regardless of the ancestral effective population size, number of microsatellite loci sampled, or the number of individuals sampled. All pooled results from the 50 loci and 20 individual sampling scenario produced estimates of $\mu$ that were within 0.3 orders of magnitude or less of the known (Tables 24, Figures 23-25). The 50 individual and 10 loci sampling scenario similarly estimated $\mu$ within 0.4 orders of magnitude of the known (Tables 5-7, Figures 26-28). All pooled results from the 20
individuals and 10 loci sampling scenario produced estimates of $\mu$ that were within 0.3 orders of magnitude of the known (Tables 8-10, Figures 29-31).

Estimates of $\mu$ are precise but do tend to underestimate the known (e.g., Figure 23). Estimates from sampling scenarios of 20 individuals and 50 loci and those with 50 individuals and 10 loci show this pattern (e.g., Figures 24 and 27). The sampling scenario with 20 individuals and 10 loci (Figures 29-31) yielded results that were slightly less accurate than the other sampling scenarios. Additionally, mutation rate estimates did not vary in precision or accuracy across demographic scenarios with $N_{A}=1000, N_{A}=10,000$, or $N_{A}=100,000$ (Figures 26-28).

Estimates of the time since decline $(t)$ are less reliable than those seen for the mutation rate. Posterior probability plots illustrate large margins of error (e.g., Figures 32A, B), significant deviation from the known value (e.g., Figure 32C), and considerable variation among replicate runs (e.g., Figure 32D). Results from the sampling scenario with 50 loci and 20 individuals yielded point estimates that deviated from 1.539 orders of magnitude less to 1.781 orders of magnitude more than the known (Tables 2-4, Figures 33-35). Pooled results from sixteen of 32 data sets in this sampling scenario are within $\pm 0.5$ orders of magnitude of the known. Estimates of time since decline from data sets simulated under the sampling scenario with 10 loci and 50 individuals were similarly variable, with point estimates between 1.799 orders of magnitude less than to 2.470 orders of magnitude more than the known (Tables 5-7, Figures 36-38). Twenty-six of the forty data sets simulated under this sampling scenario produced estimates of $t$ that were within $\pm 0.5$ orders of magnitude of the known. Estimates of $t$ from the 20 individual and 10 loci sampling scheme deviated by 1.740 orders of magnitude less than to 1.910 orders of magnitude
more than the known (Tables 8-10, Figures 39-41), with eighteen data sets yielding estimates more than 0.5 orders of magnitude different than the known.

Estimates of generations since decline are imprecise. Of all the sampling scenarios, estimates from pooled results with 50 individuals and 10 loci were the most consistent among runs and generally underestimated the known generations (Figures 36-38). Data sets with 20 individuals and 50 loci generally yielded underestimates of time since decline, particularly with more recent declines (1-10 generations, Figures 33-35). Results from the sampling scenario with 20 individuals and 10 loci are comparable (Figures 39-41). Modifications to the demographic scenario $\left(N_{A}=1000, N_{A}=10,000\right.$, or $N_{A}=100,000$; Figures $\left.36-38\right)$ did not result in improved accuracy or precision in estimating $t$.

## M-ratio calculations

$M$-ratios and critical $M$ values $\left(M_{C}\right)$ were determined for all simulated data sets. In accordance with Garza \& Williamson (2001), $M_{C}$ values were simulated based on the sample size, number of loci, and mutation model; calculated $M$-ratio values less than the $M_{C}$ indicate a significant population decline. In sampling scenarios with 20 individuals and 10 loci, decline was indicated for 14 out of $48\left(=29.2 \% ; 2\right.$ out of 14 data sets for $N_{A}=1,000 ; 6$ out of 17 data sets for $N_{A}=10,000 ; 6$ out of 17 data sets for $N_{A}=100,000 ;$ Table 11) of the simulated data sets. Improving sampling by increasing the number of individuals genotyped ( 50 individuals and 10 loci) had little effect on the power of this analysis, with $M$-ratios demonstrating decline in 15 out of $48\left(=31.3 \% ; 3\right.$ out of 14 data sets for $N_{A}=1,000 ; 4$ out of 17 data sets for $N_{A}=10,000 ; 8$ out of 17 data sets for $N_{A}=100,000$; Table 12) simulated data sets. However, increasing the number of loci examined (20 individuals and 50 loci) markedly improved statistical power, with $M$-ratios indicating decline in 29 out of $48\left(=60.4 \% ; 6\right.$ out of 14 data sets for $N_{A}=1,000 ; 10$ out of 17
data sets for $N_{A}=10,000 ; 13$ out of 17 data sets for $N_{A}=100,000$; Table 13) simulated data sets. Overall, 58 of $144(=40.3 \%)$ simulated data sets indicated decline using the $M$-ratio bottleneck test. Of those data sets that did indicate decline, 51 of $58(=87.9 \%)$ were simulated under conditions of $-10 \%$ or $-50 \%$ rate of decline and/or 50 or 100 generations of decline.

## $\theta$ calculations

The $\theta$ values calculated for each scenario did not demonstrate accuracy or precision throughout all simulated data sets or pooled results. $\theta_{\text {col }}$ was determined using msvar estimate modes (or in some cases medians) for $N_{e}$ and $\mu$. $\theta_{\text {col }}$ was roughly an order of magnitude greater than the known $\theta$ for 70 out of 119 (58.8\%) of the pooled results (Tables 14-22), and was generally the estimate of $\theta$ that was most divergent from the known. Estimates of $\theta_{v}, \theta_{h}$, and $\theta_{x}$ were determined using repeat number variance, expected homozygosity, and allele frequency means, respectively. These simple summary statistics provided more accurate estimates of the known $\theta$. Among these $\theta$ estimates, $\theta_{v}$ typically provided the most accurate estimate of the true $\theta$ in demographic scenarios with lower rates of decline ( $-1 \%$ to $-5 \%$ ) and more recent times since the onset of decline (up to 10 generations), whereas $\theta_{x}$ provided more accurate estimates for more extreme scenarios of decline (Tables 14-22). $\theta_{h}$ was generally less accurate than either $\theta_{v}$ or $\theta_{x}$, with a few exceptions. Sampling scenarios with 50 individuals and 20 loci demonstrated this pattern the most consistently through all three demographic scenarios $\left(N_{A}=1000,10,000\right.$, and 100,000; Tables 14-16). Sampling scenarios with 20 individuals and 10 loci did also demonstrate this pattern across demographic scenarios, but with less consistency (Tables 17-19). Sampling scenarios with 50 individuals and 10 loci lacked a consistent pattern for the demographic scenario with $N_{A}=1000\left(\right.$ Table 20). For example, when $N_{A}=1000$, the most precise estimator of $\theta$ was still either $\theta_{v}$ or $\theta_{x}$, but $\theta_{v}$ was most accurate in some sampling scenarios with high rates of
decline ( $-50 \%$ decline, 1 generations; Table 20) and $\theta_{x}$ was in some cases most accurate in sampling scenarios with low rates of decline ( $-1 \%$ decline, 5 generations; Table 20). In a few cases ( 1 generation, at $-1 \%,-5 \%$, and $-10 \%$ decline; Table 20), $\theta_{h}$ most accurately estimated the known $\theta$. Demographic scenarios where $N_{A}=10,000\left(\right.$ Table 21) or $N_{A}=100,000$ (Table 22) did generally demonstrate better accuracy from $\theta_{v}$ at lower rates of decline over less time and from $\theta_{x}$ at higher rates of decline and/or generations, as seen in previous sampling scenarios.

## DISCUSSION

## Msvar analyses

My results indicate that msvar is an unbiased estimator of the $N_{A}$ and $\mu$ parameters, but it provides imprecise and often inaccurate estimates of the $N_{e}$ and $t$ parameters. The accuracy $\left(N_{A}\right)$ and precision $\left(N_{A}, \mu\right)$ observed with sampling scenarios with 20 individuals and 50 loci and those with 50 individuals and 10 loci, as compared to those with 20 individuals and 10 loci, indicate that more intense sampling, whether by increasing the number of individuals or number of loci, provides improved statistical power for estimating these population parameters using msvar.

Girod et al. (2011) simulated microsatellite data sets with the same ancestral effective population sizes of $1000,10,000$, and 100,000 , under similar rates of decline (approximately $0.5 \%$ to $-50 \%$ ), and similar timescales (10-500 generations) using msvar. These authors found that their estimates of all parameters were imprecise and inaccurate. Here, I also found that estimates of time and the current effective population size lacked accuracy, but ancestral effective population size and mutation rate were both consistently estimated with accuracy and precision by msvar.

There were some noteworthy differences in the construction of the two studies that may account for these discrepancies. Girod et al. (2011) simulated five different data sets for each demographic scenario, estimated parameters from each data set only once, and found that parameter estimates from these data sets were notably different from each other. This approach confounds stochasticity in the coalescent process simulating the data sets with that in the analysis of the data in msvar. Genealogies for single populations can be analyzed to estimate population parameters, taking into account stochastic effects on a population, but the technique used by Girod et al. (2011) is analogous to taking samples from five different populations and expecting
them all to produce accurate and precise estimates of population characteristics as if they represented the same population. My study had one simulated data set for each demographic scenario, which was analyzed in triplicate in msvar. Thus, my results better assess the precision, accuracy, and bias inherent to msvar alone.

Girod et al. (2011) also permitted broader initial variation of all parameters in msvar in order to test the ability of the program to estimate parameters from uninformative priors. Mutation rate was estimated accurately and precisely in my study, generally within 0.5 orders of magnitude of the known. Girod et al. (2011) found much less precision in estimating mutation rate. This may be attributable to a difference in priors. Girod et al. (2011) set mutation rate priors very broadly, with a mean of -4 (known was -3 ) and a standard deviation of 2 . I set mutation rate priors more narrowly, as is common in many empirical publications (Okello et al. 2008, Bourke \& Frantz 2010, Rotheray et al. 2012), with a prior of -3 (known was -3 ) and a standard deviation of 0.5 . When each parameter is estimated by msvar, the other parameters are permitted to vary within the bounds set by the user (pers. comm., M. Beaumont). Storz and Beaumont (2002) recommended a general strategy of having broad priors for $N_{e}$ and $N_{A}$ but priors that were more informative for the mutation rate, and stated that the $N_{e}, N_{A}$, and $t$ parameters are strongly dependent on the prior for $\mu$. Analyses by Storz and Beaumont (2002) were consistent with Girod et al. (2011) in finding that broad priors would produce results dependent on variation in the mutation rate.

## M-ratio tests

$M$-ratios less than the $M_{C}$ value were detected for 58 of 144 data sets, signifying that the $M$-ratio accurately detected population decline in $40.3 \%$ of the simulations (Tables 11-13). Two $M$-ratios indicating decline were too close to the $M_{C}$ value to definitively indicate decline (-5\%
decline from an $N_{A}=1000$ for 1 generation, and $-1 \%$ decline from an $N_{A}=100,000$ for 1 generation, Table 13). Apart from these two results, nearly all (51 of 56) remaining significant $M$-ratios were detected at higher rates of decline ( $-10 \%$ or $-50 \%$ ) and/or longer time intervals (50 or 100 generations). Sampling scenarios with 20 individuals and 10 loci indicated population decline in only $29.2 \%$ of data sets (Table 11). Sampling scenarios with 50 individuals and 10 loci had similar power, with $M$-ratios indicating decline in $31.3 \%$ of data sets (Table 12). However, sampling scenarios with 20 individuals and 50 loci showed much greater power, with population decline indicated in $56.3 \%$ of data sets (Table 13). Compared to a minimal sampling scheme of 20 individuals and 10 loci, increasing the number of loci had a much greater impact on the power of the $M$-ratio test than increasing the number of individuals. The greatest rate of successful identification of population decline was seen with a sampling scenario of 20 individuals and 50 microsatellite loci and a demographic scenario of $N_{A}=100,000$, with $M$-ratios correctly indicating decline for $70.6 \%$ of simulated data sets. Although Garza and Williamson (2001) predicted that $M$-ratios would be sensitive to population decline only after 100 generations, very high rates of decline ( $-50 \%$ ) were detectable in as little as a single generation under an optimal sampling scenario ( 20 individuals and 50 loci). There are a small number of data sets that were expected to show significant signals of decline and did not (Table 11, $N_{A}=1000,5$ gen. and $50 \%, 10$ gen. and $-10 \%$; Table $12, N_{A}=1000,10$ gen. and $-10 \%, N_{A}=100,000,10$ gen. and $10 \%$ ) as sampling scenarios of fewer generations since decline or rates of decline did indicate population decline. This may be attributable to the stochastic nature of allele loss in declining populations. Alleles, including rare alleles, are presumed to be lost randomly from a declining population. By chance, rare alleles may or may not be lost as a population declines and if too few
rare alleles are lost as a population declines, this could negatively affect the sensitivity of the $M$ ratio to detect population decline.

Significant signals of population decline were seen most frequently ( $51.0 \%$ of data sets) in demographic scenarios with $N_{A}=100,000$. For data sets with $N_{A}=10,000$, population decline was detected in $39.2 \%$ of data sets and for data sets with $N_{A}=1000$, population decline was detectable in only $23.8 \%$ of data sets, indicating greater sensitivity when the ancestral population sizes are large. Population genetic theory predicts that a larger population will possess more rare alleles than a smaller population. As the $M$-ratio is dependent on the loss of rare alleles, this test is more powerful in demographic scenarios with larger $N_{A}$ values. This is consistent with other studies that have found that $M$-ratios detect population declines more reliably where there are larger ancestral effective population sizes, as compared to populations with smaller ancestral effective sizes (Kuo \& Janzen 2004, Hoban et al. 2014).

## Diversity estimates

I assessed whether various measures of the genetic diversity parameter $\theta$ could serve as meaningful proxies for monitoring population declines. $\theta_{\text {col }}$ was roughly an order of magnitude greater than the known $\theta$ for 70 out of 119 data sets, or $58.8 \%$ of the simulations (Tables 14-22). $\theta_{\text {col }}\left(=4 N_{e} \mu\right)$ was calculated using msvar point estimates for $N_{e}$ and $\mu$. The summary statistics $\theta_{v}$, $\theta_{h}$, and $\theta_{x}$ were determined using the repeat number variance, expected homozygosity, and allele frequency means, respectively; these estimates were much more similar to the known $\theta$. $\theta_{v}$ typically provided the most accurate estimates of the true $\theta$ in less extreme scenarios of decline (-1\% decline over 1-10 generations), while $\theta_{x}$ provided more accurate estimates for more extreme rates of decline ( $-50 \%$ decline over 1-10 generations; Table 15). $\theta_{x}$ is expected to reflect declines, especially strong declines, because it is calculated from allele frequencies and thus
should reflect the loss of rare alleles as a population declines (Williamson-Natesan 2005), but has been observed to be downward biased when the true $\theta$ is greater than 50 (Haasl \& Payseur 2010). The true, or known, $\theta$ for the demographic scenarios in this study were $\theta=4,40$, and 400 , so it is likely that $\theta_{x}$ would produce downward biased estimates of the known $\theta$, particularly when $N_{A}=10,000$ or 100,000 . This may provide an explanation for the lack of precision seen for $\theta_{x}$ as compared to other methods of calculating $\theta . \theta_{v}$ is determined from changes in the number of repeats observed and would be expected, like allele frequencies, to decrease as a population declines and alleles are lost. Like Haasl \& Payseur (2010), I found that $\theta_{h}$ provided overestimates of $\theta$, regardless of the sampling or demographic scenario. Considering the poor estimates of $\theta$ produced by msvar parameter estimates, use of the scaled parameter $\theta$ derived from msvar could not be recommended.

## Implications for conservation

The analyses conducted here indicate that genetic monitoring alone would not be a reliable method of estimating the current effective population size of eastern red bats. While there is circumstantial evidence for a decline in the eastern red bat population (Winhold et al. 2008), accurate census estimates are not available. Mortality due to wind turbines has been estimated (Arnett \& Baerwald 2013), but the proportion of the populations being killed at wind turbines is still unknown. Current effective population size was recently estimated for eastern red bats (Vonhof \& Russell 2015), but estimates of current effective population size are not available for other migrating bat species. Because $N_{e} / N_{c}$ for eastern red bats is also unknown, this ratio cannot be used to evaluate the impact of wind turbine mortalities on this species. Additionally, eastern red bats may face an increased risk of extinction due to its migratory behavior (Arnett \& Baerwald 2013). Impacts of climate change and habitat fragmentation further increase this risk
(Jones \& Rebelo 2013). Additional research is needed in determining what method is most efficacious in estimating a census population size for eastern red bats to monitor population impacts as well as the effectiveness of any mitigation measures designed to curtail wind turbine mortalities.

Eastern red bats have been found to have a single panmictic population (Vonhof \& Russell 2015), so any mortalities from wind turbines would not be disproportionately impacting some subpopulations over others. It is generally expected that migratory species do not have structured populations (Moussy et al. 2013). While a similar pattern of panmixia has been found for migratory Mexican free-tailed bats (Tadarida brasiliensis, Russell et al. 2005), not all migratory bat species are necessarily panmictic. Another migratory species, eastern pipistrelles (Perimyotis subflavus), has been found to have sex-biased dispersal, with significant structure among females but not males (Martin 2014).

Arnett and Baerwald (2013) estimated wind turbine mortalities from 2000 to 2011 in the U. S. and Canada to be between $143,023-287,403$ for eastern red bats, $26,004-52,255$ for the big brown bat (Eptesicus fuscus), 247,040 - 633,822 for the hoary bat (Lasiurus cinereus), 148,839-308,322 for the silver-haired bat (Lasionycteris noctivagans), 51,617-106,925 for the little brown bat (Myotis lucifugus), 45,260-93,756 for P. subflavus, and 21,282-44,087 for $T$. brasiliensis. Significant gaps do exist in our knowledge of population size and population structure for many bat species including some of those listed above, and these gaps impair our ability to assess the effects of turbine-related mortalities on bat populations. This could include potential disproportionate impacts to subpopulations suffering wind turbine mortalities at higher rates than other subpopulations.

Climate change, habitat fragmentation, and other anthropogenic disturbances have negative impacts on bat species (Jones et al. 2009). Many bat mortalities are caused by changes to the physical environment in which bats fly, such as the construction of roads through bat foraging routes (Russell et al. 2009) or nesting sites (Lesiński 2008). Noise pollution also impacts bats and may interfere with their ability to use echolocation (Simmons et al. 2004). The greater mouse eared bat (M. myotis) has been found to avoid highways when seeking prey; it is thought that noise from highway traffic interferes with passive listening for insect prey (Siemers \& Schaub 2011). In fact, sound-emitting interference has demonstrated some success as a method of deterring bats from wind turbines (Horn et al. 2008, Arnett et al. 2013). Some bat species are also suffering devastating losses due to white nose syndrome (Coleman \& Reichard 2014). Under these combined pressures, multiple bat species are facing extinction threats. Myotis septentrionalis was listed as a threatened species in April 2015, primarily because of white nose syndrome, although other impacts included in the listing are wind turbine collisions and climate change (U.S. Fish and Wildlife Service 2015). To address the many negative impacts facing bat species, better quality data on population size, structure, and losses are needed.

Population census size or current effective population size is commonly estimated as a means of genetically monitoring species (Nei \& Tajima 1981, Waples 1989, Hare et al. 2011). For many species of conservation interest, there are significant barriers to obtaining an accurate census estimate. When populations are widely dispersed across terrestrial or aquatic systems, it can impede accurate census estimates. The use of current effective population size estimates becomes important for conservation monitoring of populations potentially in decline. Coalescent estimates of $N_{e}$ are thought to be more reliable than other methods of estimating $N_{e}$ (Luikart et al. 2010). My study indicates that, even with specific knowledge of the mutation rate, estimates of
current $N_{e}$ using msvar are not reliable and routinely overestimate this parameter. Results from msvar produced estimates with very broad $90 \%$ HPD intervals spanning, in some cases, up to six orders of magnitude. The large amount of error associated with estimates of $N_{e}$ also made it difficult to detect significant population declines (i.e., $N_{e}$ significantly less than $N_{A}$ for a given simulation) using msvar. Only a few simulated data sets, typically involving decline over 50 or 100 generations, gave a significant signal of decline. Therefore, coalescent-based estimates of current $N_{e}$ alone should not be used for conservation decisions. Msvar estimates of $N_{e}$ from simulations with ancestral effective population sizes of 1000 had slightly more precise modes, but still had HPD $90 \%$ intervals so broad (from 10 to $10,000,000$ for $N_{A}=1000$ ) as to be meaningless. Consequently $N_{e}$ estimates from msvar were not demonstrably reliable at any of the population sizes tested in this study. Further simulations are needed to determine whether msvar estimates of $N_{e}$ demonstrate improved accuracy or precision over short timescales if $N_{A}$ is less than 1000.

Current effective population size, or $N_{e}$, is defined as the size of a theoretical population at Wright-Fisher equilibrium that experiences the same rate of genetic drift as the study population. The populations modeled in this study do violate one assumption of a Wright-Fisher equilibrium population in that populations were simulated to be declining in size and therefore not at equilibrium. However, coalescent analyses of populations have been demonstrated to be robust even if violations of the Wright-Fisher population model do occur (Kingman 1982, Möhle 1998). If real data were to be used it would also be necessary to consider additional violations of Wright-Fisher equilibrium, as follows. Eastern red bats are a panmictic population (Vonhof \& Russell 2015), meeting the expectation of a single closed population, but it is unlikely that mating is completely random for a species with a range from Canada to northern Mexico
(Arroyo-Cabrales et al. 2008). Additionally, it is likely that eastern red bats do have overlapping generations (Cryan et al. 2012), further violating the assumptions of a Wright-Fisher equilibrium population. Lastly, the Wright-Fisher model of an equilibrium population also presumes a haploid hermaphroditic population, neither of which is true of eastern red bats. These additional violations for a real population would not be likely to affect the quality of coalescent estimates of populations (Hein et al. 2004), so long as the sample size was significantly smaller than the total population size (Fu 2006).

To improve coalescent-based estimates of $N_{e}$, it would be important to improve computational capacity to analyze larger data sets with greater speed. Some analyses in this study required only a few hours to run, but many required weeks or months of computational time on a ten-node cluster. I intended to explore demographic scenarios with an $N_{A}$ of $1,000,000$ and sampling scenarios including 100 loci. Neither of these scenarios would run on msvar using the ten-node computer cluster used for the other data sets in this study. Msvar is not designed to handle such large data sets. Alternative computational approaches, such as approximate Bayesian computation, while requiring less computational power, are also less sensitive to detecting past population parameters (Beaumont et al. 2002). Both computational capacity and more efficient software would be necessary to take full advantage of the data potentially available from NextGen sequencing, and may provide a more reliable method to assess short-term declines of large populations.
$M$-ratios are more reliable indicators of population declines, particularly over long periods of time or with rates of decline of $-10 \%$ or more. Additionally, $M$-ratios demonstrated higher rates of detecting population decline when $N_{A}$ was large $(=10,000$ or 100,000$)$, as it would be expected for these populations to have greater numbers of rare alleles to lose.

Populations with a small $N_{A}(=1000)$ would likely have fewer rare alleles and be less sensitive to detecting population decline using $M$-ratios. However, this statistic has limited power to detect lower rates of decline over shorter time frames. $M$ - ratios also require knowledge of $\theta$, the estimation of which may involve considerable error.
$M$-ratios may provide a useful monitoring tool for other migratory species likely to have large ancestral effective population sizes. Some migratory species of conservation concern include scalloped hammerhead sharks (Baum et al. 2003), green sea turtles (Jackson et al. 2001), and caribou (Vors \& Boyce 2009). Like eastern red bats, accurately estimating $N_{c}$ or $N_{e}$ for many aquatic and terrestrial migratory species can be problematic. In order to assess impacts to widely dispersed migratory species, it is necessary to monitor species throughout their range (Vonhof and Russell 2015), and $M$-ratios could potentially provide a simple and effective means of monitoring population declines for species with historically large ancestral effective population sizes.

SNPs may be an alternative genetic marker for detecting population bottlenecks, but would require the use of highly polymorphic sites (Morin et al. 2004). However, the sole use of highly polymorphic SNP data can potentially cause ascertainment bias in data sets (Morin et al. 2012). Mitochondrial DNA has been used for long term conservation planning and identification of evolutionarily significant units (Moritz 1994), but more recently has been determined to potentially provide an incomplete assessment of a species' conservation priority (Rubinoff 2006) and may produce overestimates of current effective population size when compared to $N_{e}$ estimates from microsatellite loci (Qiu et al. 2013). Hypervariable microsatellite loci with mutation rates of $10^{-2}$ could also be used to monitor $N_{e}$ and have been found in multiple species including humans (Itsara et al. 2010) and birds (Anmarkrud et al. 2011). Their use would require
knowledge of locus-specific mutation rates for each species being studied, so that more quickly mutating loci could be identified and analyzed appropriately. Their mutation rate may also be influenced by multiple factors including environmental conditions, sex, or chromosomal location (Anmarkrud et al. 2011), and the effects of these factors on accurate $N_{e}$ estimation are not known. Detailed knowledge of their mutation characteristics would be needed for accurate estimates of $N_{e}$ from hypervariable microsatellite loci. As indicated by the results of this study and that of Storz and Beaumont (2002), knowledge of mutation rates is essential to estimating any other parameters using coalescent methods such as msvar. Other possibilities that could be pursued in future research would be estimating $N_{e}$ using large numbers of microsatellite loci, 100 or more. $M$-ratios demonstrated greater sensitivity in this study in sampling scenarios with greater numbers of loci. Felsenstein (2006) also determined that maximizing the number of loci produced better assessments of genetic parameters in coalescent analyses than maximizing the number of individuals. NextGen sequencing has the capacity to provide researchers with data from hundreds of microsatellite loci (Tucker et al. 2009). In order to take advantage of the wealth of knowledge available from these data sets, it will be vital for computational capacity to improve.

Table 1. Data simulated for this study were created in three sampling scenarios, one with 20 individuals and 50 microsatellite loci, one with 50 individuals and 10 microsatellite loci, and one with 20 individuals and 10 microsatellite loci. For each sampling scenario, each of the following demographic scenarios was simulated for analysis, resulting in a total of 48 demographic scenarios per sampling scenario:

| $N_{A}$ | $r$ | $t$ |
| :---: | :---: | :---: |
| 1000 | -1\% decline | 1 generation |
|  | -1\% decline | 5 generations |
|  | -1\% decline | 10 generations |
|  | -1\% decline | 50 generations |
|  | -1\% decline | 100 generations |
|  | -5\% decline | 1 generation |
|  | -5\% decline | 5 generations |
|  | -5\% decline | 10 generations |
|  | -5\% decline | 50 generations |
|  | -10\% decline | 1 generation |
|  | -10\% decline | 5 generations |
|  | -10\% decline | 10 generations |
|  | -50\% decline | 1 generation |
|  | -50\% decline | 5 generations |
| 10,000 | -1\% decline | 1 generation |
|  | -1\% decline | 5 generations |
|  | -1\% decline | 10 generations |
|  | -1\% decline | 50 generations |
|  | -1\% decline | 100 generations |
|  | -5\% decline | 1 generation |
|  | -5\% decline | 5 generations |
|  | -5\% decline | 10 generations |
|  | -5\% decline | 50 generations |
|  | -5\% decline | 100 generations |
|  | -10\% decline | 1 generation |
|  | -10\% decline | 5 generations |
|  | -10\% decline | 10 generations |
|  | -10\% decline | 50 generations |
|  | -50\% decline | 1 generation |
|  | -50\% decline | 5 generations |
|  | -50\% decline | 10 generations |
| 100,000 | -1\% decline | 1 generation |
|  | -1\% decline | 5 generations |
|  | -1\% decline | 10 generations |
|  | -1\% decline | 50 generations |
|  | -1\% decline | 100 generations |
|  | -5\% decline | 1 generation |
|  | -5\% decline | 5 generations |


|  | $-5 \%$ decline | 10 generations |
| :--- | :--- | :--- |
|  | $-5 \%$ decline | 50 generations |
|  | $-5 \%$ decline | 100 generations |
|  | $-10 \%$ decline | 1 generation |
|  | $-10 \%$ decline | 5 generations |
|  | $-10 \%$ decline | 10 generations |
| $-10 \%$ decline | 50 generations |  |
|  | $-50 \%$ decline | 1 generation |
|  | $-50 \%$ decline | 5 generations |
|  | $-50 \%$ decline | 10 generations |

Table 2. Msvar results showing known vs. estimated parameters for data sets with $N_{A}=1000,50$ microsatellite loci, and 20 individuals, percent decline noted. Estimates are reported as the mode (or median) of the posterior distribution with the $90 \%$ highest posterior density. All known and estimated parameter values are given on the $\log _{10}$ scale.

| time | $N_{e}$ |  | $N_{A}$ |  | $\mu$ |  | - $t$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | known | estimate | known | estimate | known | estimate | known | estimate |
| -1\% |  |  |  |  |  |  |  |  |
| 1 gen | 2.996 | 3.92 (1.32, 6.88) | 3.00 | 3.16 (2.23, 3.84) | -3.00 | -3.17 (-3.95, -2.37) | 0.00 | -0.02 (-3.19, 2.56) |
| 5 gen | 2.978 | 3.18 (1.19, 6.95) | 3.00 | 3.29 (2.33, 3.92) | -3.00 | -3.14 (-3.94, -2.37) | 0.70 | -0.78 (-3.11, 2.26) |
| 10 gen | 2.957 | 3.41 (1.08, 7.16) | 3.00 | 3.27 (2.35, 3.94) | -3.00 | -3.25 (-3.97, -2.39) | 1.00 | -0.41 (-3.31, 1.91) |
| 50 gen | 2.783 | 3.15 (1.61, 6.84) | 3.00 | 3.16 (2.29, 4.09) | -3.00 | -3.12 (-3.99, -2.41) | 1.70 | 0.16 (-3.18, 3.61) |
| 100 gen | 2.566 | 2.5 (0.49, 4.02) | 3.00 | 3.25 (2.33, 4.02) | -3.00 | -3.29 (-3.98, -2.41) | 2.00 | 2.60 (-0.39, 4.60) |
| -5\% |  |  |  |  |  |  |  |  |
| 1 gen | 2.978 | 3.93 (1.12, 7.16) | 3.00 | 3.07 (2.37, 3.96) | -3.00 | -3.06 (-3.94, -2.36) | 0.00 | -0.55 (-3.22, 2.28) |
| 5 gen | 2.891 | 3.60 (1.16, 6.94) | 3.00 | 3.01 (2.29, 3.91) | -3.00 | -3.17 (-3.96, -2.39) | 0.70 | 0.37 (-2.98, 2.36) |
| 10 gen | 2.783 | 3.15 (1.81, 6.75) | 3.00 | 3.2 (2.20, 4.38) | -3.00 | -3.12 (-3.90, -2.32) | 1.00 | 1.00 (-2.01, 5.95) |
| 50 gen | 1.914 | 1.89 (0.1, 2.76) | 3.00 | 3.08 (2.33, 3.86) | -3.00 | -3.24 (-4.01, -2.49) | 1.70 | 2.70 (0.89, 3.53) |
| -10\% |  |  |  |  |  |  |  |  |
| 1 gen | 2.957 | 4.42 (1.46, 7.33) | 3.00 | 3.14 (2.33, 3.93) | -3.00 | -3.09 (-3.96, -2.38) | 0.00 | -0.08 (-3.30, 2.17) |
| 5 gen | 2.783 | 3.86 (1.62, 6.88) | 3.00 | 3.24 (2.31, 4.02) | -3.00 | -3.22 (-3.94, -2.37) | 0.70 | -0.03 (-3.36, 3.42) |
| 10 gen | 2.566 | 3.15 (1.07, 7.00) | 3.00 | 3.24 (2.35, 4.01) | -3.00 | -3.20 (-4.00, -2.42) | 1.00 | 0.25 (-2.88, 2.39) |
| -50\% |  |  |  |  |  |  |  |  |
| 1 gen | 2.783 | 4.16 (1.30, 7.40) | 3.00 | 3.26 (2.33, 3.93) | -3.00 | -3.16 (-3.96, -2.38) | 0.00 | -0.58 (-3.40, 1.88) |
| 5 gen | 1.914 | 2.53 (-0.27, 6.01) | 3.00 | 3.11 (2.41, 4.02) | -3.00 | -3.29 (-4.02, -2.44) | 0.70 | 1.16 (-2.03, 3.07) |

Table 3. Msvar results showing known vs. estimated parameters for data sets with $N_{A}=10,000,50$ microsatellite loci, and 20 individuals, percent decline noted. Estimates are reported as the mode (or median) of the posterior distribution with the $90 \%$ highest posterior density. All known and estimated parameter values are given on the $\log _{10}$ scale.

| time | $N_{e}$ |  | $N_{A}$ |  | $\mu$ |  | $t$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | known | estimate | known | estimate | known | estimate | known | estimate |
| -1\% |  |  |  |  |  |  |  |  |
| 1 gen | 3.996 | 3.89 (2.14, 8.15) | 4.00 | 4.08 (3.31, 4.92) | -3.00 | -3.08 (-3.96, -2.37) | 0.00 | 0.12 (-3.51, 2.16) |
| 5 gen | 3.978 | 4.86 (2.40, 8.13) | 4.00 | 4.23 (3.33, 4.92) | -3.00 | -3.19 (-3.94, -2.37) | 0.70 | 0.47 (-2.58, 2.82) |
| 10 gen | 3.957 | 5.27 (2.46, 8.10) | 4.00 | 4.08 (3.33, 4.92) | -3.00 | -3.23 (-3.94, -2.37) | 1.00 | 0.35 (-2.59, 2.96) |
| 50 gen | 3.783 | 5.15 (2.87, 8.04) | 4.00 | 4.16 (3.29, 4.92) | -3.00 | -3.33 (-3.94, -2.37) | 1.70 | 1.80 (-1.88, 3.24) |
| 100 gen | 3.566 | 5.19 (2.59, 7.83) | 4.00 | 4.11 (3.27, 4.88) | -3.00 | -3.34 (-3.97, -2.41) | 2.000 | 1.12 (-1.79, 3.13) |
| -5\% |  |  |  |  |  |  |  |  |
| 1 gen | 3.978 | 5.45 (2.25, 8.20) | 4.00 | 4.19 (3.31, 4.89) | -3.00 | -3.20 (-3.94, -2.36) | 0.00 | -0.14 (-3.17, 2.19) |
| 5 gen | 3.891 | 5.15 (1.97, 7.99) | 4.00 | 4.05 (3.31, 4.89) | -3.00 | -3.03 (-3.90, -2.33) | 0.70 | 0.31 (-2.52, 2.94) |
| 10 gen | 3.783 | 4.51 (2.40, 7.81) | 4.00 | 4.13 (3.35, 4.96) | -3.00 | -3.20 (-3.93, -2.35) | 1.00 | $0.54(-2.46,3.21)$ |
| 100 gen | 1.829 | 1.6 (0.89, 2.47) | 4.00 | 4.27 (3.43, 4.97) | -3.00 | -3.12 (-4.02, -2.52) | 2.00 | 3.18 (2.50, 4.09) |
| -10\% |  |  |  |  |  |  |  |  |
| 1 gen | 3.957 | 4.89 (2.24, 8.11) | 4.00 | 4.13 (3.33, 4.92) | -3.00 | -3.20 (-3.94, -2.35) | 0.00 | -0.35 (-3.06, 2.62) |
| 5 gen | 3.783 | 4.76 (2.24, 8.00) | 4.00 | 4.07 (3.35, 4.92) | -3.00 | -3.00 (-3.92, -2.36) | 0.70 | 0.26 (-2.45, 2.88) |
| 10 gen | 3.566 | 4.97 (2.49, 8.23) | 4.00 | 4.01 (3.31, 4.88) | -3.00 | -3.26 (-3.93, -2.38) | 1.00 | 0.34 (-2.41, 2.76) |
| 50 gen | 1.829 | 2.14 (1.45, 3.01) | 4.00 | 4.15 (3.35, 4.88) | -3.00 | -3.15 (-3.98, -2.47) | 1.70 | 3.48 (2.70, 4.27) |
| -50\% |  |  |  |  |  |  |  |  |
| 1 gen | 3.783 | 5.25 (2.36, 8.04) | 4.00 | 4.20 (3.33, 4.92) | -3.00 | -3.12 (-3.96, -2.38) | 0.00 | -0.55 (-3.06, 2.92) |
| 5 gen | 2.914 | 4.97 (2.32, 8.17) | 4.00 | 4.17 (3.33, 4.92) | -3.00 | -3.27 (-3.94, -2.37) | 0.70 | 0.38 (-2.60, 2.48) |

Table 4. Msvar results showing known vs. estimated parameters for data sets with $N_{A}=100,000,50$ microsatellite loci, and 20 individuals, percent decline noted. Estimates are reported as the mode (or median) of the posterior distribution with the $90 \%$ highest posterior density. All known and estimated parameter values are given on the $\log _{10}$ scale.

| time | $N_{e}$ |  | $N_{A}$ |  | $\mu$ |  | $t$ |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | known | estimate | known | estimate | known | estimate | known | estimate |
| $-1 \%$ |  |  |  |  |  |  |  |  |
| 1 gen | 4.996 | $5.7(3.09,9.36)$ | 5.00 | $5.21(4.22,5.82)$ | -3.00 | $-3.20(-3.95,-2.35)$ | 0.00 | $-0.79(-3.17,2.57)$ |
| 5 gen | 4.978 | $5.70(3.22,9.12)$ | 5.00 | $5.17(4.28,5.85)$ | -3.00 | $-3.23(-3.91,-2.35)$ | 0.70 | $0.29(-2.54,2.95)$ |
| 50 gen | 4.783 | $6.32(3.38,9.12)$ | 5.00 | $5.03(4.25,5.84)$ | -3.00 | $-3.27(-3.94,-2.37)$ | 1.70 | $0.84(-1.79,3.06)$ |

Table 5. Msvar results showing known vs. estimated parameters for data sets with $N_{A}=1000,10$ microsatellite loci, and 50 individuals, percent decline noted. Estimates are reported as the mode (or median) of the posterior distribution with the $90 \%$ highest posterior density. All known and estimated parameter values are given on the $\log _{10}$ scale.

| time | $N_{e}$ |  | $N_{A}$ |  | $\mu$ |  | - $t$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | known | estimate | known | estimate | known | estimate | known | estimate |
| -1\% |  |  |  |  |  |  |  |  |
| 1 gen | 2.996 | 3.66 (1.20, 7.18) | 3.00 | 2.96 (2.27, 3.89) | -3.00 | -3.02 (-3.93, -2.37) | 0.00 | -0.91 (-3.37, 2.20) |
| 5 gen | 2.978 | $3.14(1.27,7.06)$ | 3.00 | 3.29 (2.36, 4.06) | -3.00 | -3.09 (-3.96, -2.38) | 0.70 | -0.17 (-3.08, 2.89) |
| 10 gen | 2.957 | 3.46 (0.82, 6.81) | 3.00 | 3.15 (2.31, 3.98) | -3.00 | -3.19 (-3.94, -2.37) | 1.00 | 0.62 (-2.78, 3.19) |
| 50 gen | 2.783 | 2.75 (0.78, 6.68) | 3.00 | 2.98 (2.09, 4.04) | -3.00 | -3.23 (-4.02, -2.44) | 1.70 | $0.91(-2.38,4.97)$ |
| 100 gen | 2.566 | 2.94 (0.94, 6.44) | 3.00 | 3.09 (1.99, 4.16) | -3.00 | -3.28 (-4.00, -2.41) | 2.00 | 0.92 (-1.73, 5.87) |
| -5\% |  |  |  |  |  |  |  |  |
| 1 gen | 2.978 | 4.71 (1.62, 7.53) | 3.00 | 3.04 (2.16, 3.82) | -3.00 | -3.14 (-3.94, -2.35) | 0.00 | 1.82 (-2.98, 2.90) |
| 5 gen | 2.891 | 3.03 (1.10, 7.27) | 3.00 | 3.14 (2.37, 4.05) | -3.00 | -3.14 (-3.96, -2.38) | 0.70 | 0.18 (-3.02, 2.69) |
| 10 gen | 2.783 | 3.43 (1.36, 7.02) | 3.00 | 3.15 (2.27, 4.02) | -3.00 | -2.99 (-3.91, -2.33) | 1.00 | 1.01 (-3.11, 3.50) |
| 50 gen | 1.914 | 1.83 (-0.10, 2.69) | 3.00 | 3.21 (2.40, 4.18) | -3.00 | -3.36 (-4.01, -2.47) | 1.70 | 2.29 (0.93, 4.09) |
| -10\% |  |  |  |  |  |  |  |  |
| 1 gen | 2.957 | 3.87 (1.16, 7.30) | 3.00 | 3.13 (2.22, 3.87) | -3.00 | -3.11 (-3.98, -2.40) | 0.00 | -0.87 (-3.42, 2.33) |
| 5 gen | 2.783 | 3.07 (0.91, 6.90) | 3.00 | 3.13 (2.18, 3.88) | -3.00 | -3.23 (-3.94, -2.36) | 0.70 | 0.47 (-3.03, 3.01) |
| 10 gen | 2.566 | 2.61 (0.53, 6.81) | 3.00 | 3.03 (2.24, 3.95) | -3.00 | -3.18 (-4.00, -2.44) | 1.00 | 0.69 (-2.69, 3.07) |
| -50\% |  |  |  |  |  |  |  |  |
| 1 gen | 2.783 | 3.67 (1.08, 7.11) | 3.00 | 3.14 (2.24, 3.89) | -3.00 | -3.10 (-3.94, -2.36) | 0.00 | -0.10 (-3.33, 2.17) |
| 5 gen | 1.914 | 2.85 (0.70, 6.78) | 3.00 | 3.08 (2.14, 3.84) | -3.00 | -3.16 (-4.00, -2.42) | 0.70 | 0.45 (-3.14, 2.86) |

Table 6. Msvar results showing known vs. estimated parameters for data sets with $N_{A}=10,000,10$ microsatellite loci, and 50 individuals, percent decline noted. Estimates are reported as the mode (or median) of the posterior distribution with the $90 \%$ highest posterior density. All known and estimated parameter values are given on the $\log _{10}$ scale.

| time | $N_{e}$ |  | $N_{A}$ |  | - $\mu$ |  | - $t$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | known | estimate | known | estimate | known | estimate | known | estimate |
| -1\% |  |  |  |  |  |  |  |  |
| 1 gen | 3.996 | 4.66 (2.47, 8.34) | 4.00 | 3.99 (3.23, 4.85) | -3.00 | -3.05 (-3.90, -2.33) | 0.00 | 0.23 (-2.98, 3.46) |
| 5 gen | 3.978 | 4.93 (3.15, 8.29) | 4.00 | 4.12 (3.22, 4.84) | -3.00 | -2.94 (-3.79, -2.23) | 0.70 | 2.48 (-1.77, 4.02) |
| 10 gen | 3.957 | 5.06 (2.64, 8.36) | 4.00 | 4.06 (3.29, 4.93) | -3.00 | -3.00 (-3.92, -2.33) | 1.00 | 2.21 (-2.33, 3.16) |
| 50 gen | 3.783 | 4.74 (2.81, 8.23) | 4.00 | 4.02 (3.29, 5.02) | -3.00 | -3.0 (-3.94, -2.36) | 1.70 | 2.03 (-2.07, 4.14) |
| 100 gen | 3.566 | 3.73 (1.65, 7.60) | 4.00 | 4.10 (3.35, 5.01) | -3.00 | -3.20 (-4.02, -2.45) | 2.000 | 0.99 (-1.86, 3.86) |
| -5\% |  |  |  |  |  |  |  |  |
| 1 gen | 3.978 | 5.19 (2.56, 8.39) | 4.00 | 4.11 (3.30, 4.94) | -3.00 | -3.12 (-3.88, -2.29) | 0.00 | -0.38 (-2.87, 3.03) |
| 5 gen | 3.891 | 4.80 (2.22, 8.36) | 4.00 | 4.18 (3.35, 4.95) | -3.00 | -3.16 (-3.96, -2.38) | 0.70 | 0.80 (-2.61, 2.57) |
| 10 gen | 3.783 | 4.91 (2.30, 8.04) | 4.00 | 4.08 (3.31, 4.92) | -3.00 | -3.01 (-3.99, -2.41) | 1.00 | 0.45 (-2.42, 3.09) |
| 50 gen | 2.914 | 3.87 (2.47, 8.27) | 4.00 | 4.10 (3.31, 4.96) | -3.00 | -3.12 (-3.96, -2.39) | 1.70 | 1.04 (-2.29, 3.11) |
| 100 gen | 1.829 | 4.08 (2.45, 8.00) | 4.00 | 4.22 (3.29, 5.01) | -3.00 | -3.14 (-4.00, -2.43) | 2.000 | 1.14 (-2.51, 3.54) |
| -10\% |  |  |  |  |  |  |  |  |
| 1 gen | 3.957 | 4.09 (1.86, 8.10) | 4.00 | 4.22 (3.33, 4.93) | -3.00 | -3.29 (-3.93, -2.36) | 0.00 | -0.31 (-3.26, 2.30) |
| 5 gen | 3.783 | 4.99 (2.23, 8.20) | 4.00 | 3.97 (3.26, 4.89) | -3.00 | -3.18 (-4.00, -2.40) | 0.70 | 1.06 (-2.55, 2.97) |
| 10 gen | 3.566 | 5.74 (2.89, 8.28) | 4.00 | 3.89 (3.16, 4.80) | -3.00 | -3.17 (-3.90, -2.33) | 1.00 | 2.52 (-1.87, 3.67) |
| 50 gen | 1.829 | 4.07 (1.69, 7.80) | 4.00 | 4.17 (3.39, 5.05) | -3.00 | -3.13 (-3.96, -2.39) | 1.70 | 1.48 (-2.05, 3.35) |
| -50\% |  |  |  |  |  |  |  |  |
| 1 gen | 3.783 | 5.41 (2.79, 8.76) | 4.00 | 3.99 (3.29, 4.96) | -3.00 | -2.97 (-3.88, -2.27) | 0.00 | 2.47 (-2.87, 3.54) |
| 5 gen | 2.914 | 4.29 (1.90, 8.03) | 4.00 | 4.04 (3.33, 4.93) | -3.00 | -3.15 (-3.95, -2.39) | 0.70 | 0.80 (-2.59, 2.66) |
| 10 gen | 1.829 | 5.23 (2.40, 8.08) | 4.00 | 3.94 (3.31, 4.93) | -3.00 | -3.04 (-3.96, -2.39) | 1.00 | $1.27(-2.53,3.00)$ |

Table 7. Msvar results showing known vs. estimated parameters for data sets with $N_{A}=100,000,10$ microsatellite loci, and 50 individuals, percent decline noted. Estimates are reported as the mode (or median) of the posterior distribution with the $90 \%$ highest posterior density. All known and estimated parameter values are given on the $\log _{10}$ scale.

| time | $N_{e}$ |  | $N_{A}$ |  | $\mu$ |  | $t$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | known | estimate | known | estimate | known | estimate | known | estimate |
| -1\% |  |  |  |  |  |  |  |  |
| 50 gen | 4.783 | 5.27 (3.57, 9.03) | 5.00 | 4.90 (4.19, 5.81) | -3.00 | -3.16 (-3.94, -2.38) | 1.70 | 1.41 (-1.67, 4.51) |
| -5\% |  |  |  |  |  |  |  |  |
| 1 gen | 4.978 | 6.06 (3.05, 9.25) | 5.00 | 4.98 (4.15, 5.75) | -3.00 | -3.01 (-3.96, -2.37) | 0.00 | -0.29 (-3.17, 2.36) |
| 5 gen | 4.891 | 6.05 (3.14, 9.18) | 5.00 | 5.03 (4.19, 5.76) | -3.00 | -3.03 (-3.94, -2.38) | 0.70 | 0.72 (-2.61, 2.64) |
| 10 gen | 4.783 | 6.67 (3.42, 9.31) | 5.00 | 5.09 (4.19, 5.80) | -3.00 | -3.16 (-3.90, -2.31) | 1.00 | 1.34 (-2.17, 3.39) |
| 100 gen | 2.829 | 2.86 (2.00, 3.59) | 5.00 | 5.26 (4.37, 5.89) | -3.00 | -3.17 (-3.99, -2.49) | 2.000 | 3.46 (2.62, 4.21) |
| -10\% |  |  |  |  |  |  |  |  |
| 1 gen | 4.957 | 6.32 (2.95, 9.20) | 5.00 | 5.08 (4.18, 5.78) | -3.00 | -3.14 (-3.94, -2.35) | 0.00 | 0.14 (-3.11, 2.75) |
| 5 gen | 4.783 | 5.74 (3.20, 9.18) | 5.00 | 5.05 (4.24, 5.83) | -3.00 | -3.20 (-3.97, -2.39) | 0.70 | 0.69 (-2.42, 3.09) |
| 10 gen | 4.566 | 5.50 (3.22, 9.39) | 5.00 | 4.96 (4.19, 5.78) | -3.00 | -3.18 (-3.98, -2.41) | 1.00 | 0.79 (-2.33, 2.88) |
| -50\% |  |  |  |  |  |  |  |  |
| 1 gen | 4.783 | 6.12 (3.26, 9.20) | 5.00 | 4.96 (4.08, 5.72) | -3.00 | -2.93 (-3.87, -2.28) | 0.00 | 0.45 (-2.90, 4.31) |

Table 8. Msvar results showing known vs. estimated parameters for data sets with $N_{A}=1000,10$ microsatellite loci, and 20 individuals, percent decline noted. Estimates are reported as the mode (or median) of the posterior distribution with the $90 \%$ highest posterior density. All known and estimated parameter values are given on the $\log _{10}$ scale.

| time | $N_{e}$ |  | $N_{A}$ |  | $\mu$ |  | $t$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | known | estimate | known | estimate | known | estimate | known | estimate |
| -1\% |  |  |  |  |  |  |  |  |
| 1 gen | 2.996 | 3.75 (1.35, 7.22) | 3.00 | 3.02 (2.16, 3.84) | -3.00 | -2.99 (-3.90, -2.33) | 0.00 | 1.00 (-3.17, 2.77) |
| 5 gen | 2.978 | 3.1 (1.30, 7.17) | 3.00 | 3.17 (2.40, 4.06) | -3.00 | -3.25 (-4.00, -2.41) | 0.70 | -0.03 (-2.84, 2.70) |
| 10 gen | 2.957 | 4.26 (2.29, 8.45) | 3.00 | $2.94(1.96,3.80)$ | -3.00 | -2.98 (-3.82, -2.24) | 1.00 | 2.57 (-1.42, 4.04) |
| 50 gen | 2.783 | 2.81 (1.11, 6.83) | 3.00 | 3.22 (2.24, 4.14) | -3.00 | -3.27 (-3.96, -2.37) | 1.70 | 0.89 (-2.19, 5.10) |
| 100 gen | 2.566 | 2.81 (1.35, 6.72) | 3.00 | 2.91 (1.77, 4.06) | -3.00 | -3.32 (-3.96, -2.38) | 2.000 | 1.27 (-1.81, 5.55) |
| -5\% |  |  |  |  |  |  |  |  |
| 1 gen | 2.978 | 4.02 (0.91, 7.00) | 3.00 | 3.21 (2.39, 4.02) | -3.00 | -3.19 (-3.94, -2.37) | 0.00 | -0.38 (-3.28, 2.43) |
| 5 gen | 2.891 | 2.83 (1.06, 7.14) | 3.00 | 3.31 (2.41, 4.07) | -3.00 | -3.14 (-3.93, -2.36) | 0.70 | 0.81 (-2.92, 2.81) |
| 10 gen | 2.783 | 3.15 (1.36, 7.09) | 3.00 | 3.19 (2.07, 3.86) | -3.00 | -3.20 (-3.95, -2.37) | 1.00 | 0.55 (-2.92, 3.24) |
| 50 gen | 1.914 | 1.27 (-0.59, 2.45) | 3.00 | 3.46 (2.60, 4.27) | -3.00 | -3.19 (-4.06, -2.52) | 1.70 | 2.32 (0.68, 3.53) |
| -10\% |  |  |  |  |  |  |  |  |
| 1 gen | 2.957 | 4.43 (1.53, 7.42) | 3.00 | 3.16 (2.35, 4.01) | -3.00 | -3.18 (-3.88, -2.29) | 0.00 | -0.10 (-3.1, 2.73) |
| 5 gen | 2.783 | 3.34 (0.98, 6.99) | 3.00 | 3.29 (2.29, 4.02) | -3.00 | -3.03 (-3.97, -2.37) | 0.70 | 0.53 (-3.17, 2.96) |
| 10 gen | 2.566 | 3.43 (1.09, 7.07) | 3.00 | 2.99 (2.22, 4.02) | -3.00 | -3.2 (-3.97, -2.39) | 1.00 | 0.55 (-2.96, 3.31) |
| -50\% |  |  |  |  |  |  |  |  |
| 1 gen | 2.783 | 3.45 (0.93, 7.02) | 3.00 | 3.21 (2.37, 4.02) | -3.00 | -3.23 (-3.96, -2.38) | 0.00 | -0.04 (-3.29, 2.49) |
| 5 gen | 1.914 | 3.60 (1.16, 7.12) | 3.00 | 2.91 (2.00, 3.73) | -3.00 | -3.21 (-3.96, -2.39) | 0.70 | 0.26 (-2.96, 2.86) |

Table 9. Msvar results showing known vs. estimated parameters for data sets with $N_{A}=10,000,10$ microsatellite loci, and 20 individuals, percent decline noted. Estimates are reported as the mode (or median) of the posterior distribution with the $90 \%$ highest posterior density. All known and estimated parameter values are given on the $\log _{10}$ scale.

| time | $N_{e}$ |  | $N_{A}$ |  | $\mu$ |  | t |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | known | estimate | known | estimate | known | estimate | known | estimate |
| -1\% |  |  |  |  |  |  |  |  |
| 1 gen | 3.996 | 5.41 (2.22, 8.51) | 4.00 | 4.20 (3.33, 5.00) | -3.00 | -3.07 (-3.90, -2.29) | 0.00 | 0.44 (-2.86, 3.65) |
| 5 gen | 3.978 | 4.56 (2.47, 8.29) | 4.00 | 4.31 (3.40, 5.02) | -3.00 | -3.13 (-3.93, -2.35) | 0.70 | $0.38(-2.68,3.26)$ |
| 10 gen | 3.957 | 5.18 (2.32, 8.11) | 4.00 | 4.20 (3.41, 5.05) | -3.00 | -3.12 (-3.90, -2.33) | 1.00 | 0.01 (-2.42, 3.40) |
| 50 gen | 3.783 | 4.96 (2.32, 8.07) | 4.00 | 4.30 (3.38, 5.05) | -3.00 | -3.03 (-3.97, -2.39) | 1.70 | 1.61 (-2.07, 3.58) |
| 100 gen | 3.566 | 3.52 (1.39, 7.47) | 4.00 | 4.41 (3.42, 5.10) | -3.00 | -3.22 (-3.98, -2.41) | 2.00 | 1.02 (-1.69, 3.89) |
| -5\% |  |  |  |  |  |  |  |  |
| 1 gen | 3.978 | 4.97 (1.91, 8.15) | 4.00 | 4.18 (3.24, 4.85) | -3.00 | -3.04 (-3.95, -2.37) | 0.00 | -0.49 (-3.27, 2.34) |
| 5 gen | 3.891 | 4.28 (1.90, 8.03) | 4.00 | 4.08 (3.28, 4.89) | -3.00 | -3.14 (-3.96, -2.39) | 0.70 | 0.76 (-2.68, 2.90) |
| 10 gen | 3.783 | $4.81(1.78,8.49)$ | 4.00 | 4.25 (3.33, 4.97) | -3.00 | -3.10 (-3.98, -2.41) | 1.00 | 0.68 (-2.57, 2.84) |
| 50 gen | 2.914 | 4.94 (2.98, 8.49) | 4.00 | 4.10 (3.13, 4.81) | -3.00 | -3.19 (-3.88, -2.31) | 1.70 | 2.22 (-1.49, 3.96) |
| 100 gen | 1.829 | 2.46 (0.76, 3.27) | 4.00 | 4.39 (3.54, 5.13) | -3.00 | -3.18 (-4.01, -2.49) | 2.000 | 2.91 (1.54, 3.92) |
| -10\% |  |  |  |  |  |  |  |  |
| 1 gen | 3.957 | 4.95 (2.22, 8.17) | 4.00 | 3.92 (3.10, 4.80) | -3.00 | -3.00 (-3.92, -2.33) | 0.00 | -0.07 (-3.32, 3.31) |
| 5 gen | 3.783 | 4.66 (2.18, 8.12) | 4.00 | 4.10 (3.25, 4.89) | -3.00 | -3.11 (-3.96, -2.37) | 0.70 | 0.15 (-2.65, 2.90) |
| 10 gen | 3.566 | 5.42 (2.29, 8.28) | 4.00 | 4.03 (3.27, 4.88) | -3.00 | -3.17 (-3.98, -2.41) | 1.00 | 0.51 (-2.60, 2.97) |
| 50 gen | 1.829 | 1.37 (-0.08, 2.59) | 4.00 | 4.13 (3.33, 5.02) | -3.00 | -3.22 (-4.06, -2.54) | 1.70 | 2.96 (1.44, 4.02) |
| -50\% |  |  |  |  |  |  |  |  |
| 1 gen | 3.783 | 4.62 (2.12, 8.18) | 4.00 | 4.07 (3.35, 5.01) | -3.00 | -3.1 (-3.94, -2.36) | 0.00 | -0.02 (-3.34, 3.35) |
| 5 gen | 2.914 | 4.97 (2.14, 8.20) | 4.00 | 4.18 (3.30, 4.91) | -3.00 | -3.19 (-3.93, -2.35) | 0.70 | 0.86 (-2.59, 2.96) |
| 10 gen | 1.829 | 2.52 (1.02, 3.77) | 4.00 | 4.17 (3.35, 4.93) | -3.00 | -3.09 (-3.97, -2.43) | 1.00 | 2.91 (1.29, 4.26) |

Table 10. Msvar results showing known vs. estimated parameters for data sets with $N_{A}=100,000,10$ microsatellite loci, and 20 individuals, percent decline noted. Estimates are reported as the mode (or median) of the posterior distribution with the $90 \%$ highest posterior density. All known and estimated parameter values are given on the $\log _{10}$ scale.

| time | $N_{e}$ |  | $N_{A}$ |  | $\mu$ |  | $t$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | known | mode | known | mode | known | mode | known | mode |
| -1\% |  |  |  |  |  |  |  |  |
| 1 gen | 4.996 | 5.57 (3.06, 9.27) | 5.00 | 5.08 (4.23, 5.83) | -3.00 | -3.01 (-3.98, -2.38) | 0.00 | -0.25 (-3.26, 2.86) |
| 5 gen | 4.978 | 4.80 (2.90, 9.27) | 5.00 | 5.03 (4.28, 5.89) | -3.00 | -3.26 (-3.99, -2.39) | 0.70 | 0.94 (-2.53, 2.87) |
| 10 gen | 4.957 | 6.09 (3.14, 9.20) | 5.00 | 5.14 (4.24, 5.83) | -3.00 | -3.08 (-3.96, -2.39) | 1.00 | 0.5 (-2.28, 3.15) |
| 50 gen | 4.783 | 6.61 (3.32, 9.16) | 5.00 | 5.04 (4.07, 5.74) | -3.00 | -3.16 (-3.92, -2.36) | 1.70 | 2.13 (-1.58, 4.47) |
| 100 gen | 4.566 | 5.57 (3.29, 9.19) | 5.00 | 4.96 (4.24, 5.85) | -3.00 | -3.17 (-3.93, -2.37) | 2.000 | 1.27 (-1.61, 3.62) |
| -5\% |  |  |  |  |  |  |  |  |
| 1 gen | 4.978 | 5.52 (3.01, 9.27) | 5.00 | 5.09 (4.24, 5.83) | -3.00 | -2.97 (-3.94, -2.36) | 0.00 | 0.15 (-3.27, 2.73) |
| 5 gen | 4.891 | 6.11 (3.36, 9.23) | 5.00 | 5.19 (4.24, 5.84) | -3.00 | -3.05 (-3.92, -2.34) | 0.70 | 0.60 (-2.66, 3.88) |
| 10 gen | 4.783 | 5.21 (3.23, 9.25) | 5.00 | 5.10 (4.20, 5.81) | -3.00 | -3.14 (-3.96, -2.37) | 1.00 | 0.66 (-2.29, 3.28) |
| 50 gen | 3.914 | 5.56 (3.23, 9.12) | 5.00 | 5.01 (4.24, 5.85) | -3.00 | -3.01 (-3.92, -2.34) | 1.70 | 1.18 (-1.67, 3.60) |
| -10\% |  |  |  |  |  |  |  |  |
| 1 gen | 4.957 | 5.96 (2.83, 9.10) | 5.00 | 5.14 (4.16, 5.75) | -3.00 | -2.95 (-3.94, -2.37) | 0.00 | 0.35 (-3.29, 3.30) |
| 5 gen | 4.783 | 5.23 (3.06, 9.11) | 5.00 | 4.89 (4.24, 5.83) | -3.00 | -3.11 (-3.96, -2.38) | 0.70 | 0.29 (-2.61, 3.11) |
| 10 gen | 4.566 | 5.57 (3.30, 9.29) | 5.00 | 5.13 (4.22, 5.81) | -3.00 | -3.25 (-3.97, -2.39) | 1.00 | 0.59 (-2.18, 3.36) |
| 50 gen | 2.829 | 2.93 (1.08, 3.86) | 5.00 | 5.08 (4.32, 5.87) | -3.00 | -3.26 (-4.02, -2.49) | 1.70 | 2.93 (1.43, 4.02) |
| -50\% |  |  |  |  |  |  |  |  |
| 1 gen | 4.783 | 5.72 (3.07, 9.28) | 5.00 | 5.12 (4.15, 5.77) | -3.00 | -3.04 (-3.96, -2.36) | 0.00 | -0.27 (-3.18, 3.05) |
| 5 gen | 3.914 | 5.89 (3.27, 9.23) | 5.00 | 5.04 (4.19, 5.81) | -3.00 | -3.04 (-3.94, -2.35) | 0.70 | 0.65 (-2.50, 3.88) |

Table 11. $M$-ratios and critical $M$ values (presented as estimated $M$-ratio, $M_{C}$ ) for all simulations listed for 20 individuals, 10 microsatellite loci. $N_{A}$ is noted for each set. $M$ ratios that are lower than $M_{C}$, indicating population decline, are highlighted in grey.

| $N_{A}=1000$ | \% decline |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | -1\% | -5\% | -10\% | -50\% |
| 1 gen. | 0.948, 0.853 | 0.949, 0.854 | 0.941, 0.865 | 0.968, 0.879 |
| 5 gen. | 0.935, 0.853 | 0.897, 0.865 | 0.851, 0.878 | 0.975, 0.950 |
| 10 gen. | 0.967, 0.857 | 0.936, 0.878 | 0.969, 0.900 |  |
| 50 gen. | 0.898, 0.879 | 0.743, 0.950 |  |  |
| 100 gen. | 0.975, 0.903 |  |  |  |
| $N_{A}=10,000$ | \% decline |  |  |  |
|  | -1\% | -5\% | -10\% | -50\% |
| 1 gen. | 0.804, 0.641 | 0.807, 0.644 | 0.851, 0.651 | 0.848, 0.697 |
| 5 gen. | 0.818, 0.643 | 0.779, 0.669 | 0.770, 0.696 | 0.804, 0.864 |
| 10 gen. | 0.778, 0.650 | 0.828, 0.695 | 0.746, 0.748 | 0.699, 0.954 |
| 50 gen. | 0.743, 0.696 | 0.788, 0.862 | 0.600, 0.954 |  |
| 100 gen. | 0.774, 0.748 | 0.612, 0.984 |  |  |
| $N_{A}=100,000$ | \% decline |  |  |  |
|  | -1\% | -5\% | -10\% | -50\% |
| 1 gen. | 0.575, 0.341 | 0.491, 0.344 | 0.529, 0.351 | 0.594, 0.401 |
| 5 gen. | 0.424, 0.346 | 0.482, 0.370 | 0.465, 0.402 | 0.555, 0.663 |
| 10 gen. | 0.476, 0.351 | 0.523, 0.401 | 0.466, 0.467 | 0.472, 0.873 |
| 50 gen. | 0.558, 0.401 | 0.485, 0.663 | 0.516, 0.873 |  |
| 100 gen. | 0.524, 0.468 | 0.373, 0.874 |  |  |

Table 12. $M$-ratios and critical $M$ values (presented as estimated $M$-ratio, $M_{C}$ ) for all simulations listed for 50 individuals, 10 microsatellite loci. $N_{A}$ is noted for each set. $M$ ratios that are lower than $M_{C}$, indicating population decline, are highlighted in grey.

| $N_{A}=1000$ | \% decline |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | -1\% | -5\% | -10\% | -50\% |
| 1 gen. | 0.924, 0.896 | 1.000, 0.898 | 0.986, 0.899 | 0.986, 0.910 |
| 5 gen. | 0.988, 0.898 | 0.946, 0.904 | 0.917, 0.909 | 0.930, 0.950 |
| 10 gen. | 0.921, 0.899 | 0.875, 0.909 | 0.957, 0.920 |  |
| 50 gen. | 0.983, 0.908 | 0.795, 0.988 |  |  |
| 100 gen. | 0.967, 0.922 |  |  |  |
| $N_{A}=10,000$ | \% decline |  |  |  |
|  | -1\% | -5\% | -10\% | -50\% |
| 1 gen. | 0.969, 0.806 | 0.844, 0.809 | 0.836, 0.811 | 0.928, 0.830 |
| 5 gen. | 0.882, 0.808 | 0.836, 0.819 | 0.882, 0.828 | 0.875, 0.902 |
| 10 gen. | 0.835, 0.813 | 0.890, 0.829 | 0.853, 0.849 | 0.858, 0.950 |
| 50 gen. | 0.926, 0.830 | 0.915, 0.901 | 0.837, 0.950 |  |
| 100 gen. | 0.854, 0.850 | 0.869, 0.950 |  |  |
| $N_{A}=100,000$ | \% decline |  |  |  |
|  | -1\% | -5\% | -10\% | -50\% |
| 1 gen. | 0.734, 0.637 | 0.703, 0.641 | 0.548, 0.644 | 0.741, 0.682 |
| 5 gen. | 0.640, 0.639 | 0.767, 0.658 | 0.664, 0.682 | 0.806, 0.815 |
| 10 gen. | 0.746, 0.645 | 0.618, 0.681 | 0.757, 0.723 | 0.625, 0.906 |
| 50 gen. | 0.719, 0.682 | 0.657, 0.815 | 0.548, 0.906 |  |
| 100 gen. | 0.725, 0.721 | 0.356, 0.907 |  |  |

Table 13. $M$-ratios and critical $M$ values (presented as estimated $M$-ratio, $M_{C}$ ) for all simulations listed for 20 individuals, 50 microsatellite loci. $N_{A}$ is noted for each set. $M$ ratios that are lower than $M_{C}$, indicating population decline, are highlighted in grey.

| $N_{A}=1000$ | \% decline |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | -1\% | -5\% | -10\% | -50\% |
| 1 gen | 0.938, 0.903 | 0.904, 0.905 | 0.942, 0.907 | 0.901, 0.920 |
| 5 gen | 1.099, 0.905 | 0.922, 0.912 | 0.949, 0.921 | 0.907, 0.967 |
| 10 gen | 0.936, 0.906 | 0.928, 0.921 | 0.934, 0.936 |  |
| 50 gen | 0.942, 0.921 | 0.900, 0.968 |  |  |
| 100 gen | 0.928, 0.936 |  |  |  |
| $N_{A}=10,000$ | \% decline |  |  |  |
|  | -1\% | -5\% | -10\% | -50\% |
| 1 gen. | 0.765, 0.763 | 0.851, 0.766 | 0.811, 0.770 | 0.796, 0.803 |
| 5 gen . | 0.813, 0.766 | 0.799, 0.783 | 0.799, 0.803 | 0.775, 0.910 |
| 10 gen. | 0.781, 0.771 | 0.793, 0.838 | 0.790, 0.837 | 0.661, 0.970 |
| 50 gen. | 0.830, 0.803 | 0.773, 0.910 | 0.564, 0.970 |  |
| 100 gen. | 0.813, 0.837 | 0.536, 0.970 |  |  |
| $N_{A}=100,000$ | \% decline |  |  |  |
|  | -1\% | -5\% | -10\% | -50\% |
| 1 gen. | 0.530, 0.531 | 0.504, 0.504 | 0.544, 0.510 | 0.513, 0.562 |
| 5 gen. | 0.784, 0.505 | 0.484, 0.530 | 0.539, 0.563 | 0.514, 0.778 |
| 10 gen. | 0.713, 0.510 | 0.527, 0.562 | 0.493, 0.626 | 0.500, 0.917 |
| 50 gen. | 0.504, 0.562 | 0.563, 0.779 | 0.404, 0.917 |  |
| 100 gen. | 0.522, 0.626 | 0.410, 0.945 |  |  |

Table 14. $\theta$ calculations for each data set for the 50 microsatellite loci \& 20 individuals sampling scenario. $N_{A}=1000, \theta_{A}=4$, generations, and percent decline noted for each scenario. $\theta=4 N_{e} \mu$ was calculated from 'known' parameters, $\theta_{\text {col }}=4 N_{e} \mu$ was calculated from msvar point estimates, $\theta_{v}$ was determined using the repeat number variance, $\theta_{h}$ was determined using expected homozygosity, and $\theta_{x}$ was determined using mean allele frequencies.

|  | $\theta$ | $\theta_{\text {col }}$ | $\theta_{v}$ | $\theta_{h}$ | $\theta_{x}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| -1\% |  |  |  |  |  |
| 1 gen. | 3.960 | 22.494 | 3.534 | 5.266 | 1.796 |
| 5 gen. | 3.805 | 4.386 | 3.716 | 6.177 | 2.219 |
| 10 gen. | 3.619 | 5.782 | 4.070 | 6.015 | 2.281 |
| 50 gen. | 2.426 | 4.286 | 3.546 | 7.007 | 2.391 |
| 100 gen. | 1.472 | 0.649 | 4.223 | 5.742 | 1.714 |
| -5\% |  |  |  |  |  |
| 1 gen. | 3.805 | 29.652 | 4.664 | 6.175 | 2.216 |
| 5 gen. | 3.115 | 10.766 | 4.031 | 6.270 | 2.046 |
| 10 gen. | 2.426 | 4.286 | 8.404 | 6.459 | 2.150 |
| 50 gen. | 0.328 | 0.179 | 2.273 | 2.927 | 0.851 |
| -10\% |  |  |  |  |  |
| 1 gen. | 3.619 | 85.518 | 4.440 | 6.528 | 2.151 |
| 5 gen. | 2.426 | 17.461 | 3.810 | 6.168 | 2.350 |
| 10 gen. | 1.472 | 3.565 | 5.028 | 6.450 | 2.230 |
| -50\% |  |  |  |  |  |
| 1 gen. | 2.426 | 40.000 | 5.812 | 6.507 | 2.192 |
| 5 gen. | 0.328 | 0.695 | 4.053 | 5.953 | 1.914 |

Table 15. $\theta$ calculations for each data set for the 50 microsatellite loci \& 20 individuals sampling scenario. $N_{A}=10,000, \theta_{A}=40$, generations, and percent decline noted for each scenario. $\theta=4 N_{e} \mu$ was calculated from 'known' parameters, $\theta_{\text {col }}=4 N_{e} \mu$ was calculated from msvar point estimates, $\theta_{v}$ was determined using the repeat number variance, $\theta_{h}$ was determined using expected homozygosity, and $\theta_{x}$ was determined using mean allele frequencies. N/A indicates a scenario that did not converge, so $\theta_{\text {col }}$ was not reported.

|  | $\theta$ | $\theta_{\text {col }}$ | $\theta_{v}$ | $\theta_{h}$ | $\theta_{x}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| -1\% |  |  |  |  |  |
| 1 gen. | 39.602 | 25.826 | 43.985 | 59.34 | 10.136 |
| 5 gen. | 38.049 | 187.094 | 41.694 | 59.935 | 10.689 |
| 10 gen. | 36.193 | 438.591 | 49.357 | 86.14 | 12.11 |
| 50 gen. | 24.261 | 264.277 | 35.076 | 64.94 | 10.791 |
| 100 gen. | 14.715 | 283.178 | 36.523 | 54.198 | 9.314 |
| -5\% |  |  |  |  |  |
| 1 gen. | 38.049 | 711.312 | 32.618 | 58.783 | 10.405 |
| 5 gen. | 31.152 | 527.303 | 36.662 | 69.391 | 11.001 |
| 10 gen. | 24.261 | 81.670 | 41.35 | 73.945 | 11.195 |
| 50 gen. | 3.283 | N/A | 34.617 | 41.746 | 8.559 |
| 100 gen. | 0.270 | 0.121 | 22.113 | 2.162 | 0.539 |
| -10\% |  |  |  |  |  |
| 1 gen. | 36.193 | 195.912 | 33.384 | 66.99 | 10.795 |
| 5 gen. | 24.261 | 230.176 | 54.761 | 68.06 | 11.22 |
| 10 gen. | 14.715 | 205.145 | 38.184 | 53.14 | 10.416 |
| 50 gen. | 0.270 | 0.391 | 25.149 | 5.022 | 1.698 |
| -50\% |  |  |  |  |  |
| 1 gen. | 24.261 | 539.585 | 39.557 | 61.574 | 10.769 |
| 5 gen. | 3.283 | 200.475 | 52.830 | 73.616 | 11.411 |
| 10 gen. | 0.270 | N/A | 35.094 | 29.040 | 6.516 |

Table 16. $\theta$ calculations for each data set for the 50 microsatellite loci \& 20 individuals sampling scenario. $N_{A}=100,000, \theta_{A}=400$, generations, and percent decline noted for each scenario. $\theta=4 N_{e} \mu$ was calculated from 'known' parameters, $\theta_{\text {col }}=4 N_{e} \mu$ was calculated from msvar point estimates, $\theta_{v}$ was determined using the repeat number variance, $\theta_{h}$ was determined using expected homozygosity, and $\theta_{x}$ was determined using mean allele frequencies. N/A indicates a scenario that did not converge, so $\theta_{\text {col }}$ was not reported.

|  | $\theta$ | $\theta_{\text {col }}$ | $\theta_{v}$ | $\theta_{h}$ | $\theta_{x}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| -1\% |  |  |  |  |  |
| 1 gen . | 396.020 | 1264.911 | 537.160 | 1421.210 | 28.660 |
| 5 gen. | 380.492 | 1180.484 | 355.500 | 873.510 | 27.300 |
| 10 gen. | 361.935 | N/A | 389.920 | 722.980 | 26.600 |
| 50 gen. | 242.612 | 4488.074 | 628.710 | 883.800 | 27.150 |
| 100 gen. | 147.152 | N/A | 390.900 | $3.661 \mathrm{E}+31$ | 27.410 |
| -5\% |  |  |  |  |  |
| 1 gen. | 380.492 | N/A | 291.720 | 367.870 | 26.532 |
| 5 gen. | 311.520 | N/A | 632.390 | 1145.300 | 28.240 |
| 10 gen. | 242.612 | N/A | 433.710 | 1019.750 | 27.650 |
| 50 gen. | 32.834 | N/A | 326.850 | 919.100 | 26.920 |
| 100 gen. | 2.695 | N/A | 270.710 | 79.088 | 12.016 |
| -10\% |  |  |  |  |  |
| 1 gen. | 361.935 | N/A | 321.620 | $3.661 \mathrm{E}+31$ | 27.020 |
| 5 gen. | 242.612 | N/A | 338.240 | $3.661 \mathrm{E}+31$ | 26.870 |
| 10 gen. | 147.152 | N/A | 460.320 | 1553.860 | 27.890 |
| 50 gen. | 2.695 | N/A | 380.040 | 180.210 | 17.720 |
| -50\% |  |  |  |  |  |
| 1 gen. | 242.612 | N/A | 456.530 | 1283.360 | 28.020 |
| 5 gen. | 32.834 | N/A | 464.190 | 1199.100 | 26.960 |
| 10 gen. | 2.695 | N/A | 316.990 | $3.661 \mathrm{E}+31$ | 24.960 |

Table 17. $\theta$ calculations for each data set for the 10 microsatellite loci \& 20 individuals sampling scenario. $N_{A}=1000, \theta_{A}=4$, generations, and percent decline noted for each scenario. $\theta=4 N_{e} \mu$ was calculated from 'known' parameters, $\theta_{\text {col }}=4 N_{e} \mu$ was calculated from msvar point estimates, $\theta_{v}$ was determined using the repeat number variance, $\theta_{h}$ was determined using expected homozygosity, and $\theta_{x}$ was determined using mean allele frequencies.

|  | $\theta$ | $\theta_{\text {col }}$ | $\theta_{v}$ | $\theta_{h}$ | $\theta_{x}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| -1\% |  |  |  |  |  |
| 1 gen. | 3.960 | 23.018 | 7.269 | 7.497 | 3.344 |
| 5 gen. | 3.805 | 2.832 | 5.419 | 7.509 | 2.862 |
| 10 gen. | 3.619 | 76.218 | 2.662 | 4.631 | 1.875 |
| 50 gen. | 2.426 | 1.387 | 4.155 | 7.670 | 2.306 |
| 100 gen. | 1.472 | 1.236 | 2.471 | 5.713 | 1.494 |
| -5\% |  |  |  |  |  |
| 1 gen. | 3.805 | 27.043 | 4.585 | 7.571 | 2.694 |
| 5 gen. | 3.115 | 1.959 | 5.761 | 6.582 | 2.406 |
| 10 gen. | 2.426 | 3.565 | 4.509 | 5.240 | 1.869 |
| 50 gen. | 0.328 | 0.048 | 3.174 | 2.941 | 0.956 |
| -10\% |  |  |  |  |  |
| 1 gen. | 3.619 | 71.131 | 3.848 | 5.278 | 2.494 |
| 5 gen. | 2.426 | 8.167 | 5.785 | 5.996 | 2.119 |
| 10 gen. | 1.472 | 6.793 | 2.749 | 6.916 | 1.794 |
| -50\% |  |  |  |  |  |
| 1 gen. | 2.426 | 6.638 | 6.413 | 8.975 | 2.806 |
| 5 gen. | 0.328 | 9.819 | 1.927 | 3.884 | 1.344 |

Table 18. $\theta$ calculations for each data set for the 10 microsatellite loci \& 20 individuals sampling scenario. $N_{A}=10,000, \theta_{A}=40$, generations, and percent decline noted for each scenario. $\theta=4 N_{e} \mu$ was calculated from 'known' parameters, $\theta_{\text {col }}=4 N_{e} \mu$ was calculated from msvar point estimates, $\theta_{v}$ was determined using the repeat number variance, $\theta_{h}$ was determined using expected homozygosity, and $\theta_{x}$ was determined using mean allele frequencies.

|  | $\theta$ | $\theta_{\text {col }}$ | $\theta_{v}$ | $\theta_{h}$ | $\theta_{x}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| -1\% |  |  |  |  |  |
| 1 gen. | 39.602 | 875.105 | 48.907 | 148.809 | 13.125 |
| 5 gen. | 38.049 | 107.661 | 35.435 | 57.470 | 11.238 |
| 10 gen. | 36.193 | 459.261 | 42.791 | 72.200 | 11.431 |
| 50 gen. | 24.261 | 340.455 | 31.193 | 72.020 | 10.731 |
| 100 gen. | 14.715 | 7.981 | 50.664 | 65.960 | 11.040 |
| -5\% |  |  |  |  |  |
| 1 gen. | 38.049 | 340.455 | 24.872 | 38.888 | 9.069 |
| 5 gen. | 31.152 | 55.215 | 34.103 | 41.180 | 8.569 |
| 10 gen. | 24.261 | 205.145 | 32.962 | 43.280 | 9.269 |
| 50 gen. | 3.283 | 224.937 | 33.028 | 86.219 | 10.106 |
| 100 gen. | 0.270 | 0.762 | 35.921 | 24.007 | 5.869 |
| -10\% |  |  |  |  |  |
| 1 gen. | 36.193 | 356.500 | 18.229 | 45.830 | 8.494 |
| 5 gen. | 24.261 | 141.925 | 48.774 | 58.350 | 10.175 |
| 10 gen. | 14.715 | 711.312 | 39.954 | 51.510 | 10.770 |
| 50 gen. | 0.270 | 0.057 | 43.737 | 4.422 | 1.219 |
| -50\% |  |  |  |  |  |
| 1 gen. | 24.261 | 132.452 | 45.936 | 66.180 | 10.970 |
| 5 gen . | 3.283 | 241.024 | 40.995 | 46.160 | 8.794 |
| 10 gen. | 0.270 | 1.077 | 39.620 | 24.310 | 6.631 |

Table 19. $\theta$ calculations for each data set for the 10 microsatellite loci \& 20 individuals sampling scenario. $N_{A}=100,000, \theta_{A}=400$, generations, and percent decline noted for each scenario. $\theta=4 N_{e} \mu$ was calculated from 'known' parameters, $\theta_{c o l}=4 N_{e} \mu$ was calculated from msvar point estimates, $\theta_{v}$ was determined using the repeat number variance, $\theta_{h}$ was determined using expected homozygosity, and $\theta_{x}$ was determined using mean allele frequencies. N/A indicates a scenario that did not converge, so $\theta_{\text {col }}$ was not reported.

| $N_{a}=100,000$ | $\theta$ | $\theta_{\text {col }}$ | $\theta_{v}$ | $\theta_{h}$ | $\theta_{x}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| -1\% |  |  |  |  |  |
| 1 gen. | 396.020 | 1452.312 | 319.620 | 783.200 | 28.200 |
| 5 gen. | 380.492 | 138.695 | 510.000 | 1841.940 | 30.310 |
| 10 gen. | 361.935 | 4093.172 | 425.720 | 795.220 | 28.250 |
| 50 gen. | 242.612 | 11273.532 | 197.000 | 1238.400 | 24.520 |
| 100 gen. | 147.152 | 1004.755 | 735.930 | 724.680 | 26.230 |
| -5\% |  |  |  |  |  |
| 1 gen. | 380.492 | 1419.254 | 432.720 | 1951.100 | 29.490 |
| 5 gen. | 311.520 | 4592.614 | 391.800 | 1134.000 | 28.490 |
| 10 gen. | 242.612 | 469.959 | 594.960 | 798.200 | 26.860 |
| 50 gen. | 32.834 | 1419.254 | 326.000 | 720.600 | 27.340 |
| 100 gen. | 2.695 | N/A | 297.860 | 56.533 | 11.494 |
| -10\% |  |  |  |  |  |
| 1 gen. | 361.935 | 4093.172 | 417.240 | 835.390 | 26.980 |
| 5 gen. | 242.612 | 527.303 | 638.300 | 1928.700 | 29.880 |
| 10 gen. | 147.152 | 835.718 | 566.920 | 1667.600 | 28.170 |
| 50 gen. | 2.695 | 1.871 | 173.250 | 169.420 | 17.344 |
| -50\% |  |  |  |  |  |
| 1 gen. | 242.612 | 1914.520 | 294.250 | 871.200 | 28.460 |
| 5 gen. | 32.834 | 2831.783 | 261.010 | 839.800 | 27.370 |
| 10 gen. | 2.695 | N/A | 420.850 | 495.790 | 25.000 |

Table 20. $\theta$ calculations for each data set for the 10 microsatellite loci \& 50 individuals sampling scenario. $N_{A}=1000, \theta_{A}=4$, generations, and percent decline noted for each scenario. $\theta=4 N_{e} \mu$ was calculated from 'known' parameters, $\theta_{\text {col }}=4 N_{e} \mu$ was calculated from msvar point estimates, $\theta_{v}$ was determined using the repeat number variance, $\theta_{h}$ was determined using expected homozygosity, and $\theta_{x}$ was determined using mean allele frequencies.

|  | $\theta$ | $\theta_{\text {col }}$ | $\theta_{v}$ | $\theta_{h}$ | $\theta_{x}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| -1\% |  |  |  |  |  |
| 1 gen. | 3.960 | 17.461 | 3.644 | 3.697 | 2.944 |
| 5 gen. | 3.805 | 4.488 | 4.034 | 7.025 | 3.844 |
| 10 gen. | 3.619 | 7.448 | 4.322 | 6.233 | 2.956 |
| 50 gen. | 2.426 | 1.325 | 2.440 | 6.311 | 2.044 |
| 100 gen. | 1.472 | 1.828 | 2.463 | 3.720 | 2.188 |
| -5\% |  |  |  |  |  |
| 1 gen. | 3.805 | 148.614 | 2.518 | 4.142 | 2.469 |
| 5 gen. | 3.115 | 3.105 | 3.826 | 6.451 | 3.381 |
| 10 gen. | 2.426 | 11.017 | 6.408 | 4.981 | 3.362 |
| 50 gen. | 0.328 | 0.118 | 4.845 | 2.074 | 0.688 |
| -10\% |  |  |  |  |  |
| 1 gen. | 3.619 | 23.018 | 3.082 | 4.002 | 2.650 |
| 5 gen. | 2.426 | 2.767 | 3.118 | 4.054 | 2.500 |
| 10 gen. | 1.472 | 1.077 | 2.130 | 4.255 | 2.150 |
| -50\% |  |  |  |  |  |
| 1 gen. | 2.426 | 14.861 | 3.002 | 6.665 | 2.237 |
| 5 gen. | 0.328 | 1.959 | 3.752 | 4.778 | 2.369 |

Table 21. $\theta$ calculations for each data set for the 10 microsatellite loci \& 50 individuals sampling scenario. $N_{A}=10,000, \theta_{A}=40$, generations, and percent decline noted for each scenario. $\theta=4 N_{e} \mu$ was calculated from 'known' parameters, $\theta_{\text {col }}=4 N_{e} \mu$ was calculated from msvar point estimates, $\theta_{v}$ was determined using the repeat number variance, $\theta_{h}$ was determined using expected homozygosity, and $\theta_{x}$ was determined using mean allele frequencies.

|  | $\theta$ | $\theta_{\text {col }}$ | $\theta_{v}$ | $\theta_{h}$ | $\theta_{x}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| -1\% |  |  |  |  |  |
| 1 gen. | 39.602 | 162.952 | 21.643 | 42.550 | 16.650 |
| 5 gen. | 38.049 | 390.895 | 45.762 | 49.450 | 20.920 |
| 10 gen. | 36.193 | 459.261 | 56.160 | 55.580 | 21.760 |
| 50 gen. | 24.261 | 219.816 | 37.046 | 65.170 | 22.331 |
| 100 gen. | 14.715 | 13.554 | 46.747 | 61.500 | 21.988 |
| -5\% |  |  |  |  |  |
| 1 gen. | 38.049 | 469.959 | 47.563 | 49.791 | 20.712 |
| 5 gen . | 31.152 | 174.606 | 47.473 | 50.260 | 19.030 |
| 10 gen. | 24.261 | 317.731 | 35.956 | 50.420 | 19.031 |
| 50 gen. | 3.283 | 22.494 | 27.684 | 42.620 | 18.490 |
| 100 gen. | 0.270 | 34.839 | 44.502 | 60.920 | 20.044 |
| -10\% |  |  |  |  |  |
| 1 gen. | 36.193 | 25.238 | 50.562 | 62.120 | 20.962 |
| 5 gen . | 24.261 | 258.262 | 31.461 | 51.090 | 17.125 |
| 10 gen. | 14.715 | 1486.141 | 51.180 | 43.880 | 16.494 |
| 50 gen. | 0.270 | 34.839 | 34.870 | 53.020 | 20.640 |
| -50\% |  |  |  |  |  |
| 1 gen. | 24.261 | 1101.691 | 41.180 | 68.490 | 22.890 |
| 5 gen . | 3.283 | 55.215 | 29.471 | 46.895 | 19.081 |
| 10 gen. | 0.270 | 619.527 | 34.034 | 41.150 | 18.120 |

Table 22. $\theta$ calculations for each data set for the 10 microsatellite loci \& 50 individuals sampling scenario. $N_{A}=100,000, \theta_{A}=400$, generations, and percent decline noted for each scenario. $\theta=4 N_{e} \mu$ was calculated from 'known' parameters, $\theta_{\text {col }}=4 N_{e} \mu$ was calculated from msvar point estimates, $\theta_{v}$ was determined using the repeat number variance, $\theta_{h}$ was determined using expected homozygosity, and $\theta_{x}$ was determined using mean allele frequencies. $\mathrm{N} / \mathrm{A}$ indicates a scenario that did not converge, so $\theta_{\text {col }}$ was not reported.

|  | $\theta$ | $\theta_{\text {col }}$ | $\theta_{v}$ | $\theta_{h}$ | $\theta_{x}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| -1\% |  |  |  |  |  |
| 1 gen. | 396.020 | N/A | 263.400 | 571.400 | 87.650 |
| 5 gen. | 380.492 | N/A | 527.780 | 530.700 | 84.660 |
| 10 gen. | 361.935 | N/A | 246.650 | 475.800 | 81.900 |
| 50 gen. | 242.612 | 515.300 | 375.230 | 615.800 | 92.210 |
| 100 gen. | 147.152 | N/A | 430.200 | 430.700 | 79.430 |
| -5\% |  |  |  |  |  |
| 1 gen. | 380.492 | 4488.074 | 258.980 | 518.500 | 79.330 |
| 5 gen. | 311.520 | 4188.514 | 262.700 | 484.300 | 81.040 |
| 10 gen. | 242.612 | 12943.746 | 712.330 | 628.500 | 95.900 |
| 50 gen. | 32.834 | N/A | 371.200 | 479.100 | 82.410 |
| 100 gen. | 2.695 | 1.959 | 413.620 | 67.120 | 31.500 |
| -10\% |  |  |  |  |  |
| 1 gen. | 361.935 | 6054.245 | 322.540 | 496.000 | 83.010 |
| 5 gen. | 242.612 | 1386.947 | 522.950 | 894.800 | 96.780 |
| 10 gen. | 147.152 | 835.718 | 318.540 | 539.800 | 86.230 |
| 50 gen. | 2.695 | N/A | 323.180 | 141.460 | 41.760 |
| -50\% |  |  |  |  |  |
| 1 gen. | 242.612 | 6195.266 | 326.300 | 508.560 | 82.810 |
| 5 gen. | 32.834 | N/A | 220.200 | 570.100 | 87.720 |
| 10 gen. | 2.695 | N/A | 464.500 | 399.700 | 79.290 |

Figure 1. Population model for the sampling scenarios created using ms. $N_{A}$ is the ancestral effective population size, $r$ is the rate of decline, $t$ is the time in generations for the length of the decline, and $N_{e}$ is the current effective population size.


Figure 2. Example posterior probability plot for $N_{A}$. Results are from a demographic scenario of $-1 \%$ decline over 100 generations from an ancestral population size of 1000 . The sampling scenario involved 20 individuals genotyped at 50 loci. The red line indicates the known parameter value; posterior distributions for the three independent runs are shown as blue, green, and purple curves.


Figure 3. Estimated vs. known $N_{A}$ for scenarios with 20 individuals, 50 microsatellite loci, and $N_{A}=1000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. The red line indicates the known value of $N_{A}$. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 4. Estimated vs. known $N_{A}$ for scenarios with 20 individuals, 50 microsatellite loci, and $N_{A}=10,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. The red line indicates the known value of $N_{A}$. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 5. Estimated vs. known $N_{A}$ for scenarios with 20 individuals, 50 microsatellite loci, $N_{A}=100,000$, and $-1 \%$ decline. The red line indicates the known value of $N_{A}$. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 6. Estimated vs. known $N_{A}$ for scenarios with 50 individuals, 10 microsatellite loci, and $N_{A}=1000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. The red line indicates the known value of $N_{A}$. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 7. Estimated vs. known $N_{A}$ for scenarios with 50 individuals, 10 microsatellite loci, and $N_{A}=10,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. The red line indicates the known value of $N_{A}$. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 8. Estimated vs. known $N_{A}$ for scenarios with 50 individuals, 10 microsatellite loci, and $N_{A}=100,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. The red line indicates the known value of $N_{A}$. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 9. Estimated vs. known $N_{A}$ for scenarios with 20 individuals, 10 microsatellite loci, and $N_{A}=1000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. The red line indicates the known value of $N_{A}$. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 10. Estimated vs. known $N_{A}$ for scenarios with 20 individuals, 10 microsatellite loci, and $N_{A}=10,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. The red line indicates the known value of $N_{A}$. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 11. Estimated vs. known $N_{A}$ for scenarios with 20 individuals, 10 microsatellite loci, and $N_{A}=100,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. The red line indicates the known value of $N_{A}$. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 12. Example posterior probability plots for $N_{e}$. Red lines indicate the known parameter value; posterior distributions for the three independent runs are shown as blue, green, and purple curves. A. Results from a demographic scenario of $-1 \%$ decline over 1 generation from an ancestral population size of 1000 and a sampling scenario of 20 individuals and 10 loci. B. Results from a demographic scenario of $-10 \%$ decline over 1 generation from an ancestral population size of 100,000 and a sampling scenario of 20 individuals and 10 loci. C. Results from a demographic scenario of $-1 \%$ decline over 100 generations from an ancestral population size of 10,000 and a sampling scenario of 50 individuals and 10 loci. D. Results from a demographic scenario of $-1 \%$ decline over 100 generations from an ancestral population size of 10,000 and a sampling scenario of 20 individuals and 50 loci.





Figure 13. Estimated vs. known $N_{e}$ for scenarios with 20 individuals, 50 microsatellite loci, and $N_{A}=1000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $N_{e}$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 14. Estimated vs. known $N_{e}$ for scenarios with 20 individuals, 50 microsatellite loci, and $N_{A}=10,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $N_{e}$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 15. Estimated vs. known $N_{e}$ for scenarios with 20 individuals, 50 microsatellite loci, $N_{A}=100,000$, and $-1 \%$ decline. Red lines indicate the known values of $N_{e}$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown with each box plot in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 16. Estimated vs. known $N_{e}$ for scenarios with 50 individuals, 10 microsatellite loci, and $N_{A}=1000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $N_{e}$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 17. Estimated vs. known $N_{e}$ for scenarios with 50 individuals, 10 microsatellite loci, and $N_{A}=10,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $N_{e}$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 18. Estimated vs. known $N_{e}$ for scenarios with 50 individuals, 10 microsatellite loci, and $N_{A}=100,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $N_{e}$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 19. Estimated vs. known $N_{e}$ for scenarios with 20 individuals, 10 microsatellite loci, and $N_{A}=1000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $N_{e}$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 20. Estimated vs. known $N_{e}$ for scenarios with 20 individuals, 10 microsatellite loci, and $N_{A}=10,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $N_{e}$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 21. Estimated vs. known $N_{e}$ for scenarios with 20 individuals, 10 microsatellite loci, and $N_{A}=100,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $N_{e}$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 22. Example posterior probability plot for $\mu$. Results are from a demographic scenario of $-1 \%$ decline over 100 generations from an ancestral population size of 10,000 . The sampling scenario involved 20 individuals genotyped at 50 loci. The red line indicates the known value parameter value; posterior distributions for the three independent runs are shown as blue, green, and purple curves..


Figure 23. Estimated vs. known $\mu$ for scenarios with 20 individuals, 50 microsatellite loci, and $N_{A}=1000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $\mu$ at each timepoint. Results are shown only for analyses that converged. Individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 24 Estimated vs. known $\mu$ for scenarios with 20 individuals, 50 microsatellite loci, and $N_{A}=10,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $\mu$ at each timepoint. Results are shown only for analyses that converged. Individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 25. Estimated vs. known $\mu$ for scenarios with 20 individuals, 50 microsatellite loci, $N_{A}=100,000$, and $-1 \%$ decline. Red lines indicate the known values of $\mu$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown with each box plot in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 26. Estimated vs. known $\mu$ for scenarios with 50 individuals, 10 microsatellite loci, and $N_{A}=1000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $\mu$ at each timepoint. Results are shown only for analyses that converged. Individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 27. Estimated vs. known $\mu$ for scenarios with 50 individuals, 10 microsatellite loci, and $N_{A}=10,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $\mu$ at each timepoint. Results are shown only for analyses that converged. Individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 28. Estimated vs. known $\mu$ for scenarios with 50 individuals, 10 microsatellite loci, and $N_{A}=100,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $\mu$ at each timepoint. Results are shown only for analyses that converged. Individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 29. Estimated vs. known $\mu$ for scenarios with 20 individuals, 10 microsatellite loci, and $N_{A}=1000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $\mu$ at each timepoint. Results are shown only for analyses that converged. Individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 30. Estimated vs. known $\mu$ for scenarios with 20 individuals, 10 microsatellite loci, and $N_{A}=10,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $\mu$ at each timepoint. Results are shown only for analyses that converged. Individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 31. Estimated vs. known $\mu$ for scenarios with 20 individuals, 10 microsatellite loci, and $N_{A}=100,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $\mu$ at each timepoint. Results are shown only for analyses that converged. Individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 32. Example posterior probability plots for $t$. Red lines indicate the known parameter value at each timepoint; posterior distributions for the three independent runs are shown as blue, green, and purple curves. A. Results from a demographic scenario of $1 \%$ decline over 1 generation from an ancestral population size of 10,000 and a sampling scenario of 20 individuals and 50 loci. B. Results from a demographic scenario of $-50 \%$ decline over 100 generations from an ancestral population size of 10,000 and a sampling scenario of 50 individuals and 10 loci. C. Results from a demographic scenario of $-10 \%$ decline over 50 generations from an ancestral population size of 1000 and a sampling scenario of 20 individuals and 50 loci. D. Results from a demographic scenario of -50\% decline over 1 generation from an ancestral population size of 10,000 and a sampling scenario of 20 individuals and 50 loci.




Figure 33. Estimated vs. known generations since decline $(t)$ for scenarios with 20 individuals, 50 microsatellite loci, and $N_{A}=1000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $t$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 34. Estimated vs. known generations since decline $(t)$ for scenarios with 20 individuals, 50 microsatellite loci, and $N_{A}=10,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $t$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 35. Estimated vs. known generations since decline $(t)$ for scenarios with 20 individuals, 50 microsatellite loci, $N_{A}=100,000$, and $-1 \%$ decline. Red lines indicate the known values of $t$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 36. Estimated vs. known generations since decline $(t)$ for scenarios with 50 individuals, 10 microsatellite loci, and $N_{A}=1000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $t$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 37. Estimated vs. known generations since decline $(t)$ for scenarios with 50 individuals, 10 microsatellite loci, and $N_{A}=10,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $t$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 38. Estimated vs. known generations since decline $(t)$ for scenarios with 50 individuals, 10 microsatellite loci, and $N_{A}=100,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $t$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 39. Estimated vs. known generations since decline ( $t$ ) for scenarios with 20 individuals, 10 microsatellite loci, and $N_{A}=1000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $t$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 40. Estimated vs. known generations since decline $(t)$ for scenarios with 20 individuals, 10 microsatellite loci, and $N_{A}=10,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $t$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


Figure 41. Estimated vs. known generations since decline $(t)$ for scenarios with 20 individuals, 10 microsatellite loci, and $N_{A}=100,000$. (A) $-1 \%$ decline, (B) $-5 \%$ decline, (C) $-10 \%$ decline, (D) $-50 \%$ decline. Red lines indicate the known values of $t$ at each timepoint. Results are shown only for analyses that converged. Results of individual runs are shown in green (run 1), yellow (run 2), and pink (run 3), with the pooled results of the three runs in blue.


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