American Genetic Association

Journal of Heredity, 2020, 491–497
doi:10.1093/jhered/esaa028
Bioinformatics Brief
Advance Access publication September 12, 2020

OXFORD

Bioinformatics Brief

AgeStrucNb: Software for Simulating and Detecting Changes in the Effective Number of Breeders (N_b)

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Received May 12, 2020; First decision June 9, 2020; Accepted July 30, 2020.

Corresponding Editor: William Sherwin

Abstract

Estimation of the effective number of breeders per reproductive event (N_b) using single sample DNAmarker-based methods has rapidly grown in recent years. However, estimating N_b is difficult in agestructured populations because the performance of estimators is influenced by the N_b / N_e ratio, which varies among species with different life histories. We provide a computer program, *AgeStrucNb*, to simulate age-structured populations (including life history) and also estimate N_b. The *AgeStrucNb* program is composed of 4 major components to simulate, subsample, estimate, and then visualize N_b time series data. *AgeStrucNb* allows users to also quantify the precision and accuracy of any set of loci or sample size to estimate N_b for many species and populations. *AgeStrucNb* allows users to conduct power analysis to evaluate sensitivity to detect changes in N_b or the power to detect a correlation between trends in N_b and environmental variables (e.g., temperature, habitat quality, predator or pathogen abundance) that could be driving changes in N_b. The software provides N_b estimates for empirical data sets using the LDNe (linkage disequilibrium) method, includes publication-quality output graphs, and outputs genotype files in Genepop format for use in other programs. *AgeStrucNb* will help advance the application of genetic markers for monitoring N_b, which will help biologists to detect population declines and growth, which is crucial for research and conservation of natural and managed populations.

Subject area: conservation genomics and biodiversity
Keywords: conservation genomics, population viability, genetic monitoring, individual-based simulation

When assessing and monitoring population viability, it is crucial to consider evolutionary metrics such as the effective population size (N_e). N_e is important because it influences the rate of loss of genetic variation, inbreeding, and the efficiency of natural selection (e.g., to maintain adaptive variation). Thus, precise and accurate estimates of N_e are crucial in evolutionary, conservation, and ecological genetics and have long been

used in conservation decision making and population genetics (Crow and Kimura 1970; Charlesworth 2009; Luikart et al. 2010; Allendorf et al. 2013; Waples et al. 2014). An alternative approach to N_e is to estimate the effective number of breeders in one reproductive cycle (N_b). Estimating N_b in natural populations is important because it can be used to estimate N_e in cases where the relationship between N_e and N_b is understood (Waples et al. 2013; Waples and Antao 2014). In addition, N_b is measurable annually (whereas N_e estimation often requires multiple years or generations between sampling events), making N_b more informative in monitoring long-lived species with annual breeding cycles (Waples and Yokota 2007; Waples et al. 2013). The potential of N_b estimation in abundance monitoring is especially promising because it generally reflects the number of successful breeding adults in a population at the time of reproduction and also provides information about reproductive behavior and breeding systems (Whiteley et al. 2015; Ruzzante et al. 2016).

Estimating N_b and its variation (among breeding cycles) is useful for predicting genetic changes and viability of populations of conservation concern. Often N_b and N_c (population census size) are not of similar magnitude or correlated among years, meaning that population census studies are not necessarily informative in determining N_b, rates of loss of variation, or inbreeding (Duong et al. 2013; Dowling et al. 2014; Pierson et al. 2018). However, N_b is not a direct replacement for N_e and inference of N_e from N_b often requires understanding the relationship between N_b and N_e as determined by a species' life-history traits (Waples and Antao 2014). A lack of correlation between N_b and N_c may be explained by environmental variables that constrain N_b, but only a handful of recent studies have investigated the relationship between environmental variables and N_b (Wood et al. 2014; Whiteley et al. 2015). These previous studies found that factors such as streamflow (Whiteley et al. 2015) and other environmental variables can influence N_b and the N_b/N_c ratio (Wood et al. 2014). Therefore, understanding the relationships between environmental variables and N_b will be a critical step in conducting risk assessments in species susceptible to environmental variation.

Here we introduce the *AgeStrucNb* software package that is based on a thoroughly tested population genetic simulation model that allows for estimation of N_b for age-structured populations (Waples et al. 2014; Waples and Antao 2014). *AgeStrucNb* allows for controlled simulation of changes in N_b over time through both one time and multi-breeding cycle events to simulate changes (including nonlinear) in N_b over time. *AgeStrucNb* takes advantage of other recent advancements, including the ability to simulate and account for linkage disequilibrium (LD) in estimates of N_b (Waples et al. 2016). *AgeStrucNb* also includes several time-saving and userfriendly features, including a powerful graphical user interface, a powerful graphing tools that allow for quick visualization of results, options for parallel processing for simulating populations and N_b estimation, routines for subsampling loci and individuals, and finally, the program utilizes Genepop (v4.6) format conventions (http://genepop.curtin.edu.au/help_input.html) allowing for quick and easy formatting of empirical input data to parameterize simulation with real data (e.g., known allele frequencies).

An important feature in the *AgeStrucNb* package includes the ability to simulate a near-constant true N_b (e.g., $N_b = 50$, or $N_b = 500$, etc.) for each of many independent simulation replicates. This allows researchers to address questions such as: What is the power of a certain number of loci and individuals to reliably estimate N_b (when N_b is a certain size; Waples and Faulkner 2009)?. Here, we consider the true N_b as the value determined using the Parentage analysis without Parents (PwoP) method found in Waples and Waples (2011).

No other software program has the ability to simulate a stable or changing N_b in age-structured populations (for any given life table or vital rates) and to conduct a power analysis of the number of loci, individuals, and cohort needed to detect a decline (or increase) in N_b. We demonstrate the usefulness of the *AgeStrucNb* software tool through 2 example uses highlighting the program's flexibility in handling empirical and simulated genetic data and for multiple purposes. In the first example, we use empirical SNP data from a population of known pedigree (and hence known N_b) to illustrate the effect of subsampling loci and individuals on the bias and precision of N_b estimates (using the LDNe estimator). In the second example, we demonstrate the use of *AgeStrucNb* as a tool to quantify the power to detect population declines in N_b over time, which will help biologists establish genetic monitoring programs. Additionally, we explore the correlation between estimated N_b (using LDNe—Waples and Do 2008) and true (deterministic) N_b (Waples et al. 2011) to understand the amount of sampling needed to accurately and precisely track true changes in N_b which is needed when correlating N_b with environmental variables that are potentially driving N_b.

Methods

Program Overview and Implementation

The *AgeStrucNb* program is composed of 4 major components to simulate, subsample, estimate, and then visualize either N_b time series data for a single population data, or single time point estimates of N_b for multiple populations (Figure 1). Simulations require a species' lifetables as part of the input configuration file. The program includes configuration files, including lifetables, for over 30 commonly studied species as simulated in Waples et al. (2014) and Waples and Antao (2014). For N_b estimation, *AgeStrucNb* takes as input Genepop formatted files allowing for simulated or empirical data. Much of the base code has had extensive debugging and validation and was utilized in previous studies (Waples et al. 2014; Waples and Antao 2014). Notable program features and additions include 1) greater flexibility in the user control of the change in true N_b through time and to hold true N_b constant, 2) linear regression model testing of statistical power (e.g., useful for when designing monitoring programs aimed at detecting population declines or increases in population size), and 3) an extensive subsampling routine that can be used to subsample on the number of single nucleotide polymorphisms (SNPs), individuals, specific cohorts.

AgeStrucNb has built-in visualization tools for creating boxplot figures on-the-fly for exploring subsampling of N_b and confidence interval (CI) related data (Figure 1). The user also has the option to create linear regression plots to explore time series data (e.g., to detect slopes significantly different from 0) and a summary statistics package to determine the number of slopes significantly different from zero. Plots are created using the Matplotlib python package, which can be used to produce publication-quality figures in a .png and .pdf file format (Hunter 2007).

AgeStrucNb is a python program (created and tested using python 3.5 and 3.6, and with limited testing on 3.7), available through both a popular python package distribution database (pypi.python.org/pypi) and a github repository (github.com/ popgengui/AgeStrucNb). The manual, installation details, and a set of simulation configuration files for multiple species can be found at github.com/popgengui/AgeStrucNb/tree/data. For efficiency and speed, the program utilizes multi-core processing to allow users to start multiple simulations, estimation or visualization sessions at once, as well as perform parallelized per-replicate simulations

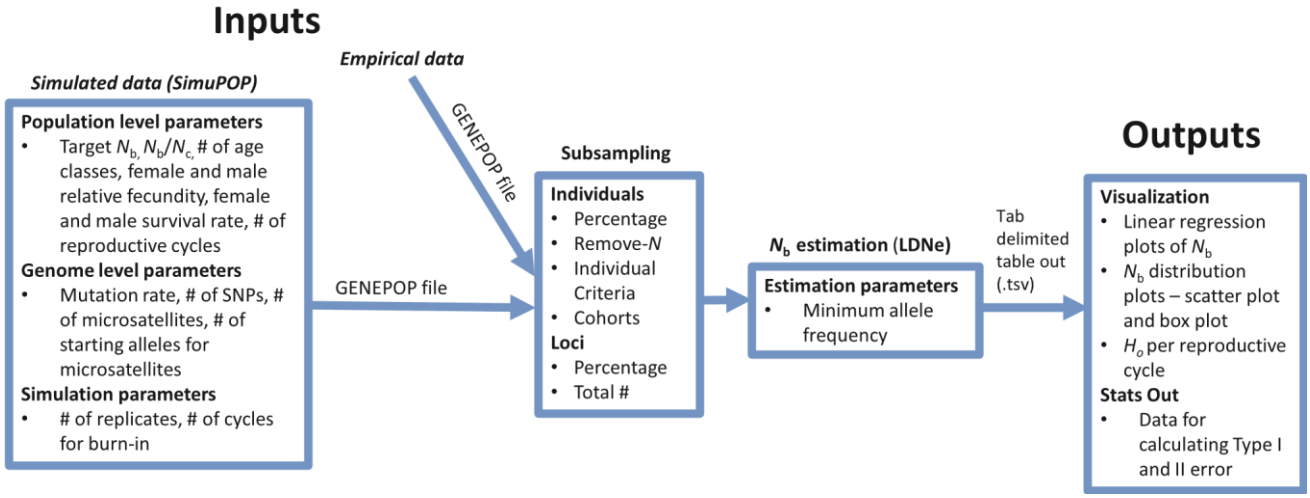


Figure 1. Workflow diagram of the *AgeStrucNb* program highlighting the main operation components (SimuPOP, subsampling, N_b estimation, and visualization). Some of the parameters for each component is show in their respective box. Input data can be either simulated (by SimuPOP) or empirical data in Genepop formatted files. The program also will output Genepop files containing either the entire population or a subsample of loci and individuals (and cohorts) for use in other programs.

and per-population N_b estimations. The program has been tested thoroughly on Linux (Ubuntu 16.04), 64-bit Windows 8.1 and 10 and had limited testing on OS X version 10.11. For a complimentary program to *AgeStrucNb* also see NeOGen: a tool for N_e simulation, estimation, and study design for species with overlapping generations (Blower et al. 2019).

Genetic Simulation With SimuPOP

AgeStrucNb uses a forward-time, individual-based population genetics simulation environment called simuPOP (Peng and Kimmel 2005; Peng and Amos 2008) to simulate age-structured genetic data for the desired study species. Demographic inputs for the simulation model include information about age and sex-specific survival rates and relative fecundity, mating system (monogamy/polygamy), the probability of male birth, and optional information about litter size. Other N_b-specific options include the ability to set a tolerance on the true N_b, the ratio of N_b/N_c, and the ability to prescribe changes in the true N_b over time either as 1-time events, a continuous decline or as multiple and varied fluctuations through time. To ensure that realized N_b is within a given threshold (e.g., within 1% of the true N_b) the program uses a trial-and-error fitting algorithm to repeatedly generate the next simulation generation and test the realized N_b (calculated using the PwoP method) is within the threshold specified by the user. The user can also enter their desired N_b from which the number of newborns (N0) and population census size (N_c) are calculated based on the input N_b/N_c ratio.

Simulations can be set to track large numbers of SNPs (tested up to 5000 in worked example 2) or microsatellite markers and including specifying the starting number of alleles. This allows for further connection and application of the program to past studies using microsatellite markers. Genetic and demographic processes are simulated and tracked based on the base unit of reproductive cycles.

At time 0, the age of each individual not in the N0 age class is drawn randomly from the stable age distribution. Because survival and reproduction are random and independent, total population size, and the number of each sex in each age class, can vary randomly around the mean values expected in a stable population. Furthermore, although primary sex ratio at age N0 varies randomly around 0.5, adult sex ratio can differ from this due to sex-specific survival rates and ages at maturity. For each newborn individual, 2 parents are selected by drawing 1 male and 1 female randomly from the pool of potential parents, with the probability of choosing a parent of age × proportional to b_x for that sex. In other words, all potential parents of the same sex and age have an equal opportunity to be the parent of each newborn (i.e., $\Phi = 1$, where Φ is the ratio of the variance to the mean reproductive success in individuals in the same age and sex class), but that is not necessarily true for individuals of different ages or sex. The value of Φ is hard to estimate empirically, but it is likely that $\Phi \neq 1$ in all cases (e.g., see Waples et al. 2018). As an optional setting, users can enforce strict adherence to sex and age class proportions using a lottery-based method that bins individuals based on age class and gender. Next, the number of individuals to be culled per age class and gender is determined by strictly maintaining age class and gender proportions relative to age and gender-specific survival rate. Individuals are then drawn randomly from each age class and gender and culled until the desired proportion is reached. Enforcing a strict age class structure allows for sustainable populations with low N_b, and thus, removing the chance for extreme events (e.g., the lost an entire gender or age class) that might not be biologically relevant (Waples and Faulkner 2009).

Simulations are optimized for speed, flexibility, and space saving. For example, users can set a simulation target for mean heterozygosity (H_E) for SNPs or microsats where the program will record genetic information for a desired number of cycles after reaching the target H_E within a certain threshold. In addition, population allele frequencies can be initialized based on a desired starting H_E. This greatly aids in speeding up simulation runs when N_b is large (e.g., Supplementary Figure S1 highlights the decay in H_E over time with respect to starting N_b).

The base python code for *AgeStrucNb* and the simulation modeling has been thoroughly tested and used extensively in multiple studies (Waples et al. 2014; Waples and Antao 2014; Luikart et al. in press). For model validation, we also conducted longer runs to track the loss of H_E over time and compared (validated) the loss rate to that expected (from theoretical equations) and the rate estimated from values from *AgeStrucNb* (Supplementary Figure S1).

Estimating N_b From Empirical and Simulated Datasets

LDNe v2 estimates N_b from genetic markers (e.g., SNPs or microsatellites) by quantifying the amount of nonrandom association (linkage disequilibrium) of alleles at independent locations on chromosomes (Waples and Do 2008). LDNe is one of the most extensively tested single

sample N_e estimator currently available (Wang 2016). Computer simulations have shown that LDNe is effective in predicting N_b in populations with N_b = 200 using only 200 SNPs and a sample of 50 individuals, and also populations with N_b = 500 using 400–5000 SNPs and >100 individuals, making it useful for estimating N_b in a range of populations (Luikart et al. in press). Other recent improvements in LDNe include increased power by lessening estimate biases in the LD method (Waples 2006) and improved jackknifed CIs (Jones et al. 2016). *AgeStrucNb* also includes further bias corrections as suggested by Eqn. 8 in Waples and Antao (2014) in cases where the ratio of estimated N_b and true N_b is not equal to 1. The most recent version of LDNe (as implemented in V2.1 of NeEstimator, released December 2017) can also utilize chromosomal information to not use pairs of loci on the same chromosome when calculating pairwise LD (to avoid the use of nonindependent loci). Therefore, *AgeStrucNb* users can use chromosomal map information for empirical and simulated datasets to remove any pairwise LD calculations for markers that reside on the same chromosome. This is an important consideration in genomic data sets where thousands of loci are used (Waples et al. 2016).

Statistical Output and Slope Significance Testing

AgeStrucNb features a stats and linear regression module that performs significance testing for a decline (or increase) in N_b point estimates from multiple time points (e.g., consecutive cohorts). Using simulated data, the program can be used to conduct power analysis to quantify the probability of detecting a negative or positive slope of a line through N_b point estimates compared to a zero slope. A range of scenarios can be simulated, including different rates of decline, and sampling of loci, individuals, and time points (cohorts). Additionally, the stats module can also be used with a single empirical data set with multiple time point estimates to test for a slope significantly different from zero.

Two parameters are estimated in the linear regression module of *AgeStrucNb*, the slope coefficient (b_1) and the intercept (b_0). This reduces the number of degrees of freedom to $n-2$ where n was the number of points (i.e., the number of cycles tested) used to calculate the regression and necessarily imposes a minimum limit on the number of sampling events of 3 breeding cycles (Neter et al. 1985). The test statistic (t*) for the slope of a linear regression is calculated for a normally distributed regression with a null hypothesis equal to zero, where $s(b_1)$ is an estimate of the variance of the slope (Equation 1; Neter et al. 1985).

$$t_* = {}^{b_1}\!/_{s(b_1)} \quad (1)$$

In most regression models b_1 is assumed to be distributed normally. This assumes the data being regressed over is roughly linear, distributed approximately normal, contains no major outliers, and independent variables. To calculate $s(b_1)$ requires calculating the mean squared error (*MSE*) of the line (Equations 2 and 3). Using t* and the degrees of freedom of the regression we can then calculate the *P*-value for that line using a Cumulative Density Function (CDF) on the T distribution (Neter et al. 1985).

$$s(b_1) = \frac{MSE}{\sum (x_i - \bar{x})} \quad (2)$$

$$\mathrm{MSE} = \frac{\sum (y_i - \quad)^2}{n - 2} \quad (3)$$

An advantage of using a CDF function is it maintains sidedness, and thus, allowing differentiation between significant positive and negative values. A *P*-value is then used to determine cases where the slope is significantly different from zero.

Empirical Examples

Example 1: Subsampling of Empirical Data

A major feature of *AgeStrucNb* is to streamline the process of subsampling data when input is either empirical or simulated datasets. *AgeStrucNb* will accept as an input a Genepop formatted file that can include any number of populations which can be treated as a timeseries for a single population or as a number of independent populations (e.g., when using empirical data for multiple locations). The N_b estimation module includes multiple optional methods of subsampling by the number of individuals (e.g., by a series of percentage values or remove-N individuals) and by the number of loci (by percentage or total number). Users can also choose to subsample individuals based on age or cohort number when calculating N_b.

We demonstrated the subsampling routine using an empirical dataset for steelhead trout (*Oncorhynchus mykiss*) from the Pahsimeroi hatchery in the Snake River Basin (Ackerman et al. 2017). Complete hatchery pedigree (parentage) information exists for the Pahsimeroi hatchery, including the genetic information for all breeding parents and their returning offspring. True N_b was calculated using the PwoP method. Multiple estimates of N_b were then calculated in *AgeStrucNb* using a panel 95 SNPs and repeated random subsets (i.e., 10%, 20%…90%) of the number of returning individuals (N = 1481). LDNe estimates were run using 100 replicates (random subsamples of individuals) per each sampling subset or 900 sampling replicates in total (Figure 2).

Ackerman et al. (2017) conducted a similar analysis on steelhead hatchery population; however, their analysis used sibship analysis in the program COLONY v2.0.5.6 to estimate N_b (Jones and Wang 2010). Ackerman et al. (2017) concluded that relatively large sample sizes (e.g., similar to the true value of N_b) were required to accurately estimate N_b using parentage analysis. There was also a downward bias in the estimates of N_b using COLONY, especially with small sample sizes. We found less obvious bias when using LDNe; however, there was a slightly increased amount of variation among N_b estimates for each subsampling. This increased variation could partly be attributed to the small SNP panel used for estimating N_b in LDNe. The major advantage of LDNe, in this case, was the greatly reduced computational time required to recreate the (subsampling used in Ackerman et al. 2017). Ackerman et al. (2017) used 90 replicate runs of the COLONY program that took a total amount of computer time ~400 h versus ~1 h needed to run 900 subsampling replicates in *AgeStrucNb*.

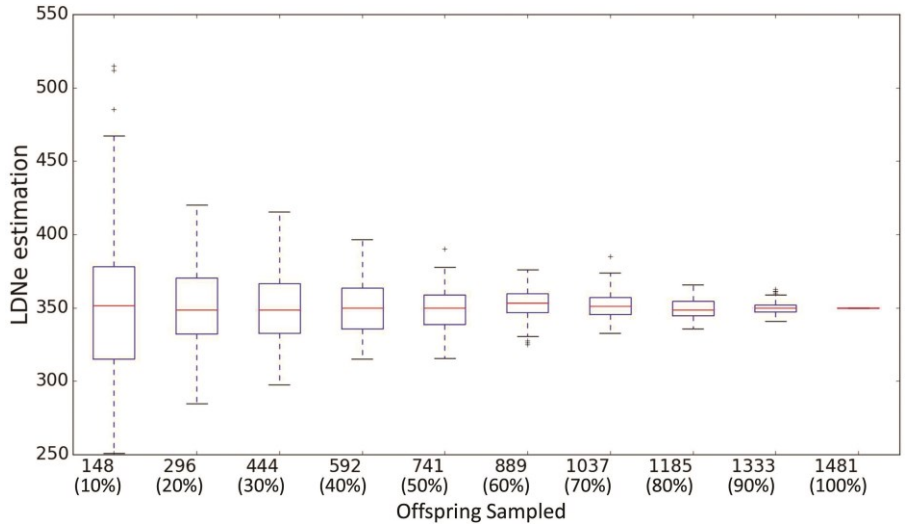


Figure 2. Output from the *AgeStrucNb* program using the visualization interface and the box-plotting option. Box-plots are for hatchery steelhead (Pahsimeroi). The total steelhead population consisted of 1,481 individual offspring with a true (pedigree-based) N_b = 353 (Ackerman et al. 2017). Subsampling across a randomly chosen set of individuals shows the variation in the LDNe estimation of N_b using 95 markers and across 10%, 20%…100% of individuals sampled. Note that even when sampling all individuals (1481) there is still error associated with the choice of sampled loci which is not depicted here.

Example 2: Establishing Monitoring Programs and Sampling Design Needs

We demonstrate the use of *AgeStrucNb* as a tool for genomic monitoring sampling design by conducting power analysis to quantify the probability of detection in a population where N_b declines by 10% per year. For our study species, we choose the short-lived wood frog (*Rana sylvatica*) that has 4 age classes and the long-lived lake trout (*Salvelinus namaycush*) that has 63 age classes. The linear regression and slope significance (described above) test was used to test for the probability of a correct detection (statistically significant negative slope) using 100 replicate simulations. Slope significance tests were calculated using 3–10 breeding cycles (and thus 3–10 N_b estimates; Figure 3) with an alpha of 0.1, which is equivalent to a false positive detection rate of ~10%. We deemed this higher false-positive rate a reasonable test based on the assumption that increased sensitivity (lower false negatives) may be desirable, even with a slightly increased rate of false-positive detections of declines. However, users can also adjust the rate of their alpha based on their risk tolerance needs. We used N_b starting values of 200 and 500 and examined the change in probability of detection over a combination of individual sample sizes (50, 100, and 200) and the number of SNP markers (100, 500, 1000, and 5000). We assumed a probability of detection ≥80% to be acceptable or desired for most monitoring programs to detect a decline. Finally, we also calculated the correlation between replicate sets (i.e., including all point estimates of a single replicate over 10 cycles) of estimated N_b correlated with the single set of true (deterministic) N_b (Table 1).

Overall, the number of cohorts sampled had a strong effect on increasing power until at least 7 cohorts were sampled. No scenarios with less than 5 cohorts samples achieved high power (>.80). For fixed numbers of individuals and loci, Larger values of N_b required an increased number of breeding cycles sampled to approach the desired 80% threshold of power. In the case of N_b = 200, 5 breeding cycles were required to reach the 80% threshold, and a minimum of 6 breeding cycles sampled was necessary for N_b = 500 to reach the same threshold. In terms of N_b, this equates to N_b = 131 for a starting N_b = 200, and N_b = 328 for a starting N_b = 500.

The number of individuals and SNPs sampled also greatly impacted the probability of detection (Figure 3). There appeared to be distinct thresholds over which the increase in the number of individuals sampled or the number of SNPs sampled did not greatly increase rates of detection. General patterns in the probability of detection were similar between species.

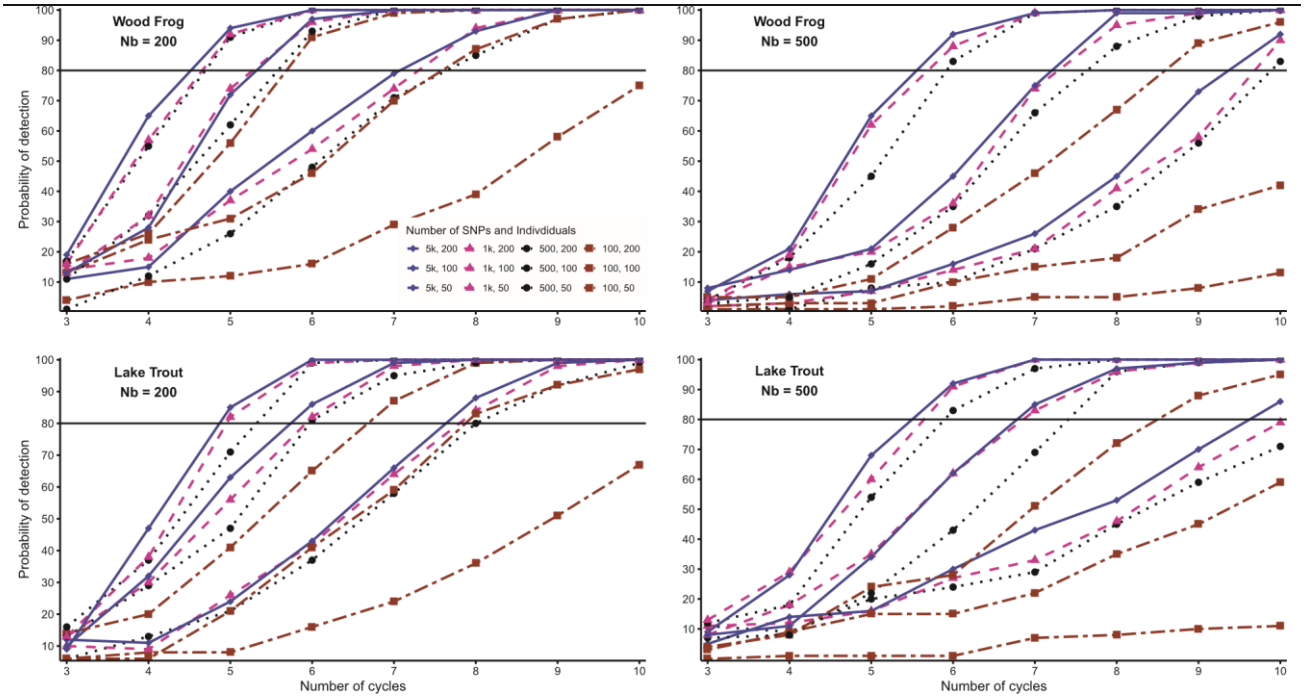
For this example, we also investigated the correlation between estimated and true N_b to determine how many SNPs and individuals need be sampled to produce tightly correlated values. This is important as a first step in determining under what conditions annual N_b might be correlated with an underlying environmental factor. If noise is too great, it could be impossible to detect an underlying correlative relationship. Using the datasets above, we correlated sets of estimated N_b over 10 breeding cycles (with a 10% decline) to the true N_b (Table 1). The range in correlation values between estimated and true N_b appeared most sensitive to the number of SNPs and individuals sampled, less dependent on the starting N_b. Values of 100–200 individuals and 1000–5000 SNPs sampled appeared to provide high accuracy (mean correlation ≥ 0.89), and moderate precision (range of correlation = 0.61–1). With ample sampling of individuals (>200) and using >500 SNPs, the precision was increased (range of correlation = 0.81–1). This simple example appears to offer hope that with adequate sampling, correlations between N_b and environmental variables will be detectable, but we leave further exploration for future use of the *AgeStrucNb* tool.

Such simulation and empirical-based tools for assessing power, precision, and accuracy will become increasingly important as managers need tools for establishing genomic monitoring programs. *AgeStrucNb* will allow managers to balance the power to detect a population decline with the cost and time associated with sampling. In general, 5–6 years of sampling time with the appropriate number of individuals (~100–200 for N_b >200) and SNPs (≥500) sampled can provide enough power to have a high probability (≥80%) to detect population declines.

Figure 3. The probability of detecting a decline in population N_b for wood frog and lake trout when initial N_b = 200 and N_b = 500 by the number of breeding cycles, individuals and number of loci sampled. Each combination of the number of SNPs (loci) sampled (squares = 100 SNPs, circles = 500 SNPs, triangles = 1000 SNPs, diamonds = 5000 SNPs) and individuals sampled (50, 100, 200) used 100 replicate simulations and a 10% decline/cycle over 10 cycles. Alpha was set to 0.1 for the *P*-value tests, representing a ~10% false positive rate. For each line type × shape combination, the difference in probability of detection plotted by each of the 3 associated lines is the relative change when using more individuals and keeping the number of SNPs the same. As an example, the solid lines with diamonds all use 5000 SNPs, but the uppermost left line with diamonds used 200 individuals, the middle line with diamonds used 100 individuals, and the furthest right line with diamonds used 50 individuals. This pattern is similar for each line type × shape combination. See online version for full color.

Table 1. The range in correlation values and the mean of the correlation values between *AgeStrucNb* (LDNe) N_b point estimates and true N_b values (derived using PwoP) for 10 breeding cycles with a 10% annual decline in N_b

		Lake trout N_b = 200		Lake trout N_b = 500		Wood frog N_b = 200		Wood frog N_b = 500	
No. of indivs. sampled	No. of SNPs sampled	Range of corr. (Min–Max)	Mean corr. value	Range of corr. (Min–Max)	Mean corr. value	Range of corr. (Min–Max)	Mean corr. value	Range of corr. (Min–Max)	Mean corr. value
50	100	−0.15–0.94	0.58	−0.61–0.75	0.14	0.19–0.92	0.63	−0.77–0.85	0.21
	500	0.49–0.98	0.81	−0.03–0.97	0.63	0.61–0.98	0.84	0.08–0.91	0.69
	1000	0.6–0.97	0.83	0.09–0.95	0.67	0.65–1	0.87	0.23–0.94	0.72
	5000	0.61–0.97	0.84	0.07–0.98	0.7	0.68–0.99	0.88	0.34–0.96	0.76
100	100	0.24–0.97	0.81	−0.09–0.93	0.56	0.61–0.98	0.84	−0.58–0.94	0.6
	500	0.76–0.99	0.92	0.53–0.97	0.85	0.87–1	0.95	0.61–0.98	0.88
	100	0.81–0.99	0.93	0.61–0.98	0.89	0.88–0.99	0.96	0.64–0.99	0.9
	5000	0.79–0.99	0.94	0.57–0.97	0.89	0.88–0.99	0.96	0.63–0.99	0.91
200	100	0.73–0.99	0.91	0.35–0.96	0.78	0.83–0.99	0.94	0.53–0.97	0.82
	500	0.9–0.99	0.96	0.81–0.99	0.94	0.93–1	0.98	0.86–0.99	0.96
	1000	0.9–1	0.97	0.85–1	0.95	0.93–1	0.98	0.86–1	0.96
	5000	0.92–1	0.97	0.89–0.99	0.95	0.94–1	0.98	0.86–0.99	0.97



Each combination of the number of individuals and SNPs sampled had 100 replicate simulation runs with 10 breeding cycles of estimated annual N_b per run that were then correlated to the set of true N_b values.

Conclusion

The increasing availability of genetic markers and lowering costs for genotyping will improve and increase future genomic monitoring programs. *AgeStrucNb* is a multi-faceted package allowing researchers and population managers to conduct simulations and power analysis to develop sensitive genetic monitoring programs for early detection of population declines (or growth). *AgeStrucNb* provides many helpful features to simulate, subsample, estimate, and visualize N_b for empirical or simulated data sets. For example, it can be used to simulate, estimate, and then conduct statistical analysis to quantify the ability to detect changes in N_b given a wide range of sampling parameters. It can also be used to subsample empirical data to then conduct power and sensitivity analysis on the number of loci and individuals sampled, and to create barplots series to visualize confidence intervals on (and precision of) N_b estimates using different subsets of individuals and loci. *AgeStrucNb* allows biologists to quickly and effectively plan the number of cohorts, loci, and individuals to sample to estimate and detect a declining N_b in agestructured species.

Supplementary Material

Supplementary material is available at *Journal of Heredity* online.

Funding

National Aeronautics and Space Administration (NASA) (NNX14AB84G) to T.C., B.T., G.L., and B.K.H.; the National Science Foundation (DEB-1639014); Montana Fish Wildlife and Parks (MT-FWP) to G.L. and B.K.H.

Acknowledgments

We thank Steve Amish for helpful discussions and ideas for monitoring N_b. Special thanks to Matt Campbell and the Idaho Department of Fish and Game – Eagle Fish Genetics Laboratory for providing the hatchery steelhead trout dataset and for insightful discussion on Nb population monitoring.

Data Availability

All source code can be found in a github repository (github.com/popgengui/AgeStrucNb). The manual, installation details, and a set of simulation configuration files for multiple species can also be found at github.com/popgengui/AgeStrucNb/tree/data.

Author Contributions

T.A. developed the original *AgeStrucNb* model and software. T.C., B.T., and B.K.H. designed and produced the *AgeStrucNb* graphical user interface and other improvements to the *AgeStrucNb* base model. All authors contributed to the intellectual development of the *AgeStrucNb* tool and writing the manuscript.

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