Conserv Genet (2010) 11:355–373
DOI 10.1007/s10592-010-0050-7

REVIEW

Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches

Gordon Luikart · Nils Ryman · David A. Tallmon · Michael K. Schwartz · Fred W. Allendorf

Received: 16 October 2009 / Accepted: 11 January 2010 / Published online: 27 February 2010

Abstract Population census size (N_C) and effective population sizes (N_e) are two crucial parameters that influence population viability, wildlife management decisions, and conservation planning. Genetic estimators of both N_C and N_e are increasingly widely used because molecular markers are increasingly available, statistical methods are improving rapidly, and genetic estimators complement or improve upon traditional demographic estimators. We review the kinds and applications of estimators of both N_C and N_e, and the often undervalued and misunderstood ratio of effective-to-census size (N_e/N_C). We focus on recently improved and well evaluated methods that are most likely to facilitate conservation. Finally, we outline areas of future research to improve N_e and N_C estimation in wild populations.

Keywords Population size estimation · Noninvasive sampling · Remote genetic monitoring · Abundance · Bottleneck · N_e/N_C ratio · Habitat fragmentation

G. Luikart (✉) · F. W. Allendorf
Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA
e-mail: gordon.luikart@mso.umt.edu

G. Luikart
Centro de Investigação em Biodiversidade e Recursos Genéticos and Universidade do, Porto (CIBIO-UP), 4485-661 Vairão, Portugal

Present Address:
G. Luikart
Flathead Lake Biological Station, University of Montana, Polson, MT, USA

N. Ryman
Division of Population Genetics, Department of Zoology, University of Stockholm, 10691 Stockholm, Sweden

D. A. Tallmon
Biology and Marine Biology Programs, University of Alaska Southeast, Juneau, AK 99801, USA

M. K. Schwartz
USDA Forest Service, Rocky Mountain Research Station, 800 E. Beckwith Ave, Missoula, MT 59801, USA

F. W. Allendorf
School of Biological Sciences, Victoria University of Wellington, Wellington 6140, New Zealand

> *Counting fish is like counting trees, except they are invisible and they keep moving.*
>
> John Shepherd (from Hilborn 2002)

> *Effective population size (N_e) is a critical parameter in population biology, but it is difficult to collect enough demographic data from natural populations to calculate N_e directly.*
>
> Robin Waples (2005)

Introduction

Population census size (N_C) and effective population size (N_e) are among the most important parameters in wildlife management and conservation because they can inform management and help predict the extinction risk of populations. N_C and N_e are difficult to estimate, especially for secretive and elusive species, ranging from fish to other aquatic organisms and forest-dwelling mammals. The first quote above exemplifies a common problem: N_C seldom

can be assessed by directly counting individuals. We often must estimate N_C because it is difficult to count all the individuals in a population,. The second quotation above exemplifies the difficulty of estimating N_e directly from demographic data such as variance in reproductive success and thus the need for indirect genetic estimators (Harris and Allendorf 1989; Frankham 1995a, b; Leberg 2005).

It is timely to review N_C and N_e estimators because the field is advancing rapidly with recent publications reporting new estimators and performance evaluations for both N_C (Boulanger et al. 2008a, b; Kendall et al. 2008; Settlage et al. 2008; Knapp et al. 2009) and N_e (Jorde and Ryman 2007; Waples and Yokota 2007; Nomura 2008; Palstra and Ruzzante 2008; Tallmon et al. 2008; Wang 2009; Waples and Do in press). We focus on estimators that are currently the most useful for conservation and wildlife management, e.g. the estimators of contemporary (current) N_e. Estimators of historical N_e have less immediate relevance to conservation (although excellent examples exist; see Alter et al. 2007), and recently have been reviewed elsewhere (Charlesworth 2009). Historical N_e also is relatively difficult to estimate.

We discuss sampling requirements, assumptions, and genetic markers for estimation of N_C and N_e, which will hopefully encourage researchers to estimate both the N_C and N_e to improve understanding of the N_e/N_C ratio in natural populations (Nunney 1993, 2002). Finally, we discuss future research to improve estimation of N_C, N_e, and the N_e/N_C ratio.

Estimation of census size (N_C)

We define population census size (N_C) as the number of adults in a study area or population, unless stated otherwise. This avoids inclusion of thousands of offspring in fecund species that have no chance of reaching adulthood or contributing demographically or genetically to their population. We emphasize it is crucial for researchers to report their definition of population size and what age or stage-classes are counted in their N_C estimation. We realize that DNA-based estimates of N_C often include pre-reproductive young because age usually cannot be determined from remotely-sampled DNA (but see below and Criscuolo et al. 2009). Researchers can potentially avoid noninvasive sampling of young animals by collecting only large fecal pellets or sampling only large hairs from hair-snares positioned high off the ground can. Finally, we note that wildlife researchers often use the term 'abundance'—the number of individuals in a particular area, similar to our definition of N_C, except that our N_C refers to the number of adults only.

Traditional capture-mark-recapture (CMR) studies use physical markers such as leg bands to mark individuals. The principle of CMR is to mark individuals in an initial capture session and then, in subsequent recapture sessions, to quantify the proportion of marked individuals. N_C is then estimated from the ratio of marked to unmarked individuals in recapture sessions, assuming that all individuals (marked and unmarked) are randomly mixed and sampled and thus all are equally catchable during the recapture sessions (Seber 1973). However, it can be impossible or dangerous to capture and handle enough animals to estimate N_C with adequate precision.

Noninvasive or remote genetic techniques allow marking of many animals by using DNA collected from hair, feathers, urine, menstrual blood, snail slime tracks, or other tissue samples. One difference between genetic mark-recapture and traditional mark-recapture is a concern about poor data quality caused by genotyping errors that can be common with noninvasive DNA samples (see reviews by Taberlet et al. 1999; Pompanon et al. 2005; Waits and Paetkau 2005). Fortunately, new laboratory techniques and N_C estimation models continually improve our ability to limit effects of genotyping errors on N_C estimates (e.g. Wright et al. 2009; Beja-Pereira et al. 2009).

One-sample N_C estimators

Unlike classical CMR that requires multiple sampling sessions, noninvasive DNA-based N_C estimates can be obtained from a single sampling session. The principal of one-sample N_C estimation is as follows: if the same multilocus genotype, i.e., individual, is observed ('captured') two or more times in the same single sampling session, all captures beyond the first are considered 'recaptures'. The ability to estimate N_C from a single sampling session is extremely helpful for species that are costly or time consuming to sample.

Rarefaction curves have been used to asses $\hat{N}_C$ (Kohn et al. 1999; Eggert et al. 2003; Frantz and Roper 2006). Kohn et al. (1999) genotyped 115 fecal samples from coyotes (*Canus latrans*) in an area near Los Angeles; they found 30 unique, 3-locus genotypes, resulting in a rarefaction index of 38 individuals in the population (Fig. 1). A rarefaction curve, also called an accumulation curve, is a plot with the number of unique multi-locus genotypes on the y-axis and the number of analyzed samples on the *x*-axis. Rarefaction assumes that all individuals will have a unique genotype, e.g. when genotyped at 6–10 microsatellite loci, and that sampling is conducted "with replacement".

Rarefaction approaches are becoming less widely used as recent maximum likelihood and Bayesian methods (Table 1) can make use of the information provided by

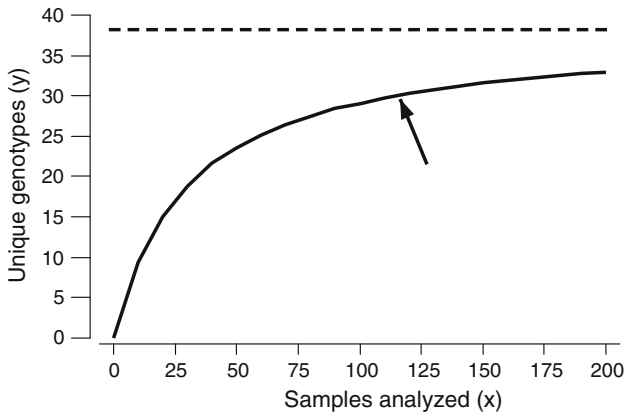

Fig. 1 Population size inference based on the rarefaction curve used to estimate the number of coyotes from feces sampled in an area near Los Angeles by Kohn et al. (1999). Plot of the average number of unique genotypes (y) discovered as a function of the number of samples (x) using the equation $y = (ax)/(b + x)$, where a is the population size asymptote, and b a constant which is the rate of decline in the value of the slope. Kohn et al. (1999) found 30 unique genotypes in 115 samples analyzed, resulting in an index of 38 individuals in the population

multiple occurrences of individual genotypes within a session (Miller et al. 2005; Petit and Valiere 2006; Lukacs et al. 2007).

Two-sample N_C estimators

Two-sample or multi-sample DNA-based N_C estimators are the same as traditional CMR demographic estimators, except that genotypes replace physical marks. There are many types of two-sample N_C estimators (Table 1). In fact, one of the most commonly used software programs for estimating N_C and survival using mark-recapture data—program MARK (White and Burnham 1999)—has more than 100 different models to estimate population parameters, including N_C (White et al. 2006). These models principally differ in the parameter of interest, initial assumptions, how an animal is encountered (e.g. recovered dead versus re-sighting of a marked animal) and how that animal has been marked (White et al. 2006). For example, models for open and closed populations exist (see below).

The kernel of multi-sample CMR estimators is the attempt to adjust raw count data by the probability that an animal was detected or captured, called capture probability (CP). Capture probability can be constant across all groups, or vary by individual, time, age or any other covariate. The literature is replete with examples emphasizing the importance of high capture probability to achieve precise N_C estimates. High capture probability is achieved if the sample size is large enough that every individual has a 'high' chance of being recaptured with a given technique such as hair or fecal sampling.

The greatest challenge to estimating N_C using capture-mark-recapture methodology often has been the heterogeneous capture probabilities among individuals in the sampled population (Lukacs et al. 2007; Boulanger et al. 2008a). Lukacs et al. (2007) proposed methods to incorporate information from multiple captures of genotypes within each sample and to adjust estimates for capture heterogeneity; the method worked well for elephant (*Loxodonta africana*) dung samples.

Reliable N_C estimates using DNA sampling generally require sufficient sample sizes and capture probabilities, low capture biases (by sex, age etc.), and demographic closure. Pilot studies are recommended to assess whether sufficient capture data can be obtained and genotyping error rates are sufficiently low to avoid bias caused by false genotypes, for example (Valière et al. 2006; Harris et al. in press). Boulanger et al. (2004) conducted simulations that suggested capture probabilities of 0.20 and population sizes of $\sim$50 were needed to generate reliable results for estimating brown bear (*Ursus arctos*) N_C from DNA sampling. In general, much higher capture probabilities are desirable and will provide more precise estimates.

Settlage et al. (2008) assessed capture probabilities and statistical power for black bears (*Ursus americanus*) in two areas of the southern Appalachian Mountains of the USA, using 9 microsatellites genotyped from hairs. For a low density area, 60 bears were identified; the capture probabilities and precision of the population estimates were acceptable if samples were collected over at least 3-5 consecutive one-week sampling periods. However, in the second high density study area, capture probability (CP) and precision were unacceptably low ($CP < 0.20$) given the sampling of 129 bears; capture heterogeneity caused by inadequate sample numbers and spatial coverage apparently contributed to the poor performance. This study illustrates the crucial importance of conducting a power analysis and considering the population density and trapping effort required to achieve sufficiently high CP and spatial coverage of sampling across a study area.

Comparing one- and two-sample N_C estimators

Petit and Valiere (2006) used simulations to evaluate the reliability of different N_C estimators that use DNA-based approaches. For equal sampling efforts, they compared population size estimates from rarefaction curves, a classical maximum likelihood CMR method, and one-sample and multiple-sample Bayesian estimators (BAYESN, an *R* script module for $\hat{N}_C$ calculation, Table 1). In a closed population without sampling heterogeneity, one-sample noninvasive $\hat{N}_C$ estimation was as reliable as classical

Table 1 N_C estimators used in genetic studies of natural populations, along with main assumptions and comments

N_C estimator or indices	Strengths	Key assumptions	Comments	Software
Sequential Bayesian	Can be used for one or multiple samples	No capture heterogeneity; No em/ immigration, deaths or births	Evaluated by Petit and Valiere (2006)	BAYESN, *R* scripts exist; Puechmaille and Petit (2007)
One sample (continuous sampling)				
Capwire	Small N_C (<100); high capture heterogeneity	Simple urn (random) sampling; Closed population; Sampling design not accounted for; Capture probability is not accounted for	Simulation & empirical evaluations of performance Works well at $N_C \leq 100$	CAPWIRE[a] Petit and Valiere N (2006)
Rarefaction (Accumulation-curves)	Index can be calculated with one sampling occasion	Closed population; Sampling design not accounted for; Capture probability is not accounted for; Not all available data are used	Performance assessed by Frantz and Roper (2006)	GIMLET
Two or more samples				
Lincoln-Peterson	Can readily combine traditional and genetic data (Bellemain et al. 2005)	Closed population All individuals are equally likely to be captured in each sampling session Capture probability not modeled	When sample sizes are low Lincoln-Peterson can outperform estimators that often require large datasets to properly select models for individual heterogeneity in capture probability	DROPOUT
CMR closed mixture models	Closed models allow heterogeneity in capture probability to be readily modeled to estimate population size	Assumptions vary depending on which of the many models used. Most try to model individual heterogeneity in capture probability as a function of time, behavioral responses to capture, and differences among individuals	For a list of all pertinent software see: http://www.mbr-pwrc.usgs.gov/software.html Reviewed in Lukacs and Burnham (2005)	MARK, CAPTURE
CMR open mixture models	Can estimate population size, survival, movement rates, and population growth rates	Assumptions vary depending on which of the many models used. Most try to model individual heterogeneity in capture probability as a function of time, behavioral responses to capture, and differences among individuals	For a list of all pertinent software see: http://www.mbr-pwrc.usgs.gov/software.html Reviewed in Lukacs and Burnham (2005)	MARK, MSURGE
Occupancy models	Basic models only require diction—nondetection data, which is relatively easy to sample for with non-invasive methods	Detection probability doesn't change as a function of previous detection (or non-detection) Occupancy of a site is constant (or random) across sampling periods Occupancy of a site is independent of other sites-	Relatively new branch of CMR Reviewed in Royle et al. 2008 Critical reference is book published by MacKenzie et al. (2006)	PRESENCE, WinBUGS

CMR. The Bayesian estimator in the case of a single sampling session was most reliable with 95% confidence intervals containing the parametric N_C in >90% of simulations. Future simulation evaluations with capture heterogeneity are needed to quantify the effect of capture heterogeneity on the reliability of one-sample estimators.

Recent empirical studies of bats (*Rhinolophus hipposideros*; Puechmaille and Petit 2007) and black bears (Robinson et al. 2009) suggest that one-sample methods work well in small populations with less than 100 individuals. Robinson et al. (2009) concluded that one-sample models provided more precise estimates than two multi-session CMR models in program MARK (Cooch and White 2006). However, the true parametric N_C was unknown, as in most empirical studies, and thus the conclusions are not certain.

Harris et al. (in press) applied both one-sample and two-sample estimators in a noninvasive fecal DNA-based study of argali mountain sheep (*Ovis ammon*) in Afghanistan. The one-sample estimates from the urn model in the CAPWIRE software were more precise (153, 95% CI: 120–202) than the conventional multi-session closed CMR estimator (172, 95% CI: 117–232) using female argali and program MARK. However, the spatial distribution of the within-session recaptures was spatially clumped, thereby violating the independence assumption underlying the continuous-time one-sample model in CAPWIRE software program (Table 1). The authors therefore rejected the estimate from CAPWIRE as having false precision, and used only the CMR closed-capture estimate of N_C. It is not surprising that fecal pellet piles from individuals were spatially clumped when considering that an individual might have repeatedly defecated in the same small geographic area. This could be a common problem for many species and one-sample estimators. Finally, CMR will often out perform one-sample (i.e. continuous capture) methods because existing one-sample approaches are less flexible in modeling the capture process.

Open and closed models

CMR models can be classified based on assumptions of demographic closure. "Closed" models assume no additions or subtractions of individuals during the time period of interest, such that no births, deaths, emigrations, or immigrations, occur during or between sampling occasions. Alternatively, open models allow changes in N_C and can also provide estimates of survival and recruitment, in addition to $\hat{N}_C$. However, open models usually require larger sample sizes and longer study times. Closed models are commonly used because they can provide reasonably precise and accurate estimates of N_C with fewer data. A primary advantage of closed models over open ones is their ability to model variation in capture probability. With open models, researchers must use the robust design (which essentially imbeds multiple closed-capture samplings within a larger open one) in order to account for capture variation—which should be common.

There have been several large-scale capture-mark recapture efforts using DNA from hair and scat. Both open and closed models have been used, although due to the nature of sample deposition with scat, these studies tend to use open models. For example, old feces deposited over several weeks can be collected, which makes capture sessions longer and therefore more likely to violate assumptions of closure.

Kendall et al. (2009) used DNA from hair snares and rubs and Huggins–Pledger closed mark–recapture models to estimate population size for grizzly bears (*Ursus arctos*) in the 33,480 km^2 in the Northern Continental Divide Ecosystem of Montana, U.S.A. They estimated the population to be 765 bears (95% CI = 715–831) which was greater than twice the existing estimate from sighting data. They could justify the closed model because their sample design involved discrete sampling sessions to collect bear hair at sites where they induced the bear to visit and deposit a sample. The confidence interval on $\hat{N}_C$ in this study is impressively small thanks to very intensive field efforts and clever and sophisticated use of available models. This landmark study is perhaps the first to estimate N_c of a wide-ranging species for an entire ecosystem.

Harris et al. (in press) illustrated the importance of considering open and closed models separately for males and females. With four sampling sessions, the closed-capture model $\hat{N}_C$ for female argali mountain sheep was 172 (95%CI: 117–232), which was ~23% higher than visual counts (index of N_C). However, the comparison of mark-recapture models suggested that males were not a closed population, and thus a reliable overall $\hat{N}_C$ could not be obtained. Males likely moved in and out of the study area and thus were unlikely sampled representatively throughout the entire male population. Even without a clear overall $\hat{N}_C$, the study yielded a female $\hat{N}_C$ and also suggested that the local population was not isolated from other argali populations, which is crucial information that could not have been confirmed with the visual count indices alone.

Prugh et al. (2005) used DNA from fecal samples collected on snowtracks to estimate the size of a coyote population in the Alaska Range with Cormack-Jolly-Seber open population models. They found that the coyote $\hat{N}_C$ and survival diminished in the first year following a crash in an important prey item, the snowshoe hare. Similarly, Marucco et al. (2009) estimated $\hat{N}_C$ and population growth (λ) over 7 years for a recolonizing wolf (*Canus lupus*) population in the Italian Alps using non-invasive genetic

sampling and open capture-mark-recapture models. Specifically, these authors used a Cormack-Jolly-Seber model to estimate apparent survival (survival of individuals that remain in the study area), recapture rates, and $\hat{N}_C$, and examined a series of 20 biological models that may have explained variance in these rates. They found that $\hat{N}_C$ more than doubled in 7 years largely due to high winter survival rates of adults.

Above we noted that open models have the advantage of being able to account for recruitment and mortality, an advantage over closed models. Yet, open models do not estimate capture probability within-years, as done by closed-captures population models. Thus many researchers have turned to robust design models to estimation both within-year capture probabilities and between year survival. In addition researchers are beginning to use a new class of models called occupancy models to calculate $\hat{N}_C$ (MacKenzie and Royle 2005; MacKenzie et al. 2005) and understand how occupancy is influenced by covariates.

Occupancy modeling

Occupancy modeling uses information on species detections at a site (given repeat site visits) to estimate site occupancy and probability of detection given presence. These data can be modeled to estimate N_C and population growth rates (MacKenzie et al. 2006). Occupancy models estimate probability of detection using an approach that is similar to Huggins closed population size models except that occupancy of the sample unit is estimated versus $\hat{N}_C$ for sample units as is done with the Huggins estimators (Huggins 1991). The Huggins model is useful for dealing with heterogeneity issues by modeling capture probability as a function of covariates (e.g. weight, length, snow depth etc.) influencing individuals or populations. One advantage of occupancy models is that they use more data than traditional population size estimators; for example, a complete absence of individuals at a site over time can be used in the data analysis for occupancy models, but not in CMR studies.

Boulanger et al. (2008a) applied occupancy models to estimate probability of detection at DNA hair snares for grizzly bears that were previously radio-collared and bears that have never been handled. They found that previously captured bears had a lower probability of detection. This is important as the authors can now account for previous capture (a source of individual heterogeneity) in their population size estimates. Thus far we know of no published DNA-based surveys that compute $\hat{N}_C$ using this method, although the approach has been used to evaluate the influence of environmental and study design factors on carnivore occupancy of different areas (Boulanger et al. 2008a).

Comparing demographic and genetic estimators of N_C

Recent studies have compared the usefulness of traditional versus DNA-based methods for estimating N_C. For example, Solberg et al. (2006) used the brown bear in south-central Sweden to compare three different methods of estimating N_C, including methods based on traditional demographic data as well as on non-invasive genetic data. The best traditional method was based on observations of bears from a helicopter. The best overall method was the genetic method using a closed population MARK estimator, as recommended in a previous study (Bellemain et al. 2005). Solberg et al. (2006) concluded that traditional field methods likely underestimated population size. They also concluded that the noninvasive genetic method was less expensive than the most reliable traditional field method (a CMR method based on observations of bears from a helicopter), and preferable from an ethical point of view.

Several studies have highlighted the use of DNA-based N_C estimates to obtain valuable data for conservation and management and have sometimes obtained improved or substantially different estimates (10–50%) from traditional CMR data or previous perceptions (Kendall et al. 2009; Guschanski et al. 2009). In addition, N_C estimates have been used to assess the effects of hunting on population size and to set harvest quotas to avoid overharvest (Immell and Anthony 2008).

Future developments in N_C estimation

Great strides have been made in the expansion of classical CMR methods to include the estimation of parameters that affect $\hat{N}_C$, such as survival, recruitment, movement, and population growth as well as habitat occupancy, and even species diversity. In addition, a great deal of effort has been invested in developing model averaging for CMR parameters, in which parameter estimates from multiple "competing" models are combined into a weighted average, to improve inferences. There is no reason these advances cannot be applied using DNA-based studies (Lukacs and Burnham 2005).

The future should see expansion of non-invasive DNA methods of $\hat{N}_C$ calculation to include other biologically important parameters that explain underlying causes of changes in N_C, and perhaps even community ecology parameters such as the estimation of species richness (Cam et al. 2002). Perhaps the greatest improvement in N_C estimation will be when we devise ways to use genetic estimates of demographic movement and effective population size to inform our CMR models.

N_C estimation will be continually advanced by new DNA extraction and genotyping technologies (Perkel 2008)

that allow analysis of more individual samples that have low quality and quantities of DNA (Beja-Pereira et al. 2009). SNPs and short microsatellites can be genotyped on samples with poorer DNA than can be genotyped with typical microsatellites of 150–250 base pairs in length (Campbell and Narum 2009; Musgrave-Brown et al. 2007). Use of more samples could increase capture probability and improve estimates of capture heterogeneity to improve accuracy and precision in N_C estimates.

Future studies could include estimates an individual's age from remotely-sampled DNA. Quantitative (real time) PCR-based techniques can now assess telomere length (chromosome end length, which is correlated with age) from very small quantities of DNA (Criscuolo et al. 2009; Ren et al. 2009). Knowing the age of individuals will facilitate N_C estimation and assessment of N_e/N_C ratios. This telomere length QPCR method will likely need to be calibrated separately for each new species or taxon perhaps using different PCR primers or primer concentrations (Criscuolo et al. 2009). Future research is needed to verify this method's reliability and transferability among taxa.

Estimation of effective size

N_e is defined as the size of an ideal population (Fisher 1930; Wright 1931) that has the same rate of change of allele frequencies or heterozygosity as the observed population. We consider the two most used concepts of N_e: variance N_{eV} and inbreeding N_{eI} (Box 1, Table 2; Schwartz et al. 1999; Leberg 2005). N_{eV} equals N_{eI} for a single isolated population of constant size. Many publications do not mention this or make the distinction between the two quantities.

N_e estimators can be difficult to compare because different N_e concepts and estimators refer to different time frames and spatial scales (Schwartz et al. 1998). Three time frames include the contemporary (recent) time frame including the past one-to-few generations, the historical time frame including the past tens-to-thousands of generations, and the ancient time frame including thousands or millions of generations in the past (Wang 2005). We discuss mainly the contemporary time frame, for which N_e estimation is generally most feasible and reliable (Box 1).

The spatial scales of N_e, global versus local, are important to consider because they can influence interpretation of $\hat{N}_e$ and the assessment of population viability. Global N_e becomes important when considering long-term viability, and the maintenance of genetic variation and adaptive potential. Local (contemporary) N_e is generally

Box 1. Kinds of N_e estimators and concepts

Effective population size is whatever must be substituted in the formula (1/2N) to describe the actual loss in heterozygosity

Sewall Wright (1969)

Contemporary N_e can be estimated using demographic (direct) methods (Caballero 1994) or genetic (indirect) methods (Table 2). Demographic estimators often overestimate the true N_e because demographic estimators seldom include all the factors, such as variance in reproductive success, which can reduce N_e compared to the N_C (but see Saura et al. 2008 for an exception)

N_e estimates can be difficult to compare because they have been applied to many different measures of genetic change (Crow and Denniston 1988). For example, the inbreeding N_e (N_{eI}) is concerned with the loss of heterozygosity. The variance N_e (N_{eV}) is concerned with change in allele frequencies through time. Other forms of N_e exist (Ewens 1982; Crow and Denniston 1988; Wakeley and Sargsyan 2009). N_{eV} and N_{eI} are most widely used, well evaluated, and most useful estimators in conservation and management

N_{eV} is generally more sensitive for early detection of population declines or bottlenecks because N_{eV} generally declines rapidly during bottlenecks whereas N_{eI} does not change until inbreeding accumulates following increased mating between relatives, which occurs only a generation after a decline (Allendorf and Luikart 2007, p.159). N_{eV} is determined primarily by the number of offspring, which are few in number in declining populations, whereas N_{eI} is influenced more by the number of parents. For example, a population that declines from infinity to 2 individuals will have a N_{eV} near 2, but N_{eI} remains near infinity for one generation. It can be difficult to understand the practical consequences of the N_{eV} vs N_{eI} distinction because they can refer to different time periods depending on if parents and/or offspring are sampled and which N_e estimator is used (see Waples 2005)

The coalescent effective size (N_{eC}) concept considers, in theory, all aspects of genetic change, whereas other forms of N_e (N_{eV} and N_{eI}) include only a single measure of the rate of genetic drift (variance in allele frequencies) or inbreeding (heterozygosity) (Wakeley and Sargsyan 2009). The coalescent N_e might sometimes be preferable because the coalescent holds for a surprisingly wide range of population models including the Wright-Fisher models. The coalescent N_e concept could be helpful, for example, when considering the gametic disequilibrium N_e, i.e., LD-N_e, (Hill 1981; Waples and Do in press) which might contain information on both genetic drift and inbreeding due to few parents. Interestingly, coalescent-based N_e estimators apparently perform well in small populations (e.g. $N_e < 50$; Berthier et al. 2002; Tallmon et al. 2004; Anderson 2005) even though the assumption of only one coalescent event per generation is likely violated in small populations

Finally, it is worth mentioning the difficulty in long-term N_e estimation (Schwartz et al. 1999; Wang 2005; Charlesworth 2009). The difficulty is exemplified by Ovenden et al. (2007) who estimated that long-term N_e was 10 fold higher than the contemporary N_e in tiger prawns (*Penaeus esculentus*) from Morton Bay, Australia. The authors suggest it would be tempting to conclude there has been a recent reduction in N_e, but are quick to point out that the difference between the two $\hat{N}_e$'s (historical and contemporary) could result from assuming a mutation rate of 10^{-3} rather than 10^{-4}; the lower mutation rate would require a higher N_e to yield the same heterozygosity. The authors estimated contemporary N_e using standard temporal methods (Waples 1989), and the long-term N_e using heterozygosity assuming mutation-drift equilibrium, closed population (over thousands of years), and the infinite alleles model of mutation

Table 2 Types of N_e estimators including demographic and genetic methods, and contemporary versus long-term N_e. Bottleneck tests do not estimate N_e but rather provide evidence of a recent change in N_e

Demographic methods	Genetic methods		
Sex ratio bias	Long-term *Ne* (global)	Contemporary *Ne*(local)	Recent Changes of *Ne*
Fluctuating population size	*H*-level at mutation drift equilibrium	Temporalvariance (*F*) 2- sample; or 1-sample 'LD' methods	Bottleneck tests e.g. 1-sample test for a deficit of rare alleles
Variance in family size			
Simulations using mating system and vital rate data (age-specific survival & birth rates, etc.)			

more important than global N_e in the short term for avoiding inbreeding depression and immediate threats to population persistence. Local N_e will approach the global N_e of a species or metapopulation as gene flow (migration) increases among local subpopulations. Interestingly, some estimators of local N_e are relatively insensitive to gene flow (England et al. in review); this could lead researchers to conclude that the long-term local population viability is low, which is not necessarily true if gene flow continues to maintain high heterozygosity and a large global N_e along with the possibility of genetic rescue. Local N_e is more likely to be confounded with global N_e when using long-term historical N_e estimators because the assumption of no immigration is more likely to be violated over the long-term than the short-term.

One-sample estimators

The most widely used and well evaluated single-sample estimator of contemporary N_e is the linkage disequilibrium (LD) method (Hill 1981), which is usually considered an estimator of N_{eI}. The LD method is less widely used than the temporal method (two-sample methods below; Palstra and Ruzzante 2008), which seems surprising because LD methods require only one sample of individuals. However, the LD method for non-overlapping generations suffered from a severe bias until recently (England et al. 2006; Waples 2006), its performance is less well evaluated, and it has not yet been extended for use in species with overlapping generations. Further, LD methods were developed later (Hill 1981) than temporal methods (Krimbas and Tsakas 1971), and Hill (1981) concluded they were of little practical use because the noise from sampling error was large relative to the LD signal (Waples 1991). Fortunately, the severe bias for non-overlapping generations has been largely corrected (Waples 2006), and the bias and precision are being quantified for a wide range of population and sampling scenarios with non-overlapping generations (Waples and Do 2008; Waples and Do in press; England et al. in review).

The principle of the LD method is that as N_e decreases, genetic drift with few parents generates nonrandom associations among alleles at different loci, i.e., gametic disequilibrium or linkage disequilbrium (LD) (Hill 1981; Waples 1991). Thus, LD can be used to estimate N_e. Unlinked loci are usually used, but linked loci can provide increased precision if recombination rates are known, and could provide information on historical N_e if recombination rates are low for a set of loci (Wang 2005). Even for unlinked loci, LD can require several generations to decay, thus LD-N_e could contain information on the effective size from one or a few generations in the past. For example, in a declining population, LD-N_e could be biased-high for 1–2 generation reflecting the large N_e 1–2 generation ago (e.g. Waples 2005, 2006; England et al. in review).

Assumptions of LD-N_e are similar to the temporal method (Tables 3, 4), and require that the source of LD is from small N_e. LD-N_e assumes random mating, although an estimator exists for monogamous mating (Waples and Do 2008). Random selfing apparently does not bias LD-N_e (Weir and Hill 1980). Robustness of LD-N_e to violation of assumptions is little explored. LD-N_e estimates are susceptible to bias because numerous processes other than small N_e can generate LD including substructure, immigration or admixture, extensive close inbreeding, and overlapping generations.

Another problem related to overlapping generations is how to interpret estimates from LD-N_e for cohorts in terms of N_e per generation. If we sample a single cohort, then we can compute N_b (number of parents or breeding adults) from 1 year using LD-N_e. If we sample multiple cohorts, then we will be estimating something between N_b and N_e. Thus, our estimate of N_e from LD-N_e could be smaller than N_e per generation because you only sampled progeny of a fraction of the parents in the generation.

Precise estimates of N_e can be obtained with non-overlapping generations by using 10–20 microsatellite loci (5–10 alleles/locus) and samples of at least 25–50 individuals, if the effective population size is less than approximately 500 (Waples and Do in press). This performance is better than the standard temporal method that, given the same number of loci and individuals, is generally reliable for N_e up to $\sim$200

Table 3 Genetic estimators of contemporary N_e, their strengths, weaknesses, key assumptions[a], and performance in natural populations

N_e estimator	Strengths	Key assumptions[a]	Comments	*Software* & references
One sample				
Linkage Disequilibrium (LD)	Uses any ~10–20 unlinked loci, and 30–50 individuals	LD signal arises only from genetic drift	Potentially strongly biased by substructure, admixture, age structure, and small samples Not clear if purely an "inbreeding N_e" estimator (N_{eI})	*LD-Ne*; Waples and Do (2008), Waples and Do (in press)
Approximate Bayesian method using LD plus 7 other summary statistics	Uses more information than the LD method; Allows prior on N_e	LD signal arises only from genetic drift effects	Same as above but less biased and more precise in theory Limited to microsatellites Computes N_e over several generations in the past	ONeSAMP; Tallmon et al. (2008)
Heterozygote excess	Estimates *Nb* from single sample if *Nb* is very small	Signal only from different allele frequencies in male & female breeders	Confidence intervals often include infinity if $N_e > 20$ unless sample >200 progeny 80 independent alleles	*Nb_HetEx*, Zhandanova and Pudovkin (2008), Balloux (2004)
Identity dis-equilibrium at 1 & 2 loci	Estimates N_e and migration rate jointly	LD signal is from genetic drift and migration	Assumes population equilibrium and known parameters for the mating system	Vitalis and Couvet (2001)
Molecular coancestry (i.e. allele sharing among sampled individuals)	Estimates *Nb* from single sample if *Nb* is very small	Nonsib pairs needed as reference for co-ancestry among individuals	Precision is poor like the heterozygote excess method The coancestry is computed from alleles identical by descent & alike in state.	Nomura (2008)
Sib identification	Applies to non-random mating populations, codominant & dominant loci	Sibs & relatedness are reliably identified. No/low immigration	Based on identification of full and half sibs Also yields information on number of parents, sex ratio, & variance in family size CI's too narrow with few loci	*Colony2*, Wang (2009)
Rarefaction of alleles	Estimates of *Nb;* precision similar to the temporal method	Progeny are produced from few adults in a large H–W equilibrium population	Rarefaction of alleles in juveniles with respect to adults by simulating production of progeny cohorts from few adults	Hedgecock et al. (2007)
Two samples				
Heterozygosity decline	Computation is simple; much theory behind heterozygosity	Decrease in heterozygosity is caused only by small N_{eI}	Heterozygosity has an high variance and thus N_e estimation has low power Linear regression between H-loss & generations between samples	Harris and Allendorf (1989), Hauser et al. (2002), Miller and Waits (2003)
Temporal *F*-statistic moments method	Computationally rapid	Allele frequency change is only from drift; No selection or migration.	Bias and precision well quantified; an unbiased estimator exists for small sample sizes and skewed allele frequencies	N_e-*estimator,* Peel et al. (2004), *TempoFs* (Jorde and Ryman 2007) uses unbiased estimator;

Table 3 continued

N_e estimator	Strengths	Key assumptions[a]	Comments	*Software* & references
Pseudo-ML (maximum likelihood) temporal method	Computationally rapid; allows for migration.	Allele frequency change arises only from drift (& migration if also estimating *m*)	Extensive simulation evaluations have been conducted Joint estimation of N_e and migration rate (*m*) for continent-island metapopulation model	*MLNE*, Wang (2001), Wang and Whitlock (2003)
ML and MCMC temporal method	Useful on mult-allelic loci	Allele frequency changes only from drift	Computationally slow; Wright-Fisher model	*MCLEEPS*, Anderson (2000)
Coalescent Bayesian temporal method	Allows prior on N_e which can improve precision	Same as just above; One coalescent event per generation	Coalescent models are computationally faster than Wright-Fisher models (as in MCLEEPS)	*TM3*, Berthier et al. (2002); *CoNe*, Anderson (2005)
Three samples				
Coalescent Bayesian	Allows prior on N_e	Same as just above	*TMVP* is like *TM3* but allows for 3 temporal samples	*TMVP*, Beaumont 2003

[a] See also Table 4

(Berthier et al. 2002; Tallmon et al. 2004; Anderson 2005) if only a few generations of drift occur between temporal samples. It is extremely difficult to distinguish between N_e's of moderate and large size (e.g. 500 and 1000 or 5000) using any N_e estimator because the genetic signal is weak compared to sampling noise (Waples and Do in press).

Precision for estimates of N_e can be improved by roughly the same amount by sampling more individuals or by sampling more microsatellite loci (Waples 1989; Tallmon et al. 2004; Waples and Do in press; England et al. in review). However, precision can often be improved more by doubling the number of individuals versus doubling the number of loci, especially for loci with few alleles such as SNPs (T. Antao, G. Luikart, unpublished manuscript). Interestingly, 180 SNP loci provide roughly the same precision as 20 microsatellite loci with 10 alleles each (Waples and Do in press). Many species soon will have hundreds of SNPs available thanks to declining costs of SNP discovery and genotyping, and availability of commercial genotyping services (Perkel 2008). Precision increases rapidly with the square of the number of loci (or alleles), which suggests a bright future for LD-N_e estimation (Waples 1991; Waples and Do in press) considering increasing availability of numerous DNA markers.

Other single-sample estimators include the heterozygote excess method (Pudovkin et al. 1996; Luikart and Cornuet 1999; Balloux 2004), the coancestry method of Nomura (2008), Wang's sibship method (2009), Tallmon et al.'s multiple summary statistic method (ONeSAMP, Tallmon et al. 2008), and a rarefaction method (Hedgcock et al. 2007) (Table 3). The heterozygote excess method has poor precision and will be seldom useful unless N_e is less than ~30 (Zhdanova and Pudovkin 2008). ONeSAMP has the greatest potential to provide improved accuracy and precision because it uses multiple summary statistics and thus more information from the data; however, it has not been thoroughly evaluated and is currently limited to use with microsatellite loci. Limited indirect comparisons (Waples and Do in press) suggest that Wang's sibship method has comparable performance to LD-N_e.

Two-sample N_e estimators

The most widely used and well evaluated estimators of N_e are those based on two samples and temporal change in allele frequencies (Krimbas and Tsakas 1971, Waples 1989; Luikart et al. 1999; Wang 2001; Tallmon et al. 2004; Jorde and Ryman 2007; Palstra and Ruzzante 2008). The temporal method (Waples 1989) requires population samples taken from two or more time points. The principle of the method is that the magnitude of allele frequency change between generations will increase as N_e decreases following a curvilinear relationship.

Table 4 Assumptions common to many N_e estimators, and approaches to avoid violating assumptions

Assumption	Likelihood & consequences of violating assumptions, and ways to avoid violations
Population is sampled at random	Likely often violated, e.g. family groups over-represented; consequences poorly understood; family over-representation could bias-low LD-$\hat{N}_e$ and temporal $\hat{N}_e$. Tests for cryptic family or spatial structure should be conducted prior to N_e estimation (e.g. Hardy–Weinberg tests, clustering as in Pritchard et al. 2000).
Loci are sampled at random	Often violated, e.g. by choosing highly polymorphic loci; Unlikely to bias contemporary $\hat{N}_e$; likely biases long-term $\hat{N}_e$ based on equilibrium heterozygosity.
Unlinked, statistically independent markers	Not violated for most loci in most studies. Future studies with 100 s of loci might violate this assumption. Strongly linked loci should not be used unless linkage is accounted for (Hill 1981). Tests for nonindependence should be conducted. Non-independent loci should be excluded, except for LD-$\hat{N}_e$ which uses inter-locus associations to estimate N_e.
No subdivision of population	Likely occasionally or often violated. Could generate gametic disequilibrium and thereby bias LD-$\hat{N}_e$. Could bias-low temporal $\hat{N}_e$ if temporal allele frequency change results from sampling different proportions of each subpopulation each time period. Test for clusters, substructure & Wahlund effects before estimating N_e (e.g. H–W tests or clustering as in Pritchard et al. 2000).
No immigration	Relaxed in temporal method of Wang and Whitlock (2003). Researchers could test for immigrants with assignment tests and remove immigrants before estimating N_e; LD-$\hat{N}_e$ and temporal $\hat{N}_e$ appear insensitive to limited immigration ($m < 0.10$) in fragmenting populations although estimates increase with immigration (m) (England et al. in review; G.L., unpublished data).
No mutation	Not violated for most loci in most contemporary N_e estimates unless mutation rate extremely high. Likely often violated for long-term N_e estimates because a mutation model and rate must be estimated (assumed).
No selection	Seldom severely violated as most loci are effectively neutral; Allozymes and SNPs in genes are less likely neutral than most microsatellites; tests for neutrality and outlier loci (e.g. Luikart et al. 2003, Worley et al. 2006) should be conducted before estimating N_e. Alternatively, N_e could be estimated jointly with selection (Bollback et al. 2008).
No overlapping generations and no age structure	Relaxed in the modified temporal methods of Jorde and Ryman (1995) and Waples (1990). Standard temporal method is biased high or low depending on the population's demographic characteristics (Jorde and Ryman 1995, 1996; Waples and Yokota 2007) but potentially less biased with several generations between temporal samples; LD-$\hat{N}_e$ likely biased (e.g. by gametic disequilibrium generated by overlapping generations). The modified approach by Jorde and Ryman (1995) provides unbiased estimates.
Stable population size	Often violated but bias-effects are poorly understood. Possibly detectable with bottleneck tests (Piry et al. 1999) and by comparing N_ev and N_{eI} estimates. Relaxed in recently developed estimators for the temporal method (Beaumont 2003) and coalescent methods (BEAST Drummond and Rambaut 2007).
All individuals sampled are from one or consecutive cohorts	Allows estimation of Nb (number of breeding parents) for a cohort via gametic disequilibrium methods. Jorde and Ryman (1995) method requires individuals from consecutive cohorts.

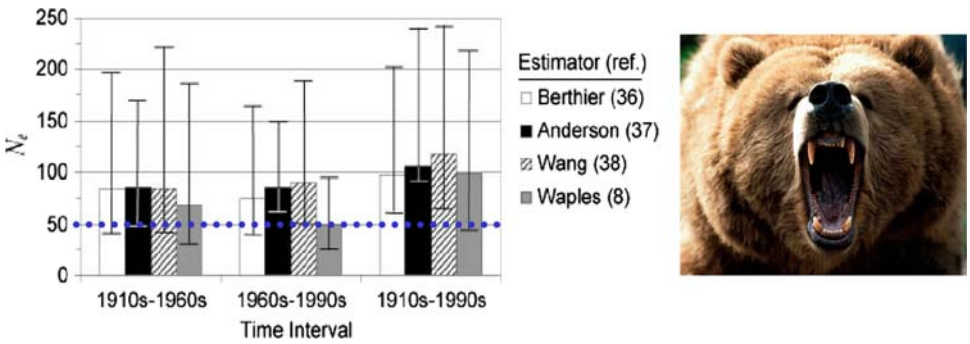

Fig. 2 Effective population size estimates from four standard temporal methods applied to microsatellite data from grizzly bears in Yellowstone National Park (Miller and Waits 2003) for three time intervals. The higher estimates from 1910s to 1990s could be biased due to fixation of alleles, which no longer drift once fixed and thereby cause underestimation of drift and overestimation of the N_e. The dotted horizontal line represents the possible true (unbiased) N_e if the estimators are biased high by ~50% as suggested possible in Waples and Yokota (2007).

The assumption of the temporal method that is most often violated is probably that of non-overlapping generations and lack of age structure (Table 4). Most natural populations of management concern have overlapping generations. Unfortunately, the standard temporal method can be severely biased when applied to species with overlapping generations (Fig. 3; Jorde and Ryman 1995, 1996; Palm et al. 2003; Waples and Yokota 2007).

Bias caused by overlapping generations is complex, difficult to predict, and depends on the species-specific survivorship pattern (the life table), the age classes sampled, and the sampling interval. For example, when simulating the amount of bias caused by ignoring the effect of overlapping generations in each of three model species, Waples and Yokota (2007) observed N_e estimates that were 50% high in the long-lived model species with low fecundity and a Type 1 survivorship curve, e.g. a large mammal (Box 2, Fig. 2). Similarly, they observed 50% low estimates in each of the two model species with moderate to high fecundity and Type 2 or Type 3 survivorship such as in some birds or highly fecund barnacles, fish, or trees. Further, bias is typically larger for samples from only one age class, e.g. when newborns are sampled at both occasions, and bias is smaller for large sampling intervals than for short ones (Jorde and Ryman 1995, 2007; Waples and Yokota 2007).

Box 2. Problems caused by biased $\hat{N}_e$

The most severe bias problem for conservation management would be if the true N_e was lower than the estimated $\hat{N}_e$. Overestimation of N_e could give a false sense of security, delay management action, lead to excessive loss of genetic variation, and perhaps extinction. A possible example comes from Yellowstone grizzly bears (*Ursus arctos horriblus*) for which the N_e could have been less than fifty ($N_e < 50$) even though standard temporal $\hat{N}_e$ estimators suggested the true N_e was ~80 (Fig. 2; Miller and Waits 2003), because the temporal method can be biased high by 50% (Waples and Yokota 2007). Such a bias could cause managers to delay management actions (e.g. translocations). Fortunately, for this example, the bias was probably less than 50% because samples were separated by several generations (1960s-1990s is approximately 3 bear generations), which apparently can reduce bias (Waples and Yokota 2007). In addition, the population is now much larger (Haroldson et al. in press)

Another common source of bias of the temporal method relates to the F statistic used for quantifying the amount of allele frequency change between sampling events (e.g. Waples 1989; Jorde and Ryman 2007; Waples and Yokota 2007). The traditional statistics are F_c (Nei and Tajima 1981) and F_k (Pollak 1983); they both produce biased estimates of N_e when sample sizes are small or allele frequencies are close to zero or one. Most studies employing the temporal method have used these traditional measures, and it is not until recently that an unbiased measure of the amount of drift and effective size, the so-called F_s measure, has been derived (Jorde and Ryman 2007).

Violation of the assumption of no immigration could bias-low N_e estimates if immigrants come from a population with divergent allele frequencies. Immigration from genetically similar populations is likely to bias-high the N_e estimate because local allele frequencies will change less rapidly as immigration will tend to maintain relatively constant allele frequencies; For example, immigration could bias-high N_e estimates in a local subpopulation of a large fragmenting population.

Precision of temporal methods is poor and generally more limiting than accuracy (bias) except when N_e is small, e.g. $N_e < 50$, such that the drift signal is strong, unless many individuals (50–100) and loci (15–30 microsatellites) are sampled. Precision can be improved roughly equally by increasing the sampling of more alleles, individuals, or generations separating samples (Waples 1989). However, likelihood methods often benefit most by sampling more individuals (Tallmon et al. 2004). Among the numerous standard temporal N_e estimators (Table 3), the likelihood based estimators are often considered less biased and more precise (Wang 2001; Berthier et al. 2002; Beaumont 2003; Anderson 2005; but see Jorde and Ryman 2007, p. 935).

Two variations of the standard temporal method allow for estimation of N_e with overlapping generations. The most general model is from Jorde and Ryman (1995), who showed that temporal change in allele frequencies depends not only on N_e but also on age structure and age-specific birth and survival rates (Fig. 3). In combination with the F_S

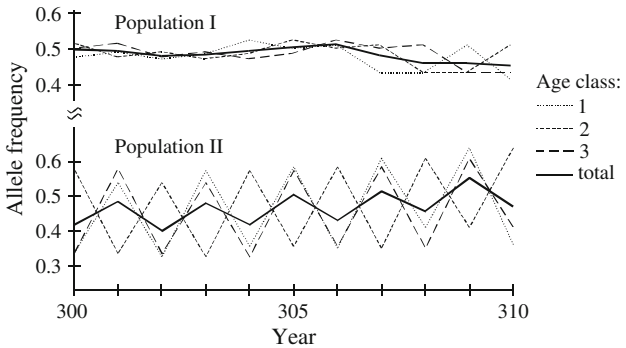

Fig. 3 Temporal allele frequency change over 10 years for separate age classes and for the total population in each of two simulated populations (I and II) with overlapping generations. Populations have different age-specific reproduction rates but have the same effective size ($N_e = 200$), total (census) sizes ($N_C = 300$), and generation interval (G = 2 years). In population I, the reproduction is evenly distributed over age-classes, whereas in II the 2-year old individuals dominate reproduction. In both cases the simulation was started in year 0 with identical allele frequencies (0.5) in all age-classes. In spite of their similar N_es, the two populations display different temporal allele frequency change, within age classes as well as for the total population. The pattern of annual shifts, particularly obvious for population II, is introduced because each age-class is similar not to the age-class in the previous year, but to the age-class to which most of the breeders of the previous year belonged, and those breeders were young several years previously. Modified from Jorde and Ryman 1995

estimator for quantifying the amount of genetic drift (Jorde and Ryman 2007), this method provides unbiased N_e estimates if age-specific survival and birth rates are available. The precision appears reasonable when using 10–20 microsatelites and samples of 50–100 individuals if $N_e < 50$–100 (Jorde and Ryman 1995; Waples and Yokota 2007). Waples (1990, 2002) developed a second modified temporal approach for species with a semelparous life history as in many Pacific salmon.

Other potentially useful temporal estimators of contemporary N_e are listed in Table 3. More research is needed to evaluate their bias, precision, and reliability in relation to the standard temporal method and to the temporal method for overlapping generations.

Examples

The feasibility of estimating contemporary N_e for an abundant and highly fecund invertebrate species was illustrated using simulations and empirical data from the tiger prawn (Ovenden et al. 2007), mentioned above. The authors genotyped eight microsatellite loci (130 independent alleles) and sampled approximately 500–600 individuals per year from 2001 to 2003 in Morton Bay, Australia. The population is relatively isolated and has largely non-overlapping generations. The moments based standard temporal method gave $\hat{N}_e$ estimates of 797 (95%CI: 366–4,182) for 2001–2002, and 1,304 (393–4,960) for 2002–2003; likelihood based temporal estimates were slightly higher. Simulation studies agreed with empirical results that these sampling conditions should give non-infinite upper confidence limits, but suggested that $\hat{N}_e$ might overestimate the true size (Ovenden et al. 2007). LD-$\hat{N}_e$ estimates were biased high in simulated and real data; however the authors did not use the recently improved (less biased) LD-N_e estimator (Waples and Do 2008).

The effects of a supplementation program on N_e were recently investigated in Chinook salmon, *Oncorhynchus tshawytscha* (Eldridge and Killebrew 2008). The authors estimated N_{eI} from demographic data and N_{eV} from genetic data using 14 microsatellites and the temporal method modified for overlapping generations and semelparous species (Waples 1990). The N_{eI} and N_{eV} estimates were different in some years but both declined from around 1,000 to near 500 (1974–1999) and were apparently similarly informative about the decline in N_e; similar results were reported in a related but independent study by Hedrick et al. (2000). The N_e/N_C ratio decreased following supplementation, due to differences in the sex ratio in the broodstock of the supplementation program. The authors suggested it was difficult to draw conclusions because of assumptions associated with the estimators. Nonetheless, this example illustrates the potential usefulness of N_e estimation and monitoring to detect declines in natural populations.

Effects on N_e of life history (trout versus salmon) and the performance of N_e estimators were investigated by Fraser et al. (2007). N_e estimates were higher but more variable within salmon populations than within trout populations as expected from salmon having larger and more variable population sizes. Linkage disequilibrium data yielded N_e estimates of similar magnitude as temporal methods in both systems. The authors emphasize the importance of temporal sampling replication and the need to consider effects of violating assumptions of contemporary N_e estimators in future research.

Comparison of N_e estimators & bottleneck tests

The LD-N_e estimator is more powerful than the temporal method for early detection of a bottleneck or fragmentation. For example, if we sample only two generations after a bottleneck to size $N_e = 100$, the power of LD-N_e is 0.80 versus only $\sim$0.60 for the temporal method when using reasonable numbers of loci and individuals (Fig. 4; T. Antao, G. Luikart, unpublished manuscript). This higher power of LD-N_e, compared to the temporal method makes the LD-N_e, method promising for detecting population declines.

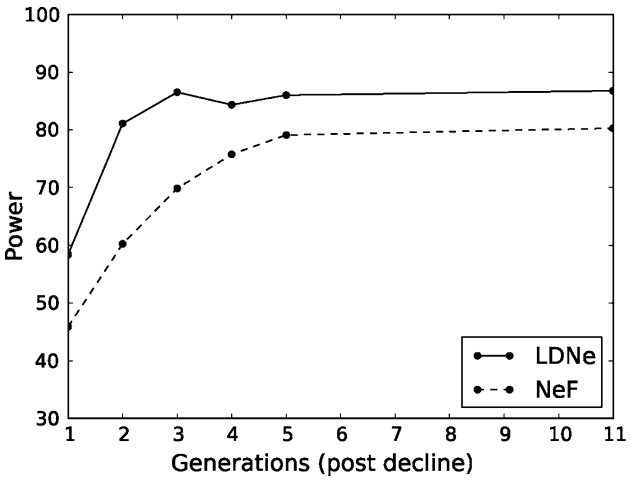

Fig. 4 Power of the linkage disequilibrium N_e estimator (N_eLD) compared to the standard temporal method (N_eF), for detecting a population decline to $N_e = 100$ after 1-to-10 generations. The decline was an instantaneous drop from $N_e = 600$. Simulations were conducted using ideal populations, and thus $N_e \approx N_C$. The temporal method gives an N_e estimate using two samples of 50 individuals (one pre-decline sample, and one post-decline sample at generation 1, or 2, or 3, etc.) and 20 loci with approximately 8 alleles per locus. The LDNe method used only one post-decline sample. Power is defined as the proportion of 1,200 independent simulations where the point estimate of N_e is less than 80% of the pre-decline size (0.80*600 = 480). From Antao, G. Luikart unpublished manuscript

One-sample N_e estimators can be used to detect a population decline if applied to two temporally-spaced samples, for example, if the second N_e estimate is lower than the first N_e estimate. Bottleneck tests based on allele frequency distributions differ from N_e estimation in that no point estimate of N_e is obtained from a bottleneck test (Table 2). The LD-N_e estimator has power similar to a bottleneck test (Cornuet and Luikart 1996) for heterozygosity excess (G. Luikart, unpublished data). Future research is needed to compare the sensitivity of bottleneck tests and N_e estimators for monitoring to detect population declines.

Future developments in N_e estimation

A novel advance in N_e estimation would involve detecting locus-specific selection while jointly estimating N_e. Soon, hundreds of loci will be used to estimate N_e. Consequently selection might increasingly influence certain loci and subsequently cause biased $\hat{N}_e$ by violating assumptions of neutrality (Table 4). New temporal N_e methods can estimate $\hat{N}_e$ and detect selection at individual loci (Bollback et al. 2008). An exciting benefit of this approach could be in advancing understanding the relative role of drift and selection in populations (e.g. Simões et al. 2009).

Another relatively novel application would be the use of N_e estimators to identify ecological factors that influence N_e, for example by testing for associations between N_e and environmental change or stress. Also new would be the use of different sets of linked loci with different recombination rates, which could allow approximate dating of past bottlenecks by using independent sets of tightly linked and loosely linked loci (Wang 2005).

Great potential for improving N_e estimators comes from Approximate Bayesian Computation (ABC). ABC is employed to estimate the N_e by using "summary statistic matching" between simulated populations and the study population of interest (Cornuet et al. 2008). The use of simulated populations allows simulation-modeling of realistic demography, age structure, and sampling, which can provide parameter estimates that are less biased than methods that do not explicitly consider demography, age structure, and sampling (Tallmon et al. 2004, 2008). ABC also facilitates the use of prior information such as $\hat{N}_C$, and the combining of multiple summary statistics (e.g. LD, temporal F statistics, heterozygote excess etc.), which can increase accuracy and precision by using more information from the data. Bayesian approaches using MCMC algorithms are promising because they use all the information in the raw data, not just summary statistics; However, they tend to be excessively computationally intensive, which makes performance evaluation difficult because a single N_e estimate for a simulated scenario could take days (but see Anderson 2005).

Perhaps the greatest overall advancement in estimating N_e will come from combining the genotyping of hundreds or thousands of loci with new computational approaches (e.g. ABC). For example, distinguishing between a moderate and large N_e is currently impossible because the signal from genetic drift is weak relatively noise from sampling only 20–30 microsatellites and 50–100 individuals. Genotyping many loci combined with new computational methods could allow researchers to resolve between an N_e of 500 and 1000 or 5000, which is currently challenging or impossible. In addition, genotyping linked loci can provide increased precision if recombination rates are known.

Future research is needed to facilitate N_e estimation in populations with overlapping generations and age structure. We suggest that investigators and reviewers acknowledge that treating overlapping generations as if they were discrete introduces a bias that might be substantial. Papers ignoring the difference should not be accepted unless the authors can argue convincingly that the effect is expected to be minor. When generations overlap, individuals generally should be aged and referred to appropriate cohorts. The ABC approach may be valuable here because there is yet no theory for how to deal with cohorts that are not consecutive in the temporal method (which is the basis for the method of Jorde and Ryman 1995). PCR-based estimates of an individual's age (Criscuolo et al. 2009) could improve our ability to obtain cohort information to estimate N_e noninvasively in age structured populations.

Future research is needed to develop estimators that account for migration under a range of metapopulation models. The estimator of Wang and Whitlock (2003) is a good example of what is needed, because it estimates N_e and m jointly and thus allows for migration. However, this estimator is limited to a specific scenario with continent-island migration at equilibrium, and thus is not a general method to account for migration when estimating temporal N_e. Estimators and evaluations are needed for a range of metapopulaton models including the stepping stone, island, and isolation by distance models, along with models including non-equilibrium scenarios such as population fragmentation, and both symmetrical and asymmetrical gene flow. Highly valuable future advances will deal with the effects of gene flow to allow estimation of local and global N_e, as well as short-term and long-term N_e.

A key improvement in interpreting estimates of N_e lies in conducting extensive performance evaluations using both simulated and empirical data from populations with known parametric N_e. Simulations could quantify effects on $\hat{N}_e$ of sampling strategies (Schwartz and McKelvey 2008), age structures, and immigration patterns for both

equilibrium and non-equilibrium populations such as fragmenting populations.

Finally, future empirical research should compute $\hat{N}_e$ using different N_e estimators for the same population (at the multiple time periods), for independent populations, and for species with different life histories. Such studies with multiple estimators, time points, and species will advance understanding of the performance of estimators and increase our understanding of the true effective size in wild populations important for conservation and management.

Estimating the N_e/N_C ratio

Knowing the approximate ratio of effective size to population size (N_e/N_C ratio) for a species might be useful for inferring N_e from N_C (or vice versa). For example, bear biologists often estimate N_C and would like to also know N_e (e.g. Box 2). Estimating only one parameter (either N_e or N_C) to infer both could save time and money. Knowing the N_e/N_C ratio could also help determine the ecological factors that reduce N_e below the N_C (Kalinowski and Waples 2002). For example, knowing if certain factors (mating system, fecundity, or survivorship) always lead to the same reduction in N_e from N_C would help researchers infer N_e from N_C. Another use of understanding N_e/N_C ratios is in predicting how management actions could increase N_e or the N_e/N_C ratio to maintain genetic variation (Cooper et al. 2009).

Inferring N_e from N_C (or vice versa) would be possible if the N_e/N_C ratio remains stable over time or for certain taxa, for example. Unfortunately, little is known about the stability of N_e/N_C ratio, although studies are accumulating.

Ardren and Kapuscinski (2003) estimated N_C and N_e over 17 years for steelhead trout (*Oncorhynchus mykiss*) from Washington State. N_e was estimated using the approach of Waples (2002) that accounts for overlapping generations and the semelparous life history of many salmonids. They reported that the N_e was constant ($N_e = 250$ or 300) while N_C declined from 113 to 50 over 17 years. This suggested the N_e/N_C ratio fluctuates and one cannot be used to infer the other in trout. It seems possible that immigration maintained the relatively high N_e. However, heterozygosity declined by 2.7% during the study (from 1977 to 1994), suggesting no immigration and that $\hat{N}_{eI}$ (which could be inferred from heterozygosity-loss) was near that estimated by the temporal method ($\hat{N}_{eV}$).

Hauser et al.'s (2002) estimates of the N_e/N_C ratio were constant ($1.8–2.8 \times 10^{-5}$) over periods of 22–48 years in snapper (*Pagrus auratus*), an exploited fish in New Zealand. Pray et al. (1996) calculated the N_e/N_C ratio in seven population size treatments of the red flour beetle (*Tribolium castaneum*) where the N_C ranged from two to 960. They found that the N_e/N_C ratio decreased as census size increased—large populations had a proportionally smaller N_e/N_C ratio than small populations. Palstra and Ruzzante (2008) reviewed N_e estimates from 83 studies using the temporal variance (N_{eV}) method; they found, like Ardren and Kapuscinski (2003) that the N_e/N_C ratio generally increased as N_C decreased among 28 salmonid populations. Similarly, Ficetola et al. (2009) recently showed that in frogs the N_e/N_C ratio increased as N_C *decreased* due to decreased variance in male reproductive success (polygamy) at low N_C.

The N_e/N_C ratio is more likely to be predictable in species with low fecundity and low variance in reproductive success than in species with high variance in reproductive success. For example, in brown bears, the N_e/N_C ratio has been estimated from 0.20 to 0.38 when using a range of variance in reproductive success in simulation models (Harris and Allendorf 1989), and when using the temporal method and microsatellite data (Miller and Waits 2003). Male and female variance in reproductive success might be relatively low in bears, thus the N_e/N_C ratio is unlikely to fluctuate extensively, although it could conceivably drop to say $N_e/N_C = 0.10$ if male or female variance in reproductive success were high, e.g. due to repeated reproductive failure of some individuals, for example. Ungulates could have higher variance in reproductive success because one male can potentially mate with many females, for example; thus N_e and the N_e/N_C ratio could fluctuate more through time than in bears. Marine fish, trees, and invertebrates have very high fecundity and variance in reproductive success such that N_e/N_C ratios can fluctuate enormously, and N_e cannot be estimated from N_C reliably.

While we can potentially infer N_e from N_C in species with a certain life history (low variance in reproductive success), it seems risky to quantitatively infer N_e from N_C (and vice versa), because cryptic reductions in N_e can occur without reductions in N_C; More empirical and simulation research is needed to understand the change in the N_e/N_C ratio (in stable and fluctuating populations), and to facilitate reliable inference of one from the other.

Reductions in N_e can occur even when census population sizes remain large if variance in reproductive success increases, for example. The only way to detect such 'cryptic' genetic bottlenecks of exploited populations is empirical observation of genetic variation over time. Genetic monitoring programs can provide a powerful means to detect loss of genetic variation if enough marker loci are used (Luikart et al. 1998; Schwartz et al. 2007).

Fortunately, both N_e and N_C often can be estimated from the same molecular genetic data. This is encouraging because it suggests we will soon accumulate more

knowledge about N_e/N_C ratios (and their stability) in natural populations. One way to approach this problem would be to sample populations regularly (annually) using a robust design approach to obtain estimates of N_e and N_C, in addition to survival estimates. It may also be beneficial to combine open N_C models, which provide recruitment and survival estimates, with two sample N_e estimators.

Future research is badly needed to improve our ability to predict N_e from N_C and vice versa. Both empirical studies and simulation studies with detailed life histories (age and sex specific birth and death rates) are needed from many taxa to address the following questions: (1) which taxa have a relatively stable N_e/N_C ratio? (2) what life history and environmental factors causes the ratio to fluctuate or change? (3) how can we design studies to estimate both N_e and N_C from the same data (e.g. noninvasive sampling).

Summary and conclusions

Estimates of N_C and N_e using one- and two-sample methods often can be obtained from the same samples of loci and individuals, e.g. 50–100 individuals and 10–20 loci. Future research should more often include estimates of both N_e and N_C, and thus the N_e/N_C ratio. The N_e/N_C ratio could vary within and among populations, and apparently increases as N_C decreases in salmonids and frogs. More empirical and simulation research is needed to determine if fluctuations are small enough in certain taxa, e.g. large carnivores, to allow inference of N_e from N_C (and vice versa).

N_C estimators are becoming increasingly sophisticated and widely used thanks largely to noninvasive DNA sampling. The availability of many models (open, closed, and occupancy), frameworks for model-testing, and performance evaluations using simulated and empirical datasets has greatly improved our ability to estimate N_C. More evaluations would help understand the sampling effort required to account for capture heterogeneity and achieve high capture probability, the two most crucial aspects in N_C estimation. The following quote from Guschanski et al. (2009) suggests that DNA-based N_C estimation will continue to expand: "Newly improved molecular methods allow fast, efficient, and relatively affordable genotyping, suggesting that genetic censusing <abundance estimation> can be widely applied to provide accurate and reliable population size estimates for a wide variety of species."

N_e estimators have greatly improved in the past few years. For example, there is now an unbiased estimator for the temporal method. Similarly, the LD-N_e estimator now gives relatively unbiased point estimates and narrower confidence intervals from a single sample than the standard temporal method using two samples. Nonetheless, future development and performance evaluations are needed because many methods including LD-N_e cannot be applied to populations with overlapping generations, age structure, or immigration. With 100 s of markers and efficient approximate Bayesian methods, it could soon become possible to jointly estimate, N_e, m, and detect selection, as well as conduct thorough performance evaluations use computer simulations.

The rapid increase in availability of DNA data and new statistical approaches is exciting and ensures that knowledge of N_e, N_C, and N_e/N_C ratios will greatly accelerate in the next years. Our ability to estimate population size parameters has vastly improved over the past decade, and will continue to improve in the future given rapid advances in genomic and computational technologies (Hauser and Seeb 2008; Haussler et al. 2009). As more organizations look to genetic data for monitoring natural populations (Schwartz et al. 2007) it becomes important understand the relative power and reliability of both N_C and N_e in detecting population declines. It is an exciting time to be a conservation geneticist.

Acknowledgements This article is based partially on work supported by the U.S. National Science Foundation Grant DEB 074218 to F.W.A, and G.L. G.L. was supported by the Portuguese-American Science Foundation, CIBIO-UP, the National Park Service (USA) and research grant PTDC/BIA-BDE/65625/2006 from the Portuguese Science Foundation (FCT). We thank R. Waples and R. Harris for many helpful citations and comments, and P. Taberlet for the citation on PCR-based aging of birds. Some ideas here arose from the Genetic Monitoring Working Group jointly supported by the National Evolutionary Synthesis Center (NSF #EF-0423641) and the National Center for Ecological Analysis and Synthesis, a Center funded by NSF (NSF #EF-0553768), the University of California, Santa Barbara, and the State of California. This work also benefited from association with the ESF Science Networking Programme ConGen.

References

Allendorf FW, Luikart G (2007) Conservation and the genetics of populations. Blackwell, Malden

Alter SE, Rynes E, Palumbi SR (2007) DNA evidence for historic population size and past ecosystem impacts of gray whales. Proc Natl Acad Sci USA 104:15162–15167

Anderson EC (2005) An efficient Monte Carlo method for estimating N_e from temporally spaced samples using a coalescent-based likelihood method. Genetics 170:955–967

Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: a workbench to detect molecular adaptation based on an F_{st}-outlier method. BMC Bioinformatics 9:323

Ardren WR, Kapuscinski AR (2003) Demographic and genetic estimates of effective population size (N_e) reveals genetic compensation in steelhead trout. Mol Ecol 12:35–49

Balloux F (2004) Heterozygote excess in small populations and the heterozygote-excess effective population size. Evolution 58:1891–1900

Beaumont MA (2003) Estimation of population growth or decline in genetically monitored populations. Genetics 164:1139–1160

Beja-Pereira A, Oliviera R, Alves PC, MKSchwartz MK, Luikart G (2009) Advancing ecological understandings through technological transformations in noninvasive genetics. Mol Ecol Resour (in press)

Bellemain E, Swenson JE, Tallmon DA, Brunberg S, Taberlet P (2005) Estimating population size of elusive animals with DNA from hunter-collected feces: comparing four methods for brown bears. Conserv Biol 19:150–161

Berthier P, Beaumont MA, Cornuet J-M, Luikart G (2002) Likelihood-based estimation of the effective population size using temporal changes in allele frequencies: a genealogical approach. Genetics 160:741–751

Bollback JP, York TL, Nielsen R (2008) Estimation of 2 Nes from temporal allele frequency data. Genetics 179:497–502

Boulanger J, Stenhouse G, Munro R (2004) Sources of heterogeneity bias when DNA mark-recapture sampling methods are applied to grizzly bear (*Ursus arctos*) populations. J Mammal 85:618–624

Boulanger J, White GC, Proctor M, Stenhouse G, MacHutchon G, Himmer S (2008a) Use of occupancy models to estimate the influence of previous live captures on DNA-based detection probabilities of grizzly bears. J Wild Manag 72:589–595

Boulanger J, Kendall KC, Stetz JB, Roon DA, Waits LP, Paetkau D (2008b) Multiple data sources improve DNA-based mark-recapture population estimates of grizzly bears. Ecol Appl 18:577–589

Caballero A (1994) Developments in the prediction of effective population size. Heredity 73:657–679

Cam E, Nichols JD, Sauer JR, Hines JE (2002) On the estimation of species richness based on the accumulation of previously unrecorded species. Ecography 25:102–108

Campbell NR, Narum SR (2009) Quantitative PCR assessment of microsatellite and SNP genotyping with variable quality DNA. Conser Genet (in press)

Charlesworth B (2009) Effective population size and patterns of molecular evolution and variation. Nat Rev Genet 10:195–205

Cooch E, White G (2006) Program MARK: a gentle introduction, 5th edn. Colorado State University. Fort Collins, CO, USA. http://www.phidot.org/software/mark/docs/book/

Cooper AM, Miller LM, Kapuscinski AR (2009) Conservation of population structure and genetic diversity under captive breeding of remnant coaster brook trout (*Salvelinus fontinalis*) populations. Conserv Genet 10:0621–1566

Cornuet J-M, Luikart G (1996) Description and evaluation of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

Cornuet J-M, Santos F, Beaumont MA, Robert CP, Marin J-M, Balding DJ, Guillemaud T, Estoup A (2008) Inferring population history with DIY ABC: a user-friendly approach to Approximate Bayesian Computation. Bioinformatics 24:2713–2719

Criscuolo F, Bize P, Nasir L, Metcalfe NB, Foote CG, Griffiths K (2009) Real-time quantitative PCR assay for measurement of avian telomeres. J Avian Biol 40:342–347

Crow JF, Denniston C (1988) Inbreeding and variance effective population numbers. Evolution 42:482–495

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214

Eggert LS, Eggert JA, Woodruff DS (2003) Estimation population sizes for elusive animals: elephants of Kakum National Park, Ghana. Mol Ecol 12:1389–1402

Eldridge WH, Killebrew E (2008) Genetic diversity over multiple generations of supplementation: an example from Chinook salmon using microsatellite and demographic data. Conserv Genet 9:13–28

England PR, Cornuet J-M, Berthier P, Tallmon DA, Luikart G (2006) Estimating effective population size from linkage disequilibrium: severe bias using small samples. Conserv Genet 7:303–308

England PR, Luikart G, Waples RS (in review) Early detection of population fragmentation using linkage disequilibrium estimation of effective population size

Ewens WJ (1982) On the concept of the effective population size. Theor Popul Biol 21:373–378

Ficetola GF, Padoa-Schioppa E, Wang J, Garner TWJ (2009) Polygyny, census and effective population size in the threatened frog, *Rana latastei.* Anim Conserv (in press)

Fisher RA (1930) The genetical theory of natural selection. Oxford University Press, Oxford

Frankham R (1995) Effective population-size/adult-population size in wildlife populations: a review. Genet Res 66:95–107

Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. Genet Res 66:95–107

Frantz AC, Roper TJ (2006) Simulations to assess the performance of different rarefaction methods in estimating population size using small datasets. Conserv Genet 7:315–318

Fraser DJ, Hansen MM, Ostergaard S, Tessier N, Legault M, Bernatchez L (2007) Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. Mol Ecol 16:3866–3889

Guschanski K, Vigilant L, McNeilage A, Gray M, Kagoda E, Robbins MM (2009) Counting elusive animals: comparing field and genetic census of the entire mountain gorilla population of Bwindi Impenetrable National Park, Uganda. Biol Conserv 142:290–300

Haroldson M, Schwartz C, Kendall K, Gunther K, Moody D, Frey K, Paetkau D (in press) Genetic analysis of individual origins supports isolation of grizzly bears in the Greater Yellowstone Ecosystem. Ursus

Harris RB, Allendorf FW (1989) Genetically effective population-size of large mammals—an assessment of estimators. Conserv Biol 3:181–191

Harris RB, Winnie JR, Amish S, Beja-Pereira A, Godinho R, Luikart G (in press) Population estimation of argali (*Ovis ammon*) in the Afghan Pamir using capture-recapture modeling from fecal DNA. J Wildl Manage

Hauser L, Seeb JE (2008) Advances in molecular technology and their impact on fisheries genetics. Fish Fish 9:473–486

Hauser L, Adcock GJ, Smith PJ, Ramirez JHB, Carvalho GR (2002) Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). Proc Natl Acad Sci USA 99:11742–11747

Haussler D et al (2009) Genome 10K: A proposal to obtain whole-genome sequence for 10,000 vertebrate species. J Hered 100:659–674

Hedgecock D, Launey S, Pudovkin AI, Naciri Y, Lapègue S, Bonhomme F (2007) Small effective number of parents (*N*b) inferred for a naturally spawned cohort of juvenile European flat oysters *Ostrea edulis*. Mar Biol 150:1173–1182

Hedrick P, Hedgecock D, Hamelberg S, Croci S (2000) The impact of supplementation in winter-run Chinook salmon on effective population size. J Hered 91:112–116

Hilborn R (2002) The dark side of reference points. Bull Mar Sci 70:403–408

Hill WG (1981) Estimation of effective population size from data on linkage disequilibrium. Genet Res 38:209–216

Huggins RM (1991) Some practical aspects of a conditional likelihood approach to capture experiments. Biometrics 47:725–732

Immell D, Anthony RG (2008) Estimation of black bear abundance using a discrete DNA sampling device. J Wildl Manag 72:324–330

Jorde PE, Ryman N (1995) Temporal allele frequency change and estimation of effective size in populations with overlapping generations. Genetics 139:1077–1090

Jorde PE, Ryman N (1996) Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. Genetics 143:1369–1381

Jorde PE, Ryman N (2007) Unbiased estimator for genetic drift and effective population size. Genetics 177:927–935

Kalinowski SJ, Waples RS (2002) Relationship of effective to census size in fluctuating populations. Conserv Biol 16:129–136a

Kendall KC, Stetz JB, Roon DA, Waits LP, Boulanger JB, Paetkau D (2008) Grizzly bear density in Glacier National Park, Montana. J Wildl Manag 72:1693–1705

Kendall KC, Stetz JB, Boulanger JB, Mcleod AC, Paetkau D, White GC (2009) Demography and genetic structure of a recovering grizzly bear population. J. Wildl Manag 73:3–17

Knapp SM, Craig BA, Waits LP (2009) Incorporating genotyping error into non-invasive DNA-based mark-recapture population estimates. J Wildl Manage 73:598–604

Kohn MH, York EC, Kamradt DA, Haught G, Sauvajot RM, Wayne RK (1999) Estimating population size by genotyping faeces. Proc R Soc Lond B 266:657–663

Krimbas CB, Tsakas S (1971) The genetics of *Dacus oleae* V. Changes of esterase polymorphism in a natural population following insecticide control—selection or drift? Evolution 25:454–460

Leberg P (2005) Genetic approaches for estimating the effective size of populations. J Wildl Manag 69:1385–1399

Luikart G, Cornuet J-M (1999) Estimating the effective number of breeders from heterozygote-excess in progeny. Genetics 151:1211–1216

Luikart G, Sherwin W, Steele B, Allendorf FW (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Mol Ecol 7:963–974

Luikart G, Cornuet J-M, Allendorf FW (1999) Temporal changes in allele frequencies provide estimates of population bottleneck size. Conserv Biol 13:523–530

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. Nat Rev Genet 4:981–994

Lukacs PM, Burnham KP (2005) Review of capture recapture methods applicable to noninvasive sampling. Mol Ecol 14:3909–3919

Lukacs PM, Eggert LS, Burnham KP (2007) Estimating population size from multiple detections with non-invasive genetic data. Wildl Biol Practice 3:83–92

MacKenzie DI, Royle JA (2005) Designing occupancy studies: general advice and allocating survey effort. J Appl Ecol 42:1105–1114

MacKenzie DI, Nichols JD, Royle JA, Pollock KH, Bailey LL, Hines JE (2005) Occupancy estimation and modeling. Academic Press, Burlington, MA, USA

MacKenzie DI, Nichols JD, Royle JA, Pollock KP, Bailey LL, Hines JE (2006) Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press. San Diego, California, USA

Marucco F, PletscherDH, Boitani L, Schwartz MK, Pilgrim C, Lebreton JD (2009) Wolf survival and population trend using non-invasive capture-recapture techniques in the Western Alps. J Appl Ecol (in press)

Miller CR, Waits LP (2003) The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): implications for conservation. Proc Natl Acad Sci USA 100:4334–4339

Miller CR, Joyce P, Waits L (2005) A new method for estimating the size of small populations from genetic mark-recapture data. Mol Ecol 14:1991–2005

Musgrave-Brown E, Ballard D, Balogh K et al (2007) Forensic validation of the SNPforID 52-plex assay. Forensic Sci Int 1:186–190

Nei M, Tajima F (1981) Genetic drift and estimation of effective population size. Genetics 98:625–640

Nomura T (2008) Estimation of effective number of breeders from molecular coancestry of single cohort sample. Evol Appl 1:462–474

Nunney L (1993) The influence of mating system and overlapping generations on effective population size. Evolution 47:1329–1341

Nunney L (2002) The effective size of annual plant populations: the interaction of a seed bank with fluctuating population size in maintaining genetic variation. Am Nat 160:195–204

Ovenden J, Peel D, Street R, Courtney A, Hoyle S, Peel S, Podlich H (2007) The genetic effective and adult census size of an Australian population of tiger prawns (*Penaeus esculentus*). Mol Ecol 16:127–138

Palm S, Laikre L, Jorde PE, Ryman N (2003) Effective population size and temporal genetic change in stream resident brown trout (*Salmo trutta* L.). Conserv Genet 4:249–264

Palstra FP, Ruzzante DE (2008) Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Mol Ecol 17:3428–3447

Peel D, Ovenden JR, Peel SL (2004) Neestimator: software for estimating effective population size, Queensland Government, Department of Primary Industries and Fisheries, Brisbane, Australia, version 12

Perkel J (2008) SNP genotyping: six technologies that keyed a revolution. Nat Methods 5:447–453

Petit E, Valiere N (2006) Estimating population size with noninvasive capture-mark-recapture data. Conserv Biol 20:1062–1073

Piry S, Luikart G, Cornuet J-M (1999) Bottleneck: a computer program for detecting recent reductions in effective population size from allele frequency data. J Hered 90:502–503

Pollak E (1983) A new method for estimating the effective population size from allele frequency changes. Genetics 104:531–548

Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: causes, consequences and solutions. Nat Rev Gen 6:847–850

Pray LA, Goodnight CJ, Stevens L, Schwartz JM, Yan G (1996) The effect of population size on effective population size: an empirical study in the red flour beetle *Tribolium castaneum*. Genet Res 68:151–155

Prugh LR, Ritland CE, Arthur SM, Krebs CJ (2005) Monitoring coyote population dynamics by genotyping faeces. Mol Ecol 14:1585–1596

Pudovkin AI, Zaykin DV, Hedgecock D (1996) On the potential for estimating the effective number of breeders from Heterrozygote-excess in progeny. Genetics 144:383–387

Puechmaille S, Petit E (2007) Empirical evaluation of non-invasive Capture-Mark-Recapture estimates of population size based on a single sampling session. J Appl Ecol 44:843–852

Ren F, Li C, Xi H, Wen Y, Huang K (2009) Estimation of human age according to telomere shortening in peripheral blood leukocytes of Tibetan. Am J Forensic Med Pathol 30:252–255

Robinson SJ, Waits LP, Martin ID (2009) Estimating abundance of American black bears using DNA-based capture-mark-recapture models. Ursus 20:1–11

Ryman N, Baccus R, Reuterwall C, Smith MH (1981) Effective population size, generation interval, and potential loss of genetic variability in game species under different hunting regimes. Oikos 36:257–266

Saura M, Caballero A, Caballero P, Moran P (2008) Impact of precocious male parr on the effective size of a wild population of Atlantic salmon. Freshw Biol 53:2375–2384

Schwartz MK, Tallmon DA, Luikart G (1998) Review of DNA-based census and effective population size estimators. Anim Conserv 1:293–299

Schwartz MK, Tallmon DA, Luikart G (1999) DNA-based methods for estimating population size: many methods, much potential, unknown utility. Anim Conserv 2:321–323

Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. Trends Ecol Evol 22:25–33

Schwartz MK, McKelvey KS (2008) Why sampling scheme matters: the effect of sampling scheme on landscape genetic results. Conserv Genet 10:441–452

Seber GAF (1973) The estimation of animal abundance and related parameters. Edward Arnold, London, p 506

Settlage KE, Van Manen FT, Clark JD, King TL (2008) Challenges of DNA-based mark-recapture studies of American black bears. J Wildl Manag 72:1035–1042

Simões P, Pascual M, Santos J, Rose MR, Matos M (2009) Evolutionary dynamics of molecular markers during local adaptation: a case study in *Drosophila subobscura*. BMC Evol Biol 9:133–135

Solberg H, Bellemain E, Drageset OM, Taberlet P, Swenson JE (2006) An evaluation of field and genetic methods to estimate brown bear (*Ursus arctos*) population size. Biol Conserv 128:158–168

Taberlet P, Waits L, Luikart G (1999) Non-invasive genetic sampling: look before you leap. Trends Ecol Evol 14:323–327

Tallmon DA, Luikart G, Beaumont MA (2004) Comparative evaluation of a new effective population size estimator based on approximate Bayesian computation. Genetics 167:977–988

Tallmon DA, Koyuk A, Luikart G, Beaumont MA (2008) ONeSAMP: a program to estimate effective population size using approximate Bayesian computation. Mol Ecol Resour 8:299–301

Valière N, Bonenfant C, Toïgo C, Luikart G, Gaillard J-M, Klein F (2006) Importance of a pilot study for non-invasive genetic sampling: genotyping errors and population size estimation in red deer. Conserv Genet 8:69–78

Vitalis R, Couvet D (2001) Estimation of effective population rate from one- and two-locus identity measures. Genetics 157:911–925

Waits LP, Paetkau D (2005) Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. J Wildl Manag 69:1419–1433

Wakeley J, Sargsyan O (2009) Extensions of the coalescent effective population size. Genetics 181:341–345

Wang J (2001) A pseudo-likelihood method for estimating effective population size from temporally spaced samples. Genet Res 78:243–257

Wang J (2005) Estimation of effective population sizes from data on genetic markers. Phil Trans R Soc B 360:1395–1409

Wang J (2009) A new method for estimating effective population size from a single sample of multilocus genotypes. Mol Ecol 18:2148–2164

Wang J, Whitlock MC (2003) Estimating effective population size and migration rates from genetic samples over space and time. Genetics 163:429–446

Waples RS (1989) A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics 121:379–391

Waples RS (1990) Conservation genetics of Pacific salmon III. Estimating effective population size. J Hered 81:277–289

Waples RS (1991) Genetic methods for estimating the effective size of cetacean populations. Rep Int Whal Commn (special issue 13):279–300

Waples RS (2002) Evaluating the effect of stage-specific survivorship on the N_e/N ratio. Mol Ecol 11:1029–1037

Waples RS (2005) Genetic estimates of contemporary effective population size: to what time periods do the estimates apply? Mol Ecol 14:3335–3352

Waples RS (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conserv Genet 7:167–184

Waples RS, Do C (2008) LdNe: a program for estimating effective population size from data on linkage disequilibrium. Mol Ecol Resour 8:753–756

Waples RS, Do C (in press) Linkage disequilibrium estimates of contemporary N_e using SNPs and highly polymorphic molecular markers: an evaluation of precision and bias. Evol Appl

Waples RS, Yokota M (2007) Estimates of effective population size in species with overlapping generations. Genetics 177:927–935

Weir BS, Hill WG (1980) Effect of mating structure on variation in linkage disequilibrium. Genetics 95:447–488

White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. Bird Study 46(Supplement):120–138

White GC, Kendall WL, Barker RJ (2006) Multistate survival models and their extensions in Program MARK. J Wildl Manag 70:1521–1529

Worley KJ, Carey J, Veitch A, Coltman DW (2006) Detecting the signature of selection on immune genes in highly structured populations of wild sheep (*Ovis dalli*). Mol Ecol 15:623–637

Wright S (1931) Evolution in Mendelian populations. Genetics 16:97–159

Wright JA, Barker RJ, Schofield MR, Frantz AC, Byrom AE, Gleeson DM (2009) Incorporating genotype uncertainty into mark-recapture-type models for estimating abundance using DNA samples. Biometrics 65:833–840

Zhdanova OL, Pudovkin AI (2008) Nb_HetEx: a program to estimate the effective number of breeders. J Hered 99:694–695