

INVITED REVIEW

Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence?

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Abstract

Genetic stochasticity due to small population size contributes to population extinction, especially when population fragmentation disrupts gene flow. Estimates of effective population size (N_e) can therefore be informative about population persistence, but there is a need for an assessment of their consistency and informative relevance. Here we review the body of empirical estimates of N_e for wild populations obtained with the temporal genetic method and published since Frankham's (1995) review. Theoretical considerations have identified important sources of bias for this analytical approach, and we use empirical data to investigate the extent of these biases. We find that particularly model selection and sampling require more attention in future studies.

We report a median unbiased N_e estimate of 260 (among 83 studies) and find that this median estimate tends to be smaller for populations of conservation concern, which may therefore be more sensitive to genetic stochasticity. Furthermore, we report a median N_e/N ratio of 0.14, and find that this ratio may actually be higher for small populations, suggesting changes in biological interactions at low population abundances. We confirm the role of gene flow in countering genetic stochasticity by finding that N_e correlates strongest with neutral genetic metrics when populations can be considered isolated. This underlines the importance of gene flow for the estimation of N_e , and of population connectivity for conservation in general. Reductions in contemporary gene flow due to ongoing habitat fragmentation will likely increase the prevalence of genetic stochasticity, which should therefore remain a focal point in the conservation of biodiversity.

Keywords: effective population size, gene flow, genetic stochasticity, genetic compensation, inbreeding depression, marine fish, metapopulation dynamics, model bias, population fragmentation, sample size, *Salmo salar*, temporal method

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Introduction

A fundamental contribution by evolutionary theory to conservation biology has been the development of a predictive framework for the fate of small populations. A population's extinction risk depends on both deterministic (natural selection, harvesting, etc.) and stochastic (e.g. environmental, demographic and genetic) processes. The latter are more pronounced at small population size, thereby

potentially accelerating extinction processes often initiated by deterministic factors. Population size can also influence the level of genetic diversity (Frankham 1996): in small populations, random genetic drift, inbreeding and the resulting accumulation of deleterious mutations are all known to lead to loss of genetic variation (Frankham *et al.* 2003). These claims have a sound theoretical basis (e.g. Lynch *et al.* 1995) but in the wild it is less clear how often a link can be established between population size and extinction risk due to genetic factors.

One important genetic factor that may affect extinction risk is inbreeding depression. The relationship between

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inbreeding depression and population size has been demonstrated empirically under controlled conditions (laboratory: Frankham 2005; Swindell & Bouzat 2006c; Wright *et al.* 2007; animals in captivity: Ralls *et al.* 1979; Ballou & Ralls 1982; Boakes *et al.* 2007) as well as in the wild (Crnokrak & Roff 1999; Keller & Waller 2002). Inbreeding depression may be one factor leading to the relatively low mean fitness of small populations (Reed 2005). Fitness loss due to decreased population size has also been linked to increased extinction risk under experimental (e.g. Wright *et al.* 2007) and natural conditions (Newman & Pilon 1997; Saccheri *et al.* 1998; Nieminen *et al.* 2001). Studies of the impact of immigration on fitness in small and endangered populations provide additional evidence for population size affecting inbreeding depression (plants: Richards 2000; prairie chickens: Westemeier *et al.* 1998; wolves: Flagstad *et al.* 2003; Vila *et al.* 2003; song sparrows: Keller *et al.* 2001; and adders: Madsen *et al.* 1999). These lines of evidence thus suggest that inbreeding depression due to reduced population size can contribute significantly to extinction risk in the wild (Frankham 2005).

The empirical evidence for other genetic factors affecting population persistence is, however, less abundant. Small populations may be more susceptible than large populations to accumulation of deleterious mutations due to the decreased efficiency of natural selection: natural selection becomes effectively neutral when $s < 1/2N$ (Wright 1943). While the theoretical basis for this relationship is well established (Lynch *et al.* 1995), strong empirical support for mutational meltdowns is thus far lacking (Gilligan *et al.* 1997; Woodworth *et al.* 2002). Purging, the selective removal of deleterious alleles when in homozygous state, has been suggested as a mechanism responsible for the scarcity of such evidence. Empirical evidence for purging is, however, also mixed (Swindell & Bouzat 2006b; Boakes *et al.* 2007) and most often comes from studies on plants (Byers & Waller 1999), where the opportunity for gene flow is relatively limited. Even under isolation, the beneficial fitness effects of purging appear to be limited (Boakes *et al.* 2007 but see Crnokrak & Barrett 2002). Importantly, immigration (even at very low levels) seems to be more efficient in countering inbreeding and may interfere with purging (Keller *et al.* 2001). Although biologically plausible, the prevalence of purging in natural (animal) populations therefore remains uncertain and difficult to predict (Leberg & Firmin 2008).

Loss of genetic diversity, and therefore quantitative trait heritability ('evolutionary potential'), is often considered the most relevant evolutionary consequence of declining population size (Franklin 1980; Frankham *et al.* 2003). Although experiments under controlled conditions provide some support (Weber 1990; Weber & Diggins 1990; Swindell & Bouzat 2005; Swindell & Bouzat 2006a), in the wild the correlation between population size and evolutionary

potential is generally weak. This may result from the complex relationship between variability in adaptive quantitative traits and neutral genetic diversity (Reed & Frankham 2001; Merila & Crnokrak 2001; McKay & Latta 2002; Willi *et al.* 2006): while neutral genetic diversity decreases with population size following a sigmoid function (Frankham 1996), the heritability for quantitative traits decreases with population size only at very small population sizes (Willi *et al.* 2006). In addition, both the complex genetic architecture of most quantitative traits and the fact that the type of selection (Willi *et al.* 2006) will vary with the type of trait, e.g. morphological or life history (Mousseau & Roff 1987; Roff & Emerson 2006), make a strong correlation between neutral genetic and quantitative trait variation less likely. Nevertheless, based on reduced neutral genetic variation, Spielman *et al.* (2004) argued that threatened taxa would have an elevated extinction risk due to loss of evolutionary potential. These considerations, along with the realization that population connectivity tends to counter genetic stochasticity, suggest that concerns about the long-term evolutionary potential of small or declining populations may be less well founded than for instance, immediate concerns about inbreeding depression (Frankham *et al.* 2003).

Genetic diversity depends on a population's genetically effective size, N_e (Wright 1931), which refers to the size of an ideal population experiencing the same rate of random genetic change over time as the real population under consideration (Crow & Kimura 1970). Real biological systems do not adhere to idealized settings (Whitlock & McCauley 1999), and thus, effective sizes are typically much smaller than census sizes. For a wide range of demographic and reproductive scenarios, N_e/N is expected to be ~ 0.5 (Nunney 1991, 1993, 1996; Nunney & Elam 1994). Over longer terms, population size fluctuations can reduce N_e further (Wright 1938; Vucetich *et al.* 1997; Kalinowski & Waples 2002; Waples 2002a). Frankham (1995) reviewed (mainly demographic) estimates of the ratio of N_e to N in wild populations and reported a median value of 0.11, identifying fluctuating population size, unequal sex ratio and variance in reproductive success as the main factors responsible for the low N_e/N ratios. Life-history aspects and reproductive biology are thus very important determinants of N_e , but additional factors may also be important (Turner *et al.* 2006). This low ratio implies that populations may become sensitive to genetic stochasticity at levels where census population estimates would provide no such indications, although the consistency of N_e/N ratios in wild populations is uncertain (e.g. Hauser *et al.* 2002; Ardren & Kapuscinski 2003).

Concurrently, efforts to establish lower population size thresholds, at which the impact of genetic factors should be minimal, have suggested $N_e = 50$ to minimize inbreeding depression and $N_e = 500$ to maintain sufficient evolutionary

potential (Franklin 1980; but see Lande 1988; Franklin & Frankham 1998; Lynch & Lande 1998). Estimating N_e can thus be an important tool in the assessment of the genetic vulnerability of endangered populations (Mace & Lande 1991). Although several demographic and genetic methods have been developed to estimate N_e (reviewed by Caballero 1994; Leberg 2005; Wang 2005), here we focus on the 'temporal' genetic method, which is based on the premise that temporal variance in neutral genetic allele frequencies is inversely proportional to the effective population size. The approach was pioneered by Krimbas & Tsakas (1971), and substantial statistical refinement has since then been achieved (Nei & Tajima 1981; Pollak 1983; Waples 1989, 1990a, b; Anderson *et al.* 2000; Wang 2001; Berthier *et al.* 2002; Laval *et al.* 2003; Wang & Whitlock 2003; Anderson 2005). Originally developed for species with discrete generations, the method has been extended to situations where generations overlap (Waples 1990a, b; Jorde & Ryman 1995; Waples & Yokota 2007) and importantly, for cases in which the assumption of complete isolation cannot be held valid (Wang & Whitlock 2003). The consequences of violating some of these assumptions have also been quantified (Waples & Yokota 2007).

Since Frankham's (1996) seminal review, an increased sophistication in molecular methods has been accompanied by a drastic increase in published genetic estimates of N_e (denoted \hat{N}_e) and its ratio to census size (N_e/N). Concomitantly, awareness is growing that natural systems (as well as statistical models for estimating N_e) can have complicated features that need to be considered in detail when estimating effective population size (e.g. Wang & Whitlock 2003; Fraser *et al.* 2007a; Waples & Yokota 2007). Accounting for these factors is imperative for gaining a better understanding of both the magnitude of effective population size and its implications for conservation and evolution. Given that monitoring of N_e is strongly advocated in conservation and management programs (Schwartz *et al.* 1999; Leberg 2005; Schwartz *et al.* 2007), there is a need for an assessment of the consistency and reliability of estimates derived from these temporal methods, and their relevance for conservation.

The purpose of this review is threefold:

- 1) Theoretical evaluations have shown that the estimation of \hat{N}_e is sensitive to several potential sources of bias. Here we extend these theoretical concerns to natural populations, using empirical estimates derived from the temporal approach to quantify such biases. We focus on model selection and sample size, and make practical recommendations for future studies.
- 2) Gene flow is known to affect processes of genetic stochasticity and thereby also N_e . We review the empirical literature on gene flow and effective population size, focusing on the effect of gene flow on N_e estimation, as

well as considerations of N_e in the context of metapopulation dynamics.

- 3) Finally, we compare temporal estimates of effective population sizes of wild populations available since 1995 with those compiled by Frankham (1995) and investigate whether populations of conservation concern are characterized by relatively low effective population sizes and/or N_e/N ratios. We quantify the genetic behaviour of populations as predicted by \hat{N}_e , placing special emphasis on gene flow. We conclude with a discussion of the value and utility of the temporal approach to the estimation of effective population size in the context of conservation.

Data selection criteria

An extensive literature search located 83 studies reporting genetic estimates of N_e using the temporal method (see Table S1, Supplementary material). These came from a total of 65 species in diverse groups including amphibians, birds, crustaceans, fish, insects, mammals, molluscs, reptiles and plants. We considered estimates for wild populations only, ignoring those supplemented by supportive breeding. Five additional studies were located that provided temporal genetic data, yet had not estimated effective population size. After calculation of \hat{N}_e , these were added to the data set, which thus includes 88 studies. Where multiple estimates applied to the same population, only estimates covering different time periods were considered. Furthermore, whenever species with overlapping generations were analysed by estimating the annual number of breeders (N_b), only estimates of N_e , derived from this value, were included in subsequent analyses. Using these selection criteria, a grand total of 780 temporal estimates of N_e and 251 estimates of the N_e/N ratio were available for subsequent analyses. Although a large component of these data pertain to salmonid fish (20% of studies; 40% of temporal estimates), we attempt to make general inferences and limit our conclusions to salmonids wherever appropriate. All statistical analyses were performed in *s-PLUS* 6.2 (Insightful).

Analytical considerations when estimating N_e

Indirect genetic approaches have the strong advantage that they can provide demographically inclusive N_e estimates and avoid some of the logistic difficulties associated with direct, ecological approaches of estimating N_e . They may suffer, however, from their own sources of biases, principally among them violations of the common assumption of closed populations characterized by discrete generations, and sample size effects. Here we focus on the last two factors, relegating the consequences of ignoring gene flow to the next section. In addition, we use empirical data to evaluate approaches for improving precision of N_e estimates.

Table 1 Overview of empirical estimates of effective population size (\hat{N}_e) and its ratio to census size (N_e/N) from published studies (n , see Table S1 for references) for different conservation categories. Average transformed parameter values \pm standard deviation (SD) are presented as back-calculated untransformed values. Additionally given is the median value derived from untransformed parameter values. Values are given for all independent data points and for a more restricted data set, assumed to be relatively free of analytical model bias

Conservation category	\hat{N}_e			\hat{N}_e/N		
	n	Average \pm SD	median	n	Average \pm SD	Median
<i>All data</i>						
Exploited	21	521 \pm 125	697	9	8.1.10 ⁻⁵ \pm 7.7.10 ⁻⁵	1.9.10 ⁻⁵
Stable	226	89 \pm 36	89	61	0.20 \pm 0.09	0.16
Conservation	58	116 \pm 26	101	32	0.28 \pm 0.15	0.20
All	305	104 \pm 39	104	102	0.19 \pm 0.11	0.16
<i>Selected data only</i>						
Exploited	9	2085 \pm 322	1235	6	1.0.10 ⁻⁴ \pm 1.0.10 ⁻⁴	2.3.10 ⁻⁵
Stable	94	271 \pm 62	265	33	0.18 \pm 0.17	0.14
Conservation	35	149 \pm 31	175	26	0.27 \pm 0.15	0.20
All	140	267 \pm 65	251	64	0.16 \pm 0.13	0.14

The importance of accounting for age structure and demographics

Empirical studies have commonly applied models of estimating N_e that assume discrete generations to species with overlapping generations. Discrete model estimates of N_e over short-terms (roughly < 4 generations) are expected to be biased when applied to age-structured species with overlapping generations (Jorde & Ryman 1995; Waples & Yokota 2007), because genetic fluctuations among cohorts will often differ from those for the population as a whole (Jorde & Ryman 1995).

We used empirical N_e values to evaluate the extent of this bias by comparing all independent estimates reported with those that correctly accounted for the species' life history only. We used the following criteria for inclusion in this subset. Estimates with discrete generation models were included when the species exhibited discrete generations, regardless of time span ($n = 33$). Discrete models applied to species with overlapping generations were used when temporal samples were spaced more than four generations apart ($n = 50$), when bias due to overlapping generations is expected to be insignificant (Waples & Yokota 2007). N_e estimates on age-structured populations covering shorter time spans ($t < 4$ generations) were included only if the analytical model (Waples 1990a, b; Jorde & Ryman 1995) took this into account ($n = 60$). This restricted data set, which may more accurately reflect effective population sizes for wild species, contained 143 temporal estimates, vs. a grand total of 304 estimates.

Our analyses reveal that N_e estimates that do not properly consider species' biology generally tend to be biased downwards. A comparison of overall values (Table 1)

shows that \hat{N}_e tends to be larger for studies that applied an appropriate analytical model ($\bar{N}_e = 267 \pm 6$) than for all empirical estimates available ($\bar{N}_e = 104 \pm 39$, one-sided t -test, $P < 0.001$). This may partly be because the majority of estimates published to date are on fish, which are generally characterized by a life history with a type III survival curve (high fecundity and high juvenile mortality), for which temporal estimates derived from short time spans are particularly sensitive to downward bias (Waples & Yokota 2007). Similar differences in N_e are also observed when only considering estimates for marine fish, all of which are characterized by a type III survival curve (t -test, one-sided, $P = 0.007$, Wilcoxon one-sided rank test, $Z = 2.0688$, $P = 0.02$). These results reiterate that proper model selection is a very important consideration for the temporal approach.

Sample size considerations for temporal N_e estimators

Sample size is known to affect not only precision, but also bias temporal estimators of N_e . This is because allele frequency shifts reflect not only random drift but also the sampling process (Waples 1989). All analytical variations of the moment-based method (Waples 1989, 1990a, b; Jorde & Ryman 1995) correct for the effect of sampling. Likelihood methods make no such explicit correction, but are expected to be more powerful, given that they make use of more of the information contained in the data, in particular when data contain many rare alleles. Although power for some of these methods has been evaluated through simulations (Berthier *et al.* 2002; Wang & Whitlock 2003), the potential of sampling-related bias in empirical studies remains relatively unexplored. Hence we examined the influence of sample size on bias in temporal \hat{N}_e by applying a

Table 2 Correlation between sample size (S) and effective population size estimates, for different groupings of sample size and three different analytical models. Given are the degrees of freedom (d.f.), correlation coefficient (r) and statistical significance (P) of linear regressions for nontransformed and $\log(N_e)$ -transformed data

N_e estimator	S	Nontransformed data, linear				$\log(N_e)$ -transformed data, linear			
		d.f.	r^2	r	P	d.f.	r^2	r	P
Moment-based	All	195	0.00	0.01	0.881	195	0.07	0.27	< 0.001
	< 50	122	0.00	0.02	0.853	122	0.23	0.48	< 0.001
	< 30	51	0.01	0.07	0.625	51	0.22	0.48	0.002
Wang (2001)	All	129	0.01	0.10	0.271	129	0.26	0.51	< 0.001
	< 50	86	0.09	0.29	0.007	86	0.25	0.50	< 0.001
	< 30	24	0.21	0.46	0.018	24	0.60	0.78	< 0.001
Berthier <i>et al.</i> (2002)	All	35	0.03	0.18	0.295	35	0.01	0.05	0.791
	< 50	18	0.26	0.51	0.021	18	0.51	0.72	< 0.001
	< 30	3	–	–	–	3	–	–	–

generalized linear model to the relationship of \hat{N}_e with sample size.

We selected three different analytical methods commonly reported in the literature: the general moment-based approach, the pseudo-maximum likelihood method of Wang (2001) and the coalescent maximum-likelihood method of Berthier *et al.* (2002). Data for the three estimator methods were analysed separately, but only estimates assuming closed populations were considered. Studies are based on a large range of sample sizes and we report on correlations between \hat{N}_e and sample size for all data combined, for the subset of N_e estimates obtained with samples sizes $S < 50$, and for those obtained with samples sizes $S < 30$.

We find that insufficient sampling may lead to (strong) bias in estimates of effective population size, in particular for methods that do not explicitly correct for sample size. Regardless of the estimator method, correlations between \hat{N}_e and S are weak when all sample sizes are considered (Table 2). However, significant correlations are detected for the likelihood methods when considering smaller sample sizes ($S < 50$, $S < 30$). This suggests that they may be more sensitive to (downward) bias than the moment-based approach when dealing with small sample sizes. Moreover, extended regression analyses (Table 2) show that a general log-linear relationship provides the best fit between sample size and \hat{N}_e .

Precision of temporal estimates of N_e

Besides the obvious importance of avoiding bias, increased emphasis is also placed on the precision of N_e estimators. This precision is suggested to principally benefit from increasing (1) sample size (2) number of marker loci and (3) number of generations between samples (Waples 1989; Waples 1990a; Luikart *et al.* 1998; Anderson *et al.* 2000;

Berthier *et al.* 2002; Wang & Whitlock 2003). Estimate precision (95% confidence interval of \hat{N}_e) is strongly determined by its own magnitude (linear regression model, $n = 376$, $r = 0.91$, $P < 0.0001$): large estimates of N_e are thus expected to be less precise than small estimates. We used empirical estimates based on microsatellite markers to assess the merit for improvement in estimate precision of sample size, number of marker loci (L), time span (t in generations), total number of alleles (N_a), total number of independent alleles (N_a-L) and locus variability (N_a per locus). Analyses were conducted separately for moment-based estimators, Wang 2001) pseudo-maximum likelihood model and a third group consisting of other likelihood methods. Relationships between variables and estimator precision were investigated using generalized linear regression models. We examined the residuals of the regression of 95% confidence intervals onto N_e estimates as the dependent variable, in order to minimize the effect of estimate magnitude on its own precision.

The main improvements of precision for the temporal approach come from considerations of the genetic markers used. For each method considered, estimator precision improved with marker diversity (Table 3). In addition, the precision of the pseudo maximum-likelihood method of Wang (2001) also benefits from an increase in time span between samples. Neither number of loci or sample size appears to affect precision of N_e estimation significantly, at least within the variable ranges considered (2–19 microsatellite loci, 6.5–1058 samples per time point). With the current technical and analytical limitations, the most efficient approach to improve the precision of a temporal estimate of effective population size may therefore be to use more variable marker loci (Table 3).

These results are in line with expectations for likelihood approaches, which are well suited for analysing highly polymorphic data (Wang 2005). However, evaluations of

Table 3 Improving the precision of temporal estimates of effective population size. Given for three different methods of estimating N_e are correlations between precision and variables (factors) thought to improve precision. For each relationship the nature of the relationship, effect on precision (direction), the degrees of freedom (d.f.), correlation coefficients (r) and significance (P) are given.

N_e estimator	Factor	Relationship	Direction	d.f.	r	P
Moment-based	Number of (microsat) loci	Linear	–	141	0.12	0.168
	Sample size	Linear	–	141	0.13	0.134
	Time span (t in g)	Linear	–	140	0.10	0.239
	Number of alleles (N_a)	Linear	+	125	0.26	0.004
	Number of independent alleles	Linear	+	125	0.27	0.002
	Allelic diversity (N_a per locus)	Linear	+	125	0.37	< 0.001
Wang (2001)	Number of (microsat) loci	Linear	–	101	0.27	0.007
	Sample size	Linear	–	101	0.00	0.976
	Time span (t in g)	Log-linear	+	101	0.44	< 0.001
	Number of alleles (N_a)	Linear	+	70	0.13	0.283
	Number of independent alleles	Linear	+	70	0.15	0.196
	Allelic diversity (N_a per locus)	Log-linear	+	70	0.30	0.010
Other likelihood methods	Number of (microsat) loci	Linear	–	64	0.30	0.014
	Sample size	Linear	–	64	0.07	0.554
	Time span (t in g)	Log-linear	+	64	0.09	0.485
	Number of alleles (N_a)	Linear	+	52	0.00	0.975
	Number of independent alleles	Linear	+	52	0.08	0.570
	Allelic diversity (N_a per locus)	Log-linear	+	52	0.30	0.026

the performance of the moment-based estimator (Waples 1989) suggest that more alleles per locus, or rather more *rare* alleles resulting in a skewed allele frequency distribution, will tend to exert an upward bias on N_e estimates, with an expected decrease in precision (wider confidence intervals). Binning of rare alleles has been suggested as a strategy to improve estimator accuracy (Waples 1990a, b), but simulations (Turner *et al.* 2001) show that estimate precision may suffer as a consequence. Empirical studies have reported mixed results of the binning strategy (Scribner *et al.* 1997; Shrimpton & Heath 2003; Brede & Beebe 2006). The simple availability of more alleles to measure drift (improving precision) may override the risk of reduced estimation accuracy (upward bias). Here, the estimator of Jorde & Ryman (2007), explicitly developed for data with many rare alleles, may be particularly useful. In summary, slightly different approaches can improve precision of different estimator methods. These should be considered seriously, given that confidence intervals themselves contain information relevant for conservation (Fraser *et al.* 2007a).

Effective population size of marine fish

The statistical concerns over analytical biases may be particularly relevant for effective population size estimation of (commercially exploited) marine fish species (Hauser *et al.* 2002; Turner *et al.* 2002; Hutchinson *et al.* 2003; Hoarau *et al.* 2005; Gomez-Uchida & Banks 2006), that are characterized by N_e/N ratios that are well below

theoretical expectations (Nunney 1991, 1993, 1996) and empirical estimates for most other natural populations (Frankham 1995; this review). These species are generally characterized by type III survivorship curves, the biological consequences of which have been explored theoretically. Hedgecock (1994) suggested that extremely low N_e/N ratios may be due to a combination of high fecundity and high juvenile mortality, generating a very high variance in reproductive success which can depress N_e substantially. This idea has since been demonstrated to be theoretically plausible (Nunney 1996; Waples 2002b; Hedrick 2005), provided that a small number of families survive to produce offspring (Hedrick 2005). Such demographic trends will first need to be compatible with aspects of the species' life history and reproductive behaviour (Poulsen *et al.* 2006). For example, empirical observations of the spawning behaviour of Atlantic cod (*Gadus morhua*) (Herbinger *et al.* 1997) do not support an extreme variance in family survival, required as a biological explanation for the reported low estimates of effective population size in this species (Hutchinson *et al.* 2003; Poulsen *et al.* 2006). Furthermore, these estimates are also difficult to reconcile with observations of high marker polymorphism, unless microsatellite mutation rates are generally much higher than currently believed (Poulsen *et al.* 2006).

We evaluated the scope for the 'Hedgecock' effect by comparing N_e/N ratios for species characterized by different survival curves. Since delineating survival curve types can be complicated, we combined type I and II ($n = 24$), considering species to be type III whenever they are

characterized by relatively high fecundity ($n = 78$). We find that species with a type III survivorship curve exhibit significantly lower N_e/N ratios than species with either a type I or type II survivorship curves (type III: 0.18 ± 0.07 vs. type I + II: 0.32 ± 0.11 , t -test one-sided, $P = 0.019$). These analyses did not consider marine fish estimates and confirm that a life history with very high fecundity and high mortality early in life can indeed result in a reduction in effective population size (Waples 2002b; Hedrick 2005). However, this reduction appears too small ($\sim 40\%$) to explain the extremely low N_e/N ratios observed in marine fish (Table 1), suggesting that additional reasons may exist for this pattern. For example, nonbiological factors such as human perturbation or commercial exploitation may act to further reduce N_e (Turner *et al.* 2006). The ultimate relevance of this explanation needs to be assessed by carefully considering other explanations that can be provided by sampling variance, violation of assumptions of applied analytical models and population structuring.

There are known problems in applying the temporal method in studies of species characterized by extremely large census sizes. First, a very large number of individuals may have to be sampled to obtain reliable estimates of N_e in cases where one can *a priori* expect this value to be very large (Nei & Tajima 1981). Second, many marine species are characterized by overlapping generations and age structure, and though analytical models are available to deal with this complication (Jorde & Ryman 1995), many studies have applied discrete generation models to such species. Our own analyses confirm that N_e estimates derived from analytical models compatible with the species' biology can be substantially larger than those that did not. Studies on marine fish effective population sizes that have applied a model designed for overlapping generations tend to report slightly less extreme N_e/N ratios (e.g. Turner *et al.* 2002; Gomez-Uchida & Banks 2006; Saillant & Gold 2006) than those that did not. Some low N_e/N ratios (e.g. Hauser *et al.* 2002; Hoarau *et al.* 2005) should be relatively free from this bias, since they considered samples taken over comparatively long time spans. Future studies of marine fish N_e should attempt to harmonize analytical considerations and sampling with what is known of the species' biology and demography. These considerations do not rule out the consequences of population structuring and metapopulation dynamics for marine effective population sizes (see below).

Recommendations for future studies

Adequate sampling is essential for an unbiased estimation of N_e with the temporal method, regardless of the estimator method (moment-based or maximum-likelihood). Although that statement seems trivial, a substantial part of the published N_e estimates may be subject to (some) downward bias due to small sample sizes. This potential bias appears

less strong for moment-based methods than for likelihood methods. The main strength of likelihood methods lies in their ability to make more effective use of the information contained in data with many low frequency alleles, typical for microsatellites (Wang 2005). Our analyses, however, show that this improved power does not extend to samples characterized by small sizes ($S < 50$), a result that is consistent with sampling recommendations made for these analytical models (Anderson *et al.* 2000; Berthier *et al.* 2002; Laval *et al.* 2003; Wang & Whitlock 2003).

The important question is then how much should one sample in empirical studies. Sample size should be large enough to override effects of sampling variance when estimating N_e , that is, to maximize the signal-to-noise ratio (Nei & Tajima 1981). Temporal methods are well known to not be equally suitable for analyses of populations of all sizes, particularly for those that are very large (Waples 1989). Although many studies have recommended sample sizes of at least 50, many more may have to be sampled for large populations. Power analyses of the temporal method for detecting population bottlenecks (Luikart *et al.* 1999) reveal that even for moderately small effective population sizes ($N_e \sim 40$ – 100), substantial numbers of loci and individuals (> 10 loci, $S > 45$) may have to be surveyed. Using simulations, Ovenden *et al.* (2007) estimated that ~ 2000 sampled individuals are required to attain sufficient power to reliably estimate an effective size of 8000. Clearly, maximizing the ratio of S to N_e , rather than sample size itself, is important for the temporal approach (Nei & Tajima 1981). However, the 'true' N_e is usually unknown for natural populations. Instead, we used generational census size as a proxy for N_e to estimate this ratio. For a subset of estimates ($n = 30$) on populations smaller than 10^5 individuals per generation, which were deemed relatively unbiased on the grounds of model selection and time span between temporal samples, we find the median S/N ratio to be ~ 0.10 . Thus, while large sample sizes may be required for populations with large census size, small samples can be permissible when working with small populations and some low N_e estimates reviewed here undoubtedly reflect biological reality. Ultimately, the onus will be on researchers to justify their sampling design in the context of what is known of the species' demography, and on journal editors to keep them to it.

Second, one should carefully consider the analytical model best compatible with the study species' biology and life history. Our review confirms that inappropriate model consideration may often lead to downward bias (Waples & Yokota 2007). When a cohort analysis (Jorde & Ryman 1995) appears warranted (as will frequently be, when short time spans are studied), sampling should be optimized to ensure that individual cohorts contain sufficiently large sample sizes. For long-lived iteroparous species, this may imply collecting comparatively large sample sizes at any

given point in time, on top of the demographic information required for this analytical approach. However, estimates derived from year-class approaches can provide information that applies directly to the time span sampled, thereby facilitating efficient genetic monitoring programs (Schwartz *et al.* 2007).

An alternative option is to increase the temporal span between samples, but this time span (> 4 generations) may be very long for some species, and this approach has its own drawbacks. First, in particular in studies with archived 'ancient' DNA samples, it is important to minimize technical artefacts, since these will likely exert a downward bias on \hat{N}_e (see Poulsen *et al.* 2006). Perhaps more importantly, when no historical samples are available for comparison, the choice to wait until enough time has passed may be incompatible with the need for genetic information due to imminent conservation concerns. Here the temporal method may simply not be the best option for estimating effective population size: instead N_e estimators based on a single population sample may be more useful (Hill 1981; Bartley *et al.* 1992; Tallmon *et al.* 2004, 2008; Waples 2006). These estimators, however, suffer from the important drawback that they can currently not account for the effects of gene flow.

Finally, current genetic approaches for estimating N_e are poorly equipped to analyse populations with dynamic demographics. The cohort model of Jorde & Ryman (1995) assumes static age structure and population size, whereas many natural populations may be dynamic in this respect, especially those undergoing declines (i.e. of conservation concern). Although single sample genetic estimators may be less sensitive to this complication, some ecological methods may be better suited to evaluate the consequences of demographic instability for N_e . Analytical models have recently been developed that account for fluctuating age structure and population size, based on demographic and environmental stochasticity (Engen *et al.* 2005, 2007). These ecological models require no genetic data but are based on (detailed) demographic information. Comparative studies using direct ecological approaches and indirect genetic methods, in particular on closed systems (e.g. Kaeuffer *et al.* 2007), will likely increase our understanding of the behaviour of N_e in natural, dynamic populations.

Consequences of gene flow for \hat{N}_e

It is a basic feature of population genetics theory that gene flow will override the effects of random genetic drift when $m > 1/4N_e$ (Wright 1931). Empirical studies have since confirmed the importance of immigration in countering adverse genetic effects (e.g. Westemeier *et al.* 1998; Vila *et al.* 2003). Furthermore, under metapopulation scenarios where dispersal is mainly a function of local density, one would expect smaller populations to have higher

immigration rates (Hanski & Gaggiotti 2004; Pulliam 1988), thereby possibly countering enhanced genetic drift. When ignored, gene flow can thus bias temporal estimates of N_e , the direction of bias depending on the relative magnitude and continuity of gene flow under natural conditions. Although the importance of confirming population isolation in the temporal approach was realized long ago (Nei & Tajima 1981), there is currently only one analytical model for jointly estimating contemporary gene flow and effective population size (Wang & Whitlock 2003). We used combined estimates derived from this model to examine the question of whether gene flow is more common into effectively smaller populations than into larger populations. The main assumptions of Wang & Whitlock's model are that immigration from a source population is constant over the time period considered and that the source population is infinitely sized (i.e. has temporally stable allele frequency distributions), even though simulations have shown that the model provides reasonably accurate results when this latter assumption is not met (Wang & Whitlock 2003).

We compiled a database of 93 joint estimates of N_e and m from published studies to test the null hypothesis that incoming gene flow is independent of population size. This hypothesis was rejected. There is a negative log-linear relationship between gene flow and population size, indicating that migration rate (m) may indeed be higher into smaller populations ($r = 0.56$, $P < 0.001$, Fig. 1a). This effect can be mainly attributed to data on anadromous fish ($n = 33$, $r = 0.77$, $P < 0.001$): without estimates from this group the null hypothesis could not be rejected. These relationships remain unchanged when over longer time scales ($g > 5$ only, $r = 0.68$, $P < 0.01$ for all data ($n = 23$); $r = 0.93$, $P < 0.001$ for anadromous fish only ($n = 14$)). Thus, even though variance around estimates of gene flow at low population sizes is often considerable, our analyses suggest gene flow as inferred in this model is generally higher into small than into large (salmonid fish) populations. As such, they lend some support the suggestion that neutral genetic diversity (and possibly effective population size) in small populations can be maintained by ongoing immigration (Ostergaard *et al.* 2003; Consuegra *et al.* 2005; Fraser *et al.* 2007b; Schmeller & Merila 2007; Watts *et al.* 2007). This conclusion, however, needs to be qualified by uncertainty about how biologically realistic the applied analytical model is.

Continuous vs. incidental gene flow

A recurrent point, emphasized when applying this method, is the need for the proper knowledge of potential sources of gene flow into the focal population (Hoffman *et al.* 2004; Johnson *et al.* 2004; Fraser *et al.* 2007a). Potential biases, as well as recommendations for a biologically meaningful application of Wang & Whitlock (2003) approach, are

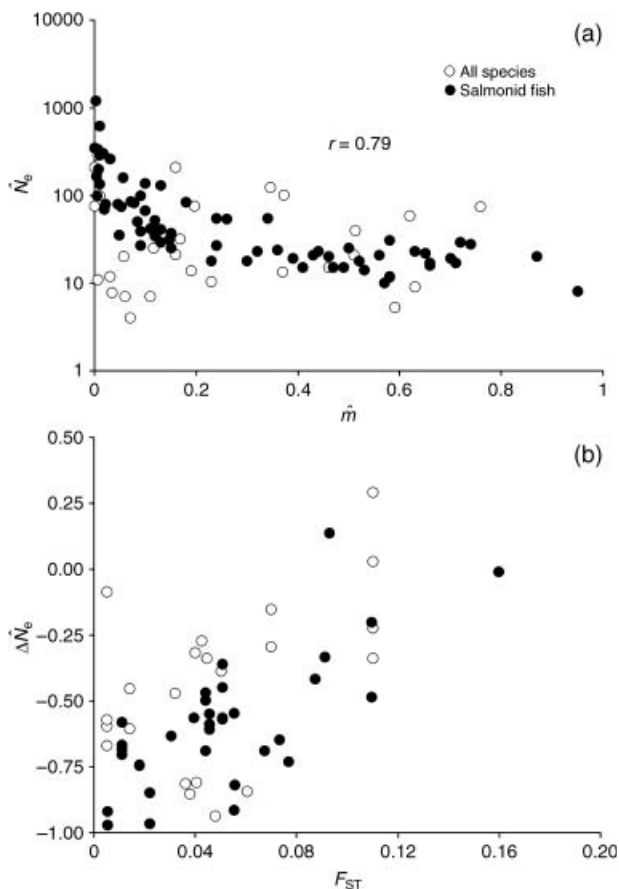


Fig. 1 Gene flow and effective population size estimation. (a) Relationship between estimates of gene flow (m) and effective population size (N_{eOPEN}) following Wang & Whitlock (2003). (b) Effect of considering gene flow on estimates of N_e as a function of genetic differentiation (F_{ST}) between focal and source populations. ΔN_e represents $(N_{eOPEN} - N_{eCLOSED})/N_{eCLOSED}$, estimated from Wang & Whitlock (2003). Correlation coefficient given for salmonid data only ($P < 0.001$). See Table S1 for references.

discussed in detail by Fraser *et al.* (2007a). Among other things, uncertainty remains regarding the causes of the frequently observed large differences between N_e estimates assuming open (N_{eOPEN}) or closed ($N_{eCLOSED}$) populations. The effects of ignoring gene flow in analyses of effective population size depend chiefly on (1) whether gene flow is incidental (discontinuous) vs. systematic (continuous) throughout the period of study (Wang & Whitlock 2003) and more trivially on (2) the extent of genetic differentiation between the focal and the source populations (Wang & Whitlock 2003; Fraser *et al.* 2007a). A downward bias is expected to occur in $N_{eCLOSED}$ (gene flow ignored) when gene flow actually occurs on an incidental basis (i.e. discontinuous) and the extent of this bias is expected to be directly proportional to the genetic differentiation between focal and source populations. Alternately, when gene flow

occurs continuously, estimating $N_{eCLOSED}$ is expected to bias \hat{N}_e upward. Although the extent of this bias may perhaps be somewhat dependent on the extent of the genetic differentiation between the focal and source populations, in practice under continuous gene flow, both populations are expected to be little differentiated.

We evaluated the likelihood of the two scenarios (upward vs. downward bias) by comparing the relative change in effective population size estimates when considering gene flow ($\Delta N_e = (N_{eOPEN} - N_{eCLOSED})/N_{eCLOSED}$) with the extent of genetic differentiation from gene flow sources as a proxy for the type of gene flow (continuous vs. incidental) (based on 56 available estimates). Our analyses suggest that there is a strongly positive correlation between ΔN_e and F_{ST} ($r = 0.56$, $P < 0.001$, Fig. 1b). This relationship becomes stronger when only considering anadromous salmonid fishes ($r = 0.72$, $P < 0.001$), arguably a group with large potential for gene flow due to their migratory life cycle (Stabell 1984; Quinn 1993). Within the salmonids, 29 of 31 estimates of ΔN_e are < 0 , indicating an upward bias in \hat{N}_e under closed conditions ($N_{eCLOSED}$). This could suggest that systematic, more or less 'continuous' incoming gene flow may generally be responsible for a reduction in the genetic drift signal. Consistent with this interpretation, the only positive estimates of N_e occur when populations are highly differentiated ($F_{ST} > 0.08$), expected only under conditions of very low and intermittent gene flow. Two studies on nonsalmonid species with an $N_{eOPEN} > N_{eCLOSED}$ (Lacson & Morizot 1991; Lessios *et al.* 1994) reported temporal instability of population structure, possibly due to (incidental) intermittent gene flow or recolonization. Ultimately, this issue may only be resolved in experiments with different scenarios of gene flow, under conditions where focal N_e can be controlled for. Although the analytical model of Wang & Whitlock (2003) may not fully capture the potential complexity of gene flow in biological systems, these analyses reiterate that contemporary gene flow, whenever biologically plausible, needs to be accounted for in empirical studies (Fraser *et al.* 2007a). Paradoxically, considerations of gene flow in the temporal method may become more important as populations become effectively larger, since genetic drift will be weaker under such circumstances.

Effective population size of metapopulations

The discussion about which effective population size estimate (open or closed) reflects the 'true' N_e may be confounded by the possibility that these estimates may actually apply to different levels in a metapopulation. Over longer terms, with constant gene flow, the local effective population size may reflect the genetic behaviour characteristic of a larger hierarchical level represented by a metapopulation (meta- N_e). Conversely, unrecognized

population substructuring may also bias estimates of effective population size downward. Under biologically realistic scenarios, the most likely effect of population subdivision is a (substantial) reduction in (meta) N_e (Whitlock & Barton 1997; Nunney 1999; Wang & Caballero 1999; Whitlock 2004). Whenever variance in subpopulation or 'deme' productivity is larger than random, meta- N_e is expected to be smaller than the sum of local effective sizes (Whitlock 2004). This may particularly be true under scenarios of asymmetrical gene flow and unequal (sub)population sizes, which might be common in natural systems (Fraser *et al.* 2004; Consuegra *et al.* 2005; Manier & Arnold 2005; Fraser *et al.* 2007a, b; Hansen *et al.* 2007; Palstra *et al.* 2007). Under such conditions the reproductive contributions of individual demes will vary greatly, effectively acting to enhance the reproductive variance of individuals within demes, hence providing an alternative explanation for low N_e and N_e/N estimates. Conversely, the genetic behaviour of individual demes will strongly depend on their relative role in a metapopulation (Wang & Caballero 1999), that is, whether they function as relative sources or sinks. Gene flow can thus strongly influence the effective size of a (sub)population, with its disregard potentially leading to strong positive or negative biases in the estimation of N_e . We reiterate that researchers should not *a priori* assume population isolation (or unity) when estimating effective population size.

The 'gene flow problem' has received relatively little attention in the literature on temporal effective population size, but may have contributed to some extremely low estimates of effective population size in marine fishes (Hauser *et al.* 2002; Hoarau *et al.* 2005; Laurent & Planes 2007). For example Hoarau *et al.* (2005) have reported N_e/N ratios of $\sim 10^{-5}$ in plaice (*Pleuronectes platessa*) in the North Sea, where population substructuring has been documented (Hoarau *et al.* 2002). Although the local population scale may be difficult to capture (Waples 1998), conclusions drawn from these studies may have been different if estimates were applied to that scale. Despite recent efforts using a combination of genetic and nongenetic approaches (e.g. Bekkevold *et al.* 2005; Ruzzante *et al.* 2006 for herring; Pampouli *et al.* 2006 for cod), population structure and dynamics remain poorly quantified for most marine systems. Increased knowledge of variance in habitat productivity (Saillant & Gold 2006) is required, as well as the extent to which gene flow (asymmetry) modulates this variance, since this will determine overall N_e . We need more empirical studies on metapopulation N_e (meta- N_e), in particular on closed systems where the number of populations or demes is known and can be sampled temporally. Under these conditions, one can start to disentangle the effects of processes operating at the metapopulation level (variance in gene flow and productivity among demes) from those acting within demes (individual reproductive

variance and other biological aspects). Without such knowledge, it will remain challenging to attach biological significance to meta- N_e estimates, in particular those for marine fish.

Concluding, the extremely low N_e/N ratios for marine fish could reflect analytical biases, as well as real biological effects of (undetected) population structuring and life history. Although future studies may succeed in removing estimator bias, ultimately the practical value of precisely estimating very large N_e also needs to be considered. Very large N_e values would indicate that genetic stochasticity may be of minor importance, as such forming a poor guidance tool for conservation and management. Studying the context and process of natural selection in large populations may perhaps provide more useful avenues for predicting evolutionary responses to factors such as fisheries pressure and environmental changes (Conover & Munch 2002; Olsen *et al.* 2004; Poulsen *et al.* 2006; Hutchings *et al.* 2007).

Relevance of effective population size for conservation

A comparison of \hat{N}_e based on conservation status

Threatened or endangered species have been shown to exhibit lower (neutral) genetic diversity than closely related nonthreatened taxa (Spielman *et al.* 2004). Threatened species may therefore be expected to exhibit relatively low effective population sizes. In addition, they may also be characterized by lower N_e/N ratios if deterministic factors, contributing to census declines, commonly act to reduce N_e to levels below biological expectations (Luikart *et al.* 1998; Turner *et al.* 2006). Increased demographic stochasticity at low census size may have similar effects by enhancing individual reproductive variance. Conversely, if environmental stochasticity is driving population declines, this may act to increase N_e/N ratios, since it affects all individuals in a population equally, thereby possibly reducing individual reproductive variance. Changes in biological interactions at low abundance (genetic compensation) would have a similar effect on N_e/N . Therefore we hypothesized that populations of conservation concern exhibited lower N_e values, and evaluated if they had lower N_e/N ratios relative to presumably stable populations. Hence we divided our data according to whether estimates correspond to populations of conservation concern or not, as well as to (formerly) commercially exploited populations.

Information on conservation status was collected from the respective publications: populations were assumed nonthreatened whenever there was no mention of conservation concerns. We only considered estimates assuming closed populations, but whenever *multiple* estimates were available for the same population-time period combination,

we selected the estimator method deemed most appropriate for the particular species (we note here that different estimators usually gave similar point estimates). Importantly, we only considered estimates that were assumed to be free of bias due to inappropriate model selection (see above). Furthermore, we ignored N_e estimates of infinity, since they most likely reflect a lack of statistical power to estimate N_e (see Waples 1989), but we did include some N_e/N ratios larger than one. Applying these restrictive criteria, the data set for this analysis contained 143 estimates of N_e and 65 estimates of N_e/N , covering a wide range of phyla, but somewhat biased towards anadromous fish (see Appendix). The question of whether effective population size differs between these three (conservation, stable, exploited) categories was first addressed with nonparametric tests (Mann–Whitney U) conducted on the untransformed data. This analysis was then repeated with transformed data (logarithmic and arcsine-square root, respectively, for N_e and N_e/N) and the significance assessed with pairwise t -tests. Average transformed values are reported back-calculated to untransformed estimates to facilitate interpretation.

We find that populations of conservation concern were characterized by smaller effective sizes: N_e (conservation concern) = range 7–1160, $\bar{N}_e = 149 \pm 31$ vs. N_e (nonconservation concern): range 19–8935, $\bar{N}_e = 295 \pm 80$ (one-sided t -test, $P = 0.0004$), though the magnitude of the mean N_e estimate (267 ± 65) suggests that, in general, genetic stochasticity is not likely of immediate concern (Table 1, Fig. 2). Commercially exploited marine fish exhibited significantly larger effective population sizes (range 560–19 535, $\bar{N}_e = 2085 \pm 322$) than the other two categories. Nonparametric tests gave similar results for the comparison of groups based on conservation concern (Mann–Whitney U -test, $Z = 3.997$, $P < 0.0001$) and when commercially exploited species are compared with stable species (Mann–Whitney U -test, $Z = -2.4315$, $P = 0.015$). Here we note that estimates for nonthreatened species were derived from (nonsignificant) longer time spans (one-sided t -test, $P = 0.07$), and can therefore, if anything, be expected to be smaller due to the effect of fluctuating population size (Wright 1938; Vucetich *et al.* 1997; Kalinowski & Waples 2002).

Contrary to effective population size, N_e/N ratios did not differ among the two conservation categories (Fig. 2, Table 1). N_e/N ratios were slightly higher, but not significantly so (one-sided t -test, $t = -1.386$, $P = 0.09$), for populations of conservation concern than for presumably stable populations (0.27 ± 0.15 vs. 0.18 ± 0.17). Marine fish populations though were characterized by substantially lower ratios ($1.0 \times 10^{-4} \pm 1.0 \times 10^{-4}$) (one-sided t -test, $t = -3.979$, $P = 0.001$). Again, nonparametric tests on the untransformed data sets gave very similar results (Mann–Whitney U -tests; $Z = -1.2899$, $P = 0.122$ for the conservation/stable

comparison and $Z = -3.8121$, $P < 0.0001$ for the exploited/stable comparison). Multigenerational ratios are often based on arithmetic mean census sizes, thereby incorporating the effect of fluctuating population size. Without those estimates, we find that N_e/N ratios are significantly higher for populations of conservation concern (0.36 ± 0.16 , median 0.37, one-sided t -test, $P = 0.039$) than for presumably stable populations (0.18 ± 0.12 , median 0.14), with results for exploited populations unchanged. When all estimates were considered, regardless of conservation status, the average N_e/N ratio is 0.16 (± 0.13 , median 0.14, $n = 65$) with, and 0.20 (± 0.15 , median 0.15, $n = 52$) without, the ratios based on the arithmetic mean census size. These results reflect the reducing effect of fluctuating population size on N_e/N (Wright 1943; Frankham 1995; Vucetich *et al.* 1997), perhaps especially for populations of conservation concern. Higher N_e/N ratios for this latter group may also imply that genetic compensation against reductions in N_e commonly operates at low census size (see below).

These estimates are slightly higher than those reported by Frankham (1995) (median value ~ 0.1), but not as high as expected theoretically (0.25–0.50; Nunney 1991, 1993, 1996). Frankham (1995) considered mainly demographic estimates, and genetic estimates can *a priori* be expected to provide lower values, since they should incorporate all demographic effects. Empirical studies that have made a direct comparison between demographic and genetic estimates generally confirm these expectations (Husband & Barrett 1992; Ardren & Kapuscinski 2003; Rowe & Beebe 2004; Schmeller & Merila 2007; Watts *et al.* 2007). Importantly, interpreting N_e/N ratios from published genetic estimates can be difficult (Waples 2005), as it is often not made explicit what generation of census sizes (N) the calculated effective sizes refer to. Inappropriate estimation of the corresponding census population size will introduce additional bias to N_e/N estimates. The general unavailability of census data in the literature precludes an evaluation of the magnitude and direction of this bias and therefore we recommend interpreting our ratios with some caution.

N_e/N ratios and population size

A 'constant' value of the N_e/N ratio, regardless of temporal fluctuations in population size, would have great practical value in conservation. However, temporal fluctuations in effective size and the N_e/N ratio are not uncommon within natural populations and can be larger than differences between populations (e.g. Miller & Kapuscinski 1997; Heath *et al.* 2002; Waples 2002a; Shrimpton & Heath 2003; Fraser *et al.* 2007b). Thus, N_e/N ratios cannot be assumed constant over time or population size. One theoretical explanation for an inverse relationship between N_e/N ratio and N is that the standardized variance in family size increases as N increases, and conversely, that this variance

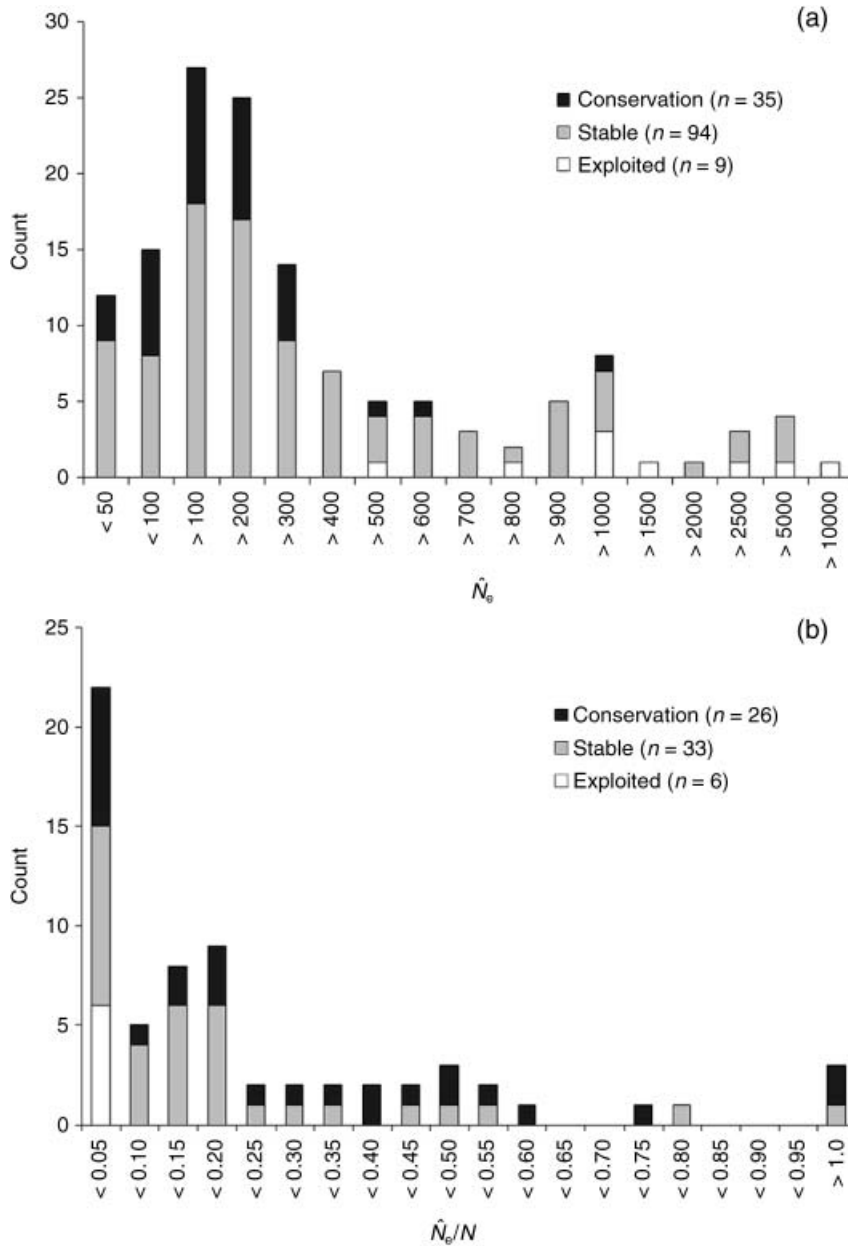


Fig. 2 Distribution of selected empirical estimates of (a) effective population size (\hat{N}_e) and (b) the ratio of effective size to census size (\hat{N}_e/N) for wild populations, sorted by different categories. Figures are based on 144 published \hat{N}_e and 66 \hat{N}_e/N values. See Table S1 for references.

decreases as N decreases (Hedrick 2005). Such genetic compensation can be viewed as a buffering mechanism against genetic adversity at low N , operating at the intrapopulation level. Initially observed under controlled laboratory settings (Pray *et al.* 1996), indications of genetic compensation in the wild have also been documented in salmonid fishes (Ardren & Kapuscinski 2003; Araki *et al.* 2007; Fraser *et al.* 2007b) and damselflies (Watts *et al.* 2007). For salmonid fish, genetic compensation can be reconciled with aspects of their breeding ecology (Fleming 1996; Fleming 1998), such as precocious mature male parr (Jones & King 1952), that may have increased fertilization success

at low breeding densities (e.g. Jones & Hutchings 2001; Jones & Hutchings 2002). Therefore, we compared N_e/N ratios to census population size (N), using empirical values reported in the literature, excluding ratios based on the arithmetic mean census size. We limited these analyses to salmonid fish ($n = 28$), since our main goal was to quantify potential density-dependent influences on N_e/N for similar mating systems.

We find that the ratio of N_e to N is indeed higher at lower census size (Fig. 3), thus suggesting that genetic compensation may be a real feature of natural (salmonid) populations. However, there are a few alternatives to

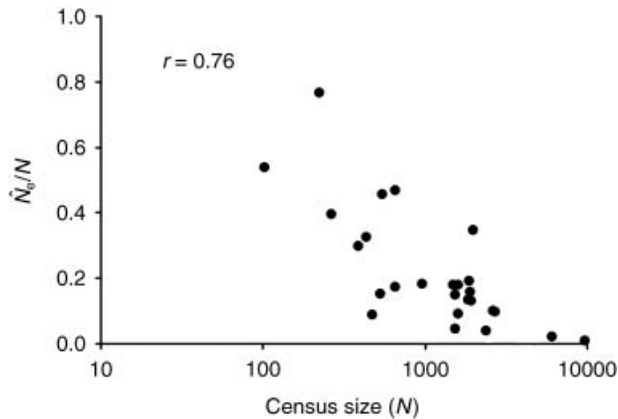


Fig. 3 Genetic compensation as an intrapopulation buffer against reductions in effective population size (N_e). Plotted are empirically derived ratios of N_e/N against harmonic mean census size (for $N < 10000$) for salmonid fish only. See Table S1 for references.

consider. First, N_e/N ratio estimates for very large census populations may have been (additionally) reduced by the effects of population substructuring (see above). Importantly, these results may simply reflect a spurious negative correlation, caused by plotting a ratio against its denominator. Therefore we cannot currently conclude, based on these data, that genetic compensation is or is not a realistic aspect of salmonid or other populations. Many more studies are required on natural systems where variance in reproductive success can be directly measured and related to population density. The ultimate relevance of genetic compensation to conservation will hinge on appropriate knowledge of the biological and environmental factors that control intraspecific competition and reproductive success.

Does \hat{N}_e predict genetic stochasticity?

The practical relevance of effective population size in conservation genetics is its predictive value for adverse genetic processes (Frankham *et al.* 2003). Since endangered species have smaller effective population sizes, one could expect them to be more sensitive to genetic stochasticity. This expectation, however, requires validation with empirical data, and here we do so by relating published N_e estimates to the amount of neutral genetic diversity (average multilocus heterozygosity ΔHe) and its rate of loss of over time (ΔHe). We did not consider number of alleles, despite their sensitivity to sudden population declines (Nei *et al.* 1975; Maruyama & Fuerst 1985; Luikart *et al.* 1998; Garza & Williamson 2001), because the theoretical relationship with effective population size is complicated by additional factors (Maruyama & Fuerst 1985).

It is worth noting that these genetic properties are related to different definitions of effective population size. Whereas genetic drift (and random loss of alleles) is determined

by variance effective size ($N_{e(v)}$), loss of genetic diversity (heterozygosity) and inbreeding are predicted by inbreeding effective size ($N_{e(i)}$) (Kimura & Crow 1963). The former is a function of the sampling process (size of offspring generation), whereas the latter is determined by the size of the parental generation. These two forms of effective size are equal when population size is constant over time, but are offset in populations that drastically fluctuate in size (Crow & Denniston 1988).

Joint estimates of \hat{N}_e and genetic metrics were selected from the literature using the following criteria. For each population, we only considered noninfinite temporal estimates for the longest time span covered. Whenever N_e was estimated using several different estimators, we selected the method considered most applicable. For the analyses of loss of genetic diversity (ΔH_E), we included short-term ($t < 4$ generations) estimates, to increase the number of data points, but assessed consistency with a more restricted data set ($t > 4$ generations). Relationships between effective population size and genetic metrics were investigated using generalized linear regression models.

Importantly, theoretical predictions of genetic stochasticity based on N_e assume that the population under consideration is closed to gene flow. Ongoing gene flow can change the impact of genetic stochasticity, as well as influence \hat{N}_e (see above), but is often assumed to be negligible. We simultaneously tested the validity of this assumption by conducting analyses with all data points and with only those that were obtained from isolated populations (i.e. those for which no potential sources of contemporary gene flow could be inferred from the information in published studies).

For the gene diversity analyses we only considered studies using microsatellite markers and estimated gene diversity as the average of (two) individual time point estimates. We compiled 86 combined estimates of N_e and H_e from a wide taxonomic range (26 isolated and 60 open populations). Analyses reveal that genetic diversity as measured by heterozygosity is strongly correlated with \hat{N}_e when only closed populations are considered (log-linear, $r = 0.73$, $P < 0.001$, Fig. 4a). This correlation weakens, though remains significant, when all populations, including those considered open to immigration, are included in the analysis (log-linear, $r = 0.34$, $P = 0.002$, Fig. 4a).

We compiled 126 individual population estimates of N_e and rate of loss of genetic diversity (H_T/H_O , calculated on a rate of decrease per generation basis), again from a wide range of organisms (for 32 isolated and 96 open populations). The second, more conservative data set only contained 59 combined estimates applying to time scales longer than 4 generations (21 isolated, 38 open populations). Loss of genetic diversity over time was evaluated by fitting a model of the expected rate of loss of heterozygosity, based on effective population size ($\Delta He = H_T/H_O = [1 - 1/2N_e]^t \approx e^{-(t/2N_e)}$;

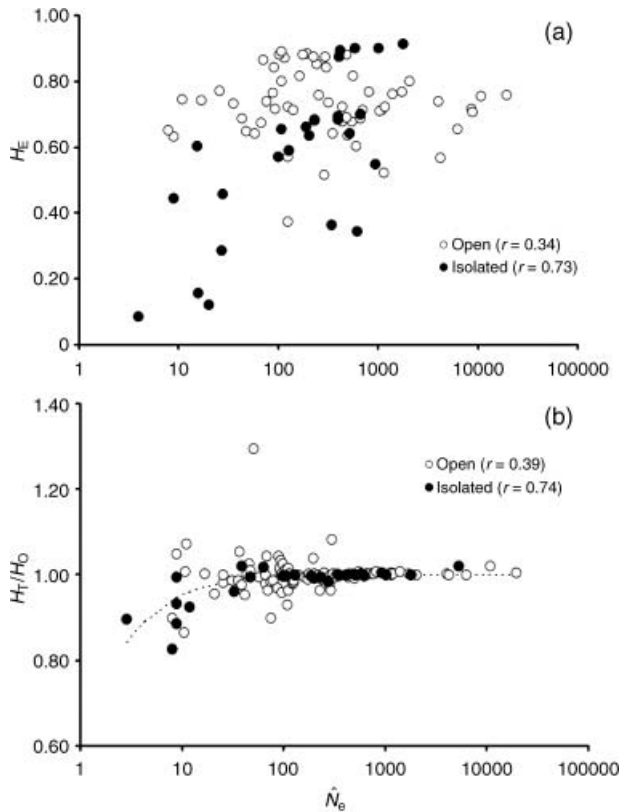


Fig. 4 Genetic stochasticity as a function of effective population size. Given are the relationships between empirical estimates of N_e and (a) average multilocus heterozygosity (H_E) and (b) generational rate of loss of genetic diversity over time (H_T/H_0). Shown are data points for isolated populations (black circles) and populations open to gene flow (open circles) along with the correlation coefficients when all points are considered jointly (following 'Open') or when only isolated populations are considered isolated (following 'Isolated'). See text for additional information and sample sizes.

Crow & Kimura 1970) to the observed temporal rate of change in genetic diversity.

\hat{N}_e generally provides a good indication of the rate of relative loss of genetic diversity, provided that the populations considered are isolated. This is particularly so for small populations, where values of change in gene diversity can be seen to fluctuate widely (Fig. 4b). These findings are similar when only considering the more robust genetic estimates ($t > 4$ generations): the predictive value of \hat{N}_e for rate of decrease in heterozygosity was highest for estimates of isolated populations in the more restricted data set ($r = 0.78$, $P < 0.001$). Genetic diversity therefore only appears to be lost substantially when populations are both small and isolated.

The main conclusion from these analyses therefore is that temporal estimates of N_e only provide relevant information on the neutral genetic processes they are theoretically

expected to predict, when the focal population receives no immigrants. Effective population size correlated poorly with genetic diversity and its loss over time when 'open' estimates were considered: gene flow appears to uncouple N_e from genetic stochasticity. This can be due to its buffering effect on genetic stochasticity, or impact on N_e itself, or a combination of these two. This latter explanation may be most likely, since temporal approaches infer N_e from measures of genetic drift. Therefore, generally speaking, effectively small populations may be less susceptible to genetic stochasticity than expected.

On the other hand, there may be a common discrepancy between $N_{e(I)}$ and $N_{e(V)}$ in natural populations. The genetic metrics considered are predicted by $N_{e(I)}$ (Kimura & Crow 1963), whereas temporal estimates reflect $N_{e(V)}$. We attempted to correct for this by considering temporal estimates for longer time spans where $N_{e(I)}$ and $N_{e(V)}$ would be roughly similar. However, a population may only acquire equilibrium N_e (when $N_{e(I)}$ and $N_{e(V)}$ are the same) when the underlying demographic and ecological dynamics remain constant for a large number of generations (Kimura & Crow 1963; Crow & Denniston 1988; Wang & Pollak 2000). Our results may suggest that this is generally not true for natural systems, and highlight the difficulty with interpreting genetic processes under nonequilibrium conditions (Hutchison & Templeton 1999; Whitlock & McCauley 1999). Population declines may be common cause of such a discrepancy, complicating the practical value of temporal estimates for conservation. This is another reason for combining the temporal method with other approaches for estimating N_e that do reflect inbreeding effective size (Hill 1981; Bartley *et al.* 1992; Waples 2006; Tallmon *et al.* 2008), thus providing more complete insights into the genetic behaviour of natural populations.

Conservation implications

The main goal of empirical studies of contemporary N_e arguably is to assess evolutionary potential and vulnerability to genetic stochasticity, under the rationale that this parameter will be informative on these processes. Based on our review, the size of an average natural population ($\hat{N}_e \sim 260$) indicates that we may often have to be concerned about the detrimental impact of genetic factors on population persistence. If one applies the most conservative thresholds for critical population size (Franklin 1980; Franklin & Frankham 1998), the majority of natural populations ($\sim 70\%$ of published estimates with $\hat{N}_e < 500$) appear to currently lack sufficient evolutionary potential for long-term adaptation. Additionally, a substantial part ($\sim 8\%$ of all published estimates have $\hat{N}_e < 50$) of natural populations may suffer from adverse effects of inbreeding depression, which is in accordance with studies based on fitness-related traits (Crnokrak & Roff 1999). This picture

obviously becomes more dramatic when considering higher threshold values for N_e (e.g. Lande 1988): populations with contemporary effective sizes in the low thousands appear to be very uncommon (~7%). On the one hand, this may indicate that genetic stochasticity constitutes an important component of population persistence for the majority of wildlife. On the other hand, theoretical predictions of genetic stochasticity as a function of population size assume complete population isolation, but immigration and gene flow may be common attributes of natural systems. Therefore, concerns of genetic stochasticity may often be somewhat overblown, since predictions will only apply when populations are truly isolated.

Our findings are likely not based on a systematic bias in sampling natural populations: N_e tends to be smaller for populations of conservation concern, but these do not constitute the majority of published estimates (~25%). Publication bias, upward and downward, cannot, however, be discounted. The effective size of many 'stable' populations may be too large to enable estimation with 'temporal' genetic methods (using conventional sample sizes), but populations impacted by genetic stochasticity due to low N_e , may also generally become extirpated before they can be sampled (Frankham 2005). Furthermore, although a large component of empirical estimates are for salmonid fish, quantitative and qualitative patterns remain consistent when omitting these. Therefore, our reported values may indeed reflect sizes typical for wild populations. Hence, for an average population, concerns of immediate threats to population persistence due to inbreeding depression (Newman & Pilson 1997; Saccheri *et al.* 1998; Nieminen *et al.* 2001) may be comparatively small. Encouragingly, commonly applied sampling designs appear generally powerful enough to detect levels of N_e at which inbreeding depression starts to become severe (Luikart *et al.* 1999). As such, temporal methods will remain an important tool in genetic monitoring, provided that due consideration is given to the potential sources of bias discussed. Concerns about genetic stochasticity ultimately need to be framed in the context of what is known about the prevalence of gene flow in natural systems.

Our review confirms that genetic stochasticity will only become important when populations have become both effectively small and isolated from the beneficial effects of gene flow. Under these circumstances (neutral) genetic diversity can indeed decay quickly (Fig. 4, Srikwan & Woodruff 2000; Johnson *et al.* 2004). Intrapopulation biological mechanisms may operate to buffer against reductions in N_e when census sizes decline, but by themselves will not replenish genetic variation lost and, ironically, may also interfere with conservation efforts to increase effective sizes. Regardless, quantitative genetic variance is lost at a much slower rate than neutral genetic variation (Willi *et al.* 2006): a substantial amount of trait heritability

and hence evolutionary potential may therefore still be maintained after populations have become effectively small. This maintenance will ultimately be of little protection against fitness losses due to inbreeding depression at small population size.

Population connectivity is perhaps a more important factor than N_e when one attempts to evaluate extinction risk due to genetic factors. Gene flow (at very low levels) can relieve inbreeding depression (e.g. Vila *et al.* 2003) and generally contribute to genetic diversity, even without strongly influencing local heterozygosity (Barton *et al.* 2002; Willi *et al.* 2006; but see Fig. 4). Our own analyses show that gene flow can uncouple N_e estimates from processes of genetic stochasticity, perhaps mainly due to a positive effect on local N_e itself. Genetic variance may generally be maintained at hierarchical levels higher than the local population, reemphasizing the importance of population connectivity. Surprisingly little still is known about the evolutionary dynamics of metapopulations (including its effective size, meta- N_e). Human-induced fragmentation can obliterate such crucial population connectivity. Although the genetic consequences of population fragmentation will vary per species (Ouborg & Vantreuren 1994; Mundy *et al.* 1997; Srikwan & Woodruff 2000; Henle *et al.* 2004; Schmuki *et al.* 2006; Vandergast *et al.* 2007), dispersal ability can influence subsequent local persistence (Hoehn *et al.* 2007). Where population fragmentation persists, gene flow potential can be further reduced due to population divergence as a consequence of (changes in) selection regimes underlying quantitative traits (Hendry 2004; Nosil *et al.* 2005) and/or enhanced genetic drift (Johansson *et al.* 2007; Willi *et al.* 2007a), which may ultimately result in selection against immigrants (Hendry 2004). This would limit the scope for genetic rescue (but see Willi *et al.* 2007b), making early detection of population fragmentation imperative. These considerations reemphasize the utility of establishing wildlife linkages as a conservation strategy (see also Nunney 2001).

In summary, the practical relevance of N_e as an indicator of the role of genetic stochasticity in population persistence will require that some important biases are dealt with in its estimation, as well as an increased understanding of the mechanisms by which genetic variance is maintained in metapopulations. Similar to its countering effect on genetic stochasticity within populations, gene flow may often have a strong influence on (estimating) local N_e : temporal genetic methods derive N_e from measures of genetic drift, itself forming one major component of genetic stochasticity. Paradoxically, although very little gene flow can confound temporal estimates of N_e , such low levels may hardly impact deterministic evolutionary processes. Researchers should be cautious in assuming that the focal population constitutes one single panmictic unit, since violation of this assumption implies that estimates may not apply to local

scales (Nei & Tajima 1981), thereby complicating inferences of genetic stochasticity and evolutionary potential based on \hat{N}_e . This uncertainty of scale reiterates that linking population genetic parameters with ecological and evolutionary processes is important (Waples & Gaggiotti 2006; Palsboll *et al.* 2007) but remains challenging. Such issues will, however, become less relevant with ongoing habitat fragmentation, where the evolutionary fate of endangered populations will progressively start to depend on local population size and less on connectivity (e.g. Johnson *et al.* 2004). Consequently genetic factors will likely become more important for population persistence and hence species extinction. Concluding, considering genetic stochasticity should remain an important focus in the conservation of biodiversity.

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This study is an extension of Friso Palstra's PhD research in Daniel Ruzzante's laboratory. Friso Palstra is interested in elucidating mechanisms that shape the evolution and ecology of (aquatic) organisms and their relevance and application to conservation and management. Daniel Ruzzante is interested in conservation genetics, biodiversity and phylogeography of aquatic organisms.

Supplementary material

The following supplementary material is available for this article:

Table S1. Overview of empirical studies of effective population size in natural populations, for different groups. Furthermore, we indicated, for each statistical analysis, the sources of used data (black circles and white circles). White circles refer to estimates that were used some general analyses, but were excluded in more restrictive data sets. Total numbers of (combined) data points used in analyses are given below each column. † refer to reanalyzed data sets.

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