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**Identification of biologically meaningful management units
for Baltic Sea key organisms (5-10 species)**

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Introduction

This task has focused on two questions relating to the identification of biologically appropriate management units. First, we have addressed the issue of distinguishing general patterns for the distribution of genetic variation and the existence of barriers to migration in seven fish species in the Baltic Sea. Further, we have examined in detail the expected bias of estimates of the genetically effective population size (N_e) when sampling from populations subject to immigration, i.e., when applying an estimation procedure designed for isolated populations to real data from species characterized by considerable amounts of gene flow/immigration, as is typical for those of the Baltic Sea.

The potential existence of a pattern for distribution of genetic variation that is common to multiple species would help in understanding the evolutionary forces shaping genetic profiles and aid when designing management programs for separate species. To allow for maintenance of short and long term evolutionary potential of targeted stocks, sustainable fisheries require biologically appropriate management units to prevent over harvest and loss of genetic variation. Genetic guidelines on the management of natural populations almost exclusively refer to distinct and isolated populations, whereas marine fish populations, like those of the Baltic Sea, typically exhibit a more or less continuous distribution over large geographical areas. Under such conditions it is important to identify population segments that are reasonably self sustaining and genetically homogeneous, resembling the panmictic unit that is generally suggested as the basis for biologically sound management. Such management reduces the risk for mixed harvest from multiple populations/genetic units that potentially leads to over harvest of separate populations (Laikre et al. 2005a,b).

In parallel with the largely empirical species comparison work we have also addressed, from an analytical perspective, the question of how gene flow affects estimates of genetically effective population size (N_e), i.e. the quantity that determines the rate of inbreeding and genetic drift. Recent empirical estimates of effective size have indicated that many marine populations may be much more vulnerable to inbreeding and drift than previously anticipated. Many marine species are characterized by large population sizes, high levels of genetic variation, and weak differentiation. A series of recent papers on exploited marine species report surprisingly small estimates of effective size, however. For example, Hauser and Carvalho (2008) reviewed 28 N_e assessments for marine fishes and found that estimates of effective size are between two and five orders of magnitude smaller than census size (N_C), with an average N_e/N_C ratio of 10^{-4} , which is only a small fraction of those reported for most other species. They also noted that most point estimates were in the range from hundreds to low thousands, and within the range where loss of genetic variability could be a justified management concern. The primary purpose of our present analysis was to address the question whether this is a valid concern, or if the small N_e/N_C ratios observed in many marine species are more likely reflecting statistical errors when ignoring the effect of migration.

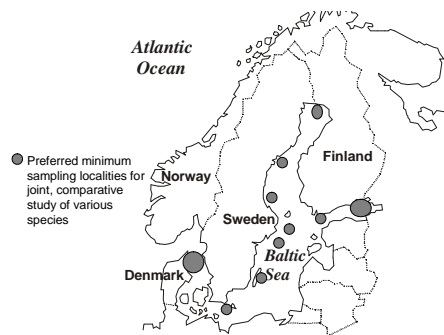
Identification of management units

This work has progressed along two different lines where we *i*) compared genetic variability patterns among five fish species testing for the existence of general

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patterns for the distribution of genetic variation and for boundaries to gene flow common to multiple species, and *ii*) conducted more detailed analyses of two species from 18 sampling localities along the Swedish east coast.

Comparing genetic patterns across species



In this study we compared spatial genetic structures from five Baltic fish species collected at the same sites covering the Baltic (see map), and looked for general patterns of genetic variation and divergence. Genotypic data for these comparisons were contributed by several partners of the BaltGene project as well as from previous studies. The key issue was to use samples collected largely at the same localities (see map). Further, to the extent

possible the analysis should be based on microsatellites with a preferred minimum of 8-10 loci per species, and the targeted number of individuals per site was at least 20 but preferably 40-50. We identified five fish species meeting these criteria reasonably well, i.e. northern pike (*Esox lucius*), whitefish (*Coregonus lavaretus*), herring (*Clupea harengus*), three spined stickleback (*Gasterosteus aculeatus*), and ninespine stickleback (*Pungitius pungitius*).

The results of this comparative study is described and discussed in greater detail in the BaltGene 1.1 and 3.1 task reports. With respect to the questions relevant to the present issue, the major conclusion is that no broad picture can be distinguished across species for the distribution of genetic variation or for the existence of barriers to gene flow (migration). The only obvious similarity is that the Strait of Öresund represents a strong barrier to gene flow in all species, thus further reinforcing the conclusion of Johannesson & André (2006) that the Baltic Sea populations are genetically isolated and distinct from those further west in the Atlantic Ocean. The apparent lack of other similarities across species with respect to connectivity and distribution of genetic variability suggests that assessment of biologically appropriate management units must be conducted on a species by species basis.

Assessing spatial scale for management for two coastal fish species

A more detailed study of the distribution of genetic variation was performed for perch (*Perca fluviatilis*) and whitefish (*Coregonus maraena*, a subspecies of *C. lavaretus*) collected from 18 sites along the Swedish coast of the Baltic Sea with sample sizes of c. 45 fish per site and species (Olsson et al. 2011a,b). In contrast to the above five-species comparison this study revealed rather similar genetic variability patterns that also conformed with those from a recent survey of pike (*Esox lucius*) in the Baltic Sea by Laikre et al. (2005b). The reason for this discrepancy may be that larger sample sizes are necessary than what could be obtained for the five-species comparison, or that patterns are easier to detect when focusing on species with a more restricted spatial distribution and/or more similar evolutionary background. In particular, the perch, pike, and whitefish are all coastal freshwater species that have recently adapted to the salinity in the Baltic Sea.

In all the three species (perch, pike, and whitefish) the pattern for distribution of genetic variation is strongly affected by isolation by distance (IBD); that is, most of

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the gene flow occurs between neighboring sites, and individuals or populations become progressively more dissimilar at increasing geographic distance. The overall degree of genetic divergence is smaller for the whitefish than for the others, but as noted by Olsson et al. (2011*b*) there seems to be an overall coherence in the genetic structure between the three species assessed, suggesting a rather general pattern for coastal species. In spite of the strong dependence on isolation by distance for all three species, there is evidence of at least regional differentiation. Both whitefish and perch, for example, exhibit a similar pattern in terms of barriers to gene flow with a strong latitudinal separation in the Baltic Sea, and a marked barrier to gene flow separates the sites between the two major basins in the Baltic Sea (the Gulf of Bothnia and the Baltic Proper) in both perch and whitefish. Spatial autocorrelation analyses indicate that genetic patch size is strikingly similar for perch and pike (c. 100 km), suggesting a spatial scale for management of about 100 km (Olsson et al. 2011*a,b*).

Estimating effective size when sampling from a subdivided population

Most studies estimating effective population size apply the temporal method that provides an estimate of the variance effective size through the amount of temporal allele frequency change under the assumption that the study population is completely isolated. This assumption is frequently violated, and the magnitude of the resulting bias is generally unknown. Particular problems occur when sampling from a structured population where high levels of gene flow may obscure identification of population boundaries and multiple populations may be included in the samples collected for measuring allele frequency change. We studied how gene flow and sampling from multiple populations affects estimates of effective size obtained by the temporal method, and provide analytical expressions for the expected estimate under an island model of migration. We show that the temporal method tends to systematically underestimate both local and global effective size when populations are connected by gene flow, and the bias is sometimes dramatic. Use of estimates of variance effective population size when a population is not genetically isolated can provide inflated expected rates of loss of genetic diversity. This phenomenon may explain the frequently reported unexpectedly low effective population sizes of marine populations that have raised concern regarding the genetic vulnerability of even exceptionally large populations.

A detailed description of this work is provided in Ryman et al. (2011, manuscript) that is about to be submitted to a scientific journal. An excerpt of the most important results, with a minimum of references, is given below.

Background

There is current concern over accelerating rates of loss of genetic variation in natural populations, potentially resulting in increased risks of extinction and reduced capacity for future evolution. Reasons for such erosion include declining population size and progressive isolation of populations that were formerly connected or continuously distributed. In order to obtain quantitative assessments of present rates of loss of genetic diversity, estimating the genetically effective size of natural populations has become increasingly common in the fields of conservation and evolutionary genetics.

The expected rate of loss of heterozygosity of a population is $1/(2N_{eI})$ per generation, where N_{eI} is the inbreeding effective size of the population. There is also a variance effective size (N_{eV}) that determines the amount of gene frequency change

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from one generation to the next, but the two quantities N_{el} and N_{eV} are identical when population size is constant. Many simplified models of population structure only use the concept "effective size" (N_e) without making the distinction between N_{el} and N_{eV} .

When assessing the effective size of natural populations most studies apply the so-called temporal method that provides an estimate of the variance effective size (N_{eV}) through the amount of allele frequency change over one or more generations. The temporal method assumes that the study population is completely isolated and that any observed genetic change is entirely due to genetic drift caused by restricted effective size. In the real world, however, many or most populations are only partially isolated, i.e. they belong to a population system and are connected to neighboring ones through migration. Violation of the assumption of complete isolation constitutes a common source of bias, the direction and magnitude of which is generally unknown, and investigators applying the temporal method tend to ignore or minimize the effect of migration in the discussion of their results.

There are several challenges involved in the estimation of effective size of a population that belongs to a population system. First, allele frequency shifts caused by immigration into the focal population may erroneously be interpreted as genetic drift and thus bias the estimate of N_{eV} . Further, when genetic differentiation is weak between the subpopulations that constitute the population system (the global population) it may be difficult to identify population boundaries and target the focal population for sampling. Many marine organisms, for example, are characterized by high migration rates and low levels of divergence between populations. A sample from the wild may easily include multiple populations, and samples collected at different occasions may consist of individuals from more or less disjunct population segments.

Finally, there are two effective sizes to be considered, i.e. that of a local population and that of the population system as a whole ($N_{eV,tot}$), and statistical power for detection of subdivision becomes an issue when differentiation is weak. In the absence of extensive studies designed to delineate genetic population structure it may be difficult to tell the difference between a subdivided population and a randomly mating one. An investigator sampling from a seemingly large and genetically homogeneous population may actually be dealing with a population system without being aware of it. In such situations an estimate of N_{eV} can be strongly misleading because, depending on the subpopulations included in the sample, it may refer to a local subpopulation affected by immigration, the global population, or something in between.

The problem of using the temporal method for estimating effective size of a population under migration has been addressed by Wang and Whitlock (2003). They devised an approach for simultaneous estimation of N_{eV} and the immigration rate (m) that can be applied to situations where allele frequencies estimates are available for the immigrant gene pool as well as for the focal population. They also discuss some of the general effects of ignoring migration when estimating N_{eV} for the special case of a local population receiving immigrants from an infinitely large donor population. There is, however, no theory that quantifies the expected bias in the estimate of N_{eV} in terms of the characteristics of the global population and the number of subpopulations included in the sample.

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We provide expressions for the expected value of the estimate of N_{eV} when applying the temporal method to samples from a population system where the component local populations (subpopulations) are connected by migration, focusing on the traditional island model of migration. Rather than estimating migration rates, our main interest is to quantify the amount of bias when disregarding migration, paying special attention to situations of high gene flow where population structure may be difficult to detect or delineate, and where multiple subpopulations may be included in the samples used for measuring the temporal change of allele frequencies.

Methods

The derivations underlying the conclusions of this paper are presented in an appendix. The main text only includes some of the key expressions and is meant to be readable without accessing the appendix.

We consider a diploid organism with discrete generations and a population system of s subpopulations, each of effective size N ($N_{eV}=N$), where migration occurs as in an island model of migration. The forces of mutation and selection are ignored. In each subpopulation a proportion m of the genes are derived from the population system as a whole (including the focal subpopulation), and the rest $(1-m)$ are from the subpopulation itself. Conceptually, each subpopulation in generation t contributes an infinite number of progeny to a migrant gene pool. In the next generation ($t+1$) any particular subpopulation consists of a mixture of genes drawn from the migrant gene pool (proportion m) and from the focal subpopulation (proportion $1-m$). The population model is demographically deterministic and genetically stochastic where s , N , and m are fixed quantities, whereas alleles are sampled binomially from both the focal subpopulation and the migrant gene pool, corresponding to the case of “stochastic migration and fixed migration rate” discussed by Sved & Latter (1977).

Temporally spaced samples for estimation of N_{eV} are taken in two consecutive generations (t and $t+1$), and those samples may include individuals from one or more subpopulations that may, or may not, be the same at both occasions. Sampling from multiple or different subpopulations is meant to mimic mixed sample compositions obtained unintentionally for reasons such as a poorly known population structure or subpopulation boundaries that are difficult to identify. In other situations the investigator may deliberately collect mixed samples in an attempt to assess global rather than local effective size.

Within subpopulations the allele frequency change from one generation to the next is determined by the joint effects of drift and migration, and estimates of N_{eV} will be biased if one assumes (erroneously) that the change is due to drift alone. We derive expressions describing how the expected variance of allele frequency shift over one generation is affected by characteristics of the population system, i.e., the number of subpopulations (s), their effective size (N), the migration rate (m), and the amount of divergence among subpopulations (F_{ST}). We focus primarily on estimates of N_{eV} based on allele frequency changes measured over a single generation (t to $t+1$), because the logic for understanding short term changes constitutes the basis for interpreting those observed over longer periods of time. Also, most empirical studies employing the temporal method measure changes over only one or a few generations.

We conducted computer simulations to verify the analytical results, using a slightly modified version of EasyPop as described in a separate appendix. This computer

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program was also used for simulations of estimates of N_{eV} obtained when measuring allele frequency change over multiple generations, and for assessing the effects of small sample sizes.

The equations describing the expected value of N_{eV} are not derived assuming migration-drift equilibrium (steady state). When illustrating the expected behavior of the estimator graphically, however, we have assumed approximate equilibrium conditions to reduce the number of independent parameters.

Results

For the infinite island model ($s=\infty$) with "stochastic migration and fixed migration rate" the equilibrium value for F_{ST} when migration and drift are in balance is

$$F_{ST} = \frac{1}{2N(1-(1-m)^2) + 1 - m} \quad (1)$$

where m is the proportion of immigrants into each subpopulation and N ($=N_{eV}$) is their effective size.

When the number of subpopulations is finite, there is strictly speaking no equilibrium value for F_{ST} in the absence of mutation, since all subpopulations will eventually become fixed for the same allele whenever $m>0$. However, long before such fixation occurs F_{ST} will approach a steady state (quasi equilibrium) where

$$F_{ST} \approx \frac{1}{\frac{s}{s-1} 2N(1-(1-m)^2) + 1 - m}. \quad (2)$$

While local effective populations sizes remain N regardless of migration rates, the effective size of the total population ($N_{eV,tot}$) is

$$N_{eV,tot} = \frac{sN}{(1-(1-m)F_{ST})} \quad (3)$$

which depends on the migration rate as well as on the degree of genetic differentiation among subpopulations (F_{ST}). The equilibrium value for this total $N_{eV,tot}$ is undefined when there is no migration ($m=0$), because F_{ST} then approaches unity (cf. equation 1) and the denominator for $N_{eV,tot}$ becomes zero. On the other hand, when migration occurs ($m>0$) the total effective size is generally larger than, or roughly equal to, the summed effective sizes of the local subpopulations, sN (Fig. 1a). The N_{eV} estimates that we discuss below should be compared with the corresponding parametric values in Figure 1a.

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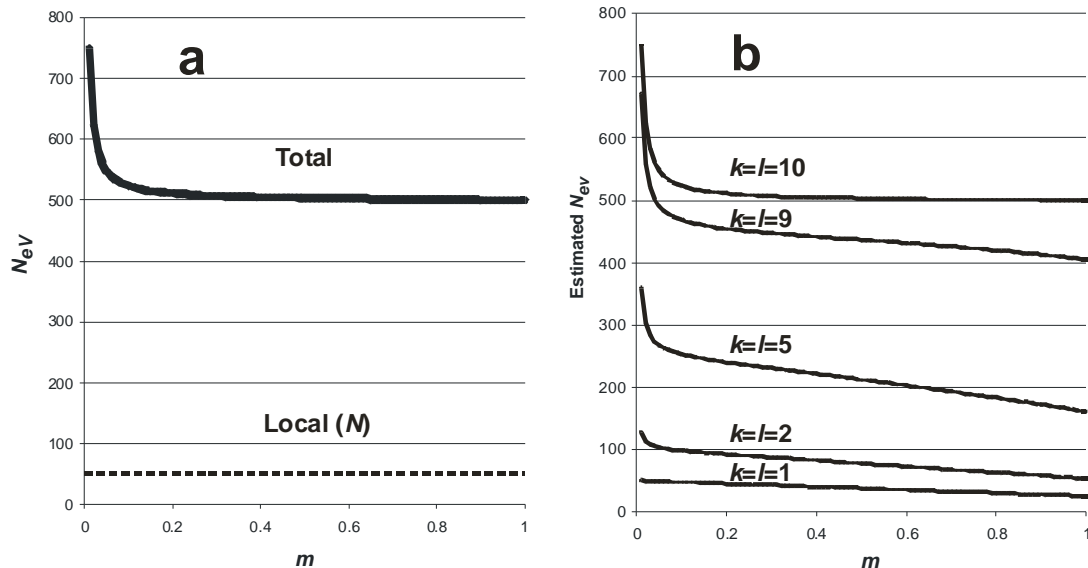


Figure 1. True (a) and expected estimated (b) variance effective size (N_{eV}) at different migration rates (m) for an island model population system in approximate migration-drift equilibrium with $s=10$ subpopulations of effective size $N=50$. The expected estimates in (b) refer to a situation where the same 1, 2, 5, 9, or 10 subpopulations were sampled in two consecutive generations ($k=l$; see text for details).

Applying the above model to an empirical situation of N_{eV} estimation we must consider the expected result of a series of possible scenarios, particularly when sampling from a system of interconnected populations where population structure is unclear or unknown. For the temporal method we consider two samples of n_t and n_{t+1} diploid individuals, respectively, that are drawn one generation apart. Scoring the samples at a set of independently segregating gene loci we denote the sample allele frequencies at the i th locus on the two occasions to be p_t and p_{t+1} , respectively. From these sample frequencies we calculate a measure (estimate) of the amount of allele frequency change (assumed to represent genetic drift only) over the generation interval as

$$F = \frac{\sum (p_{i,t} - p_{i,t+1})^2}{\sum \left(\frac{(p_{i,t} - p_{i,t+1})}{2} \right) \times \left(1 - \frac{(p_{i,t} - p_{i,t+1})}{2} \right)} \quad (4)$$

where the summations are over all loci (and over all alleles if there are more than two alleles per locus).

Under sampling plan II, the quantity (4) can be corrected for the expected contribution from sampling to yield an unbiased estimator of genetic drift

$$F^* = \frac{F[1 - 1/(4\tilde{n})] - 1/\tilde{n}}{(1 + F/4)[1 - 1/(2n_{t+1})]} \quad (5)$$

where \tilde{n} is the harmonic mean of the sample sizes n_t and n_{t+1} in generations t and $t+1$, respectively. With a single generation passing between the two sample events an estimate of the variance effective size in generation t is then given by

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$$\hat{N}_{eV} = \frac{1}{2F^*}. \quad (6)$$

This paper explores how this estimator of N_{eV} behaves when migration occurs among subpopulations, and when individuals from different subpopulations enter the samples. For the purpose of this presentation we assume that k out of the s subpopulations are sampled in each of two consecutive generations. Letting l denote the amount of overlap, i.e. the number of subpopulations sampled at both occasions, we focus on the extreme cases of $k=l$ (the same k subpopulations were sampled both times) and $l=0$ (different subpopulations were sampled on each occasion). For large sample sizes ($n_t, n_{t+1} \rightarrow \infty$) we find that for the case when the same k subpopulations are sampled each generation ($k=l$) the expected value of equation (6) is

$$E(\hat{N}_{eV}) \approx kN \times \frac{1 + \left[\left(\frac{m}{2} \right)^2 - \left(1 - \frac{m}{2} \right)^2 \right] \frac{s-k}{k(s-1)} F_{ST}}{1 - (1-m)F_{ST} + m^2 \frac{s-k}{s-1} 2NF_{ST}} \quad (7)$$

which holds for any value of F_{ST} (equilibrium or not). When equilibrium is attained, equation (1) or (2) can be substituted for F_{ST} , and we evaluate equation (7) for various numbers of actual (s) and sampled (k) subpopulations, and for different levels of migration (m).

Equation (7) indicates that when sample sizes are large and the same k subpopulations are sampled at both occasions ($k=l$), the expected estimate of N_{eV} is the combined size of the subpopulations included in the sample (kN) multiplied by a “reduction factor” that lowers the estimate below kN . The reducing effect of this correction grows stronger as m increases and k decreases (Fig. 1b). Another implication of (7), which will be discussed in greater detail below, is that global effective size ($N_{eV, tot}$) is systematically underestimated unless all the s subpopulations are included in both samples (Fig. 1b).

When completely different subpopulations are included in the two samples ($l=0$) the expected value of equation (6) is

$$E(\hat{N}_{eV}) \approx kN \times \frac{1 + \frac{1-m}{s-1} F_{ST}}{(1 - (1-m)F_{ST}) + (((1-m)^2 + 1)(s-k) + 2k(1-m)) \frac{1}{s-1} 2NF_{ST}}, \quad (8)$$

which also holds whether equilibrium has been attained or not. The general structure of this expression is similar to that of the previous one (7), except that the reducing effect of the “reduction factor” is far more powerful (below). In the next few sections we consider first the behavior of the expected estimate of N_{eV} for particular combinations of k and l at large sample sizes (equations 7 and 8), and next the effects of small sample sizes and of multiple generations between measurements.

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Both samples include one single subpopulation

Here we consider first the case of an infinite island model ($s=\infty$). When both samples only include individuals from one and the same subpopulation at both occasions ($k=l=1$), we find by inserting $k=1$ into equation (7) and substituting equation (2) for F_{ST} , that under migration-drift (quasi) equilibrium the estimated N_{eV} is expected to go from $\hat{N}_{eV}=N$ (when $m=0$) to $\hat{N}_{eV}=N/2$ (when $m=1$). That is, in the absence of migration, equation (6) estimates the local effective population size (N), as it should. With migration, on the other hand, the estimate \hat{N}_{eV} becomes *lower* than N and reaches $N/2$, or a slightly higher value under a finite island model ($s<\infty$), when the global population is panmictic ($m=1$). In other words, with high levels of migration, sampling a single subpopulation for estimating N_{eV} is expected to downwardly bias the estimated local effective size, and even more so if the estimate is thought to represent the effective size of the total population ($N_{eV,tot}=sN$). The reason for this latter, perhaps unexpected, finding is considered in detail in the Discussion. Here, we note that this behavior of \hat{N}_{eV} is verified by results of computer simulations, as depicted in Figure 2a. For the finite model used as an example in Figure 2, with parameters $s=10$ and $N=50$, the expected estimate for $m=1$ is $E(\hat{N}_{eV})=26.3$ (equations 2 and 7), i.e., slightly higher than $N/2=25$ expected under an infinite island model.

Local N_{eV} is always seriously underestimated when the samples include one subpopulation in generation t and another one in $t+1$. For a large number of subpopulations in equilibrium, expression (8) reduces to $\hat{N}_{eV} = k/(4F_{ST})$. For $k=1$ this corresponds to an expected estimated N_{eV} of 0.25 at $m=0$ and $N=26.3$ at $m=1$, in agreement with computer simulations of this scenario (Fig. 2b). Hence, and as is to be expected, under panmixia ($m=1$) it does not matter whether we sample the same or different subpopulations in t and $t+1$, and the estimate will be biased downwards in either case. As shown in Appendix 1 this symmetry holds for any value of k , i.e., when $m=1$ the expressions for \hat{N}_{eV} are identical for $l=0$ and $l=k$.

Both samples include multiple subpopulations

When each sample includes members from multiple subpopulations and the contribution from each subpopulation is the same at both occasions ($k=l>1$), we predict from equation (7) a larger N_{eV} estimate than in the case of one and the same subpopulation being sampled ($k=l=1$). Evaluation of equation (7) reveals that at high migration rates there is a downward bias such that the expected estimate is conspicuously smaller than kN , the combined size of the subpopulations sampled. Further, the expected value of \hat{N}_{eV} is smallest in the limiting case of $m=1$ (Fig. 1b; Fig. 2c, solid line), similar to when sampling the same population in both generations. The analytical results are supported by the results from computer simulations when using large sample sizes (Fig. 2c, triangles; see below for smaller sample sizes).

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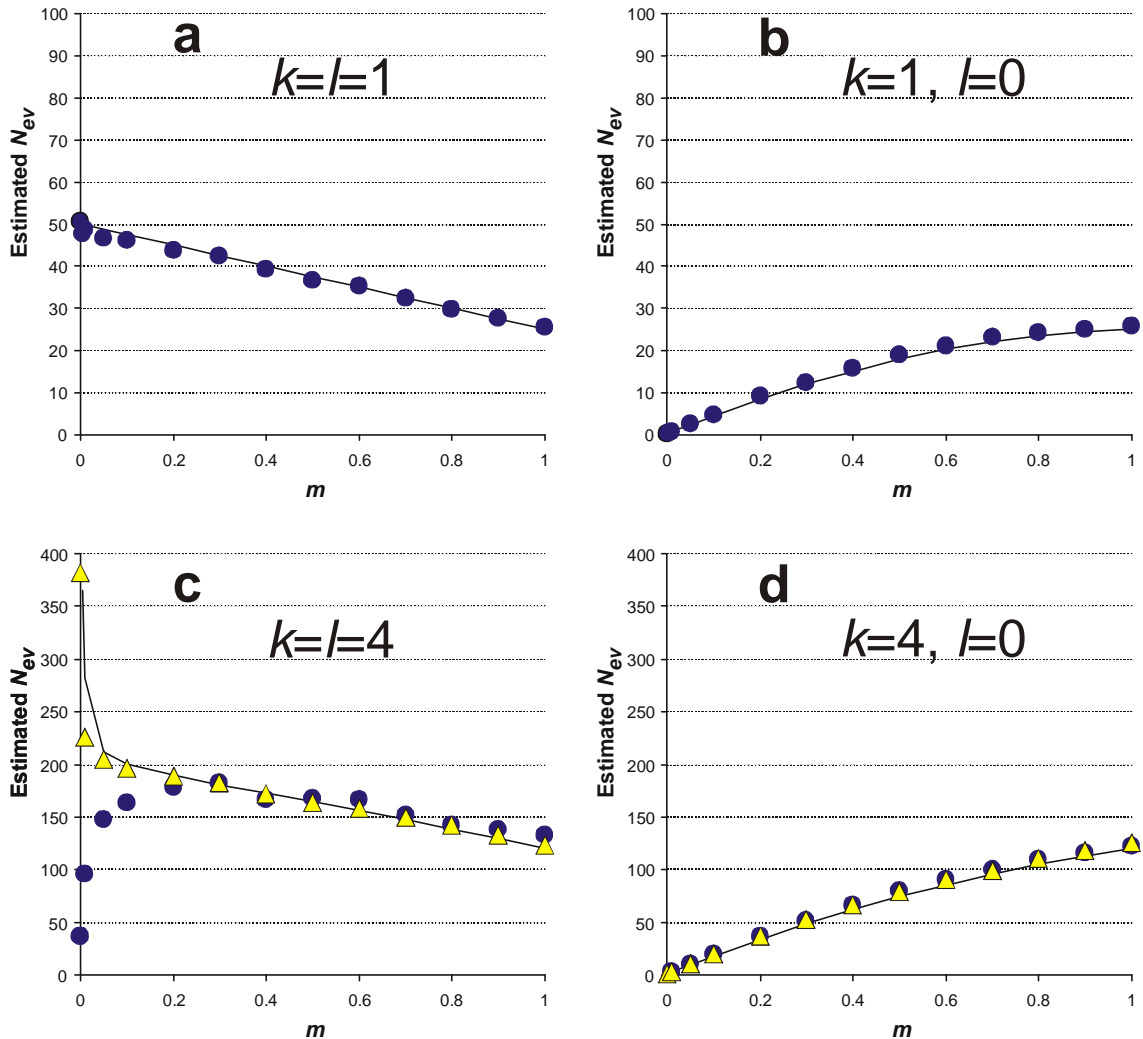


Figure 2. Expected and simulated estimates of N_{ev} at different migration rates (m) for an island model population system in approximate migration-drift equilibrium with $s=10$ subpopulations of effective size $N=50$. The populations sampled in t and $t+1$ are either the same ($k=l$) or different ones ($l=0$), l symbolizing the amount of overlap. Solid lines represent derived expected values (equations 7 and 8) and symbols simulated results. Note the different scales of the y-axes. **a,b:** N_{ev} was estimated by sampling from a single subpopulation in each generation ($k=1$). The populations sampled in t and $t+1$ are either the same (**a**; $k=l=1$) or different ones (**b**; $l=0$). Simulated sample size is $n=100$ diploid individuals. **c,d:** As above, except that $k=4$ subpopulations are sampled in each generation and random sampling of a total of $n=80$ (circles) or $n=800$ (triangles) individuals from the four subpopulations available for sampling (corresponding to an average of $n/k=20$ or $n/k=200$ individuals sampled from each subpopulation). The four subpopulations sampled in $t+1$ are either the same (**c**; $k=l=4$) or different from those in t (**d**; $l=0$).

As a numerical example we may consider the expected estimate for $m=1$ in the case of a global population with $s=10$ subpopulations of effective size $N=N_{ev}=50$ where four of them are sampled in each generation ($k=l=4$) (Fig. 2c, solid line). At migration-drift equilibrium we have $F_{ST}=0.009$ (equation 2), and inserting these values into equation 7 (or the simplified A10) yields an expected estimate of $\hat{N}_{ev}=125$. Considered as an estimate for a local population, e.g., in a situation where including multiple subpopulations in the samples was accidental, the estimate is biased upwards as compared to the true value of $N=50$, whereas it is

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biased downwards if considered as an estimate of the total population, if sampling multiple subpopulations was a deliberate attempt at estimating $N_{eV,tot}$ ($sN=500$). As implied by equation 7 (and A10), the magnitude of this latter systematic downward bias relates to the proportion of subpopulations sampled; it is most pronounced when sampling a single one ($k=l=1$), and it disappears when the global population as a whole is available for sampling ($k=l=s$). This means that the estimate will approach the effective size of the total population in the limiting case of all subpopulations being sampled ($k=s$). In other words, and as noted above, unbiased estimation of the global effective size requires sampling from all the constituent subpopulations, and not just from a limited subset (cf. Fig. 1b).

The extreme, and sometimes potentially unrealistic, scenario when multiple but completely different subpopulations are included in the two samples ($k>1$, $l=0$) yields predicted estimates that are similar to those considered above when a single subpopulation is sampled in generation t and another one in $t+1$ ($k=1$, $l=0$). That is, the expected estimate at migration-drift equilibrium is approximated by $k/4$ at $m=0$ and by $kN/2$, or a somewhat higher value when s is finite, at $m=1$, as verified by computer simulations (Fig. 2d). Hence, when different subpopulations are sampled at the two occasions, whether a single one each time or several different ones, the quantity that is estimated by the temporal method is largely a reflection of the degree of genetic differentiation among populations and is therefore strongly dependent on migration rate. As an estimate of effective size, local or total, this quantity will be biased downwardly for all m .

There are of course intermediate situations of samples including contributions from multiple subpopulations, some of which are the same and others different, as could occur for example during a reanalysis of older samples taken on different occasions and not covering exactly the same geographic area. In general, such situations should lead to results intermediate between those for the extreme situations ($l=0$ vs. $l=k$) considered here.

Effect of sample size

The situation is more complicated than indicated above when more than a single subpopulation is included in each of the samples. This is so because the relative contribution from the different subpopulations then becomes an issue. The expected value of estimated N_{eV} is derived assuming that the number of genes sampled approaches infinity. When sample size is finite, however, a variance component due to sampling from multiple populations is introduced that is not accounted for in the temporal method, where sampling from an isolated population is assumed, and this additional source of variation introduces a downward bias in the estimate of N_{eV} .

As an example of the effect of sample size on the estimate of N_{eV} we may consider the limiting case of $m=0$. The subpopulation allele frequencies will eventually become fixed at 0 or 1, and there is no allele frequency change due to genetic drift that can contribute information on effective size. Irrespective of the fixed allele frequencies, however, temporally spaced *samples* from a mixture of the same k subpopulations ($k>1$) may exhibit different allele frequencies if the proportionate contribution from the different subpopulations varies between sampling occasions. Such an allele frequency difference between samples may

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be erroneously interpreted as a signal of genetic drift. It can be shown, for example, that when $m=0$ the expected estimate of N_{eV} at equilibrium is $E(\hat{N}_{eV}) = n/2$ regardless of the true value of N_{eV} , where n is the total number of individuals sampled from the same k subpopulations in each generation.

This bias is apparent in the results of the computer simulations depicted in Figure 2c for the case of a total population with $s=10$ subpopulations where four of them are sampled ($k=l=4$) in each generation. We simulated total sample sizes of $n=80$ and $n=800$, implying that an average of $n/k=20$ and $n/k=200$ individuals, respectively, are sampled from each subpopulation. As seen from the figure, the effect of sample size is negligible at high migration rates, as expected, because when m is large the subpopulation allele frequencies are similar and the variance component due to sampling from multiple subpopulations is minor. The sample size is critical at low migration rates, however. Drawing a total of $n=800$ individuals provides a fairly accurate picture of the expected estimate of effective size calculated from equation (7), which approaches infinity as m goes towards zero (Fig. 2c, triangles and solid line). In contrast, when m is small an additional downward bias occurs for $n=80$, and in the limiting case of $m=0$, when the total N_{eV} of the four sampled subpopulations is infinity, the simulated estimate is only $\hat{N}_{eV} \approx 40$ (Fig. 2c, circles).

When different populations are sampled in t and $t+1$ the effect of sample size (n) on the expected estimate of N_{eV} is negligible (Fig. 2d; $k=4$, $l=0$), the apparent reason being that differences in relative contribution from the various subpopulations is not an issue when different ones are sampled at the two occasions. Again, at $m=1$ the expected estimate of N_{eV} for $l=0$ is identical to the corresponding quantity obtained when the same four subpopulations are included in both samples ($k=l=4$; Fig. 2c).

Discussion

There are several problems involved when using the temporal method for estimating local or global effective size in population system, and particularly so when differentiation is weak (Fig. 2). Sampling from one or more specific local populations is expected to underestimate their combined effective size when measuring change over a single generation. Measuring over longer periods can introduce an upward bias, the magnitude of which depends on the length of the sampling interval and the effective size of the global population.

When the parameter of interest is global effective size, all the subpopulations must be sampled to prevent underestimation, which may be difficult in many situations. This is true also in the case of complete panmixia ($m=1$) if reproduction is spatially or temporally structured in a way that results in a distinct grouping of the breeders that constitute the unit for sampling efforts. Further, at high levels of differentiation (little migration) inadequate sample sizes may introduce a downward bias also when all the subpopulations are sampled. Thus, applying the temporal method for assessing the rate of loss of heterozygosity due to restricted effective size may seriously overestimate the "genetic risks" when sampling from a population where the genetic structure is unclear or poorly known.

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Marine populations

Many marine species are characterized by large population sizes, high levels of genetic variation, and weak differentiation. A series of recent papers on exploited marine species report surprisingly small estimates of effective size, however. For example, Hauser and Carvalho (2008) reviewed 28 N_e assessments for marine fishes and found that estimates of effective size are between two and five orders of magnitude smaller than census size (N_C), with an average N_e/N_C ratio of 10^{-4} , which is only a small fraction of those reported for most other species. They also noted that most point estimates were in the range from hundreds to low thousands, and within the range where loss of genetic variability could be a justified management concern.

The genetic structure of many marine species is typified by multiple subpopulations (spawning areas) with a high degree of migration between them, potentially similar to the island migration model examined here. It is generally assumed that the most important explanation to the strikingly small N_e/N_C ratios observed in marine species is a high variance in reproductive success, which is possible in organisms with a potential for high fecundity and a long lifespan. As an alternate explanation to the high incidence of low N_e/N_C ratios we suggest that N_e has been estimated as N_{eV} , resulting in a mismatch between the estimates with N_{eV} referring largely to the local population and N_C to census size of the much larger metapopulation. In the review by Hauser and Carvalho (2008), for example, 24 of the 28 estimates were, in fact, obtained by the temporal method for estimating N_{eV} .

Management implications

The tendency of N_{eV} to overstate the impression of genetic vulnerability has crucial implications for management. Assuming, for example, that populations of $N_e < 50$ are considered at risk for excessive loss of genetic variation, and that a manager has difficulties to determine if a population is isolated or not. How should an estimate of $\hat{N}_{eV} \approx 30$ be dealt with? Automatically presuming that N_{eV} exaggerates the genetic threat, and taking no action, may be harmful to the focal population, whereas erroneously assuming that heterozygosity is lost at a rate of $1/(2\hat{N}_{eV})$ and launching a "rescue" program might divert resources from populations in stronger need of support. In such situations immediate remedial action may not be warranted unless there are independent indications that this population has lost genetic variation, for example by exhibiting a lower heterozygosity or fewer alleles than neighboring ones. An alternative step could be to initiate a genetic monitoring program that keeps track of potential changes of the amount of genetic variation.

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