Demographic and Genetic Estimates of Effective Population and Breeding Size in the Amphibian *Rana temporaria*

DIRK S. SCHMELLER* AND JUHA MERILÄ

Ecological Genetics Research Unit, Department of Biological and Environmental Sciences, P.O. Box 65, FI-00014, University of Helsinki, Finland

**Abstract:** Genetic methods for estimating effective population size (\(N_e\)) or the effective number of breeders (\(N_b\)) have become popular, but comparisons of these estimates with demographic estimates of \(N_e\) and \(N_b\) are rare, especially in anurans. We used three genetic (linkage disequilibrium, temporal moments, Bayesian coalescent-based method) and three demographic models, the latter considering number of breeding individuals, sex ratio, reproductive skew, and other demographic data, to estimate \(N_e\) and \(N_b\) in two subarctic populations (T and P) of the common frog *Rana temporaria*, subject to long-term capture-recapture studies. Demographic estimates of \(N_e\) based on total population size (\(N_{e[T]} = 44.5-56.9; N_{e[P]} = 68.8-93.7\)) deviated markedly from the genetic estimates obtained using the linkage disequilibrium method (\(N_{e[T]} = 97.1; N_{e[P]} = 13.2\)). The demographic estimates of \(N_b\) taking into consideration sex ratio and variance in reproductive success (\(N_{b[T]} = 10.1-39.7; N_{b[P]} = 3.9-21.3\)), were higher than the genetic estimates (\(N_{b[T]} = 3.7-5.4; N_{b[P]} = 3.5-3.9\)). The main factors affecting the effective size estimates were sex ratio and reproductive skew. The discrepancies between corresponding \(N_e\) and \(N_b\) estimates highlight the sensitivity of both demographic and genetic estimates on their underlying assumptions. Yet the ratios of effective or breeding effective size to the census population size were similar to those reported earlier for anurans, reinforcing the view that the discrepancy between actual and effective breeding sizes in anuran populations is typically very large.

**Keywords:** anura, effective population size, explosive breeder, generation time, microsatellites

Estimaciones Demográficas y Genéticas del Tamaño Poblacional Efectivo y Reproductivo en el Anfibio *Rana temporaria*

**Resumen:** Los métodos genéticos para estimar el tamaño poblacional efectivo (\(N_e\)) o el número efectivo de reproductores (\(N_b\)) se han vuelto populares, pero las comparaciones de estas estimaciones con estimaciones demográficas de \(N_e\) y \(N_b\) son raras, especialmente en anuros. Utilizamos tres modelos genéticos (desequilibrio de enlaces, momentos temporales, método Bayesiano basado en coalescencia) y tres demográficos que consideraban el número de individuos reproductores, sesgo reproductivo y otros datos demográficos, para estimar \(N_e\) y \(N_b\) en dos poblaciones subárticas (T y P) de la rana común *Rana temporaria*, sujetas a estudios de captura y recaptura. Las estimaciones demográficas de \(N_e\) con base en el total de la población (\(N_{e[T]} = 44.5-56.9; N_{e[P]} = 68.8-93.7\)) fueron marcadoramente diferentes de las estimaciones obtenidas utilizando el método de desequilibrio de enlaces (\(N_{e[T]} = 97.1; N_{e[P]} = 13.2\)). Las estimaciones demográficas de \(N_b\), considerando la proporción de sexos y la varianza en el éxito reproductivo (\(N_{b[T]} = 10.1-39.7; N_{b[P]} = 3.9-21.3\)), fueron mayores que las estimaciones genéticas (\(N_{b[T]} = 3.7-5.4; N_{b[P]} = 3.5-3.9\)). Los principales factores que afectaron las estimaciones de tamaño efectivo fueron la proporción de sexos y el sesgo reproductivo. Las discrepancias entre estimaciones de \(N_e\) y \(N_b\) correspondientes resaltan la sensibilidad de ambas estimaciones genéticas y demográficas.

*Current address: Umweltforschungszentrum Leipzig-Halle GmbH, Department of Nature Conservation Research, Permoserstrasse 15, D-04318 Leipzig, Germany, email ds@die-schmellers.de

Paper submitted June 3, 2005; revised manuscript accepted May 3, 2006.
Introduction

The rate of loss of genetic variability and increase of in-breeding within a population is defined by the effective population size \( N_e \), a concept developed by Wright (1931, 1938). Specifically, the effective size of a population is the size of an ideal population that has the same properties with respect to genetic drift as the population of interest (Wright 1931, 1938). Three different definitions of \( N_e \) exist, corresponding to predicted changes in the population’s genetic variance (variance \( N_e \)), heterozygosity (inbreeding \( N_a \)), or allele frequencies (eigenvalue \( N_r \)). Given the intimate connection between \( N_e \) and a population’s persistence probability, it is a parameter of central importance in population and conservation genetics and can be used as an indicator of a population’s viability and endangerment (e.g., Frankham et al. 2002).

The size of effectively breeding individuals in one population during one reproductive season \( N_b \) is a concept derived from \( N_e \). The two measures are directly connected because \( N_b \) times the generation time approximates \( N_e \) (Waples 1990a, 1990b). For \( N_b \), only one season of data collection is needed; thus, \( N_b \) may be a cheaper and less time-consuming way to probe the viability and endangerment of wild populations than \( N_e \).

Both measures can be estimated either directly from demographic data or indirectly from genetic markers. Because the data needed for accurate demographic estimates are logistically hard to obtain, genetic methods have assumed an increasing role in attempts to estimate \( N_e \) and \( N_b \) in the wild (e.g., Frankham 1995). In contrast to a demographic approach, genetic data can be easily gathered with modern molecular techniques, but the estimation from these data is subject to a number of restrictive assumptions (e.g., Beaumont 2003). New statistical methods for estimating \( N_e \) or \( N_b \) from marker data have been developed recently and existing ones have been made more accurate and efficient (Krimbas & Tsakas 1971; Nei & Tajima 1981; Pollak 1983; Waples 1989; Williamson & Slatkin 1999; Anderson et al. 2000; Wang 2001; Berthier et al. 2002). Despite these developments, use of both genetic and demographic approaches is still rare (e.g., Begon et al. 1980; Husband & Barrett 1992; Bouteiller & Perrin 2000; Storz et al. 2002; Arden & Kapuscinski 2003).

Such studies, however, could highlight discrepancies between direct and indirect methods and help determine factors influencing \( N_e \) and \( N_b \), knowledge essential for conservation and management decisions.

We sought to estimate \( N_e \) and \( N_b \) in two subarctic populations of the common frog \( Rana temporaria \) and to compare these estimates with census-based estimates of population sizes. To this end, we first used accurate demographic data obtained from capture-recapture studies of the same two populations to estimate census population sizes and demographic \( N_e \) and \( N_b \). Three different demographic models: sex ratio of breeding individuals (Wright 1938), reproductive skew (Kimura & Crow 1963), and a demographic estimate (Nunney & Elam 1994) were used. We compared the estimates from these models with indirect genetic estimates obtained with the one-sample method based on linkage disequilibrium (Hill 1981) and two two-sample methods based on temporal moments (Krimbas & Tsakas 1971; Waples 1989) and Bayesian coalescence-based computations (Berthier et al. 2002).

The latter method has been used rarely in empirical investigations, although it is expected that likelihood methods substantially improve the accuracy of effective population size estimates (Beaumont 2003).

Methods

Study Species and Populations

The common frog \( R. temporaria \), is a medium-sized anuran and geographically the most widely distributed amphibian species of Europe (Gasc et al. 1997). Adults in our two study populations (ponds) in subarctic Finland (approximately 69° 03′ N, 20° 47′ E) reach maturity at 4–6 years old and can live up to 11–15 years (ter Schure et al. 2002; Alho 2004). Females lay one clutch of 1500–3000 eggs per year, and all mature males and females attend the pond every year. Most of the laid eggs produce tadpoles, but only a small proportion (usually <5%) of these reaches metamorphosis. The main causes of tadpole mortality in these study populations are catastrophic deaths attributable to extreme weather conditions and to some extent to fish and bird predation. In some years, parts of the ponds may dry out and large numbers of the tadpoles—trapped in drying pockets of ponds—die from desiccation. Due to short growth seasons (75–125 days) and low ambient temperatures (mean July temperature 11.2° C; Järvinen 1987), a large proportion of tadpoles in deeper ponds may perish because they are not able to complete their development before the arrival of winter and ice cover. Nothing is known about mortality...
in between metamorphosis and maturity, but adult survival is high and similar in both sexes after maturity has been reached (approximately 70% survival from year to another; Alho 2004).

**Demographic and Genetic Data Collection**

We sampled two ponds at Kilpisjärvi in 1999. The first pond (hereafter pond T) was small (530 m²) with a maximum depth of about 60 cm. The second pond (hereafter pond P 220 m²) was deeper (1.6 m) and situated on the edge of the same marsh as pond T. We used drift fences and pitfall traps placed around each pond to trap adults migrating to these breeding sites. We marked and sexed each individual and collected a small tissue sample from each (for use in DNA analysis). A total of 58 and 80 adults were collected from pond T and P, respectively (Table 1). Capture-recapture analyses show that the adult individuals are site tenacious and do not move in between these two ponds (Alho 2004). To obtain temporarily separate samples, we captured metamorphs leaving the ponds at the end of the breeding season of 1999 in the same drift fences and pitfall traps in which the adults were captured. Altogether 375 and 25 metamorphs left ponds T and P, respectively.

<table>
<thead>
<tr>
<th>Population</th>
<th>$A_i$ ($I_{Ai}$) females</th>
<th>$b_i$ ($I_{bi}$) males</th>
<th>Number of adults</th>
<th>$N_{offspring}$</th>
<th>r</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond T</td>
<td>10.53 (0.01)</td>
<td>1.31 (0.52)</td>
<td>43</td>
<td>375</td>
<td>0.35</td>
<td>9.9</td>
</tr>
<tr>
<td>RS</td>
<td>8.33 (0.05)</td>
<td>1.35 (0.19)</td>
<td>15</td>
<td></td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Pond P</td>
<td>10.50 (0.03)</td>
<td>0.24 (3.29)</td>
<td>55</td>
<td>25</td>
<td>0.45</td>
<td>9.9</td>
</tr>
<tr>
<td>RS</td>
<td>8.40 (0.05)</td>
<td>0.36 (1.85)</td>
<td>25</td>
<td></td>
<td>0.69</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: $A_i$, average age; $b_i$, fecundity; $I_{Ai}$, standardized variance of $A_i$; $I_{bi}$, standardized variance of $b_i$. $N_{offspring}$, number of metamorphs produced in each of the populations; T, generation time, estimated as explained in methods. Number of adults and their sex ratio (r) are given separately for all and reproductively successful individuals (RS).

We used three different equations to obtain demographic estimates of $N_e$ and $N_h$: the sex ratio effective breeding size ($N_h$[sex ratio]; Wright 1938), the reproductive skew effective size ($N_h$[RS]; Kimura & Crow 1963), and the demographic effective size ($N_e$[demo]; Nunney & Elam 1994). The parameters used in the demographic estimation of $N_e$ are presented in Table 1. The sex ratio effective size was estimated as

$$N_{sex\ ratio} = \frac{4N_mN_f}{N_m + N_f},$$

where $N$ is the number of mature male ($m$) or female ($f$) individuals (Wright 1938). Applying this model to data from one season yields an $N_e$ estimate. The same equation accounts for differential reproductive success of sexes if only the number of reproductively successful males and females is used:

We performed polymerase chain reaction amplifications in a total volume of 10 μL under conditions as described in Palo et al. (2003) and scored alleles with the GeneScan 3.1 and Genotyper 2.5 software (ABI-systems, Foster City, California). Allelic diversity, observed heterozygosity, and unbiased estimates of expected heterozygosity (Nei 1987) were calculated for each population with the software Genetix 4.05 (Belkhir 2004). We assessed heterozygote excess within populations at each locus by estimating $F_{IS}$. The 95% confidence intervals (95% CI) of $F_{IS}$ and $F_{ST}$ were determined by bootstrapping (10,000 replicates).

The program Microchecker (van Oosterhout et al. 2004) was used to test for null alleles and erroneous genotyping due to stuttering. The program Fstat 2.9 (Goudet 2001) was used to calculate linkage disequilibrium. We could not detect any erroneous genotyping due to stuttering, and our tests did not reveal significant linkage between loci ($p < 0.001$ for 1% nominal level). Nevertheless, some loci did show deviations from an expected zero $F_{IS}$ and were excluded from the $N_e$ estimation (Table 2).
Table 2. Microsatellite information content for loci and samples of *R. temporaria* from two ponds.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Locus</th>
<th>Stuttering N</th>
<th>Null alleles</th>
<th>Pond T</th>
<th>Pond P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>present</td>
<td>NAF</td>
<td>F\textsubscript{IS}</td>
<td>95% CI</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>RRDL590</td>
<td>6</td>
<td>yes</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>RtCa2-09</td>
<td>5</td>
<td>no</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>Rt2-Ca2-22</td>
<td>3</td>
<td>no</td>
<td>0.000</td>
<td>−0.169</td>
</tr>
<tr>
<td>Rt2-Ca25</td>
<td>16</td>
<td>yes</td>
<td>0.086</td>
<td>0.229</td>
</tr>
<tr>
<td>Rttempμ4</td>
<td>7</td>
<td>no</td>
<td>0.012</td>
<td>0.030</td>
</tr>
<tr>
<td>Rttempμ7</td>
<td>7</td>
<td>no</td>
<td>0.035</td>
<td>0.088</td>
</tr>
<tr>
<td>RtμH</td>
<td>4</td>
<td>no</td>
<td>0.020</td>
<td>0.113</td>
</tr>
<tr>
<td>Rtu07</td>
<td>20</td>
<td>yes</td>
<td>0.060</td>
<td>0.190</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Abbreviations: N, number of alleles; F\textsubscript{IS}, inbreeding index; NAF, null allele frequency according to Brookfield (1996).

\textsuperscript{b}Loci used in the \( N_e \) computation.

\[
N_b(\text{sex ratio}) = \frac{4N_{rm}N_{rf}}{N_{rm} + N_{rf}},
\]  

(2)

where \( N_r \) is the number of successfully reproducing (b, breeding) males (\( m \)) or females (\( f \)). An alternative estimate accounting for variance in reproductive success and sex ratio was proposed by Kimura and Crow (1963):

\[
N_b(\text{RS}) = \frac{N_b k_b - 1}{k_b - 1 + \frac{V_b}{k_b}},
\]  

(3)

where \( N \) is the number of all sexually mature individuals at a lake of sex \( i \), \( k_b \) is the mean number of offspring over all individuals of sex \( i \), and \( V_b \) is the variance in reproductive success of sex \( i \). The harmonic mean of both sex-specific values then gives the overall \( N_b \). Nunney and Elam (1994) proposed a formula to estimate \( N_e \) accounting for sex-ratio skew, differential generation times, differential longevity, and differential fecundity of sexes:

\[
N_e(\text{demo}) = \frac{4r(1-r)NT}{\left(A_m(1-r) + A_f r\right) - \left(\frac{2r}{B_f} + (I_{ba}(1-r) + I_{rb} r)\right)} + \left(A_mI_{bm}(1-r) + A_f I_{fr} r\right),
\]  

(4)

where \( N \) is the number of adults in the population, \( r \) is the adult sex ratio, \( T \) is the generation time (defined as the average age of parents of each sex; Hill 1979), \( A_i \) is the mean adult longevity of sex \( i \), \( b_i \) is the mean fecundity of sex \( i \) per season, and \( I_{ba} \) and \( I_{rb} \) are the standardized variances of these parameters (variance/mean\textsuperscript{2}).

For comparison with the genetic estimators, we computed a \( N_e/N \) ratio, where \( N \) represents the census size of a population. For comparing single-season estimates (\( N_b \)) and \( N_e \), we multiplied \( N_b \) by the generation time as derived from capture-recapture analyses (9.9 years) following Waples (1990a, 1990b).

Genetic Estimators of \( N_e \) and \( N_b \)

We used two two-sample methods based on temporally separated samples and the linkage disequilibrium method as a one-sample method to obtain genetic estimates of \( N_e \) and \( N_b \). The samples were adults and their offspring collected in 1999. The principal logic behind the temporal methods is that the difference in gene frequencies between the two temporally collected samples from the same population will be inversely proportional to the effective size of the population in the absence of migration and mutation (e.g., Waples 1989; Scribner et al. 1997). For the two different temporal methods employed, the moments-based (Waples 1989) and the Bayesian coalescent-based method (Berthier et al. 2002), we computed \( N_b \) based on the total number of adults as the first temporal sample and the offspring sampled in 1999 as the second temporal sample.

The linkage disequilibrium method (\( N_e(\text{LD}) \)) is based on the realization that the loss of variation is compounded by an increase in linkage disequilibrium, which reduces the frequency of novel gene combinations (Hill 1981). Therefore, measuring the associations between alleles across several loci allows for the estimation of inbreeding \( N_e \) (Hill 1981; Peel et al. 2004). This method was applied to the data as implemented in the computer program NeEstimator 1.3 (Peel et al. 2004).

The moments-based method (\( N_b(\text{TM}) \)) computes the standardized variance in the allele frequency change for each microsatellite locus and calculates the variance effective size (Waples 1989). In our study, with temporal samples from the same year, the estimates resembled a single-season \( N_b \) estimate. We applied this method to the data with the program NeEstimator.

The Bayesian coalescent-based method (\( N_b(\text{LH}) \); Berthier et al. 2002) estimates the variance effective size of two temporally separated samples from the focal population with a given number of generations between them. We used temporal samples from the same year, and our estimates resembled a single-season \( N_b \) estimate. We used the software TM3 (Berthier et al. 2002) to make the
estimation, and due to a priori knowledge of the actual population size, we specified a maximum population size of 500. The program was run with 10,000 replications because increased numbers of replications did not improve the accuracy of the estimates as inferred from the confidence intervals but increased the computation time considerably. For comparing single-season estimates ($N_b$) and $N_e$, we multiplied $N_b$ by the generation time as derived from capture-recapture analysis (9.9 years) following Waples (1990a, 1990b).

Results

Demographic Estimates

Capture-recapture data revealed a mean adult census population size of 53 in pond T and 60 in pond P over a period of 4 years (1999–2003; Alho 2004). In 1999 the population sizes were 58 and 80, respectively. The demographic model ($N_e$[demo]) estimated $N_e$ as 56.9 ($N_e/N = 0.98$) in pond T and 93.7 ($N_e/N = 1.17$) in pond P. Accounting for sex ratio ($N[\text{sex ratio}]$) only, the estimate was 44.5 in pond T ($N[\text{sex ratio}]/N$ ratio = 0.77) and 68.8 in pond P ($N[\text{sex ratio}]/N$ ratio = 0.86), whereas it was reduced to 39.7 in pond T ($N_e/N$ ratio = 0.68) and 21.3 in pond P ($N_e/N$ ratio = 0.27) considering only reproductively successful adults ($N_e[\text{sex ratio}]$). The estimates of $N_b$, considering the sex ratio of breeding individuals and the reproductive skew ($N_b[\text{RS}]$) were much lower than the census size ($N_b[\text{T}] = 10.1; N_b/N = 0.09; N_b[\text{P}] = 3.9; N_b/N = 0.04$; Table 3). The approximated $N_e$ derived from $N_b[\text{RS}]$ ($^tN_b$) yielded a $N_e$ estimate for pond T higher than the census size ($N_e = 100; N_e/N$ ratio = 1.72) but a lower value for pond P ($N_e = 38.9; N_e/N$ ratio = 0.49; Table 3).

Genetic Variability

The two populations we investigated had low but significant genetic differentiation ($F_{ST} = 0.0136$, 95% CI 0.002–0.027). The genetic variability over all loci of the adult sample (expected heterozygosity $H_e[\text{T}] = 0.617, H_e[\text{P}] = 0.643$; allelic diversity $AD[\text{T}] = 7.25, AD[\text{P}] = 7.50$) and the offspring sample ($H_e[\text{T}] = 0.649, H_e[\text{P}] = 0.628$; allelic diversity $AD[\text{T}] = 7.75, AD[\text{P}] = 5.13$) was similar in both populations.

Genetic Estimates

The moments-based temporal method yielded similar $N_b$ estimates for both ponds with similar confidence intervals ($N_b[\text{T}] = 3.7; 95% CI = 2.1–5.8, N_b[\text{P}] = 3.9; 95% CI = 1.9–7.3; Table 3). The $N_b/N$ ratios were 0.07 (pond T) and 0.05 (pond P). The Bayesian method by Berthier et al. (2002) estimated $N_b$ of pond T as 5.4 with a 95% CI of 4.2–6.9 (Table 3). In pond P, $N_b$ was estimated as 3.5 with a 95% CI of 2.3–5.4. The $N_b/N$ ratios were 0.09 (pond T) and 0.04 (pond P). The approximated $N_e$ estimates ($^tN_b$) of these two methods ($N_e[\text{T}] = 36.6 and 53.5; N_e[\text{P}] = 38.9 and 34.7$) fell in the $N_e$ confidence interval of the linkage disequilibrium method only in pond P ($N_e[\text{T}] = 97.1; 95% CI = 78.0–122.5, N_e[\text{P}] = 13.2; 95% CI = 7.0–34.6$ (Table 3).

Discussion

Our most salient finding was the fairly large range in both the demographic and genetic estimates of effective population and breeding size depending on the method (and assumptions) used in the estimations. In particular the unaccounted variance in reproductive success and sex.

Table 3. Summary of the population size estimates of $R$. temporaria from two ponds (T and P).*

<table>
<thead>
<tr>
<th></th>
<th>Pond T</th>
<th>Pond P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$</td>
<td>58</td>
<td>80</td>
</tr>
<tr>
<td>$N_b$</td>
<td>53</td>
<td>60</td>
</tr>
<tr>
<td>$N[\text{sex ratio}]$</td>
<td>44.5</td>
<td>68.8</td>
</tr>
<tr>
<td>$N_e[\text{demo}]$</td>
<td>5.7, 56.9, 0.10, 0.98</td>
<td>9.5, 93.7, 0.16, 1.62</td>
</tr>
<tr>
<td>$N_e[\text{sex ratio}]$</td>
<td>39.7, 393.0, 0.68, 6.78</td>
<td>21.3, 210.9, 0.37, 3.64</td>
</tr>
<tr>
<td>$N_e[\text{RS}]$</td>
<td>10.1, 100.0, 0.17, 1.72</td>
<td>3.9, 38.6, 0.07, 0.67</td>
</tr>
<tr>
<td>$N_e[\text{TM}]$</td>
<td>3.7, 36.6, 0.06, 0.63</td>
<td>3.5, 34.7, 0.06, 0.60</td>
</tr>
<tr>
<td>$N_e[\text{LH}]$</td>
<td>5.4, 53.5, 0.09, 0.92</td>
<td>1.3, 13.2, 0.02, 0.23</td>
</tr>
<tr>
<td>$N_e[\text{LD}]$</td>
<td>9.8, 97.1, 0.17, 1.67</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: N, observed population size in 1999; $N_b$, population size estimate from a 4-year mark-recapture study (1999–2003; Alho 2004); $N_e$[demo], demographic model (Namney & Elam 1994); $N[\text{sex ratio}]$, sex ratio effective size (Wright 1938); $N_e[\text{sex ratio}]$, sex ratio effective breeding size (Wright 1938); $N_e[\text{RS}]$, reproductive skew effective size (Kimura & Crow 1963); $N_e[\text{TM}]$, effective breeding size using temporal moments (Waples 1989); $N_e[\text{LH}]$, effective breeding size based on the likelihood approach of Berthier et al. (2002); $N_e[\text{LD}]$, linkage disequilibrium effective size (Hill 1981). The $N_e$ estimates are converted to $N_b$ estimates by dividing the estimate by the generation time of 9.9 years. The $N_e$ estimates are derived from $N_b$ estimates by multiplying the latter with the generation time 9.9, as suggested by Waples (1990a, 1990b).
Comparing genetic and demographic estimates, following, we discuss these findings and causes for the bias in \( N_e \) and \( N_b \) estimates.

**Genetic and Demographic Estimates of \( N_e \) and \( N_b \)**

Comparing genetic and demographic \( N_e \) estimates reported in previous studies illustrates obvious differences between corresponding values, with demographic estimates being up to 10 times higher than corresponding genetic estimates (Table 4). The differences between genetic and demographic estimates result from the fact that none of the models adequately reflects the relationship between all the parameters that determine \( N_e \) or \( N_b \). These parameters are, for instance, variance in reproductive success, a skewed operational sex ratio, dispersal behavior, unequal or overlapping generation times, inbreeding, population fluctuations, and numerous other factors influencing the maintenance of genetic variability in a population (e.g., Falconer 1989; Caballero 1994; Sugg & Chesser 1994; Anthony & Blumstein 2000; Kalinowski & Waples 2002).

The single most important determinant of \( N_e \) is temporal fluctuations in population size (Wright 1938; Frankham 1995; Vucetich & Waite 1999). The \( N_e \) estimates based on data from a single time point, therefore, can be misleading because they ignore population fluctuations (Nunney & Elam 1994; Waples 2005) and are subject to high intrinsic stochastic variance (Waples 2005). Both the \( N_e \) methods we used might be affected by temporal population fluctuations, but in different ways, because a recent demographic reduction affects the inbreeding \( N_e \) to a lesser extent than it affects \( N_b \) because different gene combinations might still be present (Templeton 1980; Crow & Denniston 1988).

Although the demographic method of Nunney and Elam (1994) yielded a higher estimate of \( N_e \) for pond P compared with pond T, the reverse was true for the linkage-disequilibrium method (Hill 1981, Table 3). The high \( N_e \) estimate in pond T supported a high amount of novel gene combinations, reducing the linkage disequilibrium, whereas the reverse was true for pond P. We know that the reproductive success had been particularly low in 1999, especially in pond P. Hence, the large difference between the two ponds in population size may result from differences in bottleneck histories, despite their rather similar census sizes. Also the small number of loci and small sample sizes combined with the maintenance of genetic variation due to overlapping generations and/or natal dispersal limit the increase in linkage disequilibrium and reduce the accuracy of \( N_e \) estimates (Bartley et al. 1992; Anthony & Blumstein 2000). The demographic model estimate (Nunney & Elam 1994) may not be accurate as it assumes that the demographic parameters of a population are stable over time. Long-term observations of the populations support a stable age structure (Alho 2004). Nevertheless, the sex ratio and the population size may fluctuate due to the amphibian presence-absence distribution, which strongly differs between years (Skelly et al. 2003) and results in an overestimation of \( N_e \) with the demographic model (see also Waples 2005). The effective population estimates presented here, however, do not explain the genetic variability detected in the two populations. The heterozygosity levels were similar to those in other \( R. \) temporaria (Palo et al. 2003, 2004; L sessiona, \( H_e = 0.57 \); R. ridibunda, \( H_e = 0.59 \); Zeisset et al. 2000), but a gross estimate of \( N_e \) from heterozygosity (Nei 1987), assuming an infinite allele model of mutation (IAM) and a mutation rate between \( 10^{-2} \) and \( 10^{-4} \) (Goldstein and Schlötzer 1999), suggests \( N_e \) ranged from 403 to 4027 in pond T and from 450 to 4503 in pond P. The estimates, assuming a stepwise mutation model, ranged from 727 to 7271 in pond T and from 856 to 8558 in pond P.

The strong discrepancy between these estimates of \( N_e \) and the results of the other methods used here indicates that the population unit of \( R. \) temporaria in the study region is not composed of only the investigated individuals of the two ponds; rather, it is composed of subpopulations of a much larger metapopulation. The gross overestimation of \( N_e \) calculated from heterozygosity most likely resulted from immigration of individuals originating from neighboring populations. Even though we never observed any migration between the two ponds, we cannot neglect the fact that generation times are very long and that few immigrating individuals per generation are sufficient to maintain a high genetic variability over many generations. Nevertheless, monitoring of \( R. \) temporaria in the surroundings of our study ponds (J. Merilä, unpublished data) does not support the existence of any population in the migration range of subadult and adult \( R. \) temporaria. Yet, other studies on population structure and effective population and breeding size of \( R. \) temporaria (Brede & Beebee 2004; Brede & Beebee 2006) suggest that gene flow between subpopulations of \( R. \) temporaria are high, indicating that metapopulation structure in \( R. \) temporaria are highly effective for the maintenance of genetic variability. Hence, management actions of \( R. \) temporaria have to focus on metapopulations rather than on local populations to ensure the success of conservation efforts.

We also estimated \( N_b \), the breeding effective size, of the two populations in the particular year of sampling. For natural anuran populations this was first done for \( Bufo \) \( bufo \) (Scriber et al. 1997) and has been recently taken up by Brede and Beebee (2006) for \( R. \) temporaria and \( B. \) \( bufo \) (Table 4). In our study, \( N_b \) did not reflect the number of adults that successfully spawned; rather it reflected...
<table>
<thead>
<tr>
<th>Species</th>
<th>SI</th>
<th>$N_c (\pm SE)$</th>
<th>$N_e (\text{demo})$</th>
<th>$N_e (\text{gene})$</th>
<th>Range</th>
<th>$N_e/N$</th>
<th>Marker (number)</th>
<th>$N_e$ method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Triturus cristatus</em></td>
<td>3</td>
<td>7.4$^{\pm}$ 22</td>
<td>—</td>
<td>12</td>
<td>4.7–47</td>
<td>0.16</td>
<td>MS (8)</td>
<td>MR</td>
<td>Jehle et al. 2001</td>
</tr>
<tr>
<td><em>T. marmoratus</em></td>
<td>3</td>
<td>14$^{\pm}$ 42</td>
<td>—</td>
<td>13.4</td>
<td>5.4–43.0</td>
<td>0.09</td>
<td>MS (8)</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10$^{\pm}$ 22</td>
<td>—</td>
<td>9.6</td>
<td>6–19.5</td>
<td>0.09</td>
<td>MS (8)</td>
<td>MR</td>
<td></td>
</tr>
<tr>
<td><em>Ambystoma macr/actyum</em></td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>123</td>
<td>2–∞</td>
<td>—</td>
<td>AL (6)</td>
<td>—</td>
<td>Funk et al. 1999</td>
</tr>
<tr>
<td><em>Bufo marinus</em></td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>390</td>
<td>119–812</td>
<td>0.001</td>
<td>AL (10)</td>
<td>—</td>
<td>Eastal 1985</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>346</td>
<td>104–719</td>
<td>—</td>
<td>AL (10)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>372</td>
<td>112–770</td>
<td>—</td>
<td>AL (10)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>B. calamita</em></td>
<td>2</td>
<td>1.523 ± 36.4</td>
<td>206</td>
<td>26</td>
<td>7–21</td>
<td>0.02</td>
<td>MS (8)</td>
<td>SC, $N_e$ (demo), TM</td>
<td>Rowe &amp; Beebee 2004</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>71 ± 5.7</td>
<td>40</td>
<td>—</td>
<td>—</td>
<td>0.056</td>
<td>SC, $N_e$ (demo)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>53 ± 5.9</td>
<td>24</td>
<td>9</td>
<td>4–47</td>
<td>0.17</td>
<td>MS (8)</td>
<td>SC, $N_e$ (demo), TM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>53 ± 5.9</td>
<td>24</td>
<td>9</td>
<td>5–48</td>
<td>0.17</td>
<td>MS (8)</td>
<td>LH</td>
<td></td>
</tr>
<tr>
<td><em>B. bufo</em></td>
<td>1</td>
<td>2.500</td>
<td>—</td>
<td>31</td>
<td>11–190</td>
<td>0.012</td>
<td>MIS (3)</td>
<td>SC</td>
<td>Scribner et al. 1997</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4,000</td>
<td>—</td>
<td>21</td>
<td>9–63</td>
<td>0.005</td>
<td>MIS (3)</td>
<td>SC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4,800</td>
<td>—</td>
<td>46</td>
<td>15–∞</td>
<td>0.010</td>
<td>MIS (3)</td>
<td>SC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>100–10,000</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>SC, LH</td>
<td>—</td>
<td>Brede &amp; Beebee 2004</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1,000</td>
<td>—</td>
<td>49</td>
<td>12–454</td>
<td>0.049</td>
<td>MS (8)</td>
<td>RM, LH</td>
<td>Brede &amp; Beebee 2006</td>
</tr>
<tr>
<td><strong>Notophthalmus viridescens</strong></td>
<td>3</td>
<td>159–210</td>
<td>137–148</td>
<td>—</td>
<td>—</td>
<td>0.65–0.93</td>
<td>—</td>
<td>—</td>
<td>Gill 1978</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>91–125</td>
<td>86–124</td>
<td>—</td>
<td>—</td>
<td>0.69–0.99</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>27–35</td>
<td>21–35</td>
<td>—</td>
<td>—</td>
<td>0.60–0.98</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6–44</td>
<td>6–38</td>
<td>—</td>
<td>—</td>
<td>0.14–6.33</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1,018–1,454</td>
<td>911–1399</td>
<td>—</td>
<td>—</td>
<td>0.63–1.37</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td><strong>Eleutherodactylus</strong></td>
<td>?</td>
<td>&gt;80,000</td>
<td>&gt;56,000</td>
<td>—</td>
<td>—</td>
<td>0.07–1.50</td>
<td>—</td>
<td>—</td>
<td>Crawford 2003</td>
</tr>
<tr>
<td><strong>Rana pipiens</strong></td>
<td>1</td>
<td>167–4,200</td>
<td>42–112</td>
<td>—</td>
<td>—</td>
<td>0.01–0.67</td>
<td>—</td>
<td>MR</td>
<td>Merril 1968</td>
</tr>
<tr>
<td><em>R. lessonae</em></td>
<td>3</td>
<td>—</td>
<td>588</td>
<td>378–1355</td>
<td>&gt;0.10</td>
<td>MS (7)</td>
<td>TM</td>
<td>—</td>
<td>Hoffman et al. 2004</td>
</tr>
<tr>
<td><em>R. arvalis</em></td>
<td>3</td>
<td>328</td>
<td>—</td>
<td>420</td>
<td>245–837</td>
<td>&gt;0.10</td>
<td>MS (7)</td>
<td>TM</td>
<td></td>
</tr>
<tr>
<td><em>R. ridibunda</em></td>
<td>3</td>
<td>—</td>
<td>1019</td>
<td>490–∞</td>
<td>&gt;0.10</td>
<td>MS (7)</td>
<td>SC, TM</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td><em>R. sylvatica</em></td>
<td>3</td>
<td>—</td>
<td>410</td>
<td>222–940</td>
<td>&gt;0.10</td>
<td>MS (6)</td>
<td>TM</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>—</td>
<td>1820</td>
<td>660–∞</td>
<td>&gt;0.10</td>
<td>MS (6)</td>
<td>TM</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td><em>R. temporaria</em></td>
<td>5</td>
<td>27.6 ± 3.4</td>
<td>25.9</td>
<td>—</td>
<td>—</td>
<td>0.94</td>
<td>—</td>
<td>$N_b$ (sex ratio)</td>
<td>Sjögren-Gulve &amp; Berg 1999</td>
</tr>
<tr>
<td><em>R. arvalis</em></td>
<td>5</td>
<td>64.6 ± 8.4</td>
<td>59.2</td>
<td>—</td>
<td>—</td>
<td>0.92</td>
<td>—</td>
<td>$N_b$ (sex ratio)</td>
<td></td>
</tr>
<tr>
<td><em>R. ridibunda</em></td>
<td>5</td>
<td>113</td>
<td>92</td>
<td>—</td>
<td>—</td>
<td>0.82</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td><em>R. sylvatica</em></td>
<td>7</td>
<td>194</td>
<td>156</td>
<td>—</td>
<td>—</td>
<td>0.81</td>
<td>—</td>
<td>—</td>
<td>Berven &amp; grupen 1990</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>136</td>
<td>101</td>
<td>—</td>
<td>—</td>
<td>0.74</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>78</td>
<td>38</td>
<td>—</td>
<td>—</td>
<td>0.49</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>89</td>
<td>51</td>
<td>—</td>
<td>—</td>
<td>0.58</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td><em>R. temporaria</em></td>
<td>1</td>
<td>118</td>
<td>86</td>
<td>23–∞</td>
<td>0.729</td>
<td>MS (8)</td>
<td>TM, LH</td>
<td>—</td>
<td>Brede &amp; Beebee 2006</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>18</td>
<td>12</td>
<td>6–28</td>
<td>0.667</td>
<td>MS (8)</td>
<td>TM, LH</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

---

*a*Abbreviations: SI, sampling interval (in generations); $N$, census population size; $N_e$ (demo), demographic estimates of $N_e$; $N_e$ (gene), effective population size as estimated with molecular markers; range, confidence limits; $N_e/N$, ratio between effective and census population size estimates; MS, microsatellite; AL, allozymes; MIS, minisatellites; numbers in parentheses, number of markers; MR, mark recapture; SC, egg clutch/spring sampling; $N_e$ (demo), demographic estimate (Nunney & Elam 1995); TM, temporal moments (Waples 1989); LH, likelihood method (Berthier et al. 2004); $N_b$ (sex ratio), demographic estimate (Kimura & Crow 1963).

*b*Mean of two yearly estimates.
only the number of adults successfully producing meta-
morphs. Hence, \( N_b \) took into consideration the strong 
selection in the particular year of sampling, with a mortal-
ity rate from egg to metamorph of 99.5–99.9%. The high 
mortality rate also suggests that the reproductive output 
of single pairs could be zero despite successful spawning, 
increasing the variance of reproductive success consider-
dably. Differences between the \( N_b \) estimates were high 
(\( N_b^{[T]} = 3.7–39.7 \); \( N_b^{[P]} = 3.5–21.3 \)). The highest 
estimate of \( N_b \) was the cause of the unaccounted variance 
in reproductive success because, aside from the sex ratio 
especially, reproductive success and its variance are of 
importance in estimating \( N_b \) (e.g., Caballero 1994).

Only the model of Kimura and Crow (1963) accounts 
for the variance of reproductive success and sex ratio 
and might represent a robust estimate of \( N_b \). In comparison 
with this model the lower values of the genetic meth-
ods based on temporal samples most probably resulted 
from the violation of the discrete generation model (e.g., 
Waples 2005). The temporal samples were collected in 
the same year and therefore did not represent a genera-
tion. Because in the investigated populations individuals 
were rather long lived, the appropriate samples would 
have needed to be sampled about 10 years apart. The 
time period of our sampling, therefore, was too short 
to allow for correction of the bias generated by overlying 
generations because the cumulative genetic drift is hardly 
large enough (e.g., Waples 2005).

### Approximating \( N_e \) from \( N_b \)

Generally, an approximation of \( N_e \) from a single season 
estimate is prone to large errors, depending on the quality 
of the original data. Errors in estimating generation time 
can be especially crucial due to the multiplication with 
\( N_b \). Nevertheless, our generation time estimates should 
be accurate, allowing for the approximation of \( N_e \) from 
\( N_b \) by multiplying the latter with the generation time \( t \) 
(Waples 1990a, 1990b). Yet other factors need to be 
taken into consideration when approximating \( N_e \) from 
\( N_b \). An approximation of \( N_e \) from \( N_b \) estimated with the 
model of Kimura and Crow (1963) and the sex ratio ef-
efective breeding size (Wright 1938) yielded \( N_e \) estimates 
higher than the census size, especially in pond T. The 
everestimation showed that in the investigated popula-
tions sex ratios were not stable over time and that the 
variance of reproductive success between years was not 
constant, with low variances in optimal years and high 
variances in catastrophic years.

### \( N_e/N \) and \( N_b/N \) Ratios

Frankham (1995) found that the ratio of effective to cen-
sus size in animal populations ranged between 0.05 and 
0.80, with a mean of 0.11. For stable populations em-
pirical values of the \( N_e/N \) ratio usually range between 0.5 
and 1 of the total census population size (Nunney & Elam 
1994; Nunney 1995), whereas in fluctuating populations 
the \( N_e/N \) ratio can be as small as 0.10 (Frankham 1995; 
Kalinowski & Waples 2002). Even lower \( N_e/N \) ratios have 
been reported for a northern pike (Esox lucius) popu-
lation (\( N_e/N = 0.03 \); Miller & Kapuscinski 1997) and a 
captive population of Drosophila melanogaster (\( N_e/N = 
0.004 \); Briscoe et al. 1992). The range of the \( N_e/N \) ratios in 
our study was large (\( N_e/N = 0.23–1.67 \)); however, \( N_e/N \) 
ratios close to 1 have been found frequently in other anu-an species (Table 4), suggesting that the difference be-
tween effective and census size can sometimes be small. 
Our \( N_b/N \) ratios fall in the mid range of earlier reported 
\( N_b/N \) ratios of amphibians, ranging from 0.001 to 0.73 
(Table 4). The lower \( N_b/N \) ratios as compared with \( N_e/N \) 
ratios suggest that the effective size of breeders can fluc-
tuate substantially between years but that years with opti-
mal conditions for reproduction may buffer catastrophic 
years in the long term. In our populations, mortality in 
the egg to the froglet stage was large due to a particularly 
short breeding season (growth season just had 89 days in 
1999). Such short growth seasons are not rare and have 
been documented numerous times since 1950 (Kilpisjärvi 
Biological station, unpublished data) and likely cause a 
low reproductive output. Nevertheless, due to a high 
adult survival rate (Alho 2004), each frog may take part 
in up to nine reproductive seasons during its lifetime, 
which clearly offers several possibilities to make up for 
unsuccessful years.

### Conclusions

Accurate measures of \( N_e \) and \( N_b \) are of high relevance 
for conservation purposes because they allow the assess-
ment of the population survival ability, population dynam-
ics, and factors explaining them. Accurate estimates of \( N_e \) 
and \( N_b \), however, are hard to obtain (Waples 2005), but 
one-sample methods (point estimates), such as linkage 
disequilibrium and heterozygote excess, and two-sample 
methods (temporal moments, Bayesian coalescent-based 
method) provide independent information and should be 
applied to the same data set to obtain the most reliable 
results on the estimates. Much care also has to be taken to 
verify that the census population size and the estimates 
match the same period of time; otherwise, a sensible inter-
pretation is obsolete. An overestimation of \( N_e \) in species 
with generally low effective population size may severely 
contrast with the actual conservation needs of popula-
tions. The variance in our estimates, however, illustrates 
the critical importance of choosing the right method to 
yield appropriate values of \( N_e \). Each estimate needs to 
be accurate within its \( N_e \) type, and estimates should be 
taken across \( N_e \) types to extract maximum information. 
Therefore, we strongly recommend the use of multiple
methods of estimating \( N_e \) and of methods representing different \( N_e \) types to increase the information value.

Acknowledgments

We thank all those who contributed to the collection of data for this study. In particular, we thank O. Kalttopää, E. Söderman, A. Laurila, and the Kilpisjärvi Biological Station. We also thank J. U. Palo, G. Hinten, T. Beebee, and three anonymous referees for useful comments on earlier versions of this manuscript. This study was supported by the Academy of Finland, the Kone Foundation (Finland), and the University of Helsinki Science Foundation.

Literature Cited


Belkhir, K. 2004. GENETIX, logiciel sous WindowsTM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.


Krimbas, C. B., and S. Tsakas. 1971. The genetics of \( Dacus oleae \) V.