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

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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 ($N_{e[T]} = 44.5\text{-}56.9$; $N_{e[P]} = 68.8\text{-}93.7$) 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 ($N_{b[T]} = 10.1\text{-}39.7$; $N_{b[P]} = 3.9\text{-}21.3$), were higher than the genetic estimates ($N_{b[T]} = 3.7\text{-}5.4$; $N_{b[P]} = 3.5\text{-}3.9$). 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_r) se han vuelto populares, pero las comparaciones de estas estimaciones con estimaciones demográficas de N_e y N_r son raras, especialmente en anuros. Utilizamos tres modelos genéticos (desequilibrio de enlaces, momentos temporales, método Bayesiano basado en coalescencia) y tres demográficos que consideraron el número de individuos reproductores, sesgo reproductivo y otros datos demográficos, para estimar N_e y N_r 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 ($N_{e[T]} = 44.5\text{-}56.9$; $N_{e[P]} = 68.8\text{-}93.7$) fueron marcadamente 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_r considerando la proporción de sexos y la varianza en el éxito reproductivo ($N_{r[T]} = 10.1\text{-}39.7$; $N_{r[P]} = 3.9\text{-}21.3$) fueron mayores que las estimaciones genéticas ($N_{r[T]} = 3.7\text{-}5.4$; $N_{r[P]} = 3.5\text{-}3.9$). 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_r correspondientes resaltan la sensibilidad de ambas estimaciones genéticas y*

142

demográficas en sus suposiciones fundamentales. Sin embargo, la relación entre el tamaño poblacional efectivo o reproductivo y el tamaño poblacional censal fue similar al reportado para anuros previamente, lo que refuerza la opinión de que la discrepancia entre el tamaño censal y el efectivo de poblaciones de anuros es muy grande.

Palabras Clave: anura, microsatélites, reproductor explosivo, tamaño poblacional efectivo, tiempo generacional

Introduction

The rate of loss of genetic variability and increase of inbreeding 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_e), or allele frequencies (eigenvalue N_e). 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 1990*a*, 1990*b*). 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; Ardren & 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

Table 1. Demographic data from the two *R. temporaria* populations used in estimating N_e.[*]

Population	$A_i(I_{A_i})$		$b_i(I_{b_i})$		Number of adults		$N_{offspring}$	r	T
	females	*males*	*females*	*males*	*females*	*males*			
Pond T									
all	10.53 (0.01)	8.33 (0.05)	1.31 (0.52)	1.35 (0.19)	43	15	375	0.35	9.9
RS					34	14		0.42	
Pond P									
all	10.50 (0.03)	8.40 (0.05)	0.24 (3.29)	0.36 (1.85)	55	25	25	0.45	9.9
RS					13	9		0.69	

[*]*Abbreviations: A_i, average age; b_i, fecundity; I_{A_i}, standardized variance of A_i; I_{b_i}, 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).*

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 m^2) with a maximum depth of about 60 cm. The second pond (hereafter pond P, 220 m^2) 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. Under the assumptions that (1) each female lays a clutch of approximately 2000 eggs, (2) all the eggs were fertilized, and (3) 36 and 51 clutches were laid in ponds T and P in 1999, respectively; these figures correspond to a mortality of about 99.5% and 99.9% between fertilization and metamorphosis.

We used eight highly polymorphic microsatellite loci to estimate allele frequencies in different generations. We also used the genetic data to determine the reproductive success of adults by genetic tagging, employing the assignment program PaPa (Duchesne et al. 2002; the full data will be published elsewhere). The DNA for the microsatellite analysis was extracted with standard SDS—proteinase K digestion treatment followed by NaCl purification and isopropanol precipitation (Bruford 1992). The 138 adults and 400 metamorphs were genotyped at the following loci: RtCa2–09, Rt2Ca2-22, Rt2Ca25 (T. J. W. Garner, unpublished data), RRD590 (Vos et al. 2001),

RtμH (Pidancier et al. 2002), RtU07 (Berlin et al. 2000), Rtempμ4, and Rtempμ7 (Rowe & Beebee 2001). 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).

Demographic Estimators of N_e and N_b

We used three different equations to obtain demographic estimates of N_e and N_b: the sex ratio effective breeding size (N_b[sex ratio]; Wright 1938), the reproductive skew effective size (N_b[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

$$N(\text{sex ratio}) = \frac{4N_m N_f}{N_m + N_f}, \qquad (1)$$

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_b estimate. The same equation accounts for differential reproductive success of sexes if only the number of reproductively successful males and females is used:

Table 2. Microsatellite information content for loci and samples of *R. temporaria* from two ponds.[a]

Locus	Stuttering N	Null alleles present	NAF	Pond T F_{IS}	Pond T 95% CI	Pond P F_{IS}	Pond P 95% CI
RRD590	6	yes	0.078	0.094	(−0.117–0.299)	0.357	(0.171–0.519)
RtCa2-09	5	no	0.039	0.212	(−0.003–0.401)[b]	0.027	(−0.146–0.193)[b]
Rt2-Ca2-22	3	no	0.000	−0.169	(−0.396–0.058)	−0.275	(−0.464––0.079)
Rt2-Ca25	16	yes	0.086	0.229	(0.093–0.358)	0.156	(0.045–0.260)
Rtempμ4	7	no	0.012	0.030	(−0.142–0.190)[b]	0.035	(−0.109–0.170)[b]
Rtempμ7	7	no	0.039	0.088	(−0.153–0.305)[b]	0.193	(−0.021–0.389)[b]
RtμH	4	no	0.020	0.113	(−0.155–0.364)[b]	−0.003	(−0.195–0.183)[b]
RtU07	20	yes	0.060	0.190	(0.063–0.306)	0.076	(−0.021–0.164)

[a]*Abbreviations: N, number of alleles; F_{IS}, inbreeding index; NAF, null allele frequency according to Brookfield (1996).*
[b]*Loci used in the* N_e *computation.*

$$N_b(\text{sex ratio}) = \frac{4N_{rm}N_{rf}}{N_{rm} + N_{rf}}, \qquad (2)$$

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):

$$N_b(\text{RS}) = \frac{N_i \bar{k}_i - 1}{\bar{k}_i - 1 + \frac{V_{k_i}}{\bar{k}_i}}, \qquad (3)$$

where N is the number of all sexually mature individuals at a lek of sex i, $\bar{k}_i$ is the mean number of offspring over all individuals of sex i, and V_{k_i} 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:

$$N_e(\text{demo})$$
$$= \frac{4r(1-r)NT}{\left[\begin{array}{c} \left(A_m(1-r) + A_f r\right) - \left(\frac{2r}{b_f}\right) + \left(I_{b_m}(1-r) + I_{b_f}r\right) \\ + \left(A_m I_{A_m}(1-r) + A_f I_{A_f}r\right) \end{array}\right]}, \qquad (4)$$

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_{A_i} and I_{b_i} are the standardized variances of these parameters (variance/mean^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 (1990*a*, 1990*b*).

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[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[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[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 (1990*a*, 1990*b*).

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[sex ratio]) only, the estimate was 44.5 in pond T (N[sex ratio]/N ratio = 0.77) and 68.8 in pond P (N[sex ratio]/N ratio = 0.86), whereas it was reduced to 39.7 in pond T (N_b/N ratio = 0.68) and 21.3 in pond P (N_b/N ratio = 0.27) considering only reproductively successful adults (N_b[sex ratio]). The estimates of N_b considering the sex ratio of breeding individuals and the reproductive skew (N_b[RS]) were much lower than the census size ($N_{b[T]}$ = 10.1; N_b/N = 0.09; $N_{b[P]}$ = 3.9; N_b/N = 0.04; Table 3). The approximated N_e derived from N_b(RS) (t^*N_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 $H_{e[T]}$ = 0.617, $H_{e[P]}$ = 0.643; allelic diversity $AD_{[T]}$ = 7.25, $AD_{[P]}$ = 7.50) and the offspring sample ($H_{e[T]}$ = 0.649, $H_{e[P]}$ = 0.628; allelic diversity $AD_{[T]}$ = 7.75, $AD_{[P]}$ = 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 ($N_{b[T]}$ = 3.7; 95% CI = 2.1-5.8, $N_{b[P]}$ = 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 (t^*N_b) of these two methods ($N_{e[T]}$ = 36.6 and 53.5; $N_{e[P]}$ = 38.9 and 34.7) fell in the N_e confidence interval of the linkage disequilibrium method only in pond P ($N_{e[T]}$ = 97.1; 95% CI = 78.0-122.5, $N_{e[P]}$ = 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).*

	Pond T				*Pond P*			
	N_b	N_e	N_b/N	N_e/N	N_b	N_e	N_b/N	N_e/N
N	58				80			
N_{MR}	53				60			
N(sex ratio)	44.5				68.8			
N_e(demo)	5.7	56.9	0.10	0.98	9.5	93.7	0.16	1.62
N_b(sex ratio)	39.7	393.0	0.68	6.78	21.3	210.9	0.37	3.64
N_b(RS)	10.1	100.0	0.17	1.72	3.9	38.6	0.07	0.67
N_b(TM)	3.7	36.6	0.06	0.63	3.9	38.6	0.07	0.67
N_b(LH)	5.4	53.5	0.09	0.92	3.5	34.7	0.06	0.60
N_e(LD)	9.8	97.1	0.17	1.67	1.3	13.2	0.02	0.23

*Abbreviations: N, observed population size in 1999; N_{MR}, population size estimate from a 4-year mark-recapture study (1999-2003; Alho 2004); N_e(demo), demographic model (Nunney & Elam 1994); N(sex ratio), sex ratio effective size (Wright 1938); N_b(sex ratio), sex ratio effective breeding size (Wright 1938); N_b(RS), reproductive skew effective size (Kimura & Crow 1963); N_b(TM) effective breeding size using temporal moments (Waples 1989); N_b(LH), effective breeding size based on the likelihood approach of Berthier et al. (2002); N_e(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).

ratio led to a large upward bias in the estimates. In the 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_e 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; Lesbarreres et al. 2005; Brede & Beebee, 2006) and anuran populations (*Hyla aborea*, $H_e = 0.52$, Arens et al. 2000; *R. lessonae*, $H_e = 0.57$, *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^{-3} and 10^{-4} (Goldstein and Schlötterer 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* (Scribner 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 4. Synopsis of amphibian studies from which N_e and N_e/N estimates are available.[a]

Species	SI	N (±SE)	N_e(demo)	N_e(gene)	Range	N_e/N	Marker (number)	N_e method	Reference
Triturus cristatus	3	74[b] ± 22	—	12	4.7–47	0.16	MS (8)	MR	Jehle et al. 2001
T. marmoratus	3	147[b] ± 42	—	13.4	5.4–43.0	0.09	MS (8)	MR	
	2	104[b] ± 22	—	9.6	6–19.5	0.09	MS (8)	MR	
Ambystoma macrodactylum	3	—	—	123	2–∞	—	AL (6)	—	Funk et al. 1999
Bufo marinus	1	—	—	390	119–812	0.001	AL (10)	—	Easteal 1985
	1	—	—	346	104–719	—	AL (10)	—	
	1	—	—	372	112–770	—	AL (10)	—	
B. calamita	2	1,523 ± 36.4	206	26	7–21	0.02	MS (8)	SC, N_e(demo), TM	Rowe & Beebee 2004
	2	1,523 ± 36.4	206	45	29–879	0.03	MS (8)	LH	
	2	71 ± 5.7	40	—	—	0.56	—	SC, N_e(demo)	
	2	53 ± 5.9	24	9	4–47	0.17	MS (8)	SC, N_e(demo), TM	
	2	53 ± 5.9	24	9	5–48	0.17	MS (8)	LH	
B. bufo	1	2,500	—	31	11–190	0.012	MIS (3)	SC	Scribner et al. 1997
	1	4,000	—	21	9–63	0.005	MIS (3)	SC	
	1	4,800	—	46	13–∞	0.010	MIS (3)	SC	
	1	100–10,000	—	—	—	—	—	SC	Brede & Beebee 2004
	1	1,000	—	49	12–454	0.049	MS (8)	TM, LH	Brede & Beebee 2006
	1	852	—	34	9–405	0.040	MS (8)	TM, LH	
Notophthalmus viridescens	3	159–210	137–148	—	—	0.65–0.93	—	—	Gill 1978
	3	91–125	86–124	—	—	0.69–0.99	—	—	
	3	27–35	21–33	—	—	0.60–0.98	—	—	
	3	6–44	6–38	—	—	0.14–6.33	—	—	
	3	1,018–1,454	911–1399	—	—	0.63–1.37	—	—	
Eleutherodactylus	?	>80.000	> 56.000	—	—	0.07–1.50	—	—	Crawford 2003
Rana pipiens	1	167–4,200	42–112	—	—	0.01–0.67	—	MR	Merrell 1968
	3	—	—	588	378–1355	>0.10	MS (7)	TM	Hoffman et al. 2004
	3	328	—	420	245–837	>0.10	MS (7)	TM	
	3	—	—	1019	490–∞	>0.10	MS (7)	SC, TM	
	3	—	—	410	222–940	>0.10	MS (6)	TM	
	3	—	—	1820	660–∞	>0.10	MS (6)	TM	
R. lessonae	5	27.6 ± 3.4	25.9	—	—	0.94	—	N_b (sex ratio)	Sjögren-Gulve & Berg 1999
R. arvalis	5	64.6 ± 8.4	59.2	—	—	0.92	—	N_b (sex ratio)	
R. ridibunda	1	—	—	48.4	19.4–164.8	—	MS (5)	—	Zeisset & Beebee 2003
	1	—	—	15.8	6.5–43.0	—	MS (5)	—	
R. sylvatica	7	113	92	—	—	0.82	—	—	Berven & Grudzien 1990
	7	194	156	—	—	0.81	—	—	
	7	136	101	—	—	0.74	—	—	
	7	78	38	—	—	0.49	—	—	
	7	89	51	—	—	0.58	—	—	
R. temporaria	1	118	—	86	23–∞	0.729	MS (8)	TM, LH	Brede & Beebee 2006
	1	18	—	12	6–28	0.667	MS (8)	TM, LH	

[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/string 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 mortality 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 considerably. 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 methods 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 generation. 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 overlapping 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 1990 *a*, 1990*b*). 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 effective breeding size (Wright 1938) yielded N_e estimates higher than the census size, especially in pond T. The overestimation showed that in the investigated populations 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 census size in animal populations ranged between 0.05 and 0.80, with a mean of 0.11. For stable populations empirical 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*) population ($N_e/N = 0.03$; 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 anuran species (Table 4), suggesting that the difference between 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 fluctuate substantially between years but that years with optimal 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 assessment of the population survival ability, population dynamics, 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 interpretation is obsolete. An overestimation of N_e in species with generally low effective population size may severely contrast with the actual conservation needs of populations. 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

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