

# Demo-genetic analysis of a recovering population of otters in Central Sweden

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## Keywords

otter; PVA; genetics; demographic stochasticity; extinction.

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## Abstract

We performed a demo-genetic population viability analysis on a recovering population of otters *Lutra lutra* in Central Sweden, using data on population size, survival and genetic data from microsatellites. Population data were obtained from genotyping faeces. At present, the size and genetic variability of the population is increasing. We found that survival to first reproduction was the most crucial demographic parameter, and that even slight changes downward in this parameter, might lead to a declining population trajectory. Human factors that can affect mortality are traffic, fishing equipment and traps, and we argue that efforts to minimize road kills by means of safe passages as well as careful fishing efforts in streams and lakes would reduce the risk of extinction. In general, even though the population is now growing and has no inbreeding problem, its small abundance could make it vulnerable to chance events and environmental perturbations.

## Introduction

Many species are declining today due to human activities and there is a strong interest in the prediction of the future of populations as an aid to make decisions concerning conservation efforts. Thus, the concepts and the methodologies of population viability analyses (PVA) have been developed extensively in recent years (Boyce, 1992; Beissinger & Westphal, 1998; Brook *et al.*, 2000, 2002a,b; Coulson *et al.*, 2001; Ellner *et al.*, 2002; Lindenmayer *et al.*, 2003) to provide a scientific basis for decision making. By using demographic data, predictions concerning future population sizes and risk of extinction can be derived by simulations.

However, classic PVAs only concern the changes in numbers over time, while the negative effects of loss of genetic variability are not considered (Allendorf & Ryman, 2002; Reed & Frankham, 2003). There have only been a few attempts to incorporate both demographic and genetic factors of population long-term survival (Brook *et al.*, 2002a,b; Lehmann & Perrin, 2006; O'Grady *et al.*, 2006). We will use the approach by Lehmann & Perrin (2006), who defined a global index of resistance to genetic drift and extinction,  $\lambda_T$ , which is a product of the classic intrinsic rate of growth measured as the leading eigenvalue of the demographic transition matrix,  $\lambda_P$ , and the leading eigenvalue for the corresponding genetic matrix,  $\lambda_G$ . If both these eigenvalues equal or are larger than 1, the risks of extinction and of loss of genetic diversity is low.

In this study, we present data from a species that is difficult to study, the Eurasian otter *Lutra lutra*. This species experienced a substantial decline in population size in Sweden for a number of decades – from the 1950s to 1980s (Olsson, Sandegren & Bisther, 2006). Recently, this decline has been halted and the population is currently increasing (Länsstyrelsen Gävleborg, 1998; Bisther, 2000, 2005; Bergström, Sundberg & Näslund, 2006; Hammar, 2006). The otter is mostly nocturnal, has relatively large home ranges and spends most of its time in or very close to water, thus minimizing the possibilities for direct observation. In a recent study, we have shown that estimates based on noninvasive methods such as genetic analysis of faeces come closer to the actual population size than classic methods such as snow tracking (Arrendal, Vila & Björklund, 2007). As this is often the only way to get information concerning population size and apparent survival, we are not able to obtain direct estimates of birth rates and age distribution, but have to rely on indirect derivations from genetic data. We believe that the problems facing this study are shared by many other studies with conservation implications, especially those that focus on mammals. Yet, the need for an understanding of the future population size is nonetheless urgent.

In this study, we use the demographic and genetic data obtained in Arrendal (2007) for a demo-genetic analysis of the long-term survival of an otter population in the province of Uppland, Central Sweden. We aim to identify the demographic parameters that are most important for the

growth rate of the population, as well as the effect of demographic stochasticity. In particular, we ask the following questions: Is genetic diversity increasing or decreasing in this population? How sensitive is the population to changes in fecundity and survival? How do changes in genetic diversity combine with changes in demography? What is the most likely future in terms of population size and genetic diversity of this population? Will the population continue to increase or suffer from inbreeding?

## Materials and methods

### Background

Data were collected during the winters of 2002–2004 in the Uppland region in Central Sweden. We collected faeces along rivers and lakes, and extracted DNA from these faeces. Details concerning DNA extraction methods, microsatellite loci used and amplification success are given in Arrendal *et al.* (2007). To minimize the error in genotyping due to dropout and misprinting, we replicated heterozygotes twice and homozygotes three times. We always got the same result for the homozygotes and thus concluded that these were real. Dropout rate averaged 7.7% for the complete genotypes. Sexing was made by means of markers developed for the Y chromosome. Based on the capture–recapture data of faeces, we estimated population size using the program CAPWIRE (Miller, Joyce & Waits, 2005). Owing to the high variability of the microsatellites, we were able to identify individuals with a high certainty (Arrendal *et al.*, 2007). In short, we obtained complete genotypes from 22 individuals in 2002, 48 individuals in 2003 and 43 individuals in 2004. In addition, we had incomplete genotypes from one individual in 2002, seven individuals in 2003 and nine individuals in 2004. Based on CAPWIRE, this corresponds to 24 individuals in 2002 (range 23–25), 57 individuals in 2003 (range 55–59) and 58 individuals in 2004 (range 52–65). The smaller number in 2002 can entirely be explained by the fact that a smaller area was covered that year. This means that we could estimate survival rates using the presence/absence of individuals between years. Birth rate was estimated as the rate of new individuals entering the area between years. This might overestimate birth rate if some of the new individuals are adult immigrants. We therefore used two estimates of birth rate.

### Genetics

The genetic data were collected using noninvasive sampling of faeces during the years 2002–2004 (see Arrendal *et al.*, 2007 and Arrendal, 2007 for details on sampling and laboratory analyses). For this study, we were most interested in effective population size. There are three different effective population sizes, which describe different things. Inbreeding effective size,  $Ne_I$ , is mainly concerned with the past history of the population (Ewens, 2004), while the variance effective population size,  $Ne_V$ , and eigenvalue effective size,  $Ne_E$ , concerns the future of the population. In

particular,  $Ne_E$  is a measure of the rate of fixation of alleles, that is, rate of loss of genetic variation. Therefore, this estimate is the one used in the estimation of the demo-genetic eigenvalue (Lehmann & Perrin, 2006). Still, as the other two effective sizes give relevant information concerning the population, we also present them.

The variance effective population size was calculated as in Waples & Yokota (2007) for overlapping generations

$$Ne_V = \frac{g}{2F} \tag{1}$$

assuming an almost exhausted population census (violating this did not affect the results qualitatively), where  $g = L/T$ , where  $L$  is the interval between censuses and  $T$  is generation time).  $F$  is defined as

$$F = \frac{1}{a} \sum_{i=1}^a \frac{(P_{i1} - P_{i2})^2}{(P_{i1} + P_{i2})/2 - P_{i1}P_{i2}} \tag{2}$$

where  $a$  is the number of loci and  $P_{i1}$  is the allele frequency of the  $i$ th locus at the first sample. We used the most common allele in all cases. We used this method rather than the more elaborate one of Jorde & Ryman (1995) as we lack data on cohorts.

The inbreeding effective size ( $Ne_I$ ) was calculated as follows:

$$Ne_I = \frac{1}{2(1 - [1 - F_{IS}])} \tag{3}$$

where  $F_{IS}$  is the inbreeding of individuals relative to its subpopulation. Eigenvalue effective population size,  $Ne_E$ , was calculated as

$$Ne_E = \frac{1}{Ne_I} = \frac{h_t - h_{t+1}}{h_t} = 1 - \lambda_G \tag{4}$$

where  $h_t$  is gene diversity (probability that two alleles drawn at random are different) at time  $t$ . Thus, the eigenvalue,  $\lambda_G$ , can be calculated simultaneously from equation (4). To account for overlapping generations, we applied the same correction factor as for  $Ne_V$ , that is  $g = L/T$ .

### Demography

Survival was estimated in MARK (White & Burnham, 1999) using a mark–recapture model. Based on the estimated adult survival rate ( $s_x$ ) we estimated life expectancy ( $E$ ) after reaching maturity,

$$E = \sum_{x=2}^t L_x, \text{ where } L_x = (l_x + l_{x+1})/2, \text{ and } l_x = s_0, s_1, \dots, s_x \tag{5}$$

$t$  is time in years and  $x$  is age. The maximum observed age of females was 6 years (Arrendal, 2007), and therefore, we used that as a maximum age. This leads to  $E = 2.59$ , and thus we assumed that females reproduce on average three times after reaching maturity. From Arrendal (2007) we had two measures of annual birth rate ( $b =$  number of daughters born per female and year); if there is no net

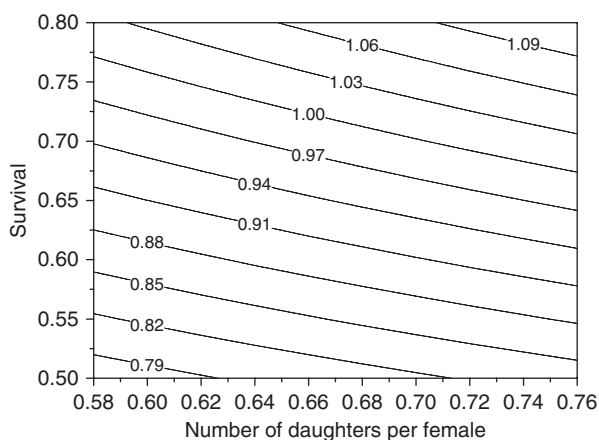
immigration then  $b = 0.68$ , assuming a net input of four females reduces this to 0.6. Over the years, only two females were observed to change area and thus we are confident that an immigration rate of four females per year represents an upper limit. This leads to a generation time of 3.5 years.

From these data we constructed a Leslie matrix, assuming that the birth pulse occurs after the census time (Caswell, 2001). This matrix has four age entries, young individuals and three older age classes corresponding to the 3 years of reproduction. As females can obviously survive longer, the last group consist of females older than 4 years. However, given the survival rate obtained, this group cannot be large compared with younger females. The matrix was analysed using different parameter combinations: (1) survival ( $s$ ) and birth rates ( $b$ ) were the same for all reproducing females, that is,  $s_0 = s_{\text{adult}} = 0.788$ , and  $b_1 = b_{\text{adult}} = 0.6$  or  $0.68$ ; (2) survival to first reproduction was lower than for adults,  $s_0 < s_{\text{adult}}$ ; (3) fecundity was lower for first-time reproducing females,  $b_1 < b_{\text{adult}}$  (see Fig. 1). From this Leslie matrix, we calculated the intrinsic rate of growth measured as the largest positive eigenvalue ( $\lambda_P$ ). We calculated elasticities (Caswell, 2001), which are the proportional change in  $\lambda_P$  resulting from proportional changes in demographic parameters (in the Leslie matrix entries).

To assess the effect of demographic stochasticity, we calculated the infinite time extinction probability for a population of size  $n$  as

$$R(n, \infty) = \left( -\frac{W[-\lambda e^{-\lambda}]}{\lambda} \right)^n \quad (6)$$

where  $W$  is Lambert's  $W$  function [i.e. the function that satisfies  $W(x) \exp(W(x)) = x$ ; Kokko & Ebenhard, 1996].



**Figure 1** Values of  $\lambda$  in relation to birth rate and survival assuming all age classes have the same survival and birth rates. Isoclines refer to parameter combinations resulting in similar values of  $\lambda$  (values along the lines).

## Results

### Genetics

Gene diversity decreased slightly but not significantly from 2002 to 2003 ( $P = 0.46$ , Wilcoxon test), but the increase between 2003 and 2004 is significant ( $P = 0.016$ , Wilcoxon test). The difference between 2002 and 2004 is not significant ( $P = 0.11$ ).

Inbreeding effective size ( $Ne_I$ ) could only be calculated for 2004 as this was the only year with a sufficient number of positive  $F_{IS}$  values.  $Ne_I$  was 33.3 (range 3.6–100), that is, 64% of census size. Variance effective population size ( $Ne_V$ ) was 9.0 for 2002–2003, 16.4 for 2002–2004 and 10.4 for 2003–2004. This resulted in a mean  $Ne_V$  of 11.92, that is, about 25% of census size.

We found an average  $\lambda_G$  of 0.86 (range 0.62–1.35 for 2002–2003), 1.36 (range 0.79–1.99) for 2002–2004 and 1.15 (range 0.97–1.52) for 2003–2004. Gene diversities (expected heterozygosity) for each year are given in Table 1, and it was clear that in most cases diversity was not lost at all, thus leading to  $\lambda_G > 1$ . This meant that eigenvalue effective population size, as defined above, could not be calculated, as this assumes a loss of genetic diversity over time.

### Demography

Using  $s = 0.788$  and  $b = 0.68$  resulted in  $\lambda = 1.066$ , while using  $b = 0.6$  resulted in  $\lambda = 1.024$ . In fact,  $b$  could be as low as 0.58 and  $\lambda$  would still be larger than 1.0 (Fig. 1). However, it was clear that if survival dropped to  $< 0.7$ , then fecundity had to be considerably larger than estimated in this population in order to keep  $\lambda > 1$ . The elasticity analysis showed that  $\lambda$  is more affected by changes in survival than in fecundity (Fig. 2) and, in particular, survival to first reproduction. The most important age class for fecundity was first-time breeders. The following numerical examples illustrate this. In order to keep  $\lambda > 1$ , first-year reproduction could be as low as 0.32 if survival was as high as for adults (0.788; assuming adult fecundity = 0.68), that is, about half the fecundity of adults. However, a change in survival from 0.788 to 0.72 (9%) lead to a required change in fecundity from 0.32 to 0.64, that is, a doubling (assuming  $b = 0.68$ ). A change in survival from 0.788 to 0.76 (3.7%) required a change in first-year fecundity of 26% (from 0.48 to 0.60).

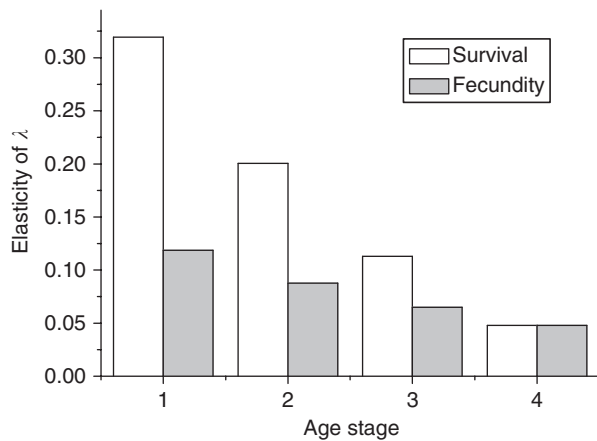
Incorporating demographic stochasticity [equation (6)], showed that the probability for ultimate risk of extinction dropped rapidly with increasing  $b$  ( $\lambda$ ; Fig. 3a). Thus, for a population size of 52 (2004) and  $b = 0.6$ , the risk of extinction was 0.084, while if  $b = 0.68$  the risk dropped to 0.0012. The effect of changes in survival to first reproduction was more drastic (Fig. 3b). A low birth rate made this effect even stronger, and survival needed to be very close to adult survival otherwise extinction was almost certain.

When we combined the demographic and genetic  $\lambda$ 's to the global index of resistance to genetic drift and extinction,  $\lambda_T = \lambda_P \lambda_G$ , we obtained using  $b = 0.68$ , a  $\lambda_T = 0.92$  for 2002–2003, and values larger than 1.0 otherwise. If we

**Table 1** Genetic diversity measures as expected heterozygosity across loci for the years 2002–2004

Locus	2002	2003	2004	2002–2003	2002–2004	2003–2004
717	0.552	0.526	0.541	−0.026	−0.011	0.015
833	0.585	0.567	0.622	−0.018	0.037	0.055
832	0.743	0.690	0.722	−0.053	−0.021	0.032
733	0.512	0.526	0.544	0.014	0.032	0.018
782	0.576	0.612	0.606	0.036	0.030	−0.006
715	0.511	0.468	0.537	−0.043	0.026	0.069
701	0.679	0.606	0.659	−0.073	−0.020	0.053
902	0.647	0.711	0.738	0.064	0.091	0.027
N	23	55	52	−0.012	0.021	0.033

The last three columns indicate the change between different years summarized with the mean value at the bottom row.



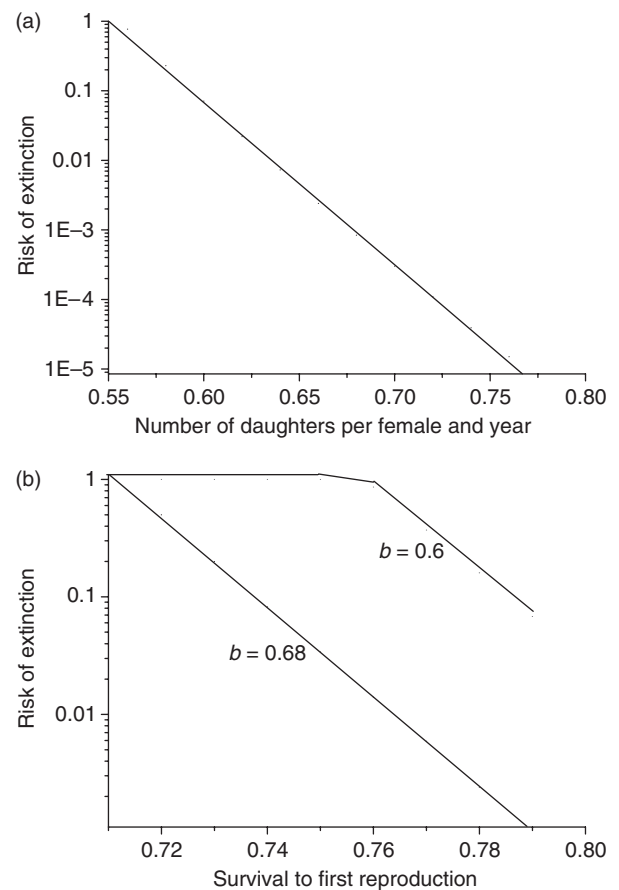
**Figure 2** Elasticity of  $\lambda$  for the survival and fecundity for different age classes.

instead used  $b = 0.6$ , we obtain a  $\lambda_T = 0.88$  for 2002–2003. For 2003–2004, this value was larger than 1.0.

### Discussion

In this analysis, we have shown that, given what we can infer from the faecal samples within and across years, the population seemed to have a possibility of increase in the future ( $\lambda_P > 1$ ). However, this was based on a number of assumptions, and the analysis of the robustness of the data showed several very important features of the population dynamics of this population. The most important factor was survival to first reproduction and it was shown that even very small changes in this parameter could drastically change the potential for an increase for this population. Viewed together with the genetic data, however, there were no indications of immediate problems to the population, through either extinction or the loss of genetic diversity.

The survival data was estimated using a limited dataset, which made the confidence interval very large, in this case between 0.46 and 1.0 (Arrendal, 2007). However, the lower data limit was unrealistic as in order to maintain or increase population size at this low survival rate, the number of daughters per female and year had to be 1.63, which would



**Figure 3** Risk of extinction in relation to (a) birth rate and (b) survival to first reproduction.

equal a litter size of about 3.2 offspring per year every year, assuming an equal sex ratio at birth. This litter size is clearly above what is known from any otter population in the wild (Erlinge, 1967; Jenkins, 1980; Mason & Macdonald, 1986; Kruuk, Conroy & Moohouse, 1991). Similarly, the fecundity could not drop below 0.35 daughters per female per year (i.e. a clutch size of 0.7), because this would require more than 100% survival.

Inbreeding and variance effective population sizes were lower than the actual number of individuals, consistent with most studies. This could indicate that genetic drift can operate rapidly and reduce genetic variability. This was contradicted by the eigenvalue effective population size, which is a measure of the rate of loss of alleles. This could only be estimated for 2002–2003, otherwise gene diversity increased and new alleles were added. This addition of alleles, likely through immigration, affected variance effective size as it is calculated here. If new alleles entered the population, then the frequency of the most common allele (used here) would change and we would have a variance over years. Likewise, inbreeding effective population size, using  $F_{IS}$ , assumed that we have no substructure, and that a positive  $F_{IS}$  actually reflected inbreeding. Positive  $F_{IS}$  values might also be a result from a Wahlund effect if there was a substructure we were not aware of. Thus, the calculations of effective sizes have to be treated with caution. Here, the undefined eigenvalue effective size was the most informative as this implied that gene diversity was increasing, that is alleles were gained rather than lost. Although there is a certain uncertainty in the estimates, we can thus conclude that gene diversity is at least not decreasing over time.

The effect of survival to first reproduction was surprisingly strong. As the survival of this group of females was so essential for the long-term survival of the population, the potential risks to survival at this age class need to be identified. One such risk factor is traffic. Road-killed otters might not constitute the largest mortality factor (Madsen, 1996; Philcox, Grogan & Macdonald, 1999; Malmquist, 2000), but as it is an additive factor it may slow down population growth and expansion. For otters found dead, road kill is the largest mortality factor in all age classes (Hauer, Asorge & Zinke, 2002). Another potentially important mortality risk is fishing equipment such as nets and fishtraps of various kinds that can trap otters under water resulting in drowning. There are no figures on this, which can be a result of poor reporting frequency. A general recommendation for management is to focus on increasing the survival prospects of females reaching their first reproductive event, as this group of individuals makes a pivotal contribution to the long-term population dynamics. Practical means of reaching this goal are otter-safe road passages and information to amateur fishermen about concern with regard to fishtraps.

Even though genetic diversity currently seems to increase, it is essential that this population is not isolated but has connections with other populations. An isolated population will lose genetic variation due to inbreeding over time (Björklund, 2003). That is, even if individuals seem to actively avoid inbreeding in any given generation, relatives might still mate in later generations and this will increase the risk for expression of recessive deleterious mutations and an overall loss of genetic variability (Björklund, 2003), which often results in depressed fitness (DeRose & Roff, 1999; Vucetich & Waite, 1999; Nieminen *et al.*, 2001; Brook *et al.*, 2002a,b; Reed & Frankham, 2003).

Ideally, a PVA should incorporate good demographic data as well as information on the variance of vital rates

(Lande, 2002; Saether & Engen, 2002) and there is currently intense debate over the validity of PVAs given the quality of data (e.g. Belovsky *et al.*, 2002). In many cases, such as in this study, a complete dataset is hard or even impossible to obtain. Nonetheless, using simple models and making simulations over a set of realistic parameter values, we were able to obtain qualitative information on the factors that are likely to affect population growth in this population. This can help to clarify the importance of the various factors that may underlie population viability, and thus increase the knowledge base upon which conservation-based management decisions can be applied. Although we were forced to make many assumptions in this study, the results were fairly robust to many of these assumptions. Furthermore, as some background data for this species were known, these assumptions were made within reasonable limits.

In conclusion, we found that the population in question, even though it was fairly small, had low risk of extinction in the short term, and that there was little risk of substantial loss of genetic variability due to drift and inbreeding. Additionally, both the number of individuals and distribution can increase in this area, and as overall population size tends to increase in Sweden, genetic diversity might even increase with time as migration among areas increase. However, the population must still be considered vulnerable due to the strong effect of survival to first reproduction and environmental stochasticity on population viability.

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