A population matrix model and population viability analysis to predict the fate of endangered species in highly managed water systems

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Abstract
Matrix models and population viability analysis (PVA) have become useful tools to understand population attributes and dynamics. Demography analysis gives valuable information for the management of threatened species, and can be used to create action plans for their conservation. PVA is particularly useful in those species with small population sizes difficult to sample. By calculating the individual fate of each member of the population, PVA simulates temporal population changes and estimates extinction risk over a time period. Here we use these models to analyse the population of axolotl Ambystoma mexicanum, which has decreased more than six times within only 5 years. Its natural environment (the Xochimilco aquatic system within Mexico City) has deteriorated significantly in the last decades. The matrix analysis showed large oscillations in the axolotl population growth rate (damping behaviour), which could explain the fast density reduction in only few generations. Younger ages (eggs and larvae) showed the highest sensitivity and elasticity values, suggesting that the lack of food sources such as zooplankton or the increased predation by exotic carp and tilapia are capable to reduce axolotl density. PVA shows low extinction probabilities using laboratory data for younger ages. However, a small reduction in egg or larvae survival rate is capable to increase extinction probabilities to 100% in 20 years. Based on these results, we found that the best strategy to restore the axolotl population is to increase the survival rate of eggs and larvae by restoring the habitat, eradicating introduced fish and improving water quality, rather than implementing a reintroduction programme.

Introduction
Amphibian declines are now a major concern for many ecologists, policy makers and conservation agencies (Biek et al., 2001). A broad analysis suggests that the possible causes of the decline are related to anthropogenic actions such as habitat destruction, disease, species introduction, exploitation or global warming (Beebee & Griffiths 2005; Pounds et al., 2006). However, the apparent decline of some amphibian species could also be the result of high population fluctuations related to their population attributes (Alford, Dixon & Pechmann, 2001). In these cases, the analysis of these attributes is imperative in order to elucidate the fate of an endangered population in an intensively managed system, by knowing which age classes are more vulnerable to modifications, and consequently, to identify major threats for a particular population.

Matrix models have become a useful tool to understand population attributes and dynamics (Caughley, 1994). The information obtained from a matrix analysis helps to elucidate the relationship between population structure and changes in population size over time. Also, simulations based on matrix models create scenarios of the population behaviour under different environmental conditions (Smith & Trout, 1994). Therefore, matrix models are useful to evaluate population conditions and to understand the effect of particular threats on different age classes. This tool also helps to estimate the effectiveness of different strategies employed to restore endangered populations based on the increment of survival rates by habitat transformation and the increment of population number by individual translocation or artificial breeding (Hunter, 1996).

Ideally, the construction of a matrix needs data obtained from large populations. However, the population sizes of threatened amphibians are frequently small. Thus, other approaches such as random population variation can be more useful (Heppel, Caswell & Crowder, 2000). Random population variation can affect the vital rates of natural
intensively managed native system. As well as the best paths for its conservation within its environment, it is necessary to elucidate the extinction risk of the axolotl (Ambystoma mexicanum), an important organism for culture and for biological diversity. These low densities represent a recent reduction. As it is an aquatic salamander that has been observed to live in highly altered systems, result of living in highly altered systems, its conservation is crucial for species with low population sizes, small distribution ranges and multiple threats as a result of living in highly altered systems.

An example of this type of species is the Mexican axolotl *Ambystoma mexicanum*, a neotenic salamander endemic to the Mexican High Plateau. Its current distribution comprises only two water bodies: Xochimilco and Chalco. Both systems used to be unified in a vast wetland that covered the entire area now occupied by Mexico City and were fed by ground water that reached surface levels in the rainy season (Alcocer-Durand & Escobar-Briones, 1992).

From the first indigenous settlements to the present day, most of the wetland has been reclaimed and destroyed, isolating Xochimilco and Chalco from one another. Now, urban areas surround the remnants of both systems that have modified hydrological dynamics and deteriorated water quality. The water is no longer coming from water springs but from water treatment plants (Solís et al., 2006). Finally, there have been at least 10 fish species introduced (L. Zambrano, pers. obs.). For many years, Chalco was completely desiccated, losing most of its native diversity. All these environmental changes are detrimental to native species, and consequently the axolotl has recently been moved to ‘Critically Endangered’ under the IUCN Red List. Despite these conditions, the axolotl has been able to survive within Xochimilco with a population density below 0.006 org m$^{-2}$ (Graue, 1998). Because there was no census in nature before the mentioned research it is not known if these low densities represent a recent reduction. As it is an important organism for culture and for biological diversity, it is necessary to elucidate the extinction risk of the axolotl as well as the best paths for its conservation within its intensively managed native system.

Here, we construct a matrix model of *A. mexicanum* using data from field samples and from animals raised in experimental tanks placed within the remaining wetland of the Xochimilco area. We analysed the condition in each life stage of the axolotl population and performed a PVA to get the probabilities of extinction of the axolotl within Xochimilco.

**Materials and methods**

Research was conducted in Xochimilco in the southern portion of Mexico City, a water body based on a seasonal wetland beside a perennial water system formed by 182 km channel web (1 m depth on average). Canal size varies from 3 to 100 m wide and up to 3 km long. Within the canal systems, Xochimilco has urban sections, agricultural and cattle areas (Solís et al., 2006). The body of water is typical of a tropical high mountain system (2200 m a.s.l.) with large changes in water temperature during the day (from 11 to 20 ºC, L. Zambrano, pers. obs.) and marked seasonality, rainy from June to October and dry from November to May, capable of modifying water quantity and quality.

**Sampling**

Axolotls were sampled from 8 AM to 1 PM for three seasons in a 2-year period: January–April 2002 (collecting once a week), September–November 2002 (collecting once a week), and March–November 2003 (collecting every other week). In each sampling season, 62 channels and eight lakes were covered. This resulted in 50 collecting sessions over the 2-year period. Each channel was treated as a transect, with a sampling point established every 200 m. At each point one throw of a traditional ‘throw-net’ or ‘atarraya’; 5.8 m diameter and 1/2 in. mesh size, was used. Axolotl density was calculated as the number of animals captured per area sampled within the throw net, which covered 26.4 m$^2$ on each throw. Each axolotl collected was weighed (g), measured for total length (TL; mm) and released at the point of capture.

**Matrix model construction**

Demographic analysis stems from the calculation of vital rates (i.e. fecundity and survival for each age category). In species with cryptic life histories, such as the axolotl, the calculation of vital rates by direct observation is problematic and data must be obtained from different sources such as laboratory studies or previously published works.

Axolotl fecundity ($F_{0i}$) was estimated using data from the mean oviposition value of 14 females in laboratory conditions. Size-specific fecundity was estimated using a regression of the number of eggs produced versus female size.

To obtain the survival rate of hatchlings in the first year we followed 14 females lay for 12 months. Egg lays were placed in experimental tanks within a rustic laboratory without walls and windows within Xochimilco behind one of the canals. Conditions and food source were emulated by constant water tank replacement with water from the system. Survival rate was measured as the mean proportion of axolotls that reach 1 year of age from the total of organisms hatched.
An estimate of the survival rate of axolotls (p_j) older than 1 year was obtained from free-living animals in Xochimilco. First, every organism was classified into a particular age category (a_j) by measuring its TL (mm). The age of every size was calculated with a length-age (Bertalanffy, 1960) growth model adjusted with data obtained from the average of 25 axolotls that were measured every 2 days from hatching to 7 months old in the tanks in Xochimilco. The growth curve was tested with two sets of seven axolotls each. One set comprised animals that were cultivated in controlled conditions of both temperature (=21 °C) and food (Daphnia every 3 days). The survival probabilities of each individual of one age category (j) to the next one (j+1) was estimated with the rate \( N(j+1)/N(j) \). This is only valid under the assumption that the size structure in natural conditions is stable.

**Projection matrix**

Population dynamics were simulated with

\[
\mathbf{A} \times \mathbf{n}_t = \mathbf{n}_{t+1}
\]

where \(\mathbf{n}_t\) is the vector of proportions for each age category at time \(t\) and \(\mathbf{A}\) is the Leslie matrix, containing transition probabilities from one age category to the other \(p_{ij}\) and fecundity values of each category \(F_{ij}\). Each element of \(\mathbf{A}\) is represented as \(a_{ij}\).

Population asymptotic properties can be obtained with

\[
\mathbf{A} \times \mathbf{n}^* = \lambda \times \mathbf{n}^*
\]

where \(\mathbf{n}^*\) is the population’s stable proportion vector and \(\lambda\) is the growth rate. At \(\lambda<1\) the population decreases, \(\lambda>1\) the population increases and \(\lambda = 1\) the population does not change. The power method was used to obtain \(\lambda\) and \(\mathbf{n}^*\) (Caswell, 2001).

**Sensitivity and elasticity analysis**

Matrix sensitivity and elasticity were also explored to indicate how changes in variables such as the survival rate in each class affects the obtained matrix demographic indices (Caswell, 2001). A values are directly related to the population dynamics and to \(\lambda\), so any change in \(a_{ij}\) is reflected in demographic vital rates. The sensitivity of a matrix relates changes in \(a_{ij}\) to variations in the growth rate. Each matrix value is associated with a sensitivity value:

\[
s_{ij} = \frac{\partial \lambda}{\partial a_{ij}} = \frac{v_i \cdot w_j}{\langle w, v \rangle}
\]

where \(s_{ij}\) is the sensitivity of element \(a_{ij}\), \(v_i\) is the \(i\)th element of the reproductive value vector (left dominant eigenvector), \(w_j\) is the stable proportion of age class \(j\) and \(\langle w, v \rangle\) is the scalar product of both vectors. A higher influence of \(a_{ij}\) on growth rates means higher \(s_{ij}\) values.

Values of \(p_{ij}\) lie between 0 and 1, while values of \(F_{ij}\) only have zero as a lower limit. The relation of \(\log(p_{ij})\) to changes in \(\log(\lambda)\) is the matrix elasticity, in which entries are proportions and can be summed to add one (de Kroon et al., 1986). Elasticity values \(e_{ij}\) show the proportional effect of a fluctuation in \(a_{ij}\) on the growth rate:

\[
e_{ij} = \frac{a_{ij} \times \partial \lambda}{\lambda \times \partial a_{ij}}
\]

(4)

**Bootstrap resampling methods**

Statistics for the growth rate and elasticities were obtained by choosing at random an element \(a_{ij}\) of \(\mathbf{A}\) and modifying its value in a range from 0 to 10% (also chosen randomly) in order to get a new matrix \(\mathbf{A}_1\), from which \(\lambda\) and elasticities were recalculated. This process was repeated 1000 times to get the average values, standard errors and confidence intervals. Intensive resampling methods are a good option for analysing data with unknown statistical distributions (Dixon & Pechmann, 2005).

**Environmental restoration and organism reintroduction simulations**

The effect of environmental modification on the axolotl survival rate was simulated by modifying the transition values of eggs to 1-year individuals \(a_{21}\) and of 1-year to 2-year individuals \(a_{32}\). The original value \(a_{21} = 0.0015\) was modified upwards three times with an increment of 0.0003 and four times downwards, by the same value each time. The other transition \(a_{32} = 0.7972\) was increased four times and decreased five times by the amount of 0.03 each time.

Reintroduction of individuals into the population was simulated with \(\mathbf{A} \times \mathbf{H} \times \mathbf{n}_0 = \mathbf{n}_{t+1}\), a formula originally proposed for harvesting (Caswell, 2001). In its original form \(\mathbf{H}\) (the harvest matrix) has values from 0 to 1 in the diagonal, which show the percentage of extraction in each age category, and zeros in the other entries. Diagonal values higher than one simulate reintroduction rather than harvesting. Reintroduction rates of eggs and 1-year individuals were simulated simultaneously. Percentages of reintroduction from the total population explored for each category were 2, 4, 6, 8 and 10%.

**PVA**

We performed a PVA to quantify the extinction probability of axolotls in Xochimilco. Contrary to basic demographic models that simulate temporal population changes through the application of \(a_{ij}\) to the size of each age category, PVA models are based on stochastic demography, which relies on the application of \(a_{ij}\) to each individual of the population. That is, population size at \((t+1)\) is the result of evaluating the probability \(P\) that each individual will survive to the next time unit, and \(1-P\) is the individual probability of death.

Because both survival and fecundity rates are considered as probabilistic processes for each individual, the population dynamics results are different from traditional projection analysis. These new assumptions allow for the possibility...
of a small population to become extinct by pure chance alone.

Intensity of fecundity events ($F_{ij}$), the proportion of reproductive individuals in each age category and $p_{ij}$ were used as parameters to build a multinomial cumulative distribution. This summarizes the probability of occurrence of the possible fates for each individual in each age category. Two types of distributions were constructed. The first distribution is made from the sum of all the fates ($p_{ij}$) corresponding to an age category, including death. The value of each $p_{ij}$ in the sum is equivalent to the probability of occurrence of that fate. The particular fate of an individual is obtained with the location of a random number along the cumulative distribution. If an individual survives, then its reproductive effort is simulated with the second cumulative distribution, which is the sum of the probabilities of occurrence of reproductive events of different magnitude ($F_{ij}$), including no reproduction. The fate of an individual is obtained as described above.

Based on these distributions, random numbers were used to simulate the individual fate of each member of the population (Caswell, 2001). Yearly extinction probabilities were calculated for 50 year periods under three scenarios: reduction in half of the survival rate values of every category, an overall reduction in half in fecundity values of every category, or reduction in half of both values of every category. The extinction probabilities over time were calculated by the average of the proportions of 50 model iterations.

**Results**

The axolotl population density was measured as 0.0012 org m$^{-2}$, almost six times lower than previous sampling results using the same technique (Graue, 1998). Based on the von Bertalanffy growth curve, axolotls have a fast increase in size during the first year, achieving up to 20 cm of TL. Within the second year the axolotl grows close to 10 cm, and the growth rate gets smaller from the third year. The axolotl maximum size ($L_\infty$) was determined to be 39.5 cm (Fig. 1). Sizes of all axolotls from the populations in controlled conditions fit properly within the matrix categories. Based on this analysis axolotls collected in Xochimilco seem to be no older than 3 years (\( <33.5 \) cm). Thus, the matrix model was built only for the first 3 years.

**Matrix model**

The matrix model showed high mortality during the first year and a large egg production in the second and third years.

\[
A = \begin{pmatrix}
0 & 0 & 760.52 & 1000 \\
0.0015 & 0 & 0 & 0 \\
0 & 0.7972 & 0 & 0 \\
0 & 0 & 0.0526 & 0 \\
\end{pmatrix}
\]

Finite growth rate has high oscillations, or damping behaviour, during many generations (Fig. 2), but once the population is stable it shows a slow depletion (\( \lambda_{\text{average}} = 0.9922 \pm 0.0137 \)). Estimates of survival up to the first year from experimental tanks and field observations clearly show mortality should be higher than these results in nature (\( \lambda = 0.70 \)). Therefore, the original matrix value of \( p_{21} = 0.0015 \) was systematically modified in order to obtain the growth rate under natural conditions. These simulations indicate that $P$ value could be three times lower ($p_{21} = 0.0005$).

The sensitivity matrix suggests that $p_{21}$ has the highest influence on growth rate. Transitions in the following years are the second most influential transitions.

\[
S = \begin{pmatrix}
0 & 0 & 0.0004 & 2.38 \times 10^{-5} \\
185.25 & 0 & 0 & 0 \\
0 & 0.4156 & 0 & 0 \\
0 & 0 & 0.4601 & 0 \\
\end{pmatrix}
\]

Elasticity analysis also suggests that survival in the first two stages has the highest influence on the proportional
variation in growth rate, followed by fecundity in the second year (i.e. age at first reproduction).

$$e = \begin{pmatrix} 0 & 0 & 0.2965 & 0.0275 \\ 0.3241 & 0 & 0 & 0 \\ 0 & 0.3242 & 0 & 0 \\ 0 & 0 & 0.0275 & 0 \end{pmatrix}$$

**Population viability**

The prediction of the population extinction probability from the matrix model with the original laboratory and field sample data is low. However, a slight decrease in the survival rate or fecundity increases the possibility of extinction (Fig. 3). A small reduction in the survival rate increases the possibility of extinction within 20 years by 70%, while a decrease in fecundity only increases the possibility of extinction by 30%. The probability of extinction is close to 100% in a 50-year period.

**Species management**

According to the sensitivity matrix, simulation of better conditions for oviposition and refuge for eggs and juveniles, reflected by an increased survival rate in stages $P_{21}$ and $P_{32}$, showed a rise in population growth rate (Fig. 4a). In fact, just a small increase in the egg survival rate of 3% produces an increase in $\lambda$ values higher than 1. A matrix simulation of the introduction of axolotls raised in captivity up to the first year also produces an increase in the growth rate. To achieve $\lambda$ values higher than 1, it is necessarily to introduce more than 8% of the total eggs or juveniles existing within the system (Fig. 4b).

**Discussion**

**Population and matrix model**

The population size seems to be very small and is composed mainly of 1-year-old individuals. This suggests that predator pressure by introduced carp, tilapia, aquatic birds, snakes and fishing keeps the population size low as in other systems with ambystomids (Hoffman, Larson & Samora, 2004; Reid, 2005; Rubbo, Shea & Kiesecker, 2006). It is also possible that there is not enough suitable habitat for axolotls to survive longer. A short-term survey in 2005 suggested that the abundance might be even lower (0.0001 m$^{-2}$) than that reported here. Nevertheless, the abundance of young individuals indicates that the population is successfully recruiting, which is an essential process if the population is to recover.

The matrix model produced a highly oscillatory behaviour, which could be explained by massive reproductive events from few classes. Some years, most of the classes have reproduction activities, leading to a big increase in the growth rate, but the next year most of the organisms are not capable of reproducing and the growth rate is reduced to almost zero. High oscillations in the growth rate could explain the changes in axolotl density in different years. After more than 100 generations, the growth rate stabilizes at a value slightly lower than unity. Therefore, a simplistic analysis of the matrix growth rate values suggests a population of axolotls that are not on the edge of extinction even though densities have been drastically reduced. However, fecundity values were obtained from laboratory conditions, so it is possible that they could be lower in field conditions.

Nevertheless, the axolotl population faces a modified environment, so the demography is unlikely to be the same as that occurring under less restrictive conditions, such as an environment without predators and with a suitable habitat.
For example, the population age structure could be shifted due to predators (Rubbo et al., 2006). Indeed, the maximum age achieved in the lab can be as much as five times higher than that in field conditions. Reproductive categories and egg production in different age classes would also vary, yielding different demographics in different habitats.

As expected, sensitivity and elasticity analyses showed that the growth rate is mainly influenced in early life stages due to high egg and juvenile mortality. The next most important processes are survival to ages two and three. However, it is surprising that the survival rate affects the growth rate more than reproduction pulses.

**PVA**

In spite of the apparently bad condition of the axolotl populations, the population viability analysis suggests low extinction probabilities. This is possibly related to the axolotl’s great reproductive capacity, which can help a small axolotl population to recover in few generations once the conditions are more favourable to its survival. However, the early stage of the matrix that has the highest sensitivity values was built from experimental tanks where eggs and larvae were free from predators that normally exist in the channels, such as fish or insects. Under normal conditions, it is probable that the survival rate with natural predators such as native fish or insects would be similar to that registered in the experimental tanks. However, exotic tilapia is a proven predator of axolotl larvae in experimental tanks (L. Zambrano unpubl. data). Over the last decade this fish population has exploded within the channels (Valiente Riveros, 2006), and tilapia is now considered a pest, with government programmes designed to reduce its presence in Xochimilco (Zambrano, Reynoso & Herrera, 2003). Therefore, it is highly possible that the survival rate of axolotls in early stages has been reduced in recent years, in which case extinction probabilities increase from 0 to 100% in 20–50 years.

**Population recovery strategies**

Numerical simulations show that both reintroduction and environmental modification can stimulate population growth rates to reach values higher than unity. The choice of the best strategy depends on costs and technical feasibility. For example, the matrix model shows that a 3% change of $P_{21}$ makes $\lambda$ values higher than unity. To increase the survival rate, the modification of the environment should be related to spatial heterogeneity (Perkins & Hunter, 2006). Although most ambystomids leave their eggs without any parental care (Bruce, 2003), axolotls need a plant structure for egg laying (L. Zambrano, pers. obs.), and many salamander females have a preference for a particular structure or plant species for egg laying (Thompson, Gates & Taylor, 1980). Preference could be related to an increase in the survival rate of the offspring due to protection and food availability. Therefore, it is possible to increase the survival rate of early axolotl stages by increasing macrophyte coverage of particular species. This hypothesis must be tested both in the laboratory and in the field.

Reintroduction of eggs and 1-year individuals grown in aquariums also causes $\lambda$ to grow. Although this strategy may be technically and economically feasible, it must be regularly applied for many years, as it does not intrinsically help the population to grow, as it results in a subsidized population. Further issues associated with reintroduction are related to genetic problems and potential diseases brought to the natural populations from reproductive colonies (Swanson, Peters & Kyle, 2006; Tweed et al., 2006), which could lead to more damage than benefit to the axolotl population in Xochimilco. If this strategy is implemented, diseases and genetic variability must be seriously considered.

From our results we suggest that the species recovery plan should involve habitat management and restoration before any other measure such as reintroduction. The two most important threats axolotls face are related to first stages: habitat transformation, including a drastic modification of riparian habitat due to urbanization, and a population explosion of introduced fish (tilapia Oreochromis niloticus), which are predators of axolotl larvae (L. Zambrano unpubl. data). Therefore, to ensure the conservation of this population in the channels of Xochimilco, it is necessary to restore the system considering both threats so as to ensure survival in early stages.

**PVA for endangered species**

PVA is an important tool for the management of endangered species and information generated by these models must be considered for conservation policies. These models can be used to predict the fate of a species in short and middle term, but can also help to implement conservation actions on a particular species. These models can be improved with more data, but most endangered organisms such as the axolotl have few and scattered populations, making it difficult to obtain the ideal amount data. The best way to fill the gaps generated by these small populations is by using the available literature, knowing the biology of the species and transforming this information in numbers. It is important to create different scenarios modifying those variables that can be more sensitive in the matrix. Then, it would be possible to test these scenarios experimentally or in the field. This can be a good way to create conservation policies for an endangered organism such as the axolotl.

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