40 years of Natterjack toad Conservation in Europe

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The need for knowledge

Work on *Bufo calamita* in the UK began in the 1970s when it was realised that more than 75% of previously existing populations had become extinct since the start of the 20th century (Beebee 1976).

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Natterjacks are entirely confined to Europe and range from Iberia, where they are abundant and widespread, east and northwards as far as Belarus and Estonia. They are absent from Italy and the Balkans, the other major southern refugia where many animals and plants survived the Pleistocene glaciations. In Iberia Natterjacks occupy a wide range of habitats and can be found at altitudes as high as 2500 meters in southern Spain. Elsewhere they are mainly a lowland species and are closely associated with open, unforested habitats including river valleys, gravel pits, coastal marshes, sand dunes and lowland heaths. These restrictions result in increasingly fragmented populations towards the northern range edges. Genetic studies confirmed that Iberia, but also south-west France, were successful refugia for Natterjacks during the last glacial maximum around 20 000 years before present (BP). Genetic diversity declines progressively from that area towards the northerly and easterly range limits. It is also clear that some Natterjacks must have survived the more recent but less severe and shorter Younger Dryas cooling about 11 000 years BP in localities further north than the south of France, and that separate populations from that time colonized the west and east of the British Isles (Rowe et al. 2006).

As in many Anuran species Natterjack toad age- and size-related life-history traits vary along latitudinal and altitudinal gradients, i.e. latitudinal variation of size follows roughly a converse Bergmann cline, but some populations do not fit to this pattern. Demographic life-history traits were studied in twelve populations (eleven in Spain and one in Germany) representing a variation of adult size from 39 mm to 95 mm snout-vent length, a latitudinal gradient from 37° to 50°, and an altitudinal gradient from sea level to 2,270 m. Skeletochronology was used to estimate age as number of lines of arrested growth and lifetime pattern of growth in breeding adults. At southern latitudes toads matured and reproduced earlier than those at northern latitudes, but had a reduced potential reproductive lifespan due to lower longevity. Similarly, age at maturity and longevity increased at elevation exceeding 2,000 m, but female potential reproductive lifespan (PRLS) did not increase with altitude, as it did in northern latitudes. Spring and summer breeding Natterjacks at the German locality differed with respect to longevity and potential reproductive lifespan by one year in favor of the early breeders, increasing the fitness of this cohort under current climate conditions. Integrating available evidence, lifetime fecundity of Natterjacks decreases at the upper altitudinal range because PRLS is about the same as in lowland populations but females are smaller. In contrast, small size of northern females was compensated for by increased PRLS which minimized latitudinal variation of lifetime fecundity. However, altitudinal effects on life-history traits do not mimic latitudinal effects. Life history trait variation along the altitudinal gradient seems to respond directly to the contraction of the annual activity. As there is no evidence for increasing mortality in highland populations, reduced lifetime fecundity may be the ultimate reason for the inability to colonize elevation exceeding 2,500 m.

Age-adjusted adult size depended mainly on the size achieved between metamorphosis and first hibernation or aestivation which in turn was influenced by local factors. The first-year size corresponds to the duration of the aboveground activity period, temperature during the activity period, and the type of shelter sites and hibernacula available in the habitat. After attaining sexual maturity, growth rates did not differ among populations. Interactions of multiple environmental factors during the first year of life determine age at maturity, adult size and size variation among populations. Local body size and potential reproductive lifespan co-vary to optimize lifetime fecundity throughout the geographical range. The presence of a small-sized population in southern Spain does not fit to the pattern predicted by a converse Bergmann cline, but is compatible with the hypothesis that body size variation among Natterjack populations may be the evolutionary byproduct of optimized lifetime fecundity.

Natterjacks have a considerable potential for adapting to local conditions. Thus, natterjacks are among the few amphibian species that have been capable of adapting locally to survive moderately saline water, which is otherwise known to cause severe physiological stress in amphibians. Nevertheless, natterjack populations naturally breeding in brackish ponds in south-western Spain have evolved an increased tolerance to salinity compared to populations breeding in freshwater ponds (Gomez-Mestre & Tejedo 2003). Moreover, natterjacks also seem to have been able to adapt to geographic differences in the presence of other amphibian competitors, since individuals from populations with a long history of competition with *Bufo bufo* tadpoles had also evolved greater competitive ability than individuals from areas where *B. bufo* was absent from (Gomez-Mestre & Tejedo 2002).

The delimitation of the spatial equivalent of isolated populations or interacting sets of local populations is crucial for conservation management of Natterjacks. The individual variation of annual migratory capacity within local populations allows delimiting core habitats of and connectivity among local populations. The migratory behaviour of adult Natterjack toad was monitored using radio telemetry at eight localities in Spain, France, Germany and the UK covering a latitudinal range from 41° to 54°N. Radio telemetry data were used to model the adults' capacity for dispersal assuming exclusively unidirectional movements. Migratory range was not sex-biased, but was three times lower in population inhabiting sandy areas than in those on clay soils, probably due to the scarcity of moist shelters causing more frequent and more distant movements. For conservation management of local Natterjack populations, we use the migratory capacity of the 50% most sedentary individuals to delimit the core area around a given breeding site. To estimate the potential genetic connectivity between neighbouring local populations, we use the minimum migratory capacity of the 5% of individuals which moved most. Estimates obtained for populations in central Europe and the UK indicate a core area of 600 m around the breeding site and a maximum distance of 2,250 m between the breeding ponds to maintain connectivity. Thus, the principal conservation problem in the UK is that most populations are isolated by distance and prone to local extinction. In contrast, core areas of populations in Spain extend to distances of about 5 km and connectivity is maintained up to 12 km distance between neighbouring breeding ponds.

Behavioural regulation of body temperature (t_b) was monitored in 38 free-ranging adults in two populations (Spain, Germany) using temperature-sensitive transmitters implanted to the abdominal cavity. In field t_b varied between +0.3 °C and 32.2 °C in Spain and between +0.5 °C and 37.4 °C in Germany. Maximum t_b measured during an experimental trial was 38.8 °C. Thermoregulatory behavior differed considerably between populations. In Germany, toads avoided environmental temperature extremes by burrowing actively into moist sandy soil (2-90 cm deep), whereas in Spain, toads hid exclusively in mammal burrows or pre-existing subterranean cavities providing moist and temperature-buffered microhabitats. Frost avoidance in Spain included frequent changes of shelter sites by aboveground dispersal, distinguishing this population from conspecifics in Germany evading frost by burrowing. The control of water balance superposed behavioral efforts to optimize t_b identifying Natterjacks as thermal conformers. Upper thermal tolerances of tadpoles do not differ geographically between Spanish and Swedish populations (39.7 °C and 39.8 °C, respectively). This high thermal tolerance appears to be adaptive to the shallow sunny ponds where natterjacks breed (Duarte et al. 2012, H. Duarte, B. Rogell and M. Tejedo, unpublished data). However, the temperatures reached at the breeding ponds may be only slightly lower than upper thermal tolerances, especially at southern populations, that may be exposed to physiological thermal stress in the coming years due to the predicted increase in the frequency of heat waves by global warming. Other consequences of global warming include the increasing drying conditions expected during the early terrestrial life, particularly at its southernmost range. Experimental results indicate similar drought tolerances in juveniles of Natterjack populations across their geographical distribution (M. Tejedo and C. Iriarte, unpublished data).

Transmitter implantation in *B calamita*. Photo: Ulrich Sinsch.

Conservation in action

In Britain conservation action has mostly entailed the maintenance of ephemeral ponds and removal of invasive scrub, eventually reintroducing a grazing regime using domestic livestock to maintain early successional habitat conditions.

The first attempts to reintroduce Natterjacks to sites in Britain where they had recently gone extinct started in the 1970s. These early efforts failed but since 1980 there have been increasing successes (Denton et al. 1997; Griffiths et al. 2010), such that by 2009, 19 out of 27 translocations where the level of success could be judged were successful (i.e. a minimum of adults returning to breed successfully), a success rate of about 70%. Translocations have been carried out by transfer of spawn, the equivalent of eight strings but made up of small sections from multiple strings to maximize genetic diversity, for two consecutive years. It has remained easier to re-establish Natterjacks at coastal dune sites (85% success) than on heathland sites (57% success). Successful translocations now outnumber native populations in the UK, of which there are 13 as defined by genetic studies. However, native populations are, on average, four times larger than successfully translocated ones. More than 77 % of all UK Natterjacks are still in native populations. In northern France, the expansion of the port of Le Havre in the Seine estuary has resulted in the irreversible destruction of terrestrial habitats and amphibian breeding sites. A decision was taken to translocate resident Natterjacks and the capture of migrating or erratic toads was conducted in 2001 thanks to about 5 km of drift fence and pitfall trap system and 160 plywood or carpet boards used by toads as artificial shelters widespread in the 400 hectares of the area. More than 5000 toads (adults and juveniles) were translocated in 3 areas where Natterjacks were still or historically present. The operation success was estimated in this latter area only by yearly survey of potential breeding sites and adult radiotracking. Annual survival was 25% in these adults, i.e. half the value estimated in native populations, but repeated breeding over several years indicates a successful translocation.

The existence of a 37-year data set on a population in southern England provided an opportunity to model the impact of long-term habitat management on Natterjacks (Di Minin & Griffiths 2011). The management interventions were consistent with an increase in carrying capacity over this period. As with many amphibian populations, habitat management aimed at improving juvenile survival is likely to have the most positive effects on population viability. However, even with ongoing management, fluctuating Natterjack populations may remain vulnerable to extinction for several decades unless efforts can be made to offset reductions in recruitment caused by pond desiccation.

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In Iberian Peninsula, gene flow, genetic diversity and genetic structure have been investigated in 13 populations, including the isolated ones in the Basque coast, in order to determine the best *conservation* management strategy. Significant differentiation between isolated and the rest of the analysed populations was shown, with various genetic diversity and isolation (Iraola et al. unpublished). Since both populations have to be considered as independent units for conservation purposes, it was examined whether genetic depression may increase population vulnerability to anthropogenic disturbance. Lethal concentrations of a common herbicide (glyphosate) were determined for the two genetically depressed populations, two 'healthy' ones and crosses between both types of populations. Tadpoles from low genetic diversity populations tolerated lower doses of herbicide, whereas intermediate values were obtained with crossbreed tadpoles which suggest a genetic effect (Cabido et al. 2010, 2011). In addition, immune response (phytohemagglutinin test) of adults from one of the genetically depressed populations was found to be lower than those from a 'healthy' one (Cabido & Garin-Barrio, unpublished). In conclusion, genetic diversity could determine the capacity to tolerate anthropogenic environmental stress or health status of different populations and it should be taken into account in conservation plans.

Conclusion

The Natterjack toad remains one of the best-studied amphibians within Europe and much of the research conducted has had a direct conservation focus. Long-term collaboration between researchers and conservation practitioners in different countries has been a hallmark of the programme, and the exchange of data and experiences has been of mutual benefit.

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Taking the pulse of the planet's biodiversity: a new tool for tracking changes in amphibian abundance

The Living Planet Index : A call for support

The Living Planet Index (LPI) is a measure of the state of the world's biological diversity based on population trends of vertebrate species from around the world. The Amphibian Survival Alliance (ASA), in collaboration with the Zoological Society of London and WWF, aims to develop a new index of amphibian population change. To find out more about the LPI download the fact sheet here static.zsl.org/files/1-2-1-living-planet-index-1062.pdf or contact Jaime Garcia Moreno (jaime.garciamoreno@iucn.org) or Phil Bishop (phil.bishop@iucn.org) to find out how you can get involved in this innovative initiative.

The 2nd International Symposium on the Conservation of Amphibians: *Bufo calamita*

By Ion Garin-Barrio

The 2nd International Symposium on the Conservation of Amphibians: *Bufo calamita,* took place on 17 and 18 December 2010 in the Aquarium of Donostia-San Sebastián, Spain. The symposium was organized by the research group at the Aranzadi Society of Sciences, Department of Herpetology. This team currently drives the monitoring work on the surviving populations of *Bufo calamita* on the Cantabrian Coast. At the meeting the research group worked closely with a large number of specialists in the conservation of threatened Natterjack toad populations, including the Irish, British, Swedish and Estonian.

About 100 people participate in the meeting from a range of countries including but not limited Estonia, Sweden, Belgium, Germany, France, Ireland, United Kingdom and Spain. Twenty eight studies (15 oral presentations and 13 posters) were presented at the symposium, which also provided an opportunity for attendees to exchange information and create working groups. Finally, a series of general species management measures were agreed upon and are outlined below. Any comments and questions should be sent to Ion Garin-Barrio (igarin@aranzadi-zientziak.org) Herpetology-Department Aranzadi Society of Science.

Guidelines for recovery of threatened populations of the Natterjack toad (*Bufo calamita*)

- Natterjacks require open (non-forested) areas in sandy soils, and shallow ponds to breed.
- Natterjacks do well in areas in early ecological successional stages. That implies that conservation of the species needs not be restricted to protected areas, but can also be extended to apparently disturbed areas such as sand and gravel pits or farmlands. Such environments usually lack competitors and predators of *B. calamita*.
- Therefore it is important to build public awareness about the species conservation, locally modify agricultural uses to fit in the existence of suitable habitats and possibly contemplate compensatory measures.
- Reduced effective population sizes result in low genetic diversity and low heterozygosity. Steep reduction in heterozygosity becomes an issue right after a severe bottleneck. However, low heterozygosity may not be a problem *per se* for populations that have been small for a long time, because selection is likely to have purged deleterious alleles.
- Hence, we should not give up on small populations. Large, healthy populations can be restored from them.
- Recovery of relic populations or reintroductions of *B. calamita* should aim for establishing local metapopulations. This could be achieved by establishing a minimum pond network of 4-5 suitable ponds 200-500 m (max. 1 km) away from one another. Ideally this pond system would result in a stable population in the vicinity of a few hundreds of toads. Pond connectivity is important, but population nuclei should retain certain degree of independence (i.e. migration should not be too high), or otherwise the system would behave as a single population rather than a metapopulation.
- There is no fool-proof recipe for restoring *B. calamita* populations, but recommendations based on several decades of research across several countries can be made.
- Density-independent factors are important in regulating population size, and hence carrying capacity may not be critical.
- Juvenile survival is key in population viability, and measures should be taken to maximize it.
- Refuge availability is also important: sandy areas should contain patches of small to medium sized rocks for juveniles and adults to hide and hibernate/aestivate safely.
- Translocations may be needed from neighboring populations to recover populations in the brink of extinction. Adult translocations over short distances should be avoided because they are likely to attempt to return to their site of origin. Instead, translocations should be attempted using both eggs and tadpoles, and even metamorphic individuals. In any case, translocations should not be attempted unless there is good evidence that it is possible to establish a self-sustaining, stable population in the long term. Translocations should follow current IUCN guidelines.
- Monitoring of recovering or newly established populations is essential. 'Success' can be measured at three stages: (1) released individuals survive in the new site; (2) released individuals breed successfully; and (3) a viable population is established. Ideally, (3) should be the long-term goal. Demographic stochasticity is inherent to *B. calamita* population dynamics, in part because of the ephemerality of their preferred ponds. Therefore, it should be assumed that monitoring of threatened populations is a long term task. Life span is variable among populations, but natterjacks can easily live up to 7-10 years. Generation time is typically 3 years, so no conclusions can be seriously drawn before 3 years of monitoring, but 5-10 years is more realistic in terms of assessing whether a population is likely to become established. Longer-term monitoring may be needed to determine whether the population is viable and sustainable..
- In any case, it is the number of generations, and not the actual time passed that will determine the extent of monitoring required, until it can be verified whether breeding occurs consistently.
- It would be desirable to adopt the collective aim of recovering the original range of the species, taking into account the models of suitable habitat predictions when planning translocations.
- We request that the Gobierno del Pais Vasco takes action to conserve Natterjack toad populations, and more specifically, that the Diputacion Foral de Gipuzkoa takes swift and decisive conservation measures to protect the severely threatened population of *Bufo calamita* in Gipuzkoa.