Update on the ecology and conservation of the endangered and umbrella species: *Pelobates varaldii*

Arlo Hinckley[1], Alberto Sánchez[2], Alicia Talavera[3], Tahar Slimani[4]

[1] Estación Biológica de Doñana (EBD-CSIC), Conservation and Evolutionary Genetics Group, Avda. Americo Vespucio, s/n. 41092 Sevilla. Spain. C.e.: arlo@ebd.csic.es

[2] National Museum of Natural Science (MNCN-CSIC), Department of Biodiversity and evolutive ecology. Cl. José Gutiérrez Abascal, 2. 28006 Madrid. Spain.

[3] Instituto de Hortofruticultura Subtropical y Mediterránea La Mayora (IHSM-Universidad de Málaga, CSIC). 29750 Algarrobo-Costa. Málaga. Spain.

[4] Cadi Ayyad University, Faculty of Sciences Semlalia. Laboratory Biodiversity and Ecosystem Dynamics. PO Box 2390. Marrakech 40000. Morocco.

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Resumen: El sapo de espuelas marroquí *Pelobates varaldii* es un endemismo de las llanuras atlánticas del noroeste de Marruecos que cría en charcas temporales de largo hidroperíodo. Esta especie se encuentra en peligro de extinción, amenazada por la pérdida de hábitat y la introducción de especies invasoras. Estos factores la han convertido en una de las especies más amenazadas del norte de África. Este estudio, llevado a cabo en Febrero del 2015, surge como respuesta a esta tendencia negativa, con la idea de monitorizar su estado de conservación e incentivar futuras actuaciones para mejorar la viabilidad de sus poblaciones. Además, pretende exaltar el valor del hábitat reproductivo de la especie a través del concepto de especie paraguas. En las charcas donde cría la especie se han hallado otras 33 especies de anfibios y macroinvertebrados, lo que demuestra la importancia de proteger este diverso pero amenazado hábitat. *P. varaldii* parece continuar en regresión al igual que el estado de las charcas donde cría, si bien estudios a más largo plazo serán necesarios para confirmar esto con certeza.

The family Pelobatidae comprises four species distributed in the western Palaearctic region (Amphibiaweb, 2016). Only one species, *Pelobates varaldii*, is present in North Africa, where it is endemic to the coastal plains of northern Morocco (Salvador, 1996). Its distribution is limited to four isolated areas along the Atlantic coast between Tangiers and Oualidia, and its eastern limit is in Ouezzane (De Pous *et al.*, 2012). *Pelobates varaldii* is one of the most stenoecious northafrican amphibian species, as its presence is limited to the vicinity of Mediterranean temporary ponds located on loose, sandy soils at low altitudes (Beukema *et al.*, 2013). This species is threatened by habitat destruction and the recent introduction of exotic species like *Gambusia* sp. and *Procambarus* sp. (García *et al.*, 2010; De Pous *et al.*, 2012) and for this reason *P. varaldii* is classified as endangered in the IUCN Red List categories (Salvador *et al.*, 2004; Escoriza, 2013; Reques *et al.*, 2013). In this note, all known populations of *P. varaldii* where visited with the aim to study the biodiversity (through species richness) of the ponds where it breeds and review the conservation status of each of these populations.

Temporary ponds have been until recent times a forgiven habitat. But because of their relatively isolated status in comparison to permanent water bodies (rivers, marshes and lakes), their unpredictable date of flooding and their small size and shallow conditions, biodiversity in these ponds is high (Beja & Alcazar, 2003; Rhazi *et al.*, 2004). Recent studies have pointed out

Figure 1: Map of localities sampled in this study. In green and capital letters: localities where the presence of *P. varaldii* was confirmed. In red and lower case: localities where presence of *P. varaldii* was not confirmed.
Figura 1: Mapa de localidades muestreadas en este estudio. En verde y en mayúscula aparecen las localidades donde se confirmó la presencia de *P. varaldii*. En rojo y en minúscula aparecen las localidades donde no se confirmó la presencia de *P. varaldii*.

that the length of the hydroperiod and seasonality are the main factors in determining the faunal composition and the structure of temporary aquatic communities (Boix *et al.*, 2001). The most influential factor affecting biota is the desiccation of the habitat during the dry season. Species richness normally increases as the length of the flooded period in ponds increases (Boix *et al.*, 2001; Beja & Alcazar, 2003; Eitam *et al.*, 2004; Rhazi *et al.*, 2004).

Our focal species is known to breed in temporary ponds with long hydroperiods and relatively wide surfaces (Escoriza, 2013; authors, unpublished data), two variables which are known to be linked with taxonomic richness (Boix *et al.*, 2001; Beja & Alcazar, 2003). Therefore, species richness in these sites is expected to be the highest among temporary ponds.

Sweep sampling has been the chosen method as the study required to repeatedly survey many ponds over a large region, and sampling larvae may be more efficient for monitoring amphibians than sampling adults (McDiarmid, 1994), especially in such a secretive species like *P. varaldii* (Beukema *et al.*, 2013). Furthermore, larvae relative abundance estimates are successfully being used to monitor other endangered amphibians species (Bosch & Fernández-Beaskoetxea, 2014; Oliver *et al.*, 2014). Additionally, it is also the best method for sampling pond invertebrate communities (Furse *et al.*, 1981). Amphibian sampling was carried out during February 2015 which is the month with the maximum larval densities, as almost all clutches have hatched but no larvae have metamorphed and left the ponds yet (Beukema *et al.*, 2013; authors, unpublished data). In total, 33 ponds within the potential distribution range of the species were sampled, of which 12 were occupied by *P. varaldii*. Our effort has been focused on the less studied and most endangered populations, so the La Mamora forest stronghold, intensively covered in De Pous *et al.* (2012), has been sampled with the aim to have a simple representation and not pursuing to cover all of its ponds. The different sampled localities are displayed in Figure 1. All sites were surveyed by one person sweeping four times a dip net (700 cm^2 and 4mm of mesh width) during 15 s per sweep. Each sweep was done in a different zone of the pond, trying to cover different depths and areas with different percentage of emergent vegetation. Larvae of other amphibians and macroinvertebrates were also identified at the breeding site and immediately released after it. Non-identified invertebrates were stored in ethanol for its posterior classification in the laboratory. Physicochemical parameters were taken *in situ* with a Tetra test. The abundance of the species on each site was expressed in terms of catch-per-unit-effort (CPUE; mean number of individuals in four sweeps). This estimate has served to compare among populations in this study and will hopefully serve to monitore changes in these on the long term.

The following observation needs to be made on regard to the taxonomic classification: the aim of it was to catalogue species richness from taxa bigger than 4mm in the ponds where *P. varaldii* breeds, so the target is to distinguish among taxonomic units not to identify at a species level.

Table 1: Taxonomic units present in *P. varaldii* ponds. Those units that include more than one species are followed by brackets indicating the estimated number of these ones.
Tabla 1: Unidades taxonómicas presentes en las charcas en las que habita *P. varaldii*. Aquellas unidades que incluyen más de una especie van seguidas de un paréntesis con el número estimado de éstas.

Taxonomic units
Class
Gasteropoda (2 species)
Ostracoda (4 species)
Subclass
Acari
Copepoda (2 species)
Order
Anostraca
Coleoptera
Ephemeroptera
Hemiptera
Odonata (3 species)
Suborder
Zygoptera
Family
Chironomidae (2 species)
Genus
Ancylus
Dityscus (2 species)
Hydrometra
Lymnaea
Notonecta (2 species)
Species
Daphnia sp.
Procambarus sp
Triops sp.
Amietophrynus mauritanicus
Discoglossus scovazzi
Hyla meridionalis
Pelobates varaldii
Pleurodeles waltl

Species richness (macroinvertebrates and amphibians) in *P. varaldii* ponds

As shown in Table 1, 34 different species (counting macroinvertebrates and amphibians) were found in seven analysed ponds. Given the fact that the study was only carried out during February, it is likely that the real species richness of this habitat is being underestimated as many species that inhabit the area might not be present in the ponds at this time of the year. For instance *Pelophylax saharicus* and *Amietophrynus mauritanicus* are late breeders, and their larvae were absent in February. Moreover, most of the smallest invertebrates were not counted; for instance, Cladocerans and almost all Ostracods, which are known to be diverse in this type of ponds (Eitam *et al.*, 2004), and some taxonomic units could host more than one cryptic species. Still, this underestimation of the real richness gives us an idea of the biodiversity that this habitat hosts and the importance of conserving it. For instance, the amphibian species richness mean (4.1 ± 0.37 sp) was higher than in other studies performed in the Western Mediterranean: (2.2 ± 1.2 sp) (Beja & Alcazar, 2003). Finally, it shows an additional reason to conserve the species as it acts as an umbrella species (Roberge & Angelstam, 2004), by protecting this endangered species, we would protect at least four other species of amphibians and more than 29 species of invertebrates, many of which depend completely on these ponds to complete some stage of its cycle (Beja & Alcazar, 2003).

Population status, larvae abundances and threats

Tanger-Asilah

Larvae CPUE: 94, 31, 21.

Locality 1. This zone can be stated as one of the species strongholds given the high larval CPUE and the quality of its breeding habitat (high invertebrate richness). But this doesn't mean it's not threatened; its populations remain fragmented in a highly urbanized (and prone to be urbanized) habitat. It is worth to be mentioned that while in de Pous et al.'s study (2012) the CPUE of the pond is described as low, in our expedition it has been the highest obtained. This might be explained by a high interannual weather variability that generates drastic populational and development success changes. One of the adults found in this locality is shown in Figure 2.

Localities 2 and 3 seem to be quite properly conserved habitats that host dense populations given the CPUE values, but are relatively overgrazed and threatened by the national road N1 that is at 100-150 m from the ponds where *P. varaldii* breeds (shown in Figure 3) and could be having a negative effect by road killing migrant individuals during the breeding season as occurs with *Pelobates cultripes* in Madrid (authors, unpublished data). Soil ploughing and intensive plantations are occurring also in nearby areas.

Figure 2: *Pelobates varaldii*, adult from Tanger.
Figura 2: *Pelobates varaldii*, adulto de Tanger.

Finally, *Procambarus* sp. and *Gambusia* sp. were present in suitable ponds in Tanger at some kilometers from the known *P. varaldii* localities and can ripe out these populations if they expand to these localities.

Larache

Larvae CPUE: 0 (clutches), 33.

Locality 4 is overgrazed and at less than 30 m from a road, the water is slightly polluted because of cattle disturbance but three clutches of eggs could be found in it. Locality 5 is invaded by crayfish and *Gambusia* sp. as explained previously in de Pous *et al.* (2012). In it, two good quality, same sized and proximate ponds were a good indicator of the effect of both invasive species on *P. varaldii*. Pond A is permanent and has *Gambusia* sp. and *Procambarus* sp., and no *P. varaldii* larva was found (neither *Pleurodeles waltl*, only *Hyla meridionalis*) while pond B without *Gambusia* sp. but with *Procambarus* sp. had standard larvae densities of *P. varaldi* and the other sintopic amphibians. This does not mean that *Procambarus* sp. is less harmful than *Gambusia* sp.; its effect on native communities is tremendously negative (Cruz *et al.*, 2006). A possible explanation might be that densities in pond B were clearly lower than those on pond A and *Procambarus* specimens found in B were probably recent/seasonal colonizers from A. In a recent expedition by the end of December 2015 A. Hinckley found high *Procambarus* densities 37 km upstream in the river Loukkos so the invasive threat is expanding its range.

Figure 3: Breeding habitat of *P. varaldii* close to the national road (N1).
Figura 3: Habitat reproductivo de *P. varaldii* cercano a la carretera nacional (N1).

Three other localities where the species was known to occur where sampled without success in findings. The reasons are unknown but could be due to water pollution, eutrophication and sediment filling produced by soil extraction and erosion after rain. A road extension of the N1 in this area is occurring and destroying the habitat of these last localities at the moment (A. Hinckley, unpublished data).

Ouezzane

Larvae CPUE: 0.

Despite intensive sampling in the area where Guzmán *et al.* (2007) cited *P. varaldii* larvae (seven ponds sampled and some night transects), no larva/adult or suitable habitats were found here (as in DePous *et al.*'s study [2012]), (only *H. meridionalis* larvae were found in the ponds). This zone can be defined as an unsuitable intensive agricultural area with highly polluted and eutrophic ponds and no tree/shrub cover. Given the unlikely situation that a population survives here, it has to be extremely local, fragmented and endangered.

La Mamora & surroundings

Larvae CPUE:49, 27, 32.

Pelobates varaldii was found in five out of seven sampled ponds. As stated in De Pous *et al.* (2012), this population remains as the species stronghold given the broad extension of terrestrial suitable habitat and elevated number of suitable ponds (more than 100). Nevertheless, intensive logging, overgrazing and proliferation of intensive *Eucalyptus* plantations still goes on and water pollution by cattle droppings and destruction / turbidity generated by these when drinking will have a negative effect that will slowly diminish the fitness of this population. Vegetation lacked in many of the ponds and NO_3^- levels (10 mg/l) were higher (together with Ben Slimane) than in the other populations.

In addition, in the forest of Maâmora, the Khettaras (traditional drainage water systems of groundwater seepage that use several sets of wells) are real traps for amphibians, these wells are filled with rain and attract amphibians. At Assec, different species were found trapped in 2014 (*P. waltl*, *Amietophrynus mauritanicus*, *P. varaldii* and *Discoglossus scovazzi*) (T. Slimani, unpublished data).

Ben Slimane

Larvae CPUE: 3.

The CPUE given in this pond is extremely low and similar to that found in Escoriza & Ben Hassine (2013). Although more tadpoles were found (28) after a huge sampling effort, it can be stated that this population is at the border of extinction. The pond is polluted (>10mg NO_3^-/l), the very low occurrence of invertebrates confirms this, and it is surrounded by intensive wheat fields, a dump site and a road, making unlikely the long term survivorship of *P. varaldii*.

Additionally, although not found in strict concurrence with *P. varaldii*, *Azolla filiculoides* has been seen in the Ben Slimane province and could be a threat to the species. As it generates broad mats that eliminate submerged plants and algae blocking oxygen diffusion (Janes *et al.*, 1996), it is known to reduce tadpoles densities in invaded streams (Gratwicke & Marshall, 2001).

Oualidia

Larvae CPUE: 0.

No larva was found in the ponds of the area where the only specimens of De Pous *et al.* (2012) were spotted. Only one of the ponds could have a suitable hydroperiod and it is being polluted and basified through car washing and burning of wetland vegetation (*Juncus* sp.) nearby (ashes end up in the water increasing water pH). In addition, the terrestrial habitat is apparently quite suboptimal, with an increased aridity produced by overgrazing and *Eucalyptus* plantations. Other important disturbances in the area are the construction of many roads and arids extraction. Arids extraction is having a double negative effect, as it is destroying its terrestrial habitat and has filled up with sediments some of the most suitable ponds.

Future measures

Nowadays, the most urgent action is the protection of *P. varaldii* habitat and long term monitoring of the species, preferably standardizing the methodology carried out in this study to maintain a same sampling effort along the years.

Habitat protection should focus on what we consider the three strongholds for the species: the Maâmora Forest, which holds the best populations (actions like fencing some areas to avoid overgrazing and banning *Eucalyptus* plantations would maintain and improve the species microhabitats), the region of Larache (threatened by road expansion and the invasive species *Gambusia* sp. and *Procambarus* sp., the erradication of these is a priority) and the Northern populations of Tanger-Asilah prefecture (habitat protection of the stretch of coast through the generation of a microreserve that covers the three localities with breeding ponds and its surrounding terrestrial habitat). Other possible measures could be to restore the coastal vegetation in some zones to promote a corridor among localities for the species, and to collaborate with the new golf course next to the Foret Diplomatique and perhaps others.These, with the proper management (Puglis & Bonne, 2012) could act as a good breeding habitat for the surrounding populations given the presence of many ponds. Finally, making deeper some parts of the only pond of the Foret Diplomatique, as its progressive shallowness might mean the extinction of the population once it fills up completely with sediment. At the moment, it is likely that in dry years it dries up before most of the larvae metamorph.

Regarding future monitoring, we encourage local scientists and enviromental associations to start a long term monitoring program based on methodologies focused on relative larval abundance (De Pous *et al.*, 2012; Bosch & Fernández-Beaskoetxea, 2014; Oliver *et al.*, 2014), to keep on looking for basic distribution information especially between Rabat and Oualidia, where the habitat is less optimal and the urban development has and is destroying it at a higher rate. Other targets could be to search for the *P. varaldii* in protected sites inside or close to its distributional range like the reserve Cap Spartel or Merja Zerga, to assess the suitability of restoration or creation of ponds in them, to carry out studies on larval development in the field (test in different ponds if larvae metamorphosis is being completed and the rate of success in the long term), to assess how climate change is affecting the species (as in *P. cultripes* (Andreu, 2014)) and to study the impact of road killings during the breeding period, given the nearness of the national road to most of the known northern localities.

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