Amphibian micro-hotspot at the Mindelo Ornithological Reserve (Porto, Portugal)

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Resumen: La reserva ornitológica de Mindelo (Vila do Conde, distrito de Oporto), fue creada en 1957, y es pionera en la conservación de la naturaleza en Portugal. Esto permitió proteger esta pequeña área (380 ha) de la continua degradación del ecosistema costero ocasionada por diversas presiones antrópicas durante las últimas décadas. Su proximidad al mar y diversidad de paisajes proporcionan un clima y hábitat propicios para muchas especies de aves, pero también de anfibios, reptiles y otros grupos, constituyendo un micro refugio para la biodiversidad. En esta nota se proporciona un listado de las especies de anfibios observados durante la pasada década (2011-2020), y una valoración cualitativa de la abundancia de las especies encontradas. Durante este tiempo se ha observado un total de 12 anfibios, representando el 63% de los anfibios portugueses, destacando la importancia de este micro refugio para muchos de los anfibios ibéricos y la fragilidad de un espacio que necesita ser gestionado con medidas que garanticen la supervivencia de estas especies.

The Mindelo Ornithological Reserve (MOR) lays in a coastal area of ca. 380 ha. located at the municipality of Vila de Conde in North Portugal (Figure 1). Its importance for migratory birds provided the foundations to create the first Portuguese area for nature conservation in 1957. MOR acts as a refuge for biodiversity conservation because it maintains patches of the natural landscape that once dominated the coastal area in northern Portugal, which was continuously degraded and fragmented over the last decades (from the 70´s onwards) due to human activities (e.g. industry, urbanization, agriculture), also resulting in a reduction of wetlands. Thus, MOR is currently considered an important sanctuary for wildlife.

This reserve provides optimal climatic conditions for many ectotherm species, and in particular for amphibians, with buffered thermal and moisture conditions resulted from the influence of the Atlantic Ocean. Its heterogeneous landscape, mostly dominated by sand dunes, pine forest, and shrubs, with two small coastal lagoons (maintained with water coming from the two streams crossing this area), and the formation of numerous temporary ponds and puddles across the reserve, contribute to the reproduction and survival of amphibian species. All amphibians inhabiting MOR are water dependent, but show marked differences in life-history traits (e.g. oviparous and larviparous reproduction, sand borrowers and woodland species).

Researchers from CIBIO have recently showed interest in studying some of the amphibians currently present in MOR. In particular, the fire salamander (*Salamandra salamandra* Linnaeus, 1758), which was used as the reference population of the subspecies *S. s. gallaica* for genetic (Mulder *et al.*, 2016) and morphological (Alarcón *et al.*, 2020) studies. In a recent study, Peixoto *et al.* (2020) surveyed some of the pudd-

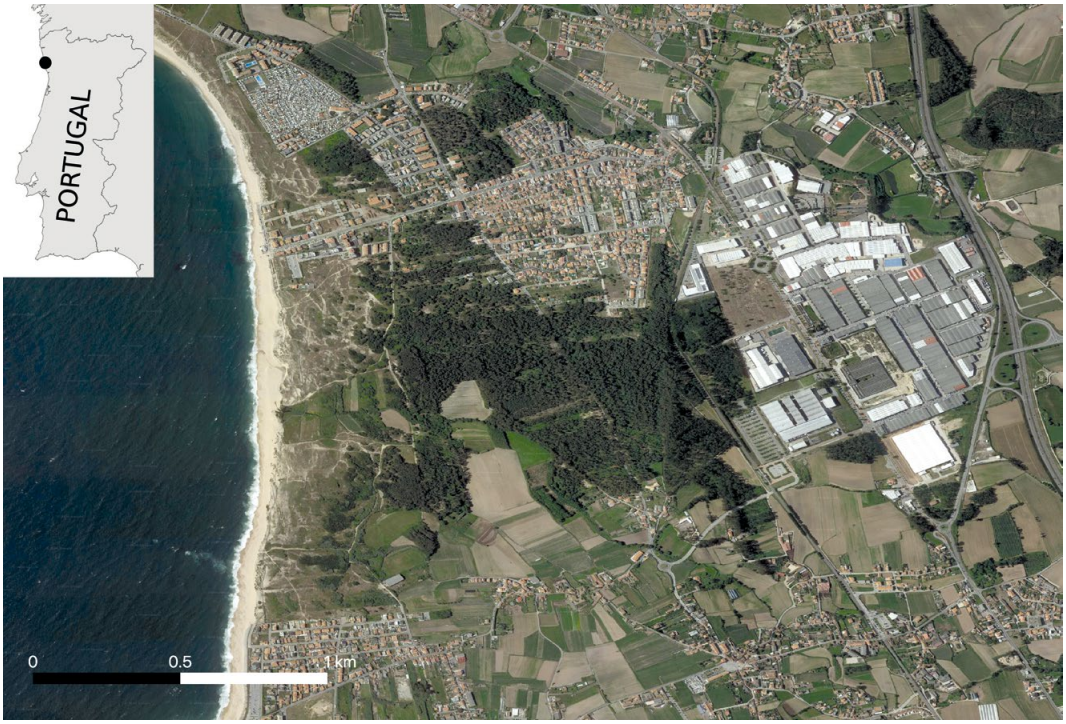

Figure 1: Map containing the Mindelo Ornithological Reserve surrounded by urban, agricultural, industrial areas and the Atlantic Ocean. The black dot in the inset represents the location of this reserve in the municipality of Vila de Conde (North Portugal).
Figura 1: Mapa de la Reserva Ornitológica de Mindelo rodeada por áreas urbanas, agrícolas e industriales y el Océano Atlántico. El punto negro representa la posición de esta reserva en el municipio de Vila do Conde (norte de Portugal).

les and ponds across this reserve to evaluate several field and laboratory protocols for amphibian detection using environmental DNA (eDNA) methods from water samples. MOR amphibian community, and fire salamanders in particular, served as an excellent study system because of the numerous temporary puddles occurring within the reserve, where amphibians can be easily found and detected with traditional sampling methods, allowing for multiple replicates to evaluate eDNA capture methods and molecular approaches. Peixoto *et al.* (2020) listed the number of species detected with both traditional and eDNA methods, however, the short-term (over a 10-day period) surveys conducted for this particular study (March 2018) might not represent the full amphibian community. Here, I list the number of species observed over sporadic visits to this reserve since 2011, also giving a qualitative assessment of each species´ abundance. These visits mostly occurred during rainy nights in the highest peak of activity and the reproductive period of the amphibians present in this region (October-November and March-May). I found a total of 12 species (eight anurans and four urodeles), representing 63% of the Portuguese amphibians (Table 1; Figure 2). This constitutes, to the best of my knowledge, the highest recorded number of amphibians in Portugal. The most abundant species, which can be found in dozens in one single night, are the natterjack toad (*Epidalea calamita*), and the Iberian painted frog (*Discoglossus galganoi*).

The fire salamander (*Salamandra salamandra*) and the western spadefoot (*Pelobates cultripes*) show high densities during nights with high levels of humidity and mild temperatures, as it often occurs across their distributions (Recuero, 2014; Velo-Antón & Buckley 2015). Three newt species inhabit this reserve: the palmate newt (*Lissotriton helveticus*), the marbled newt (*Triturus marmoratus*), and the Bosca´s newt (*Lissotriton boscai*), being the latter the least abundant of

Figure 2: From top-left to bottom-right the 12 amphibians observed in the Mindelo Ornithological Reserve: *Epidalea calamita*, *Discoglossus galganoi*, *Pelobates cultripes*, *Lissotriton helveticus*, *Triturus marmoratus*, *Salamandra salamandra*, *Lissotriton boscai*, *Pelodytes atlanticus*, *Alytes obstetricans*, *Bufo spinosus*, *Hyla molleri*, *Pelophylax perezi*. Stroke color of each figure denote the abundance: high (green) and low abundance (blue), and rare (red).
Figura 2: De arriba-izquierda a abajo-derecha, las 12 especies de anfibios observados en la Reserva Ornitológica de Mindelo: *Epidalea calamita*, *Discoglossus galganoi*, *Pelobates cultripes*, *Lissotriton helveticus*, *Triturus marmoratus*, *Salamandra salamandra*, *Lissotriton boscai*, *Pelodytes atlanticus*, *Alytes obstetricans*, *Bufo spinosus*, *Hyla molleri*, *Pelophylax perezi*. El color del trazado de cada panel denota: abundancia alta (verde), abundancia baja (azul) y especies raras (rojo).

Table 1: List of the amphibians present in Portugal, identifying the species observed in the Mindelo Ornithological Reserve (*), and the relative abundance of each species. Question marks denote species that might be extinct in the study area.
Tabla 1: Lista de anfibios presentes en Portugal, identificando las especies observadas en la Reserva Ornitológica de Mindelo (*), y la abundancia relativa de cada especie. Los signos de interrogación denotan especies que podrían estar extintas en el área de estudio.

Amphibians (Portugal)	Amphibians (MOR)	Abundance (MOR)
Alytes cisternasii		
Alytes obstetricans	*	low
Bufo spinosus	*	rare
Chioglossa lusitanica		
Discoglossus galganoi	*	high
Epidalea calamita	*	high
Hyla meridionalis		
Hyla molleri	*	rare
Lissotriton boscai	*	low
Lissotriton helveticus	*	high
Pelobates cultripes	*	high
Pelodytes ibericus		
Pelodytes atlanticus	*	low
Pelophylax perezi	*	rare
Pleurodeles waltl	?	
Rana iberica	?	
Salamandra salamandra	*	high
Triturus marmoratus	*	high
Triturus pygmaeus		

the three. The Lusitanian parsley frog (*Pelodytes atlanticus*), and the common midwife toad (*Alytes obstetricans*) show low abundances. Only very occasionally, I observed the spiny toad (*Bufo spinosus*), the Iberian green frog (*Pelophylax perezi*) and the Iberian tree frog (*Hyla molleri*). This site constitutes the northernmost locality for *P. atlanticus* (Escoriza *et al.*, 2017) and Portuguese populations of *P. cultripes* (Recuero, 2014), and the southernmost area for *L. helveticus* (Diego-Rasilla, 2014).

To complete their life cycle, amphibians present in MOR rely on the numerous shallow puddles formed across the reserve during rainy periods, which can become relative large with episodes of heavy rain or continued rainy days. With the exception of *P. cultripes*, which reproduces in stagnant water bodies that can sustain their feeding habits and larger tadpole size, all remaining anurans and the four urodeles largely frequently use small puddles for reproduction, laying their eggs (or larvae in *S. salamandra*) into these temporary and unpredictable water bodies. *Salamandra salamandra*, *P. cultripes*, *E. calamita* and *D. galganoi* have an apparently wider range and use of habitat types compared to the remaining amphibians. For instance, I often found adults of *S. salamandra* across the woodland but also covered in grains in sandy areas, which indicates the presence of nearby shelters in these particular habitats. Future efforts should be oriented to spatially characterize the distribution of each species, as well as to estimate their habitat use, population densities and species interactions.

Despite this is a protected area, MOR also contains eucalypts and small agricultural patches, allowing the entrance of vehicles used for agricultural activities. I occasionally observed roadkilled carcasses of several species (e.g. *S. salamandra*, *D. galganoi*, *E. calamita*, *P. cultripes*), likely caused by the use of these vehicles within the reserve, and coinciding with those species showing an apparently higher population density within the reserve. MOR continues to attract many professionals, amateur naturalists, and students, who regularly visit this place in search of different taxonomic groups, as well as people doing recreational activities (e.g. hiking and cycling). The herpetological value of this reserve is increased with the presence of some reptile species such as the Iberian emerald lizard (*Lacerta schreiberi*), the ocellated lizard (*Timon lepidus*), the

viperine snake (*Natrix maura*), the Montpellier snake (*Malpolon monspessulanus*), the slow worm (*Anguis fragilis*), and the presence of nearby populations of the Lataste´s viper (*Vipera latastei*).

This small emblematic area, pioneer of nature conservation in Portugal, constitutes a very important micro-hotspot of Iberian amphibians. Thus, the management of the land, and in particular the water bodies across the reserve, should take into account appropriate measures to preserve this rich amphibian community.

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Datos preliminares sobre el estado de la población de *Rana dalmatina* en el Valle de Losa (Burgos)

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Rana dalmatina es una rana parda de amplia distribución europea, cuyo hábitat óptimo está formado por bosques de frondosas de hoja caduca y sus matorrales de sustitución, con masas de agua en su interior, que son utilizadas como lugar de reproducción (Sarasola & Gosá, 2014). En la península ibérica *R. dalmatina* está presente en las provincias de Navarra, Álava, Bizkaia y Burgos, a lo largo de una franja de terreno que, de este a oeste, va desde la cuenca de Pamplona y la comarca de la Ultzama, continuando por la comarca de la Sakana (Navarra) hasta la Llana-