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Introduction

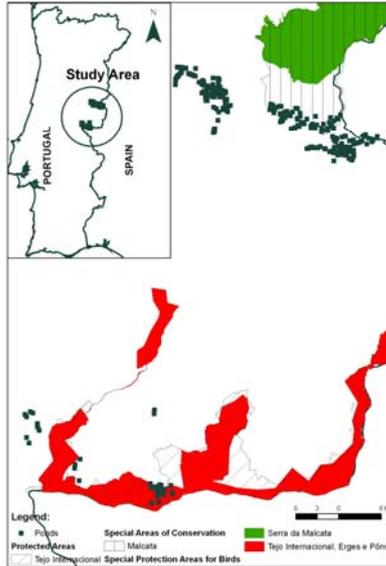
- Portuguese forest occupies 3.4 million (ha) (38%) of the national territory and this sector represents 10% of national exports;
- Eucalypt (*Eucalyptus globulus*) is one of the main species, covering nearly 26% of national territory. Originally from Tasmania, this species is largely used for pulpwood. Plantations are cut on a coppice rotation of about 10 to 16 years, usually two or three times;
- This kind of intensive management has a heavy impact on the biodiversity. Eucalypt plantations have a typical low plant diversity and complexity which is associated with impoverished fauna.
- In Portugal, studies on vertebrate biodiversity in single-species stands have provided evidence for negative impacts on avifauna and reptiles.
- Although, in Portugal, of the sixteen amphibian species present, only two are threatened, in Europe, nearly a quarter of this group is considered threatened. Habitat loss, fragmentation and degradation are the most significant threats to amphibians in Europe as well as pollution and invasive species. Since most of these species have both an aquatic and terrestrial life cycle, this group is appropriate for serving as an indicator group for broader ecosystem health.
- In this study, we evaluated the impact of eucalypt plantations on the probability of occupancy of six amphibian species in ponds using a likelihood-based method, with the software PRESENCE.

Methods

- Between April and June 2010, we surveyed 90 temporary and permanent ponds. Forty-four of these ponds were surveyed twice during this period.
- The amphibians (adults, larvae and eggs) were sampled by torchlight and dip-netting.
- Ponds were characterised using 43 variables: morphological (water depth, area and slope); aquatic vegetation (*Ranunculus* spp., other floating vegetation, *Juncus* spp., other emergent vegetation, submerged vegetation); surrounding vegetation (trees, scrubs, *Rubus* spp., grass); habitat in a radius of 1 km (agriculture, dam, oak forest, Pyrenean oak forest (*Quercus pyrenaica*), cliffs, eucalypt plantations, ash (*Fraxinus* spp.) forests, scrubland, oak and cork oak "montado", olive yard, pine tree (*Pinus pinaster*) plantations, river, wheat crops, cork oak forests, buildings); hydroperiod (permanent and temporary); substrate (stone, gravel, muddy); turbidity (transparent, turbid, very turbid); exotic predators (*Procambarus clarkii* and exotic fish); native vertebrate predators (*Natrix* spp. and terrapin (*Mauremys leprosa*)); presence of cows.

Results and Discussion

- Twelve species were identified, but five of them were not taken into consideration since they appear only in less than 7% of the ponds (*Salamandra salamandra*, *Discoglossus galganoi*, *Alytes* spp., *Bufo bufo*, *Bufo calamita*). It was not possible to analyse the data for *Hyla meridionalis* because all the models were missing numerical convergence. The models for the remaining species are described in table 1.
- The detectability (p) of some species was influenced by the turbidity of the pond water and that factor was taken into consideration when evaluating the occupancy (psi) of *Lissotriton boscai* (transparency) and *Triturus marmoratus* (very turbid).



Study Area

- This study was carried out in Castelo Branco district, Portugal;
- The climate is Mediterranean;
- The main habitats were eucalypt plantations, cork oak (*Quercus suber*) and oak (*Quercus ilex*) "montados", natural pastures, olive yards and wheat crops.
- Of the 90 ponds, 27 were occupied with exotic fish – Eastern mosquitofish (*Gambusia holbrooki*), pumpkinseed sunfish (*Lepomis gibbosus*) and largemouth bass (*Micropterus salmoides*)



- We estimated carnivore species occupancy (psi) using a likelihood-based method (MacKenzie et al. 2002), and we used the program PRESENCE v.2 software (Proteus Wildlife Research Consultants, New Zealand; <http://www.proteus.co.nz>) for defining occupancy with the single-season option (MacKenzie et al. 2006).
- This model uses multiple surveys on a collection of survey sites to construct a likelihood estimate using a series of probabilistic arguments.
- False-negative surveys can be corrected by estimating probability of detection, providing a more precise assessment of site occupancy values.

- The impact of exotic fish on amphibian species is well-documented (Yonekura et al., 2004; Denoel et al., 2005; Cruz et al., 2006). Although exotic fish were only present in 30% of the ponds we surveyed, it is clear that they have a negative impact on the amphibian occupancy.
- The habitat immediately surrounding the pond has a mixed effect in the different species considered. Grass has a positive effect on the occupancy of *Pleurodeles waltl* and *Pelobates cultripes*, while scrubland have a negative influence on the occupancy of *Pelobates cultripes* and *Pelophylax perezi*.
- Lissotriton boscai* and *Hyla arborea* were the only two species affected by the submerged vegetation.
- Pelobates cultripes* preferred ponds without any shadow, in exposed and open habitats, as did *Pelophylax perezi*.

Table 1 - Model selection analysis ($\Delta AIC < 2$) and parameter estimates of site occupancy (psi) and detectability (p) for amphibians.

| Species (common name) | Model | Detectability | Effect | Occupancy | Effect | Akaike Weight | Naive Occupancy | Psi (±SE) | P (±SE) |
|--|--|-------------------|--------|---|-------------|---------------------------|-----------------|----------------|----------------|
| <i>Pleurodeles waltl</i> (sharp-ribbed salamander) | psi(exotic fish+grass),p(.) | Not applied | | Exotic fish Grass in the surrounding habitat | - + | 0.9677 | 0.54 | 0.6271(0.0384) | 0.7841(0.0582) |
| <i>Lissotriton boscai</i> (Bosca's newt) | psi(exotic fish+ <i>Rubus</i> spp+submerged),p(transparent) | Transparent water | + | Exotic fish <i>Rubus</i> spp. in the surrounding habitat Submerged vegetation | - + + | 0.4995 | 0.53 | 0.6531(0.0425) | 0.6978(0.0834) |
| <i>Triturus marmoratus</i> (marbled newt) | psi(exotic fish),p(very turbid) | Very turbid water | - | Exotic fish | - | 0.9998 | 0.53 | 0.6315(0.0255) | 0.7897(0.0626) |
| <i>Pelobates cultripes</i> (Western spadefoot) | psi(shadow+gramineas+scrubland), p(.) | Not applied | | Pond Shadow Grass in the surrounding habitat Scrubland in the surrounding habitat | - + - | 0.7312 | 0.33 | 0.5169(0.1145) | 0.5179(0.1188) |
| <i>Hyla arborea</i> (common tree frog) | psi(submerged+exotic fish),p(.) | Not applied | | Exotic fish Submerged vegetation | - + | 0.9066 | 0.48 | 0.6465(0.0528) | 0.6347(0.0624) |
| <i>Pelophylax perezi</i> (common frog) | psi(scrubland),p(.) psi(<i>Rubus</i> spp),p(.) psi(area),p(.) | Not applied | | Scrubland in the surrounding habitat <i>Rubus</i> spp. in the surrounding habitat Pond area | - - + | 0.289 0.1471 0.1241 | 0.86 | 0.9579(0.0163) | 0.8333(0.0362) |

Conclusions

- There were no over-riding effects of forest type (native or eucalypt) on amphibian occupancy of the ponds. Instead, species occupancy was influenced by micro-scale variables and the presence of exotic fishes.
- In future studies to assess the amphibian occupancy, the 27 ponds with exotic fishes should be taken out of the study since this variable may, in fact, mask the effect of other variables.
- Although these results suggest no negative effect of eucalypt plantations on amphibian species occurrence in ponds, amphibians are at particular risk during their dry land phase, associated with juvenile dispersal and adult dispersal during the breeding season. Further studies therefore need to investigate any potential impacts on this dry land phase, especially in terms of dispersal and population fragmentation.

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