Escola Tècnica Superior
d'Enginyeria Agrària de Lleida

SIMPOSI SOBRE EL DECLIVI DE LES POBLACIONS D'AMFIBIS

SIMPOSIO SOBRE EL DECLIVE DE LAS POBLACIONES DE ANFIBIOS

SYMPOSIUM SUR LA DIMINUTION DES POPULATIONS AMPHIBIENS

SYMPOSIUM ON THE DECLINING OF THE POPULATIONS OF AMPHIBIANS

Llibre de resums

5, 6 i 7 de Març del 2004

Organitza: Departament de Producció Animal. Escola Tècnica Superior d'Enginyeria Agrària. Universitat de Lleida.

SOCIETAT CATALANA D'HERPETOLOGIA

ASOCIACION HERPETOLÓGICA ESPAÑOLA

diferentes graus de ocupaçao por um predador exótico, o lagostim-vermelho americano (*Procambarus clarkii*). Actas del VIII Congreso Luso-Español (XI Congreso Español) de Herpetologia 2004. Málaga; 125-126.

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AMPHIBIAN MORTALITY IN PENEDA-GERÊS NATIONAL PARK (N PORTUGAL)

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INTRODUCTION

Amphibian populations have been suffering widespread declines and extinctions across the world (ex. Blaustein & Wake 1990, Pounds & Crump 1994, Houlahan *et al.* 2000). Multiple causes are invoked to explain this phenomenon, including habitat loss and fragmentation (Fisher & Shaffer 1996), pollution (Hayes *et al.* 2002), competition with invading exotic species (Knapp & Mathews 2000), climatic change (Pounds 2001), increased exposure to ultraviolet-B radiation (UV-B) (Blaustein & Belden 2003) and increased prevalence and susceptibility to disease (Green & Sherman 2001) as well as interactions among these factors. So far two cases of amphibian decline have been reported for Spain, both concerning the Common Midwife toad, *Alytes obstetricans*. Toads were found affected by "red-leg disease", an opportunistic infection caused by bacteria, in the Pyreneen Mountains (Márquez 1994), and by a fungal infection due to *Batrachochytrium dendrobatidis,* a pathogen new to Europe, in Peñalara Mountains (Bosh *et al.* 2000, 2001).

The first documented case of amphibian mass mortality in Portugal occurred in the mountains of Peneda-Gerês national park, a protected and relatively untouched area in the north of the country. A large number of Marbled newts, *Triturus marmoratus*, were found dead at the shores of the ca. 0,5 ha sized Carris pond, at 1.480 m altitude (Froufe *et al.* 1999). Other amphibian species (*T. boscai*, *A. obstetricans* and *Rana perezi)* showed similar symptoms but in lower numbers (Soares *et al.* 2003). Affected adults were lethargic and displayed cutaneous ulcers and tissue haemorrhages (Apendix II of images page 79: Figs. 1-3). Affected larvae showed tissue haemorrhages and oedemas (Soares *et al.* 2003). Since iridovirus-like particles were disclosed in dermal cells underlying the ulcerated lesions of sampled diseased animals we, tentatively, consider the virus as the causative factor of the observed mass mortality episodes (Alves de Matos *et al.* 2002, and accompanying paper). We here report on the monitoring of amphibian populations of Carris pond and on the search for symptoms of amphibian disease over the entire Peneda-Gerês National Park (PGNP).

MATERIAL AND METHODS

The PNPG covers an area of 72.000 ha in NW Portugal along the border with Spain. It is a mountainous area of up to 1.500 m altitude characterized by an atlantic climate. The local conditions of altitude and relief result in a variety of micro-climates with corresponding vegetation types including deciduous oak (*Quercus robur* and *Q. pyrenaica*), coniferous and mixed forest and shrubs (heath - *Erica* sp., furze -*Ulex* sp. and brooms - *Cytisus* sp.).

Carris pond (UTM NG7929) was visited on a two-weekly basis in spring and summer and once a month autumn and winter, from 2001 to 2003. On each visit was noted the condition of all amphibians that could be seen from the shoreline and counts of dead animals. Capture-recapture methods (Heyer *et al.* 1994) were used to count adults of *T. marmoratus* during June/July. Monitoring was carried out at Batateiro pond (1.100 m altitude, UTM NG6047, at ca. 25 km distance from Carris in a straight line) along the same procedures starting August 2003. Both ponds are man made as water supply. Additionally, ponds distributed over the entire PNGP were searched for amphibians in the period from 2001 to 2003. Tissue material adjacent to skin lesions and from internal organs was collected from sick and recently died animals for virology studies and bacteriological characterization by the second author and a commercial laboratory (Laboratórios Prof. Ernestino Morais), respectively. The work was carried out following published guidelines (DAPTF 1998), and aimed to prevent contamination and spread of disease.

RESULTS

Diseased amphibians were found at two localities, namely Carris as reported earlier and at the new locality of Batateiro. In Carris pond breeding populations were observed of the Marbled newt *Triturus marmoratus* and Bosca's newt *T. boscai*, the Common midwife toad *Alytes obstetricans* and the Iberian *green frog Rana perezi*. At Batateiro pond the same species were found plus the Palmate newt *T. helveticus*. At present, proportion of recaptures is still insufficient to provide a reliable estimation of the effective of populations and, hence, the counting results will not be presented here. The counts of dead individuals are presented in Figs. 4 and 5 of Appendix II, page 80.

The presence of iridovirus-like particles was revealed in the underlying cells of the cutaneous ulcers in *T. marmoratus*, as well as the following bacteria: *Cedecea lapagei, Aeromonas hydrophila, Enterobacter agglomerans, Staphylococcus* sp., *Hafnia alvei* and *Escherichia coli* in *T. marmoratus*, *T. boscai*, and *A. obstetricans*.

DISCUSSION

This is the first report on the presence of an iridovirus-like virus in the genus *Triturus* (see also Alves de Matos *et al.*, accompanying paper). If we are right to consider an iridovirus the cause of the observed amphibian mortality, then the observed presence of bacteria in tissue samples may represent a secondary, opportunistic attack. No relation appears to exist with superficially similar cases of amphibian mass mortality at high altitude, preserved areas on the Iberian Peninsula. Mortality in the Peñalara Mountains was caused by *Batrachochytrium dendrobatidis* and the test for chytridiomycosis in a diseased *T. marmoratus* yielded a negative result (J. Bosch, *pers. comm.*).

We are uncertain about the origin of the disease. A possibility that cannot be excluded is the transfer of pathogens with the uncontrolled introduction of fish, in either of the ponds. In July 2001 we observed the presence of the American Pumpkinseed fish (*Lepomis gibbosus*) in Carris, and Batateiro pond is stocked with Brown trout (*Salmo trutta*). Unfortunately, the timing of both introductions is unknown and a temporal relationship with the first appearance of the amphibian mortality cannot be firmly established. Further

work is required to determine the role of fish as a carrier of the iridovirus, the role of the virus in the disease pathogenesis and to document the impact of fish, virus and the disease on the local amphibian populations.

ACKNOWLEDGEMENTS

Previous work was funded by Instituto de Conservação da Natureza (PNPG/ICN), Portugal.

We thank Declining Amphibian Populations Task Force (DAPTF) for a Seed Grant - "Unrestricted Awards 2004" (to C.S.) that will allow the prosecution of the work during 2004.

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