SHORT NOTES

HERPETOLOGICAL JOURNAL, Vol.5, pp. 287-289 (1995)

RECURRENT MASS MORTALITY OF LARVAL MIDWIFE TOADS *ALYTES OBSTETRICANS* IN A LAKE IN THE PYRENEAN MOUNTAINS.

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In July 1992, an episode of mass mortality of larval and metamorphic midwife toads *Alytes obstetricans,* was recorded in a lake in the Pyrenean mountains (Márquez & Lizana, 1993). We report another event of mass mortality in the same site in July 1994 and we present evidence that infection by the bacterium *Aeromonas hydrophila,* a disease commonly known as "red leg", may be responsible for the occurrence.

The decline of populations of amphibians in their natural habitats in the absence of identifiable direct causes is of increasing concern for the scientific community (Blaustein & Wake, 1990; Wyman, 1990; Wake, 1991; McCoy, 1994). Recent studies have provided solid evidence of disappearances of populations of species that were common only a few decades ago throughout hundreds of kilometres of their range, particularly in montane habitats in Western USA (Carey, 1993; Fellers & Drost, 1993). In addition to these general trends, isolated events of mass mortality of amphibians have been reported in several montane populations and in some cases an infection by the bacterium *A. hydrophila* has been reported to be the cause (Dusi, 1949; Reichenbach-Klinke & Elkan, 1965; Shotts, 1984; Nyman, 1986; Bradford, 1991). So far the evidence gathered for the amphibian populations of the Pyrenean Mountains does not allow the identification of any general trends and no episodes of mass mortality have been reported. This report documents the recurrence of high death rate, involving premetamorphic overwintering larvae of the midwife toads *A. obstetricans* in the Pyrenean Mountains.

In July 1992, the Ibón de Piedrafita was visited by the authors. This is a montane lake (1600 m a.s.l.) near the town of Tramacastilla de Tena (UTM 30TYN1630, Huesca, Aragón, Spanish Pyrenean Mountains). The lake is relatively shallow, approximately 150 m in diameter, and sustained a large number of (huge) overwintering *A. obstetricans* tadpoles in July of 1988 and 1989 (RM, personal observation). Large numbers of dead and dry tadpoles were visible on the shore of the lake in 1992. In an area of 2 m of shore the remains of over 100 dead tadpoles could be counted. Some of these we found more than 2 m away from the shore indicating that the water level had receded substantially in the last weeks. Some individuals were still alive in the water near the shore, but most of them were floating and apparently in distress. Among the recently dead and dying tadpoles, those individuals with fully developed hind legs had haemorrhagic, grossly swollen limbs and some also had areas of haemorrhage around the eyes. Metamorphic individuals were also found dead on the shore at different stages of development.

Dry, dead, adults were found under rocks, mummified in the vicinity of the water. The single fresh corpse of an adult individual found did not show signs of violent death (i.e. predation) but had an unusually conspicuous dark coloration to the thorax. The other two anuran species that bred in the same lake appeared to be unaffected. Only a single dead tadpole of *Bufo bufo* was found in 1992 and areas of the shore were teeming with juveniles of *B. bufo* and *Rana temporaria.* The only urodele that occurs in the lake, *Euproctus asper*, has never been observed in large numbers. In 1992 one apparently healthy adult individual was observed in the water during our visit. As for other aquatic vertebrates, a single species of fish is known to inhabit the lake: an introduced population of the cyprinid fish (*Chondrostoma toxostoma*). Although schools of these fishes are usually visible from the shore of the lake, we could not see any living individuals in 1992. Only one dead specimen of *C. toxostoma* was found floating near the shore. It did not show obvious signs of illness.

While no evidence of death of midwife toads was obtained in the summer of 1993, in July 1994 another episode of *A. obstetricans* tadpole die-off was observed. The dying tadpoles in the water presented similar signs to those observed in 1992. The general conditions of the lake were similar to those in 1992: large numbers of live postmetamorphic *R. temporaria* and *B. bufo* could be found on the shore, no *E. asper* were found, and only schools of fry but no adults of *C. toxostoma* could be seen from the shore.

Two samples of tadpoles were collected: a sample of eight individuals showing advanced stages of ill-heath, and a sample of five healthy tadpoles from a nearby population located in the same watershed (less than 13 km north west of the lake), which did not suffer a die-off in 1992 nor in 1994. The tadpoles were kept at 5°C for transportation to Madrid; however, the diseased tadpoles died during the trip and were analysed the day after. Blood samples (1 ml) were obtained from a vein at the insertion of the hind-limb of four diseased and three healthy tadpoles. Two samples of water (1 ml), one from each of the two sites were tested as well. All samples were diluted in 20 ml of alkaline peptone wa-

ter (APW, pH 8.4-8.6). The samples were enriched by incubation at 30°C for 24 hours. They were then plated on two growth media specific for *A. hydrophila*. The first medium (Shotts & Rimler, 1973) developed yellow colonies which were used in an additional medium, specific for *A. hydrophila* (Millership & Chattopadhayay, 1984). The colonies that grew in this second medium were further tested for oxidase production, ability to ferment glucose and growth in nutrient broth without NaCl. Positive results confirmed the identification of the yellow colonies as *A. hydrophila*.

This methodology determined that the bacteria were present in the blood of all four diseased tadpoles as well as in the water samples from both sites. Only one of the three healthy tadpoles had *A. hydrophila* in its blood. A sample of the bacterial culture was collected by scraping the tip of a sterile hypodermic needle directly on the growth medium. The needle was used to cause a small rupture in the skin of the healthy tadpoles to provoke infection. Both tadpoles died in 48 hours and one of them showed clear lesions of "red leg" (haemorrhagic areas in the swollen hind limbs and lower abdomen).

Additionally, a sample of tadpoles from the Ibón de Piedrafita was collected by the Dirección General del Medio Natural de Huesca of the Diputación General de Aragón, and analysed in the facilities of the Depto. de Patología Animal, Facultad de Veterinaria, Universidad de Zaragoza. Their independent analyses confirmed the presence of *A. hydrophila* in the blood of the tadpoles in addition to other bacteria such as *Aeromonas sobria, Hafnia alvei, Pseudomonas alcaligenes, Micrococcus albus, Alteromonas putrefaciens, Campylobacter* spp., *Bacilus* spp. and *Aeromonas* spp.

The results of our observations and analyses are in agreement with previously reported events of mortality of montane populations of anurans in North America where the bacterium *A. hydrophila* has been identified as a cause of the die-off (Dusi, 1949; Nyman, 1986). *Aeromonas hydrophila* occurs naturally in waters that provide habitat for amphibians and it is debatable whether the infection by this bacterium is the ultimate cause or whether the bacterium acts as an opportunistic pathogen, that infects animals with weakened inmunological defences due to stressors of different origins such as temperature extremes (Nyman, 1986; Carey, 1993). However, in the four nearest meteorological stations, the records of daily maximum and minimum temperatures in May, June, and July do not indicate extreme values for the year of the die-offs (1992 and 1994) when compared with the recent years where the lake was surveyed and mass mortality did not occur (1988, 1989, 1991, 1993). The maximum and minimum monthly values for the years of the die-offs are close to the average monthly extremes in the period 1971-1994. Other potential causes of stress such as an eventual change in water acidity after a fast thawing of the snow, or possible presence of toxins, remain to be considered. The present study demonstrates that die-offs can be recurrent in natural habitats, although it must be emphasized that these episodes occur in a specific lake, since other populations of *A. obstetricans* periodically visited in the near vicinity (Márquez, 1992, 1993) did not show any signs of disease in 1992 nor in 1994. This report also extends the list of species of amphibians naturally affected by "red leg" to *A. obstetricans*. Furthermore, it is of interest to consider the differences in vulnerability between coexisting species of amphibians in the lake, with *B. bufo* and *R. temporaria* being apparently unaffected by the disease in the Ibón de Piedrafita in the years of the midwife toad die-off. Perhaps the much shorter aquatic phase of these amphibians rendered them less vulnerable to the disease that affected midwife toads or to the potential stress that lowered the defences of the midwife toad tadpoles against infection by the bacterium. In any event, the potential vulnerability of other species in the genus *Alytes,* which are also characterized by long larval periods, is of some concern, considering the endangered status of the Mallorcan midwife toad *Alytes muletensis* and the efforts devoted to its captive reproduction program (Bush, 1993; Tonge & Bloxam, 1989).

Acknowledgements. We thank P. Barrachina, J. Fabo, V. Juste and the Dirección General del Medio Natural de Huesca (Diputación General de Aragón) for their help with the monitoring of the site and the collection and analysis of the samples, and for the expeditious granting of the collecting permits. C. Franco helped with the bacteriological analyses. Meteorological data was kindly provided by E. Rodríguez from the network of the Estación Pirenaica de Ecología (Jaca, Huesca). Field work was funded by project CYCIT PB 92-0091 (PI: P. Alberch) Ministerio de Educación y Ciencia (Spain). Logistic support was provided by the Estación Bio-Geológica El Ventorrillo (C.S.I.C.).

REFERENCES

Blaustein, A. R. & Wake, D. B. (1990). Declining amphibian populations: A global phenomenon? *Trends in Ecology and Evolution* **5**, 203-204.

Bradford, D. F. (1991). Mass mortality and extinction in a high elevation population of *Rana muscosa*. *J. Herpetol.* **25**, 174-177.

Bush, S. L. (1993). Courtship and male parental care in the Mallorcan midwife toad *Alytes muletensis*. PhD. Dissertation, University of East Anglia, United Kingdom.

Carey, C. (1993). Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. *Conservation Biology* **7**, 355-362.

Dusi, J. L. (1949). The natural occurrence of "Red leg", *Pseudomonas hydrophila* in a population of American toads *Bufo americanus*. *Ohio J. Sci.* **49**, 70-71.

Fellers, G. M. & Drost, C. A. (1993). Decline of anurans in National Parks in Western United States.

Abstracts of Second World Congress of Herpetology, 85.

Márquez, R. (1992). Terrestrial paternal care and short breeding seasons: Reproductive phenology of the midwife toads *Alytes obstetricans* and *A. cisternasii*. *Ecography* **15**, 279-288.

Márquez, R. (1993). Male reproductive success in two midwife toads (*Alytes obstetricans* and *A. cisternasii*). *Behav. Ecol. Sociobiol.* **32**, 283-291.

Márquez, R. & Lizana, M. (1993). Poblaciones de anfibios en declive ¿Un fenómeno global? *Quercus* **94**, 6-10.

McCoy, E. D. (1994). "Amphibian decline": A scientific dilemma in more ways than one. *Herpetologica* **50**, 98-103.

Millership, S. E. & Chattopadhayay, B. (1984). Methods for the isolation of *Aeromonas hydrophila* and *Plesiomonas shigelloides* from faeces. *J. Hyg. Camb.* **92**, 145-152.

Nyman, S. (1986). Mass mortality in larval *Rana sylvatica* attributable to the bacterium *Aeromonas hydrophila*. *J. Herpetol.* **20**, 196-201.

Reichenbach-Klinke, H. & Elkan, E. (1965). *The principal diseases of lower vertebrates. Diseases of Amphibians.* Hong Kong: TFH Publications.

Shotts, E. B. (1984). Aeromonas. In *Diseases of Amphibians and Reptiles*, 49-57. G. L. Hoff, F. L. Frye & E. R. Jacobson (Eds.). New York: Plenum Press.

Shotts, E. B. & Rimler, R. (1973). Medium for the isolation of *Aeromonas hydrophila*. *Applied Microbiology* **26**, 550-553.

Tonge, S. & Bloxam, Q. (1989). Breeding the Mallorcan midwife toad, *Alytes muletensis*. *Int. Zoo. Yb.* **28**, 45-53.

Wake, D. B. (1991). Declining amphibian populations. *Science* **253**, 860.

Wyman, R. L. (1990). What's happening to the amphibians? *Conservation Biology* **4**, 350-352.

Accepted: 6.1.95