

Management strategy to avoid chytrid fungus infection in egg clutches of the Valcheta frog *Pleurodema somuncurens*

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SUMMARY: Eggs which were removed from a chytrid-infected population of Valcheta frogs shortly after laying and then hatched in a clean environment resulted in juveniles free of the fungus.

BACKGROUND: An ex-situ colony of the critically endangered Valcheta frog *Pleurodema somuncurens* (Ceï 1969) was established in order to facilitate a species reintroduction programme. The colony was founded with forty frogs from the Valcheta stream, located in the Somuncura Plateau (40°59' S, 66°40'36 W), northern Patagonia, Argentina.

Individuals of the colony were previously infected with chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*; Arellano et al. 2017), and although an unsuccessful *Bd* cleaning treatment had been applied a few months before (Arellano et al. 2018), several clutches of eggs were laid in *Bd*-infected terrariums. We therefore tested a management strategy aimed at reducing or even avoiding possible infection of tadpoles and juveniles.

ACTION: The ex-situ colony was established in laboratory terrariums (80 x 27 cm), each containing eight frogs with a 2/1 female/male sex ratio. Eighteen months after the colony was established, four adult pairs of frogs from three different terrariums laid a total of five clutches of eggs. Before these reproductive events, a *Bd* cleaning treatment was applied without success; adults carried an average *Bd* infection load of 2.3 zoospore genomic equivalents. To avoid potential possible cross-infection, the eggs were separated from adults just after laying and placed in previously disinfected terrariums. Once the tadpoles reached Gosner stage 36 (Gosner 1960), ten individuals were randomly checked for *Bd* infection by examination of fresh unstained oral disks under a stereomicroscope. Once the tadpoles metamorphosed, ten juvenile frogs were swabbed and analyzed for infection using *Bd*-qPCR (Boyle et al. 2004, Kerby et al. 2013).

CONSEQUENCES & DISCUSSION: Each clutch consisted of about 300 viable eggs, of which 160 reached tadpole stage and 100 survived to become adults. Direct examination of the oral disks of tadpoles and qPCR analysis of juveniles were both negative for the presence of *Bd* in all ten individuals tested. Since *Bd* has not yet been observed in eggs (Bancroft et al. 2011), our results suggest that removing eggs from infected adults may be an effective management strategy to reduce or even avoid infection in individuals bred in captivity.

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