Treatment of adult Valcheta frogs *Pleurodema somuncurense* for chytrid fungus

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SUMMARY: Treatment of an ex-situ colony of Valcheta frog with chloramphenicol solution was not successful in eliminating chytrid fungus.

BACKGROUND: The Valcheta frog *Pleurodema somuncurense* is a critically endangered species endemic to the Valcheta stream, Patagonia, Argentina (40°59' S, 66°40'36 W). This almost entirely aquatic species is facing several threats, including the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*; Arellano *et al*. 2017). To facilitate a species reintroduction program, an ex-situ colony of *P. somuncurense* was established and a cleaning protocol using antibiotic solution, which had been shown to be effective in treating *Bd* fungus infections in amphibians in two cases (Smith *et al*. 2018), was applied.

ACTION: The ex-situ colony was subjected to a *Bd* cleaning protocol in January 2016 after a year of quarantine. Forty wild-caught adult frogs were divided into five groups of eight, and housed at 20 °C in laboratory terrariums (80 x 27 cm), with rock substrate (5 mm diameter), artificial plants and dechlorinated tap water. Frogs were fed twice weekly with cockroaches, mealworm larvae and crickets. To treat the *Bd* infection, a 20 ppm chloramphenicol solution (Parafarm, Argentina) was prepared by diluting 200 mg chloramphenicol powder in 2 l warm dechlorinated tap water, then adding 18 l cold water. This was applied over 15 days (modified from Young *et al*. 2012). This was novel, as the treatment was applied without removing the frogs from their original terrariums to reduce stress. Treatment solutions were changed daily and strict hygiene protocols were applied (disposable gloves were used and waste water and containers were disinfected). To monitor infection levels, ten frogs were randomly selected and swabbed before, during, and at the end of treatment, and all individuals were monitored daily for symptoms associated with *Bd*. Samples were analyzed with standard *Bd*-qPCR techniques (Kerby *et al*. 2013). Infection intensity (individual average *Bd* load) and prevalence (proportion of infected individuals) among treatments (before, during and after treatment) were compared using ANOVA and comparison of proportions respectively.

CONSEQUENCES & DISCUSSION: The treatment was not successful, and resulted in no changes in prevalence or intensity of *Bd* between pre- and post-treatment samples (n = 31, F = 1.94, p = 0.16). There was a decrease in both prevalence and intensity during the treatment (Figure 1), suggesting there may have been some effect of the antibiotic on *Bd*. However the treatment was not effective enough to clean all individuals, allowing *Bd* to return to original infection values once the treatment was complete. This may have been due to the exposure time or concentration of chloramphenicol being inadequate for this species, or the presence of substrate in the terrarium functioning as a sink source of *Bd* zoospores (Johnson & Speare 2003). Despite this, adults remained healthy and there was no mortality of individuals before (including the year of quarantine) or after the cleaning treatment. It seems possible that the species is *Bd* resistant or tolerant, at least at infection levels exhibited in the laboratory.

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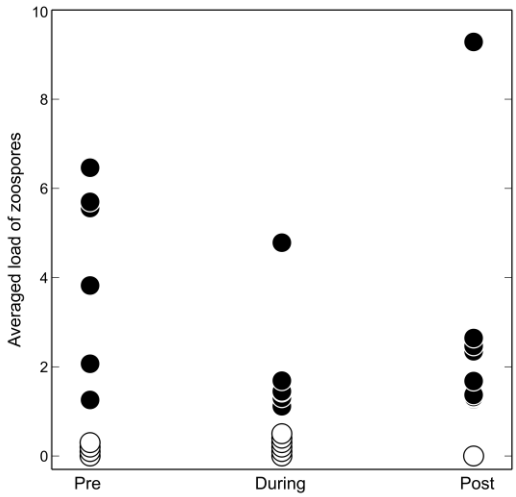

Figure 1. Individual *Bd* infection intensity (average zoospore load) and prevalence (*Bd* negative in white, positive in black) in Valcheta frogs pre-, during and post-treatment with chloramphenicol.

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