Annual assessment of Chytrid fungus prevalence amongst endangered native Fijian ground frog populations on Viwa – Fiji Islands

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Final Report

Submitted by
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INTRODUCTION

The Fijian ground frog (*Platymantis vitianus*) [FGF] is an iconic, endemic amphibian species of the Fiji Islands with strong eco-tourism and biodiversity values. It is listed as endangered under the current World Conservation Union (IUCN) Red List Category B1ab (v) because of its severely fragmented distribution and declining *in-situ* population. The distribution of FGF throughout Fiji remains known inadequately, although Watson (1960) and Ryan (1984) suspected that it was once found on Viti Levu, Kadavu, Beqa and Moturiki. Confirmed populations are noted from Waisali Forest Reserve (Vanua Levu), Nakauvadra, Viti Levu (Tuiwawa pers. comm.), and four other mongoose-free islands in the mid-eastern parts of Fiji Islands including, Viwa Island in Tailevu; Ovalau and Gau in the Lomaiviti group; and Taveuni (Narayan, 2010).

Figure 1.0 Map of Fiji Islands showing the two mainlands, Vitilevu and Vanua Levu. Viwa Island is shown marked by a red arrow. (Map by fijiislandvacationmap.com)

During the past three decades, a number of amphibian populations have undergone severe declines, some of which have resulted in extinction (Blaustein and Wake, 1990; Pimm et al., 1995; Stuart et al., 2004). Globally, many of these declines have been linked to the emergence of Chytridiomycosis, a disease caused by the recently discovered fungal pathogen Chytrid fungus *Batrachochytrium dendrobatidis* (Berger et al., 1998; Daszak et al., 2003; Kriger and Hero, 2007; Hero and Morrison, 2003). New Zealand's first case of this disease, chytridiomycosis, was diagnosed in a population of golden bell frogs *Litoria raniformis* (Waldman, 2001).

The fungus is found in the keratinized skin of amphibians (Longcore, 1999). In adults this is contained to the stomach, legs and toe pads of the animal. In larvae the mouth parts are usually the only tissue affected. The fungus is not normally lethal to larval stages;

however in metamorphing and adult stages, it can be fatal (Blaustein, 2004). The exact mechanism by which *B. dendrobatidis* kills is still largely unknown. The only consistent symptoms of the fungus are excessive skin cell loss and lesions in animals with heavy infections.

OBJECTIVES

The high prevalence of Chytrid fungus in Australia and New Zealand and its prevalence in cool and moist habitats are strong indicators for investigating the prevalence of this disease within isolated natural frog populations in Fiji Islands. Currently, chytridiomycosis is the only explanation, for which supporting evidence is available, for the global ''enigmatic'' declines and disappearances of frog populations and species (Skerratt et al., 2007). The discovery of Chytrid fungus in native Fijian ground frog (*Platymantis vitiana*) [FGF] populations in Fiji Islands could be the major missing piece of the conservation puzzle solving the cause of recent native frog population declines in Fiji Islands. Currently, the only known causes of *in-situ* FGF population declines are loss of habitat through human activities and natural disasters and competition and predation of both adult and froglets by the non-native cane toads (Rhenilla marina) [Narayan pers. comm.].

Thus the aim of this study was to determine the prevalence of Chytrid fungus amongst a sub-population of FGF on Viwa Island (60 ha island present 900 m east off the coast of mainland Viti Levu).

METHODS

Frog surveys

Surveys (n = 2) took place during a breeding season (August throughout to March) and non-breeding season (April to July, n = 1) on Viwa Island. Sampling was conducted in the selected natural habitat sites of the endangered Fijian ground frog near ponds, forested landscape and agricultural areas on Viwa Island. We sampled frogs along 100 m transects (n = 6) with each selected natural habitat and captured at least 10 adult FGF per transect.

Frogs were captured using clean, unused 20 - 25 cm plastic bags. We sampled each frog for Chytrid fungus by firmly running a cotton swab (Medical Wire & Equipment, MW 100-100; Kriger *et al.* (2006) 10 times over each of the following locations: (i) the frog's dorsal surface; (ii) the frog's sides, from groin to armpit; (iii) the ventral surface; and (iv) the undersides of the thighs. Additionally, five outward strokes of the swab were employed on the undersides of each frog's feet, for a total of 70 strokes. Swabs were then be replaced in their original container (a plastic tube), stored on ice in a cooler upon return from the field, and frozen at -20°C. All frogs were handled with unused non-powdered latex gloves to prevent disease transmission between animals, and released immediately after sampling. To ensure that no frogs were inadvertently sampled twice, sampling of frogs did not commence until all frogs at a given section of each transect

were caught, and no further sampling was done at that section of transect after frogs were released.

Laboratory analysis
Swabs were analysed for the presence of *B. dendrobatidis* using quantitative (real-time) polymerase chain reaction techniques (qPCR) described by Boyle *et al.* (2004), and employing the changes described by Kriger *et al.* (2006). Thus, all samples that tested positive in the initial singlicate qPCR assay were re-analysed using a triplicate assay and a full set of *B. dendrobatidis* standards, in order to confirm the initial result and accurately quantify the number of *B. dendrobatidis* zoospores present.

RESULTS

30 Nov, 2010

Report on Chytrid (Bd) PCR testing from Fijian ground frog (*Platymantis vitiana*) Swab samples sent October 2010

We conducted the real time PCR using three replicates/sample as recommended by Hyatt *et al* (2007).

Results:
Total samples received: 300
Negative samples: All samples were negative for chytridiomycosis

References:
Hyatt, A.D., Boyle, D.G., Olsen, V., Boyle, D.B., Berger, L., Obendorf, D., Dalton, A., Kriger, K., Hero, M., Hines, H., Phillot, R., Campbell, R., Marantelli, G., Gleason, F. & Colling, A. (2007). Diagnostic assays and sampling protocols for the detection of Batrachochytrium dendrobatidis. Diseases of Aquatic Organisms 73, 175-192.

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Conclusions

It is concluded that Fijian ground frog population in Viwa, Fiji Islands is currently free from the pathogenic fungal disease Chytridiomycosis. This negative result could possibly be due to the high mean ambient temperatures throughout the year in Fiji Islands, which hinders the growth of chytrid fungus within forested natural habitats.

Therefore, currently the major threats to the endangered Fijian ground population on Viwa Islands are habitat destruction and invasive cane toads. Thus it would be worthwhile to trial the removal of cane toads from FGF natural habitats on Viwa Island.

Appendix (photos)

Chytrid swabbing of a Fijian ground frog specimen

Viwa Island youths assisting Dr. Edward during the Chytrid survey

Landcare Research Lab technician conducting chytrid testing on Fijian ground frog DNA samples

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Budget statement

Allocated budget:	6000 Pounds
Expenditure:	3000 Pounds Field Surveys 3000 Pounds Laboratory Analysis
Total RSG funding	6000 pounds.