Prevalence of the Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) in the Australian Alps

David Hunter[1], Rod Pietsch[1], Nick Clemann[2], Michael Scroggie[2], Gregory Hollis[3] and Gerry Marantelli [4]

1 – NSW Department of Environment & Climate Change, 11 Farrer Place, Queanbeyan, New South Wales 2620

2 – Arthur Rylah Institute for Environmental Research, (PO Box 137) 123 Brown Street, Heidelberg, Victoria, 3084

3 – Department of Sustainability and Environment, 120 McCarthys Spur Rd, Noojee, Victoria, 3833

4 - Amphibian Research Centre, PO Box 959, Merlynston, Victoria 3058

Department of **Environment & Climate Change** NSW

State Government Victoria Department of Sustainability and Environment

AUSTRALIAN ALPS NATIONAL PARKS

Report to the Australian Alps Liaison Committee: January 2009

Summary

Over the past three decades, many amphibian species along the eastern ranges of Australia have been declining at an alarming rate. The Australian Alps region has been no exception, with all endemic frog species declining to a level warranting listing as nationally threatened. There is considerable evidence implicating a disease (chytridiomycosis) as the cause of these declines. This disease is caused by infection with the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), which is believed to have been introduced into the Australian environment a few decades ago. We screened for amphibian chytrid fungus infection in two frog species across the Australian Alps; the common eastern froglet (*Crinia signifiera*), and the alpine tree frog (*Litoria verreauxii alpina*). We were particularly interested in identifying the extent to which the common eastern froglet, a species that has not declined in recent years, is a reservoir host for the amphibian chytrid fungus in the Australian Alps. We were also interested in identifying rates of infection in populations of the alpine tree frog, a subspecies that has disappeared from much of its historic range, to infer the likely susceptibility of this subspecies to this pathogen. This study found that apparently robust populations of both the common eastern froglet and the alpine tree frog carry high infection rates (typically > 80%) of the amphibian chytrid fungus, suggesting that for both taxa, many extant populations currently have a high level of population resilience to this pathogen, at least for the populations that we sampled. This result also identifies both frog taxa as substantial reservoir hosts for the amphibian chytrid fungus in the Australian Alps, which has implications for the management of other threatened frog species in this region. We did not detect the amphibian chytrid fungus on either species at one site sampled in Kosciuszko National Park, suggesting that this site may be pathogen free. Owing to the relative isolation of this site, it is possible that the amphibian chytrid fungus has not reached this site. The presence of 'naïve populations' offers a valuable opportunity to understand the impact of the amphibian chytrid fungus and develop management actions aimed at recovering species such as the southern corroboree frog (*Pseudophryne corroboree*) and Baw Baw frog (*Philoria frosti*) that continue to be threatened by this pathogen.

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Acknowledgements

Assistance in the field was provided by Gabriel Wilks (NSW DECC). The Australian Alps Liaison Committee funded the screening of the swabs. Murray Evans (Environment ACT) provided valuable assistance in implementing this project. Field work and reporting was funded by the NSW Department of Environment and Climate Change and the Victorian Department of Sustainability and Environment.

Cover Photo: Mismatch amplexus between a male common eastern froglet and male alpine tree frog (Photo: David Hunter).

Section 1. Introduction

Amphibian declines and extinctions have occurred at an alarming rate over the past three decades (Stuart *et al*. 2004), with the current rate of amphibian extinctions far exceeding historic extinction rates as indicated by the fossil record (McCallum 2007). While there are a number of causal agents implicated in these declines (see Alford and Richards 1999 for review), the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, which causes the disease chytridiomycosis, is most notable for its widespread impact and association with these declines (Berger *et al*. 1998, Daszak *et al*. 2003, Lips *et al*. 2006, Skerratt *et al*. 2007). Amphibian declines attributed to chytridiomycosis have occurred on every major continent where amphibians occur (Berger *et al*. 1998, Rachowicz *et al*. 2005, Lips *et al*. 2006). All frog species endemic to the mainland Australian Alps have undergone substantial declines and range contractions (Osborne *et al*. 1999), with chytridiomycosis considered the most significant causal factor in some of these declines (Hunter *et al*. in press).

To date, both genetic (Morehouse *et al*. 2003) and pre-decline screening for infection (Berger *et al*. 1998) suggest that the amphibian chytrid fungus only recently arrived in the Australian environment. While the data supporting the ‘novel pathogen hypothesis’ is considered insufficient by some authors to resolve this (McCallum 2005, Rachowicz *et al*. 2005), it is argued by Skerratt *et al*. (2007) that the current data is sufficient for managers to consider amphibian chytrid fungus as both newly emerging and the primary cause of many recent amphibian declines and extinction. Hence, Skerratt *et al*. (2007) suggest that conservation managers should respond to this disease in a swift and proactive manner.

Another unresolved issue with respect to the impact of amphibian chytrid fungus is the mechanisms by which this pathogen could be causing species to decline to critically low densities or extinction. Simple host/pathogen models predict that a highly virulent pathogen will have limited capacity to cause population decline because infected individuals would be expected to die before infecting others (Anderson 1979). Hence, factors other than just the interaction between the susceptible host and the pathogen are typically required for a virulent pathogen to cause significant population decline. The most common factor enhancing the capacity for a virulent pathogen to spread through a population is the presence of non-susceptible reservoir host species in the shared environment (Gog *et al.* 2002). Furthermore, reducing the impact of disease in wildlife populations often requires the control of reservoir host species in critical habitats (Caley and Hone 1994, Lloyd-Smith *et al.* 2005).

In this study, we investigated rates of infection with the amphibian chytrid fungus in populations of the common eastern froglet (*Crinia signifera*) and the alpine tree frog (*Litoria verreauxii alpina*) in sub-alpine bog environments across the Australian Alps. The common eastern froglet has shown no sign of major decline, and a recent study found that this species is an abundant reservoir host for the amphibian chytrid fungus in areas occupied by the critically endangered southern corroboree frog (*Pseudophryne corroboree*) (Hunter *et al.* 2007). Conversely, the alpine tree frog has contracted from much of its former range over the past two decades (Osborne *et al.* 1999). Interestingly, the alpine tree frog appears to have contracted from areas where it was historically in close contact with the common eastern froglet (i.e. used the same microhabitats around breeding pools, David Hunter and Gerry Marantelli personal observations). One hypothesis for this observation is that the decline of the alpine tree frogs is due to disease caused by the amphibian chytrid fungus, and that the impact of this pathogen is much greater where the common eastern froglet can operate as a reservoir host and increase rates of infection in the alpine tree frog. This project was undertaken as an initial stage in assessing this hypothesis, and identifying the broader distribution of the amphibian chytrid fungus in the Australian Alps. The specific aims of this study were to:

1. Determine the distribution and infection rates of the amphibian chytrid fungus in alpine tree frog and common eastern froglet populations across the mainland Australian Alps.

2. Determine the likelihood that the apparent allopatric distribution between the alpine tree frog and the common eastern froglet in most areas where they occur is due to the common eastern froglet acting as a reservoir host for the amphibian chytrid fungus.

3. Attempt to locate frog populations in the mainland Australian Alps that are presently free of the amphibian chytrid fungus.

Section 2. Methods

2.1 Study Species

2.1.1 Common Eastern Froglet

The common eastern froglet (*Crinia signifera*) (Figure 1) is a small frog species found throughout much of eastern and south-eastern Australia, including the Australian Alps region to an altitude of 2000 meters. It breeds in a variety of aquatic habitat types, from small bog pools and seepages, to large dams. Breeding predominately occurs in spring and early summer. Despite many other frog species in the Australian Alps suffering dramatic declines since the mid to late 1980's (Osborne *et al.* 1999), the common eastern froglet has remained in very high abundance throughout its previous known range in this region (David Hunter personal observations).

Figure 1. Male common eastern froglet (*Crinia signifera*), Kosciuszko National Park (Photo: David Hunter).

2.1.2 Alpine Tree Frog

The alpine tree frog (*Litoria verreauxii alpina*) (Figure 2) is a high altitude subspecies related to the more widespread whistling tree frog (*Litoria verreauxii verreauxii*), which is found throughout much of eastern Australia (Barker *et al.* 1995). This species breeds in a variety of habitat types, from small pools to large dams, from mid spring to early summer. Historically, the alpine tree frog was found throughout much of the mainland Australian Alps; however, since the mid to late 1980's, this species has contracted and disappeared from much of its known historic range (Osborne *et al.* 1999).

Figure 2. Female alpine tree frog (*Litoria verreauxii alpina*), Kosciuszko National Park (Photo: David Hunter).

2.2 Sampling Sites

Figure 3 shows the location of sites sampled in this study. Sites were chosen to represent the broader geographic region of the mainland Australian Alps where the study taxa occur. Sampling was undertaken in 2006 during spring and early summer.

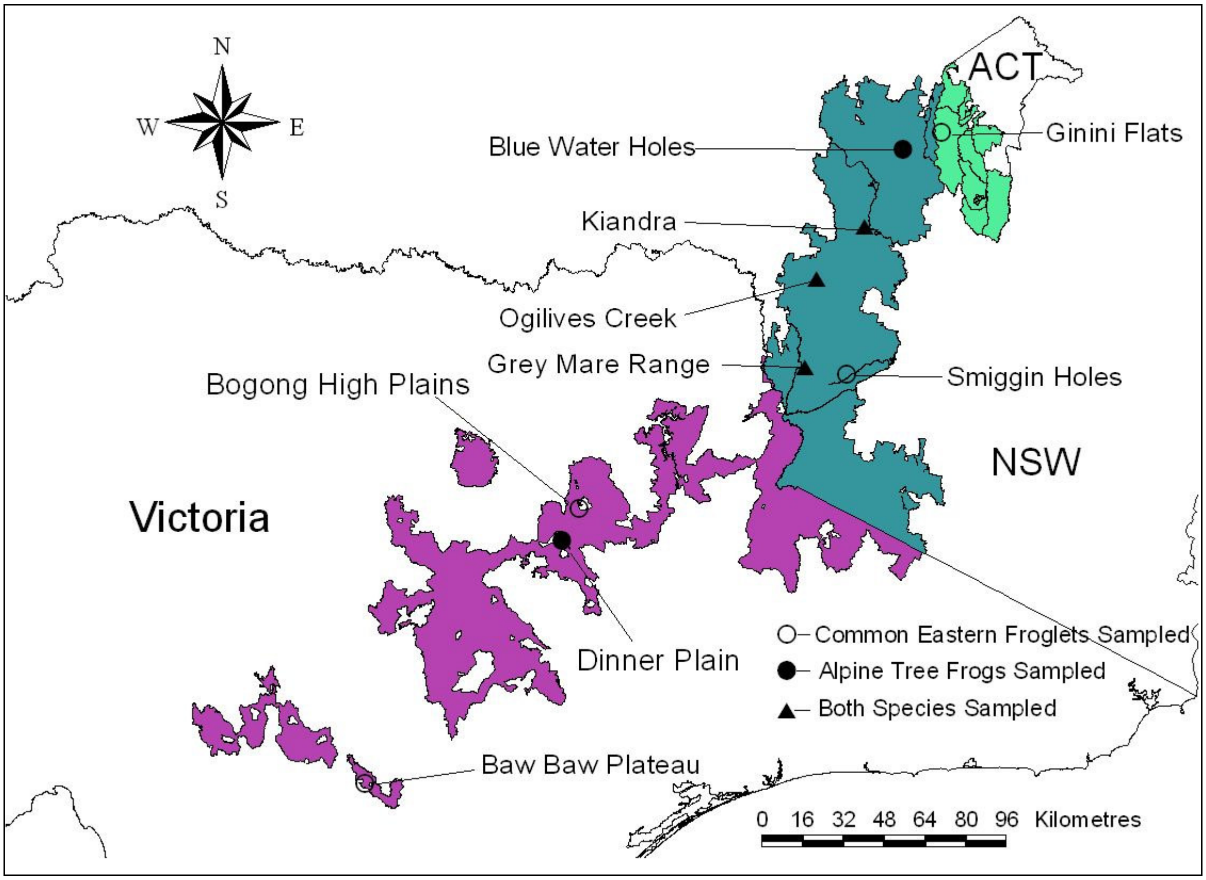

Figure 3. Location of sites in the mainland Australian Alps region where sampling was undertaken for the amphibian chytrid fungus in alpine tree frog and common eastern froglet populations. Shaded areas represent areas within National Parks in the Australian Alps region.

2.3 Field Swabbing Procedures

Alpine tree frogs and common eastern froglets were hand captured either during the day, or at night by spotlight. For both frog species, the swabbing procedure involved holding the frog by the back legs and wiping three times on each of the feet, hands, inside and outside of the thighs, stomach and back region. The swabs were stored in a cool location until delivery to the CSIRO Australian Animal Health Laboratory in Geelong. The swabs were screened for the presence of amphibian chytrid fungus DNA using Taqman real-time PCR assay (see Boyle *et al.* 2004 for details of this procedure).

The following procedures were undertaken to minimise disease transmission between sites and between individual frogs within sites:

- Before entering the sites, all equipment that came into contact with frogs was sterilised with 70 percent ethanol and completely dried for at least four hours.
- Each frog was handled using a new pair of disposable rubber gloves and a new plastic snap lock bag. Both items were immediately discarded after the frog was processed, and a new set used for the next frog.

2.4 Statistical Analysis

Uncertainty around the total proportion of adults testing positive for infection with *B. dendrobatidis* was estimated using a Bayesian approach with uninformative priors. The 95% credible intervals were propagated using Markov Chain Monte Carlo methods with 100,000 samples after the first 10,000 samples were discarded. This was undertaken using the WinBUGS software package, version 1.4 (Spiegelhalter *et al.* 2003).

Section 3. Results

Except for the Grey Mare Range site in the Kosciuszko National Park, the amphibian chytrid fungus was detected in all populations examined for both the alpine tree frog and the common eastern froglet (Table 1). At Grey Mare Range, no amphibian chytrid fungus infection was recorded for either species (Table 1). For sites where infection was detected, rates of infection in both the alpine tree frog and common eastern froglet were generally very high (Table 1). The exception to this was for the common eastern froglet results on the Baw Baw Plateau where relatively lower infection was observed (Table 1). The spore count per infected swab from the Baw Baw Plateau was also generally lower than the spore counts observed at all other sites where infection was recorded (Figure 4). Overall, inhibition of samples was generally low, except for the Baw Baw Plateau samples where one third were inhibited (Table 1).

Table 1. Results for amphibian chytrid fungus sampling from common eastern froglet and alpine tree frog populations. Calculation for proportion positive and 95% credible intervals excluded inhibited samples.

Site	Species	No. Sampled	No. Positive	No. Inhibited	Proportion Positive	95% Credible Intervals
Kiandra	alpine tree frog	19	17	0	0.89	0.68 - 0.97
Blue Water Holes	alpine tree frog	9	9	0	1.00	0.68 - 1.00
Ogilives Creek	alpine tree frog	15	14	1	1.00	0.78 - 1.00
Mt Hotham	alpine tree frog	20	16	0	0.80	0.59 - 0.92
Grey Mare Range	alpine tree frog	6	0	0	0.00	0.00 - 0.41
Kiandra	common eastern froglet	20	16	0	0.80	0.59 - 0.92
Smiggin Holes	common eastern froglet	26	25	0	0.96	0.81 - 0.99
Ogilives Creek	common eastern froglet	6	4	0	0.67	0.28 - 0.90
Grey Mare Range	common eastern froglet	14	0	0	0.00	0.00 - 0.22
Bogong High Plains	common eastern froglet	6	5	0	0.83	0.42 - 0.96
Ginini Flat	common eastern froglet	22	15	3	0.79	0.57 - 0.91
Baw Baw Plateau	common eastern froglet	35	10	10	0.40	0.23 - 0.59

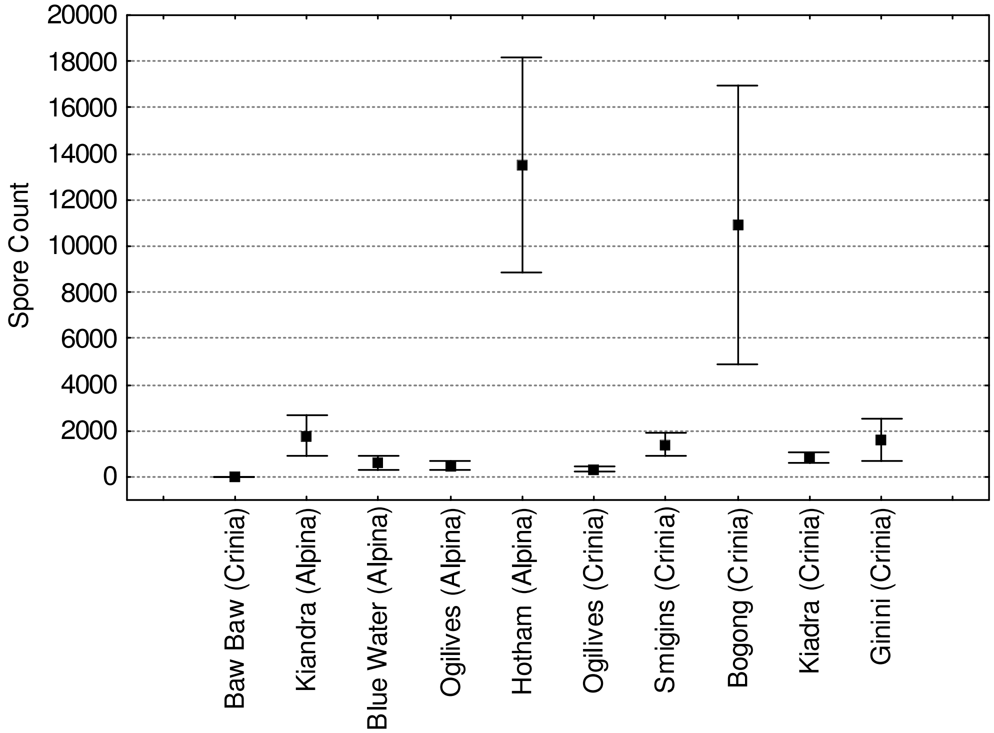

Figure 4. Spore counts on swabs that tested positive for the presence of the amphibian chytrid fungus. Central point is the mean and error bars are standard errors.

Section 4. Discussion

4.1 Distribution and prevalence of the amphibian chytrid fungus in common eastern froglet and alpine tree frog populations across the Australian Alps

The results of this study suggest that the amphibian chytrid fungus is present throughout much of the mainland Australian Alps. This is consistent with the broad distribution already identified for this pathogen along the eastern ranges of Australia (Berger *et al.* 1998, Kriger *et al.* 2006). Assuming that the amphibian chytrid fungus only entered the Australian environment three decades ago (Skerratt *et al.* 2007), the comprehensive spread of this pathogen into the Australian Alps would have been facilitated by the high degree of connectedness among frog populations through this region, and down to lower altitude areas. Other vectors for this pathogen, including humans, may have also contributed to its rapid spread.

Of particular interest in our results is the extremely high rates of infection for the amphibian chytrid fungus in the majority of common eastern froglet and alpine tree frog populations sampled (Table 1). Since the actual rates of infection in these populations are likely to be greater than we identified, due to infection not being detected in some individuals (Hyatt *et al*. 2007), infection in populations of both species may often reach 100 percent. This level of infection has been observed in other frog species (cf. Hanselmann *et al*. 2004), and may reflect conditions in the Australian Alps being conducive to maintaining infection with this pathogen. The amphibian chytrid fungus prefers cool temperatures and moist conditions (Berger *et al*. 2004). Consequently, it is likely that habitats preferred by frogs in the Australian Alps present ideal conditions for this fungus.

Of the sites where infection was recorded, infection rates in the common eastern froglet population on the Baw Baw Plateau were relatively low (Table 1). Additionally, this site had lower spore counts than those observed for the other sites (Figure 1). These results suggest that the extent of infection on individual frogs may have been limited in some way at this site. While a number of studies have identified spatial and temporal variation in detectable infection rates for this pathogen (Berger *et al*. 2004, Kriger *et al*. 2006), it is difficult to suggest a possible causal mechanism for this result as the timing of sampling and the habitats were consistent among sites. This aspect of our results requires further investigation to identify possible causal mechanisms.

The most substantial variation in infection rates observed among sites was the failure to detect any infection for either the common eastern froglet or the alpine tree frog at the Grey Mare Range site. Given the consistently high levels of infection observed elsewhere for both species, this result suggests that this site may be free of infection with the amphibian chytrid fungus. It cannot be discounted that other factors reduced detectable levels of infection at this site; however, this seems unlikely as infection was highly detectable in nearby sites. This result is discussed further in section 4.3.

4.2 The common eastern froglet as a reservoir host for the amphibian chytrid fungus, and the often allopatric distribution between this species and the alpine tree frog

Our study supports the results of an earlier study that identified the common eastern froglet as a major reservoir host for the amphibian chytrid fungus (Hunter *et al.* 2007). The present study has also identified the alpine tree frog as a significant reservoir host for this pathogen. This is interesting because the timing and pattern of decline in the alpine tree frog is similar to the decline of other frog species along the eastern ranges of Australia for which chytridiomycosis has been implicated as the primary causal agent (Berger *et al.* 1998, Skerrat *et al.* 2007).

A notable feature of the decline in the alpine tree frog is that it appears to have contracted from areas where it historically co-occurred with the common eastern froglet (David Hunter personal observations); areas where these taxa co-occur are now rare. Our results do not appear to support the hypothesis that this range contraction is due to the common eastern froglet operating as a reservoir host for the amphibian chytrid fungus, as the results suggest that extant populations of the alpine tree frog are also relatively resistant at the population level, and a reservoir host for this pathogen. Factors that may explain the apparent range contraction in the alpine tree frog, and which should be examined further, include: the alpine tree frog was initially susceptible to the amphibian chytrid fungus, but has subsequently attained resistance (c.f. Retallick *et al.* 2004); the common eastern froglet and alpine tree frog support different strains of the amphibian chytrid fungus (c.f. Berger *et al.* 2005); the common eastern froglet has a competitive advantage over the alpine tree frog in the presence of the amphibian chytrid fungus.

Regardless of the interaction between the common eastern froglet and the alpine tree frog, both species are currently reservoir hosts for the amphibian chytrid fungus across the Australian Alps. A number of authors have suggested the potential importance of reservoir hosts in the decline of amphibians due to infection with the amphibian chytrid fungus (Daszak *et al.* 1999, McCallum 2005, Woodhams and Alford 2005). The ecology of both frogs and the amphibian chytrid fungus are conducive to a multi host/single pathogen system resulting in increased infection of susceptible species with increasing abundance of non-susceptible/reservoir host species. This is due to the amphibian chytrid fungus being a generalist pathogen (Berger *et al.* 1998), the fact that amphibians often congregate for breeding and their tadpoles share non-species specific aquatic environments, and that this pathogen has a free-swimming zoospore stage that can live independent of frog hosts for up to seven weeks in the aquatic environment before infecting a new host (Johnson and Speare 2003). Given the likely importance of reservoir host species in declines caused by the amphibian chytrid fungus, any activity that may increase the distribution or density of the common eastern froglet or the alpine tree frog into areas occupied by other threatened frog species (i.e. corroboree frogs and Baw Baw frog) should be considered a potentially threatening process.

4.3 Amphibian chytrid fungus-free frog populations in the Australian Alps

While the amphibian chytrid fungus appears ubiquitous through much of eastern Australia, and assuming the novel pathogen hypothesis explaining the emergence of this pathogen is correct (see McCallum 2005 for discussion of this hypothesis), it is possible that relatively isolated frog populations have remained pathogen free. This suggestion is most likely on off shore islands and in high altitude areas isolated by steep / dissected country (i.e. “sky islands” *sensu* Koumoundouros *et al.* in press) that would restrict the movement of vectors for the amphibian chytrid fungus. The results suggesting that the Grey Mare Range site may be free of amphibian chytrid fungus is consistent with this, as this site is relatively isolated by distance and terrain. While it cannot be discounted that the Grey Mare Range population was previously infected and then lost infection, this is seems unlikely since all other apparently robust populations of both species maintain high rates of infection (Table 1).

The presence of pathogen-free frog populations in the Australian Alps may provide a valuable opportunity for threatened frog recovery programs. The national threat abatement plan for the amphibian chytrid fungus (DEH 2006) recommends that for species threatened with extinction from chytridiomycosis, captive breeding and reintroduction should be undertaken to maintain the interaction between frog and pathogen until the impact of the amphibian chytrid fungus has reduced sufficiently to allow the frog population to be self-sustaining. While some frog species appear to have attained greater population resilience to the amphibian chytrid fungus (cf. Retallick *et al.* 2004), there has been no demonstration as to how this resilience has developed. The presence of apparently naïve frog populations would allow comparative studies to be undertaken to identify the mechanisms that have allowed some frog species to co-exist with the amphibian chytrid fungus. Such studies would provide greater capacity to develop management actions aimed at enhancing the resilience of species that have remained susceptible, such as the southern corroboree frog and Baw Baw frog.

4.4 Management implications

The following management recommendations are aimed at limiting the spread and impact of the amphibian chytrid fungus in the Australian Alps:

- Any action that increases the abundance of the common eastern froglet or alpine tree frog in areas occupied by other threatened frog species should be considered a threatening process.
- Unless for specific research into the amphibian chytrid fungus, common eastern froglets and alpine tree frogs from the Australian Alps should not be transported from one area to another, as this will effectively spread the pathogen.
- Areas suspected or known to be free of amphibian chytrid fungus infection should be considered a valuable resource and treated with the highest level of quarantine.

4.5 Further Research

The following research actions arising from this study are recommended as a means to increase our understanding of how the amphibian chytrid fungus has impacted frogs in the Australian Alps, and how we may recover species like the southern corroboree frog that continue to be threatened by this pathogen:

- Quantify the current distribution and breeding habitat use of the common eastern froglet and alpine tree frog across the Australian Alps.
- Undertake further sampling for the amphibian chytrid fungus across the Australian Alps with the specific aim of locating other pathogen-free populations.
- Assess whether strains of the amphibian chytrid fungus vary among areas and frog species in the Australian Alps.
- Compare the virulence of the amphibian chytrid fungus to frogs from naïve and exposed populations as a means of inferring whether increased resistance has developed since the arrival of this pathogen.

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Appendix 1. Report from the CSIRO Animal Health Laboratory where the swabs were analysed.

DIAGNOSTIC REPORT:

CSIRO
Livestock Industries
Australian Animal Health Laboratory
Project: *Bioimaging and Ecohealth*
5 Portarlington Road
Geelong Vic 3220
Private Bag 24
Australia (61) 0352275419, fax (61) 0352275555

DATE: Monday 18th December, 2006

SPECIMEN: Swabs
SAN: SAN 06-04065 AND SAN 07-02376
ASSAY: Real time Taqman PCR for the amphibian chytrid *Batrachochytrium dendrobatidis*
Reference: FIR06/53-1

METHODS

Samples were analysed by Taqman real-time PCR assay (*Diseases of Aquatic Organisms (2004)* ***60****:141-148*). All samples were analysed in triplicate.
An internal control was included in the assay to test for inhibitors in the samples

RESULTS

AAHL #	Species	Site	Date	No. Zoospore	Result
1	L.v.alpina	Kiandra	18.10.06	1**	?
2	L.v.alpina	Kiandra	18.10.06	3015	+
3	L.v.alpina	Kiandra	18.10.06	-	-
4	L.v.alpina	Kiandra	18.10.06	5	+
5	L.v.alpina	Kiandra	18.10.06	-	-
6	L.v.alpina	Kiandra	18.10.06	42.4	+
7	L.v.alpina	Kiandra	18.10.06	0.8**	?
8	L.v.alpina	Kiandra	18.10.06	37.8	+
9	L.v.alpina	Kiandra	18.10.06	9303	+
10	L.v.alpina	Kiandra	18.10.06	12	+
11	L.v.alpina	Kiandra	18.10.06	13777	+
12	L.v.alpina	Kiandra	18.10.06	119	+
13	L.v.alpina	Kiandra	18.10.06	147	+
14	L.v.alpina	Kiandra	18.10.06	37.2	+
15	L.v.alpina	Kiandra	18.10.06	249	+
16	L.v.alpina	Kiandra	18.10.06	232	+
17	L.v.alpina	Kiandra	18.10.06	769	+
18	L.v.alpina	Kiandra	18.10.06	3.6*	?
19	L.v.alpina	Kiandra	18.10.06	2465	+
20	L.v.alpina	Blue Water Hole	6.10.06	1.3	+
21	L.v.alpina	Blue Water Hole	6.10.06	6.2	+
22	L.v.alpina	Blue Water Hole	6.10.06	2696	+
23	L.v.alpina	Blue Water Hole	6.10.06	89	+
24	L.v.alpina	Blue Water Hole	6.10.06	564	+
25	L.v.alpina	Blue Water Hole	6.10.06	327	+
26	L.v.alpina	Blue Water Hole	6.10.06	23.5	+
27	L.v.alpina	Blue Water Hole	6.10.06	1239	+
28	L.v.alpina	Blue Water Hole	6.10.06	402	+
29	L.v.alpina	Ogilvies	18.10.06	(1064)	?
30	L.v.alpina	Ogilvies	18.10.06	4.6	+
31	L.v.alpina	Ogilvies	18.10.06	8	+
32	L.v.alpina	Ogilvies	18.10.06	1098	+
33	L.v.alpina	Ogilvies	18.10.06	(667)	?
34	L.v.alpina	Ogilvies	18.10.06	-	#
35	L.v.alpina	Ogilvies	18.10.06	(465*)	?
36	L.v.alpina	Ogilvies	18.10.06	61.4	+
37	L.v.alpina	Mt Hotham	22.11.06	369	+
38	L.v.alpina	Mt Hotham	22.11.06	18.4	+
39	L.v.alpina	Mt Hotham	22.11.06	0.6*	?
40	L.v.alpina	Mt Hotham	22.11.06	26009	+
41	L.v.alpina	Mt Hotham	22.11.06	4382	+
42	L.v.alpina	Mt Hotham	22.11.06	16623	+
43	L.v.alpina	Mt Hotham	22.11.06	50136	+
44	L.v.alpina	Mt Hotham	22.11.06	(5475)	+

45	L.v.alpina	Mt Hotham	22.11.06	70194	+
46	L.v.alpina	Mt Hotham	22.11.06	274	+
47	L.v.alpina	Mt Hotham	22.11.06	7250	+
48	L.v.alpina	Mt Hotham	22.11.06	23.8	+
49	L.v.alpina	Mt Hotham	22.11.06	687	+
50	L.v.alpina	Mt Hotham	22.11.06	29588	+
51	L.v.alpina	Mt Hotham	22.11.06	-	-
52	L.v.alpina	Mt Hotham	22.11.06	-	-
53	L.v.alpina	Mt Hotham	22.11.06	-	-
54	L.v.alpina	Mt Hotham	22.11.06	17368	+
55	L.v.alpina	Mt Hotham	22.11.06	104797	+
56	L.v.alpina	Mt Hotham	22.11.06	311	+
57	L.v.alpina	Grey Mare	11.11.06	-	-
58	L.v.alpina	Grey Mare	11.11.06	-	-
59	L.v.alpina	Grey Mare	11.11.06	-	-
60	L.v.alpina	Grey Mare	11.11.06	-	-
61	L.v.alpina	Grey Mare	11.11.06	-	-
62	L.v.alpina	Grey Mare	19.10.06	-	-
63	C.signifera	Grey Mare	11.11.06	-	-
64	C.signifera	Grey Mare	11.11.06	-	-
65	C.signifera	Grey Mare	11.11.06	-	-
66	C.signifera	Grey Mare	19.10.06	-	-
67	C.signifera	Grey Mare	19.10.06	-	-
68	C.signifera	Grey Mare	19.10.06	-	-
69	C.signifera	Grey Mare	19.10.06	-	-
70	C.signifera	Grey Mare	19.10.06	-	-
71	C.signifera	Grey Mare	19.10.06	-	-
72	C.signifera	Grey Mare	19.10.06	-	-
73	C.signifera	Grey Mare	19.10.06	-	-
74	C.signifera	Grey Mare	19.10.06	-	-
75	C.signifera	Grey Mare	19.10.06	-	-
76	C.signifera	Grey Mare	19.10.06	-	-
77	C.signifera	Ogilvies	18.10.06	(187)	+
78	C.signifera	Ogilvies	18.10.06	(256*)	-
79	C.signifera	Ogilvies	18.10.06	698	+
80	C.signifera	Ogilvies	18.10.06	35.5	+
81	C.signifera	Ogilvies	18.10.06	0.6*	?
82	C.signifera	Ogilvies	18.10.06	735	+
83	C.signifera	Smiggin Holes	19.10.06	190	+
84	C.signifera	Smiggin Holes	19.10.06	9601	+
85	C.signifera	Smiggin Holes	19.10.06	1892	+
86	C.signifera	Smiggin Holes	19.10.06	1253	+
87	C.signifera	Smiggin Holes	19.10.06	1057	+
88	C.signifera	Smiggin Holes	19.10.06	823	+
89	C.signifera	Smiggin Holes	19.10.06	2.4	+
90	C.signifera	Smiggin Holes	19.10.06	2480	+
91	C.signifera	Smiggin Holes	19.10.06	306	+
92	C.signifera	Smiggin Holes	19.10.06	2329	+
93	C.signifera	Smiggin Holes	19.10.06	2.4	+
94	C.signifera	Smiggin Holes	19.10.06	6.1	+
95	C.signifera	Smiggin Holes	19.10.06	-	-
96	C.signifera	Smiggin Holes	19.10.06	887	+
97	C.signifera	Smiggin Holes	19.10.06	3868	+
98	C.signifera	Smiggin Holes	19.10.06	945	+
99	C.signifera	Smiggin Holes	19.10.06	460	+
100	C.signifera	Smiggin Holes	19.10.06	28.3	+
101	C.signifera	Smiggin Holes	19.10.06	288	+
102	C.signifera	Smiggin Holes	19.10.06	50	+
103	C.signifera	Bogong H.P.	21.11.06	35095	+
104	C.signifera	Bogong H.P.	21.11.06	117	+
105	C.signifera	Bogong H.P.	21.11.06	1077	+
106	C.signifera	Bogong H.P.	21.11.06	9729	+
107	C.signifera	Bogong H.P.	21.11.06	8542	+
108	C.signifera	Bogong H.P.	21.11.06	-	-
109	C.signifera	Kiandra	18.10.06	-	-
110	C.signifera	Kiandra	18.10.06	3051	+
111	C.signifera	Kiandra	18.10.06	14.5	+
112	C.signifera	Kiandra	18.10.06	456	+
113	C.signifera	Kiandra	18.10.06	575	+
114	C.signifera	Kiandra	18.10.06	104	+
115	C.signifera	Kiandra	18.10.06	283	+
116	C.signifera	Kiandra	18.10.06	66.5	+
117	C.signifera	Kiandra	18.10.06	-	-
118	C.signifera	Kiandra	18.10.06	-	-
119	C.signifera	Kiandra	18.10.06	1400	+
120	C.signifera	Kiandra	18.10.06	1192	+
121	C.signifera	Kiandra	18.10.06	1282	+
122	C.signifera	Kiandra	18.10.06	-	-
123	C.signifera	Kiandra	18.10.06	2228	+
124	C.signifera	Kiandra	18.10.06	1625	+

125	C.signifera	Kiandra	18.10.06	195	+
126	C.signifera	Kiandra	18.10.06	258	+
127	C.signifera	Kiandra	18.10.06	520	+
128	C.signifera	Kiandra	18.10.06	313	+
129	C.signifera	Ginini Flat	18.10.06	-	-
130	C.signifera	Ginini Flat	18.10.06	(640)	+
131	C.signifera	Ginini Flat	18.10.06	10	+
132	C.signifera	Ginini Flat	18.10.06	-	#
133	C.signifera	Ginini Flat	18.10.06	9.2	+
134	C.signifera	Ginini Flat	18.10.06	-	-
135	C.signifera	Ginini Flat	18.10.06	-	-
136	C.signifera	Ginini Flat	18.10.06	14725	+
137	C.signifera	Ginini Flat	18.10.06	-	-
138	C.signifera	Ginini Flat	18.10.06	(380)	+
139	C.signifera	Ginini Flat	18.10.06	500.9**	?
140	C.signifera	Ginini Flat	18.10.06	(339)	+
141	C.signifera	Ginini Flat	18.10.06	177	+
142	C.signifera	Ginini Flat	18.10.06	954	+
143	C.signifera	Ginini Flat	18.10.06	-	#
144	C.signifera	Ginini Flat	18.10.06	2719	+
145	C.signifera	Ginini Flat	18.10.06	396	+
146	C.signifera	Ginini Flat	18.10.06	-	#
147	C.signifera	Ginini Flat	18.10.06	12.42	+
148	C.signifera	Ginini Flat	18.10.06	545	+
149	C.signifera	Ginini Flat	18.10.06	1889	+
150	C.signifera	Ginini Flat	18.10.06	(625*)	?
4	C.signifera	Baw Baw plateau - Village flat	10/10/2006	0	-
5	C.signifera	Baw Baw plateau - Baraganth flat	10/10/2006	0	-
6	C.signifera	Baw Baw plateau - Mandarra flat	10/10/2006	3**	?
7	C.signifera	Baw Baw plateau - Village flat Big Hill	12/10/2006	136	+
8	C.signifera	Baw Baw plateau - Village Dam Valley	12/10/2006	0	-
9	C.signifera	Baw Baw plateau - Freeman Flat	7/10/2006	0	-
10	C.signifera	Baw Baw plateau - Gumear flat	7/10/2006	0	-/#
11	C.signifera	Baw Baw plateau - Currawong flat	7/10/2006	0	#
12	C.signifera	Baw Baw plateau - Dam Valley lower	12/10/2006	0	#
13	C.signifera	Baw Baw plateau - Pudding basin	10/10/2006	0	#
Swabs labelled with					
14	*C.signifera*	Baw Baw plateau - Freeman flat	7/10/2006	0	-
15	*C.signifera*	Baw Baw plateau - Pudding basin	10/10/2006	0	-
16	*C.signifera*	Baw Baw plateau - moondarra flat	10/10/2006	0	-
17	*C.signifera*	Baw Baw plateau - Currawong flat	7/10/2006	0	#
18	*C.signifera*	Baw Baw plateau - Gwinear flat	7/10/2006	0	#
19	*C.signifera*	Baw Baw plateau -Village lower Dam Valley	12/10/2006	0	-
20	*C.signifera*	Baw Baw plateau - Baragwantha flat	10/10/2006	0	-
21	*C.signifera*	Baw Baw plateau - Village Dam Valley	12/10/2006	0.9*	?
22	*C.signifera*	Baw Baw plateau - Village Big Hill	12/10/2006	1**	?
Swabs labelled with circled #3					
23	*C.signifera*	Baw Baw plateau - Baragwantha flat	10/10/2006	0.2**	?
24	*C.signifera*	Baw Baw plateau - Freeman Flat	7/10/2006	0	#
25	*C.signifera*	Baw Baw plateau - Currawong flat	7/10/2006	1	+
26	*C.signifera*	Baw Baw plateau - Dam Valley	12/10/2006	1	+
27	*C.signifera*	Baw Baw plateau - Pudding basin	10/10/2006	0	-
28	*C.signifera*	Baw Baw plateau - Gurtnegi flat?	7/10/2006	0	-
29	*C.signifera*	Baw Baw plateau - Mandarra flat	10/10/2006	3	+
30	*C.signifera*	Baw Baw plateau - Big Hill	12/10/2006	0	#
Swabs labelled with circled #4					
31	*C.signifera*	Baw Baw plateau -Big Hill	12/10/2006	0	#
32	*C.signifera*	Baw Baw plateau - Baragwantha flat	10/10/2006	13	+
33	*C.signifera*	Baw Baw plateau - Pudding basin	10/10/2006	0	-
34	*C.signifera*	Baw Baw plateau - Dam Valley Tanks	12/10/2006	0.08**	?
Swabs					
35	*C.signifera*	Baw Baw plateau - Big Hill	12/10/2006	44	+
36	*C.signifera*	Baw Baw plateau - Dam Valley	12/10/2006	0	-
Swabs labelled with circled #6					
37	*C.signifera*	Baw Baw plateau - Big Hill	12/10/2006	0	#
No circled number on this swab:					
38	*C.signifera*	Baw Baw plateau - McMillans flat	10/10/2006	0.2*	?

Positives are those samples that return positive data in all three wells. Samples that return a low number of zoospore equivalents in only one well (*) or two wells** (from a total of three) are defined as "indeterminate" (?) and should be re-examined from further/additional samples. Samples exhibiting inhibition of the internal positive control are indicated with #. Several samples exhibited inhibition at 1/10 dilution. These were repeated at 1/100. Results for the repeated samples are in parenthesis to indicate they have been re-assayed. Note that these results still may have *, **, ? or # even at the 1/100 dilution.

Authorised by: Alex Hyatt