Herpetological Review, 2011, 42(1), 53–57.

Dynamics of Chytridiomycosis in a Tasmanian Frog Community

Most previous studies of the prevalence of chytridiomycosis (*Batrachochytrium dendrobatidis*, *Bd*) in wild frog populations in Australia have been in tropical or subtropical environments, in areas with predominantly summer rainfall (Kriger and Hero 2007; Kriger et al. 2007; McDonald et al. 2005). As Tasmania is both more temperate and does not have a summer-dominated rainfall pattern, seasonal patterns in prevalence in Tasmania may not be the same as in other parts of Australia. In tropical and subtropical areas of Australia, *Bd* prevalence and clinical disease are both higher in the colder and drier winter months (Berger et al. 2004; Kriger and Hero 2006; Retallick et al. 2004). In a broad-scale latitudinal study of chytridiomycosis in the *Litoria lesueuri* complex in eastern Australia, Kriger et al. (2007) found that prevalence and intensity of *Bd* infection increased with latitude, with an order of magnitude increase in the intensity of infection (as measured by zoospore equivalents) from 17° to 37°S. This latitudinal variation appeared to be related to a negative association between infection intensity

MOHAMED NAJJIB BIN ABDUL AZIZ
School of Zoology, University of Tasmania, Hobart 7001, Australia
LEE F. SKERRATT
Amphibian Disease Ecology Group, School of Public Health
Tropical Medicine and Rehabilitation Sciences, James Cook University
Townsville, Queensland, Australia
HAMISH McCALLUM*
School of Zoology, University of Tasmania, Hobart 7001, Australia
Current address: School of Environment, Griffith University
Brisbane 4111, Australia

*Corresponding author; e-mail: H.McCallum@griffith.edu.au

and temperature in the warmest quarter of the year and a positive association between infection intensity and rainfall in the driest quarter. They suggested that temperate frog populations might therefore be particularly susceptible to chytridiomycosis, especially as substantial population declines have occurred in at least 11 frog species in south-east Australia (Hero et al. 2006). However, the highest latitude they examined was 37.26°S, close to the Victoria-New South Wales border, which is still in a region of predominantly summer rainfall.

Chytridiomycosis has only recently been reported from Tasmania (Obendorf 2005; Obendorf and Dalton 2006; Pauza and Driessen 2008). It has been detected in samples from a number of sites surrounding the World Heritage Area in south-west Tasmania, in tadpoles of all four frog species surveyed (*Litoria burrowsae, L. ewingii, Crinia signifera*, and *C. tasmaniensis*; Pauza and Driessen 2008). There are concerns that it may threaten Tasmania's three endemic anurans (*Litoria burrowsae, Bryobatrachus nimbus* and *Crinia tasmaniensis*; Pauza and Driessen 2008) and also the threatened green and gold frog, *Litoria raniformis* (Obendorf and Dalton 2006). There has not, however, been any quantitative examination of prevalence or intensity of infection in adult frogs, as distinct from tadpoles, in Tasmania. Our objective in this brief study was to address this gap in knowledge by estimating prevalence and intensity of *Bd* infection, and possible effects of *Bd* infection on survival and capture probability in two Tasmanian frog species.

Our study site was in Knocklofty Reserve, Hobart, Tasmania. The reserve usually has several small ponds with the Brown Tree Frog *Litoria ewingii*, the Spotted Marsh Frog *Limnodynastes tasmaniensis*, and the Common Froglet, *Crinia signifera* (http://www.friendsofknocklofty.org/fauna.html; accessed 5 July 2010). These species are common in Tasmania and also are found in southern mainland Australia (Barker et al. 1995). At the time of the study, southern Tasmania was in severe drought, with 24-month cumulative rainfall to 1 March 2008 in the lowest 5% of historical totals (http://www.bom.gov.au/climate/drought/archive/20080304.shtml; accessed 5 July 2010). Only one water body, a small flooded quarry at 42.881°S, 147.303°E, still held water at the time of the study in Knocklofty Reserve. All data collection was therefore concentrated at that site.

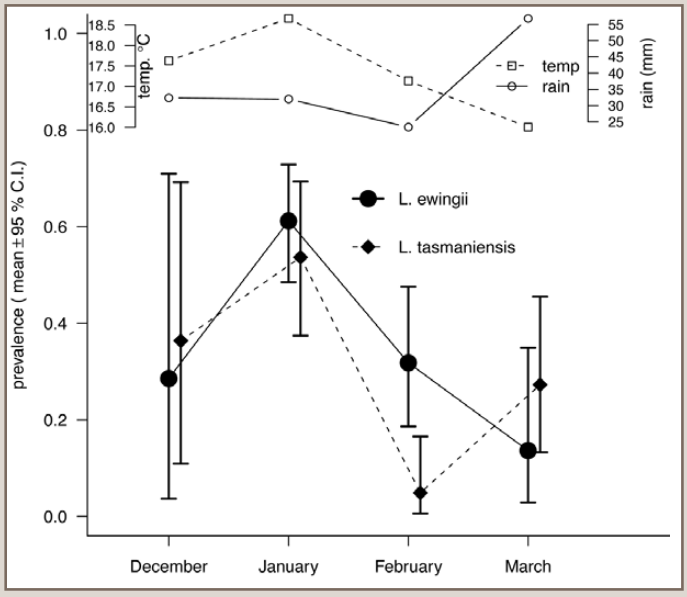

FIG. 1. Prevalence of *Batrachochytrium dendrobatidis* infection in *Litoria ewingii* (solid circles) and *Limnodynastes tasmaniensis* (diamonds) per monthly survey period, December 2007 to March 2008, from Knocklofty Reserve, Tasmania, Australia. Rainfall (circles) and mean temperature (squares) in the 30 days preceding each sampling period are shown.

We conducted fieldwork from December 2007 to March 2008, with monthly field surveys of 4–5 days, conducted in late afternoon and early evening. Frogs were captured by hand after searching under rocks and logs around the edge of the pond. On first capture, adult frogs were measured using calipers and individually marked using toe "tipping." No more than 2 mm of the toe tip was removed and a maximum of one toe tip per foot or 4 toe tips per individual were removed. To minimize spread of disease between individuals, all frogs were placed into individual plastic bags immediately after capture and field gear such as scissors and callipers were disinfected in 70% ethanol between uses on individual frogs. Individual frogs were handled with a new pair of latex disposable gloves. The first time an individual frog was captured during one of the monthly survey periods, it was swabbed for *Bd* detection using a standardized technique as described by Kriger et al. (2006), using individually packed sterile dry swabs (MW100-100, Medical Wire and Equipment Company, Bath, UK). Frogs were swabbed 10 times on their ventral and dorsal surfaces, 10 times on each side from the fore to hind limbs and five times on each foot.

Swabs were processed using real-time quantitative TaqMan PCR (qPCR) as described by Boyle et al. (2004) and Hyatt et al. (2007) with modifications as described by Garland et al. (2009). DNA extracts from swabs were diluted and added to PCR reactions and analyzed in groups of 22 swabs, and in each group, a negative control swab (negative extraction control - NEC) and a positive extraction control swab (PEC) were processed. The TaqMan analyses were performed in triplicate for the sample extracts, NEC, no template controls (NTC) and PEC. Standards (100, 10, 1, 0.1 zoospore equivalents) were analyzed in quadruplicate. Analyses were performed on the Rotor-GeneT 6000 (Corbett Research) using Gene-Disc 100 tubes and a set threshold of 0.01 to determine the PCR cycle at point of detection (Cycle threshold (Ct)). PCR master mix and diluted sample extract, diluted NEC, PEC, water (NTC), or standard, were added to the tubes with a CAS-1200T pipetting robot (Corbett Robotics). NEC, PEC, NTC and standards were analyzed together by qPCR for each group of 22 swabs.

Sample extracts obtaining a negative result for the TaqMan assay were reanalyzed for inhibition by assessing amplification of the TaqMan exogenous internal positive control. Single analyses were performed. If the Ct value of the IPC was above 32 then the extract was diluted to 1 in 100 and a second TaqMan

TABLE 1. Recapture rates and *Batrachochytrium dendrobatidis* (*Bd*) infection status at time of first capture for *Litoria ewingii* and *Limnodynastes tasmanienisis* at Knocklofty Reserve, Tasmania, Australia. Frogs caught in the last of the four sampling periods could not have been recaptured in a subsequent sampling period, hence the percentage of frogs recaptured in the final column is based on frogs captured in the first three sampling periods only. Frogs with uncertain infection status on first capture have been omitted (*L. ewingii*: N = 11; *L. tasmaniensis*: N = 17).

Species	*Bd* infection status at first capture	N	*Bd* prevalence	No. frogs captured in first three sampling periods	No. (%) recaptured in a subsequent sampling period
Litoria ewingii	Negative	77		62	1 (2)
	Positive	56	42%	56	6 (11)
	TOTAL	133		188	7 (6)
Limnodynastes tasmaniensis	Negative	59		50	21 (42)
	Positive	27	31%	23	7 (30)
	TOTAL	88			
Crinia tasmaniensis	Negative	0		0	0
	Positive	1	100%	1	1 (100)

assay (in triplicate) was performed on the inhibited sample. Samples where 1 or 2 only of the triplicate qPCR reactions were positive were regarded as inconclusive and not included in any analyses.

Program MARK (Cooch and White 2010) was used to model capture-mark- recapture data, examining whether *Bd* infection affected frog survival or capture probability. Initially, capture probability models were constructed with fully saturated survival models. Secondly, survival and transition rates were modelled using the best supported capture models from the first step and evaluated using AICc weight. Model selection uncertainty was accounted for by model averaging (Burnham 2002; Burnham and Anderson 2004). Prevalence was analyzed using logistic models implemented in R (R Development Core Team 2007). When the same individual was captured on multiple occasions in the same survey period, it was treated as a single observation and was scored as positive if it was clearly positive (3 of the 3 replicate qPCR reactions) on any of the capture occasions and negative if it was clearly negative (0 of 3 qPCR reactions) on at least one of the capture occasions, even if inconclusive (see above) on some of the others. For analysis of infection intensity (zoospore counts), the maximum count per individual per sampling period was used in the analysis.

At the time of first capture, 42% of *L. ewingii* (N = 133) tested positive for *Bd*, whereas 31% of *L. tasmaniensis* (N = 88) tested positive (Table 1). However, a Fisher's exact test did not reject the null hypothesis of equal prevalence in the two populations (P = 0.09, 95% CI for odds ratio 0.33, 1.11). The one *C. signifera* individual captured was infected with *Bd*.

Overall, *L. tasmaniensis* had a much higher recapture rate (38%) than *L. ewingii* (6%). A logistic model of recapture probability in subsequent sampling intervals as a function of species and infection status at the time of first capture showed that there was a significant interaction term (χ^2 = 5.52, 1 df, P = 0.019). A borderline significantly higher (P = 0.052 ; Fisher's exact test) proportion of infected *L. ewingii* were recaptured (10.7%) as compared to noninfected individuals (1.6%). In *L. tasmaniensis*, there were more recaptures of noninfected individuals (42%) as compared to infected frogs (30%), although a Fisher's exact test did not reject the null hypothesis of equal recapture probability between these species (P = 0.44).

Although the prevalence of infection differed between *L. tasmaniensis* and *L. ewingii* captured in each of the four monthly survey periods (Figure 1; logistic model of prevalence as a function of species and collection month had a significant interaction term; χ^2 = 11.2, 3 df, P = 0.01), inspection of the data suggested that prevalence was similar for both species in December, January, and March, but was significantly lower in *L tasmaniensis* than in *L. ewingii* in February. Whereas the mean temperature in the 30 days preceding the sampling period appeared to be positively associated with prevalence (χ^2 = 29.8, 1 df, P = 0.00001; see Fig. 1), a model including month, species and their interaction fitted the data significantly better than a model including species, temperature and an interaction (χ^2 = 17.3, 4 df, P = 0.002), indicating that temperature was not a sufficient explanation for the observed differences in prevalence between months and species. Similarly, although the interaction between species and the total rainfall in the 30 days preceding the sampling period was associated with prevalence (χ^2 = 4.35, 1 df, P = 0.03), it was not sufficient to explain the observed differences in prevalence (χ^2 = 43.0, 4 df, P ≈ 0).

Capture-Mark-Recapture modelling was only possible for *L. tasmaniensis* because the recapture rate for *L. ewingii* was too low to provide sufficient data. Models with constant capture probability and models with capture probability dependent on infection status were almost equally well supported (AICc weights 0.33 and 0.36, respectively). Models with survival dependent on disease status were more weakly supported (total AICc weight 0.19) than those in which survival was independent of disease status (Total AICc weight 0.44). Model-averaged estimates of *L. tasmanienisis* monthly survival were

therefore almost identical for diseased individuals (0.60, SE = 0.16) and non-diseased individuals (0.59, SE = 0.13), whereas the model-averaged capture probability for diseased individuals was 0.41 (SE = 0.13), in comparison with 0.55 (SE = 0.16) for non-diseased individuals. Models including an effect of temperature on either recapture (Total AICc weight 0.18) or survival (Total AICc weight 0.25) were not well supported.

Geometric mean infection intensity (as measured by zoospore counts) in infected frogs was 24.0. There was no evidence that the monthly variation in this intensity differed between the species ($F = 1.50$, $df = 3, 87$; $P = 0.22$) or that the intensity of infection differed between the species overall ($F = 0.77$, $df = 1, 7$; $P = 0.38$). However, the difference in infection intensity between months was of borderline significance ($F = 2.70$, $df = 3, 87$, $P = 0.051$).

We found evidence that the proportion of infected frogs that were recaptured, relative to the proportion of uninfected frogs that were not recaptured, differed significantly between *L. ewingii* and *L. tasmaniensis*. Several previous studies have used the proportion of frogs recaptured as a proxy for survival but such an effect can be due to differences in survival or catchability. In this case, the difference in recapture rates appeared primarily to be due to higher recapture rates of infected *L. ewingii* compared to uninfected *L. ewingii,* which suggests that there may be behavioral differences between infected and uninfected frogs rather than reduced survival of infected frogs. Whether it is infection status, perhaps by making frogs less active, which increases their probability of recapture, or whether frogs with certain behaviors (perhaps those which are most closely associated with the water body) are more likely to be both infected and recaptured cannot be determined from our results. Overall, the recapture rate of *L. ewingii* was much lower than that of *L. tasmanienisis*. We can only speculate on why this should have been the case, but it is likely to be the result of behavioral differences between the two species.

Using mark-recapture methods, we were unable to detect any effect of *Bd* on the survival of *Limnodynastes tasmaniensis*, despite a relatively high prevalence of *Bd* infection. However, we conducted our study in summer only and there may be mortality due to *Bd* in other seasons. Mortality mainly occurs in winter and spring in northern Australia (Berger et al. 2004; Murray et al. 2009). Akaike weights for models with constant recapture probability and recapture probability depending on disease status were approximately equal and there is insufficient evidence to reject a null hypothesis that catchability is equal between infected and uninfected *L. tasmaniensis*. However, the significant difference between the two species in the effect of infection on recapture probability cautions that infection may influence the probability of capture. Not only does this mean that differences between species in the proportion of frogs recaptured as a function of infection status may not be indicative of differences in mortality induced by the pathogen, but it also means that prevalence in any sample may not be an unbiased estimate of prevalence in the frog population as a whole (Jennelle et al. 2007). This has potential implications beyond our study. With the exception of Retallick et al. (2004) and Murray et al. (2009), neither of which found any indication that infection by *Bd* affected catchability, we know of no other study that has explicitly investigated possible differences in catchability between infected and uninfected frogs. This means that all conclusions drawn about prevalence in populations based on prevalence in samples of adult frogs where catchability of infected frogs has not been assessed need to be treated with caution.

Interestingly, we found that the monthly changes in apparent prevalence differed between our two study species. Although prevalence peaked in both species in January and declined by February, in *L. ewingii* it continued to decline in March, whereas in *L. tasmaniensis* it rose again. This may be a result of behavioral differences between the two species, but it does suggest that the dynamics of infection in these two species, although they were captured around the same water body, are not tightly linked. Little is known about how *Bd* is transmitted in the field. In experimental situations, transmission through water containing zoospores has been demonstrated several times (Berger et al. 1998; Rachowicz and Briggs 2007; Rachowicz and Vredenburg 2004). If this were the predominant means of transmission in the field and species were similar in their contact rate with water, susceptibility to infection and mortality and recovery rates, one might expect that temporal patterns in prevalence would be synchronous in all species sharing the water body in which transmission occurred. Another possibility is that transmission may occur through direct contact between individual frogs, particularly through shared use of shelter sites (Rowley and Alford 2007). If this is the predominant route of infection and there are differences in habitat use between species, then disease dynamics in different species would be less tightly coupled than if transmission occurred predominantly through a shared water body. Our results are consistent with either infection occurring predominantly through a frog-to-frog contact or differences in contact rate with water, susceptibility to infection, mortality and recovery rates.

We detected substantial levels of *Bd* infection in post-metamorphic *L. tasmaniensis.* In a broadscale survey of clinically ill frogs, Berger et al (2004) found six adult *L. tasmaniensis* to have died from chytridiomycosis. Obendorf and Dalton (2006) also reported *Bd* infection in *L. tasmaniensis* but their study investigated only tadpoles. In contrast, Woodhams et al. (2007) attempted to experimentally infect four Australian frog species (*Litoria caerulea, Mixophyes fasciolatus, Litoria chloris* and *L. tasmaniensis*) with *Bd* spores. Only *L. tasmaniensis* failed to become infected and had 100% survival over the course of the experiment. Furthermore, they found evidence that peptide skin secretions of *L. tasmaniensis* inhibited growth of *Bd* in vitro. These conflicting results highlight the importance of using caution when extrapolating laboratory experimental results to the field.

Comparison of infection levels, both in terms of intensity and prevalence, is problematical between sites, species and

seasons, even if a standardized swabbing protocol has been applied in each case. However, it is worth noting that the intensity of infection in our study in both species (approximately 30 zoospore equivalents) was relatively low in comparison with the mean intensity of about 1000 zoospore equivalents reported by Kriger et al (2007) and Kriger and Hero (2006) in *L. wilcoxii* at a similar mean temperature. Pathogenesis of *Bd* is dependent on intensity of infection and may explain the lack of mortality observed in this study (Voyles et al 2009).

Acknowledgments.—This project was funded by an Australian Research Council Discovery Grant (DP0451402) and was carried out under University of Tasmania Animal Ethics Permit A0009732 and a Scientific permit from the Wildlife Management Branch, Department of Primary Industries and Water, Tasmania. We are indebted to David Obendorf for assisting with site selection and frog identification, to Shelly Lachish for assisting with the MARK analysis, Stephen Garland for conducting the PCR analysis at James Cook University and Danait Belay, Sophie Karpiniec, Josiane Eve, Nabil Taff and Jiaxin Li for assisting with the fieldwork.

Literature Cited

Barker, J., G. Grigg, and M. Tyler 1995. A Field Guide to Australian Frogs. Surrey Beatty & Sons, Chipping Norton, New South Wales, Australia.

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, H. B. Hines, K. R. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc. Natl. Acad. Sci. USA 95:9031–9036.

———, ———, H. B. Hines, G. Marantelli, A. D. Hyatt, K. R. McDonald, L. F. Skerratt, V. Olsen, J. M. Clarke, G. Gillespie, M. Mahony, N. Sheppard, C. Williams, and M. J. Tyler. 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Aust. Vet. J. 82:434–439.

Boyle, D., D. Boyle, V. Olsen, J. Morgan, and A. Hyatt. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis. Aquat. Org. 60:141–148.

Burnham, K. P. 2002. Model Selection and Multi-model Inference: A Practical Information-theoretic Approach. Springer, New York.

———, and D. R. Anderson. 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociological Methods Research 33: 261–304.

Cooch, E., and G. White 2010. Using MARK—a gentle introduction. http://www.phidot.org/software/mark/docs/book/ (accessed 7 July 2010).

Garland, S. A. Baker, A. D. Phillott, and L. F. Skerratt. 2009. BSA reduces inhibition in a TaqMan® assay for the detection of *Batrachochytrium dendrobatidis*. Dis. Aquat. Org. DOI:103354/dao02053

Hero, J.-M., C. Morrison, G. Gillespie, J. D. Roberts, D. Newell, E. Meyer, K. R. McDonald, F. Lemckert, M. Mahony, W. Osborne, H. B. Hines, S. Richards, C. Hoskin, J. Clarke, N. Doak, and L. Shoo. 2006. Overview of the conservation status of Australian frogs. Pac. Conserv. Biol. 12:313–320.

Hyatt, A. D., D. G. Boyle, V. Olsen, D. B. Boyle, L. Berger, D. Obendorf, A. Dalton, K. Kriger, M. Hero, H. Hines, R. Phillott, R. Campbell, G. Marantelli, F. Gleason, and A. Colling. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Dis. Aquat. Org.73:175–192.

Jennelle, C. S., E. G. Cooch, M. J. Conroy, and J. C. Senar. 2007. State-specific detection probabilities and disease prevalence. Ecol. Appl. 17:154–167.

Kriger, K. M., and J. M. Hero. 2006. Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. J. Zool. 271:352–359.

———, and ———. 2007. The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. Divers. Dist. 13:781–788.

———, H. B. Hines, A. D. Hyatt, D. G. Boyle, and J.-M. Hero. 2006. Techniques for detecting chytridiomycosis in wild frogs: comparing histology with real-time Taqman PCR. Dis. Aquat. Org. 71:141–148.

———, F. Pereoglou, and J.-M. Hero. 2007. Latitudinal variation in the prevalence and intensity of chytrid (*Batrachochytrium dendrobatidis*) infection in eastern Australia. Conserv. Biol. 21:1280–1290.

McDonald, K. R., D. Méndez, R. Müller, A. B. Freeman, and R. Speare. 2005. Decline in the prevalence of chytridiomycosis in frog populations in north Queensland, Australia. Pac. Conserv. Biol. 11:114–120.

Murray, K. A., L. F. Skerratt, R. Speare, and H. McCallum. 2009. The impact and dynamics of disease in species threatened by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*. Conserv. Biol. 23:1242–1252.

Obendorf, D. L. 2005. Application of field and diagnostic methods for chytridiomycosis in Tasmanian frogs. Central North Field Naturalists Inc. Tasmania, Australia, Launceston, Tasmania.

———, and A. Dalton. 2006. A survey for the presence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in Tasmania. Pap. Proc. Royal Soc. Tasmania 140:25–29.

Pauza, M., and M. Driessen. 2008. Distribution and Potential Spread of Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis* in the Tasmanian Wilderness World Heritage Area. Department of Primary Industries, Parks, Water and Environment, Tasmania, Hobart. Available from http://www.dpiw.tas.gov.au/inter.nsf/Attachments/LJEM-7BY3CR/$FILE/Chytrid%20Report%20Feb%202008.pdf (accessed 7 July 2010)

R Development Core Team 2007. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Rachowicz, L. J., and C. J. Briggs. 2007. Quantifying the disease transmission function: effects of density on *Batrachochytrium dendrobatidis* transmission in the mountain yellow-legged frog *Rana muscosa*. J. Anim. Ecol. 76:711–721.

———, and V. T. Vredenburg. 2004. Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. Dis. Aquat. Org. 61:75–83.

Retallick, R. W. R., H. McCallum, and R. Speare. 2004. Endemic infection of the amphibian chytrid fungus in a frog community post-decline. PLoS Biology 2:e351.

Rowley, J. J. L., and R. A. Alford. 2007. Behaviour of Australian rainforest stream frogs may affect the transmission of chytridiomycosis. Dis. Aquat. Org.77:1–9.

Voyles, J., S. Young, L. Berger, C. Campbell, W. F. Voyles, A. Dinudom, D. Cook, R. Webb, R. A. Alford, L. F. Skerratt, and R. Speare. 2009. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. Science 326:582–585.

Woodhams, D. C., K. Ardipradja, R. A. Alford, G. Marantelli, L. K. Reinert, and L. A. Rollins-Smith. 2007. Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. Anim. Conserv. 10:409–417.