

**FINAL REPORT TO DEPARTMENT OF THE ENVIRONMENT, WATER,
HERITAGE AND THE ARTS ON WORK COMPLETED FOR RFT 43/2004,**

“Experimental research to obtain a better understanding of the pathogenesis of chytridiomycosis, and the susceptibility and resistance of key amphibian species to chytridiomycosis in Australia.” May 2009

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Executive Summary

Introduction

The call for tenders RFT43/2004 designated 6 objectives to be addressed. In the proposal we submitted in response to RFT 43/2004, we described 9 projects, each of which addressed some or all of these objectives. All of the projects were designed to share some resources with the projects we designed in our response to RFT 42/2004, and some were partially or wholly subsumed within the work to be carried out in that submission. Similarly, some of the 9 projects outlined in our proposal for RFT43/2004 addressed data needed in our response to RFT 42/2004. Both reports therefore refer to each other where appropriate.

The tenders were called in 2004 and projects were initially designed to be completed within three years. Some projects were delayed by the need to recruit qualified staff and students, and funds were not expended as fast as initially anticipated, so the projects were extended for a fourth year. All are now complete, although some data collected remain to be written up.

RFT 43/2004 identified six major objectives. Objectives 1, 2, 4, 5, and 6 are highly interrelated and were addressed by a series of linked projects. Objective 3 was addressed by a more clearly separate project. We will present Objectives 1, 2, 4, 5, and 6, and then summarise the findings dealing with those. We will then present Objective 3 and a summary

Objectives identified in RFT 43-04

1. Build on our understanding of the pathogenesis of chytridiomycosis including the host and environmental factors that determine the ultimate outcome of infection, i.e., death, persistent infection with no obvious effect, and cure.
2. Investigate populations of frog species that have undergone widespread chytrid associated decline to:
 - i. identify the factors that maintain these populations, and
 - ii. develop and assess management activities that can reproduce these factors in other populations, particularly threatened species.
3. Investigate the manifestation of chytridiomycosis by determining the physiological mechanisms associated with morbidity and mortality of affected frogs.
4. Determine whether populations that have recovered after chytrid-associated decline are susceptible to future severe effects from chytridiomycosis.
5. Determine the resistance of key frog species (how key frog species are determined must be clearly identified by the tenderer in their proposal) to chytridiomycosis using a standard laboratory model that relates to risk in the wild, particularly for species that are currently chytrid-free, or species that occur in areas where the status of chytrid is unknown.
6. Search for and examine evidence of resistance to chytrid and research techniques to increase resistance in at risk species (how at risk species are determined must be clearly identified by the tenderer in their proposal), including captive breeding and selection for restocking.

Results addressing Objectives 1, 2, 4, 5, and 6

We addressed these objectives simultaneously, using a combination of comparisons among natural populations, field experiments, laboratory experiments, and chemical analysis of host antimicrobial peptides.

We found that host and environmental factors interact in complex ways to determine the probability of acquiring infections by *B. dendrobatidis* and the ultimate outcome of those infections. Host behaviour, in the form of social behaviour and microenvironmental use, can have a strong effect on the probability of acquiring infections. Radio- and harmonic radar-tracking studies of three species (*Litoria genimaculata*, *L. nannotis*, and *L. wilcoxii/jungguy* complex) showed that encounter rates among individuals within species varied in a way that could explain differences in the probability of becoming infected. We examined unoccupied frog retreat sites for the presence of *B. dendrobatidis* DNA using swabbing samples analysed using diagnostic quantitative PCR, and found no evidence that infective stages of the pathogen remain for even short periods after retreat sites are occupied by frogs. In laboratory experiments, we demonstrated that frogs can acquire infections from contact with water containing infective zoospores. It thus appears that either direct contact among individuals, or contact with bodies of water containing zoospores, are the usual routes by which frogs acquire infections. The species that suffered the strongest effects during initial outbreaks of chytridiomycosis, *L. nannotis*, usually spends the day in communal retreat sites in crevices in rocks in the spray zone of fast moving water. This provides an ideal environment for the transmission of the pathogen, since frogs are close together and often make physical contact or make contact with substrate immediately after it is vacated by other individuals, and the films of moisture usually present in these locations provide a means for the aquatic infective zoospores of *B. dendrobatidis* to remain viable and move from host to host. The species (*L. genimaculata*) that suffered intermediate impacts at the population level has intermediate levels of exposure to transmission via simultaneous occupation of retreat sites and contact among individuals, while *L. wilcoxii/jungguy* complex has the lowest levels. Our results make it clear that knowledge of species' social behaviour and retreat site selection can improve our ability to predict their vulnerability to acquiring *B. dendrobatidis* infections, and thus their vulnerability to epidemic outbreaks of chytridiomycosis.

In addition to affecting the probability of transmission, our tracking and experimental data indicate behaviour affects the persistence of infections and the fate of infected individuals. Previous work by our group (Woodhams et al 2003) demonstrated that elevated body temperatures can cure frogs of infection by *B. dendrobatidis*. We found that relatively short term (2 week) patterns of body temperature variation can explain a substantial proportion of both interspecific and intraspecific variation in the prevalence of infection. Across all three species, individuals who increased their body temperatures above 25 degrees C were only 1/3 as likely to carry *B. dendrobatidis* infections as those who did not. The proportion of individuals that attained those temperatures was highest in the species (*L. wilcoxii/jungguy* complex) that has the lowest natural prevalence of *B. dendrobatidis* infections, intermediate in *L. genimaculata*, which has intermediate prevalences, and lowest in *L. nannotis*, which has the highest prevalences in the field.

To examine in more detail whether and how frog behaviour affects the transmission of *B. dendrobatidis* and the fate of infected individuals, we performed a series of experiments in standard laboratory environments. One series of experiments examined whether frogs can detect and avoid water containing *B. dendrobatidis* zoospores. He hypothesised that this might happen either because the process of zoospores encystment and infection may

produce local irritation to frogs' skins, or because frogs might detect chemical cues given off by *B. dendrobatidis*, as they have been demonstrated to do for some aquatic predators. The results of these experiments indicate that two species we examined (*L. caerulea* and *L. genimaculata*) have a significant tendency to avoid water containing *B. dendrobatidis* zoospores. Some individuals of each species show this very strongly, while others do not, but overall they show a statistically significant ability to avoid infected water. This may account for some of the lower vulnerability to chytridiomycosis that these species exhibit in the field. In a second set of experiments we constructed a set of standard environmental chambers that allowed frogs to choose both temperature (along a gradient from cool to very warm) and humidity (high or low) environments. We placed infected and uninfected *L. genimaculata*, *L. caerulea*, and *L. jungguy* in these enclosures and monitored their behaviour and intensity of infection in infected individuals. We found no evidence that individuals infected with *B. dendrobatidis* altered their choice of diurnal resting sites. This suggests that none of these species exhibits the phenomenon known as "behavioural fever", in which some ectothermic animals alter their behaviour to elevate their body temperatures in response to infections. However, both infected and uninfected individuals of all three species chose microenvironments that would slow the development of *B. dendrobatidis* infections or cure infections, because they chose relatively high temperatures and low humidities at least some of the time. Any future experiments conducted to examine the potential vulnerability of species to chytridiomycosis should use experimental environments similar to ours. Confining experimental frogs to containers that offer only a constant environmental temperature (usually between 17 and 23 °C, exactly within the optimum range for rapid growth of *B. dendrobatidis*, and saturated humidity, also ideal for *B. dendrobatidis* proliferation) provides the optimal conditions for growth of *B. dendrobatidis*. The results of experiments conducted under such conditions are likely to indicate that species are vulnerable to chytridiomycosis when they may actually be invulnerable under field conditions, where they can choose warmer and/or dryer microenvironments.

The results of this part of our study imply that elevation is not the only macroenvironmental factor that can determine the outcome of emergence of chytridiomycosis in a naïve population; it is likely that cloud cover and canopy cover also have effects, since they will alter the access of species to warmer, dryer retreat sites. Species that occur at a narrow range of elevations are more likely to survive in parts of their range with more open canopies or less cloud cover. This prediction was confirmed by our documentation of several generally unknown frog populations during this research. We carried out surveys of streams at high elevations, well above 400m, but outside the western boundary of the Wet Tropics rainforest. We initially documented populations of the endangered Waterfall Frog, *Litoria nannotis*. These populations occur at high local densities, much higher than the densities of this species at rainforest sites it has recolonised after the local extinctions caused by chytridiomycosis in the early 1990s. They presently coexist with high prevalences of infection with *B. dendrobatidis*, and repeated sampling has demonstrated that many individuals coexist with infections for extended periods. Although their history is not known, it is likely that these populations did not suffer to the same extent as rainforest populations during the initial outbreaks of chytridiomycosis. Tracking and environmental monitoring at these sites has confirmed that at them frogs experience dryer and hotter conditions than in adjacent rainforest sites. This indicates that surveys and conservation efforts should not overlook habitats peripheral to, and even believed to be outside the range of, species under threat from chytridiomycosis. When, as is the case for rainforest stream frogs, environmental conditions at the core of species' ranges coincide with conditions favourable to chytridiomycosis, peripheral populations may be highly important for the continued

survival of species, giving them access to environmental conditions that provide refuge from the disease. The importance of the idea that peripheral populations in environmental refuges may be critical for the survival of species is reinforced by our discovery, during this research, of a previously unknown population of the Armored Mistfrog, *Litoria lorica*. This species was only known from closed-canopy rainforest sites at high elevations. It disappeared from all known sites during the epidemic outbreaks of chytridiomycosis in the early 1990s. It had not been seen since 1992, and although it was listed as critically endangered, was widely presumed to be extinct. We carried out a survey in June 2008 along a section of high elevation stream in dry sclerophyll forest outside the western boundary of the Wet Tropics on the Carbine Tableland, and discovered a population of this species, occurring at high local densities, occupying several kilometres of the stream. Swab sampling and diagnostic qPCR revealed very high rates of infection of frogs, greater than 80% in winter. Repeat sampling in 2009 showed that individuals are persisting with the infection for extended periods and the population density remains relatively constant. The area has been protected under Queensland legislation, and further research is planned to increase our understanding of how this species, and a dense sympatric population of *L. nannotis*, persist despite very high prevalence of *B. dendrobatidis*.

It has been well documented that the prevalence of infections by *B. dendrobatidis* fluctuates seasonally. It can reach very low levels during the warm summer months, but can then increase rapidly during the winter months. Because frogs appear to acquire infections either through direct contact among individuals or through contact with water containing infective zoospores, the source of this rapid increase in prevalence has been puzzling. It suggests that there is a source of zoospores other than frogs. Our examinations of frog retreat sites established that zoospores do not appear to persist in them. We also sampled a wide range of environmental substrates and potential alternative hosts. Preliminary results suggested that *B. dendrobatidis* might survive on the exoskeletons of stream-dwelling prawns and crayfish, but experimental work and extensive sampling, plus re-examination of our original data, refuted this. However, in Wet Tropics rainforests and many other habitats containing permanent water, the tadpoles of frogs are present all year. Tadpoles are known to carry infections of *B. dendrobatidis* in their keratinised mouthparts. We therefore carried out a series of detailed field studies on tadpoles. We focused primarily on tadpoles of the waterfall frog, *L. nannotis*. This species breeds in an extended period over summer, so that hatchling tadpoles enter the stream between approximately September and March in most years. Most tadpoles remain in the stream through winter and metamorphose in the following summer, also over several months. We found the prevalence and intensity of infection fluctuated seasonally, in a manner similar to that seen in frogs but with a different mechanistic explanation. Hatchling tadpoles enter the stream in large numbers over the summer period, and are initially uninfected. Prevalence increases with residence time, so that by April/May it approaches or reaches 100%. Experiments with tadpoles maintained in containers in the stream showed that isolated individuals can acquire infections through contact with stream water, and that individuals at higher density acquire infections more rapidly than isolated individuals, so infections will build up more rapidly where tadpole populations are dense.

Because the infection occurs only in the mouthparts, infected tadpoles do not grow and develop as rapidly as uninfected conspecifics, but mortality is rare; they can survive, feed, and grow even with extensive mouthpart damage. By April/May of each year, the high densities of tadpoles, combined with high prevalences of *B. dendrobatidis* infection, form a major source of infective zoospores in the aquatic environment. It is likely that the rapid increase in prevalence among frogs at this time is in part a consequence of contact with

water containing zoospores shed by infected tadpoles. In midwinter many infected tadpoles shed their mouthparts; prevalences decrease and some individuals lose their infections entirely. Uninfected individuals metamorphose earlier than infected ones, so there is always a source of infective zoospores when breeding resumes in spring. The nature of this annual cycle means that even if prevalence drops to zero or near zero in terrestrial frogs over summer, infected tadpoles form an interspecific reservoir that maintains the pathogen in the population, and frogs reacquire infections from water as temperatures drop and tadpole prevalence increases in autumn. This result indicates that it will be difficult or impossible to eliminate *B. dendrobatidis* once it is established in a population of frogs with overwintering tadpoles; even if the infection was eliminated from all terrestrial individuals, it would persist in tadpoles and re-emerge. Successful treatment would require simultaneous elimination of the pathogen from terrestrial juveniles and adults and aquatic tadpoles. It may be possible to eliminate the infection by treatment of the terrestrial stages of frogs that occur in isolated populations and do not have overwintering tadpoles.

Although host behaviour, environment use, and intraspecific reservoirs clearly play a major role in determining susceptibility to acquiring infections and developing disease, our research also showed that aspects of the innate immune system have strong effects. It has been known for some time that frogs secrete a complex mixture of peptides from skin glands, and that some of these have antimicrobial properties (AMPs). Earlier work at James Cook University showed that, at the level of species, the effectiveness of these antimicrobial peptides against *B. dendrobatidis* was correlated with the probability of species declining during outbreaks of chytridiomycosis. The work carried out under Tender 43/2004 expanded on that earlier work by examining how the effectiveness of AMPs varies among individuals within species across sites with different histories of effects from chytridiomycosis and across seasons.

High elevation populations have been subjected to strong natural selection because they declined during outbreaks of chytridiomycosis in the 1990s. We hypothesised that if AMPs are responsible in part for the recovery of these populations, frogs in them should now have more peptides, or more effective peptides, than those in populations that did not decline during the initial epidemics of chytridiomycosis. We found that the effectiveness of AMPs varies among sites in a manner consistent with this hypothesis. In both *Litoria genimaculata* and *L. rheocola*, a greater proportion of individuals from high elevation populations secreted peptides that inhibited the growth of *B. dendrobatidis* *in vitro*. We found no evidence for changes in the effectiveness of peptides per unit mass against *B. dendrobatidis*, indicating that frogs have responded to selection by producing larger quantities of the same peptides, rather than by evolving new peptides. We also found that the proportions of frogs producing effective peptides are greater in winter, when the prevalence of infection by *B. dendrobatidis* is higher and environmental factors that prevent the development of disease are less effective. The composition of peptides also varies between winter and summer, and peptides are more effective per unit mass against *B. dendrobatidis* in winter than in summer. This probably is not a response to selection via chytridiomycosis, but is likely to reflect a long evolutionary history in which frogs are more vulnerable to many infections during the cooler months, when their metabolic activity, and thus the rate of response of their adaptive immune system, is lower, and opportunities for attaining elevated body temperatures are reduced.

In addition to the work summarised above, we carried out a set of targeted field and laboratory studies on microhylid frogs. There are at least 20 species of microhylids in the Wet Tropics region, including many species with relatively limited ranges, some of which

are listed because of small range or population size. No species or populations are known to have declined. Only a single individual has been reported to be infected with *B. dendrobatidis* in nature, and none have been demonstrated to be infected in captivity. We examined a total of 595 samples from different individuals of nine species of direct-developing Australian microhylids for the presence of *Batrachochytrium dendrobatidis* infection. Of these, 336 were historic samples collected between 1995 and 2001 and 259 were swab samples collected during 2005-2008. One hundred and two of the historic samples were examined histologically, and the remaining 493 samples were examined using diagnostic quantitative PCR. None of the 595 samples showed evidence of infection by Bd. If these data are regarded as a single sample representative of Australian microhylids, the upper 95% binomial confidence limit for the prevalence of infection in frogs of this family is 0.0062 (less than 1%). Even if only the data from the more powerful diagnostic qPCR tests are used, the upper 95% confidence limit for prevalence is 0.0075, well under 1%. Our data thus strongly suggest that Australian microhylids have an extremely low prevalence of Bd in nature, and are either not susceptible, or are only slightly susceptible, to chytridiomycosis. They, and perhaps some other direct-developing species, may be highly resistant to Bd because they possess antimicrobial symbionts in skin flora or in skin secretions as a means to reduce fungal infestations that would otherwise overwhelm terrestrial egg clutches.

To determine whether very powerful antimicrobial peptides might be responsible for protecting microhylids from infection by *B. dendrobatidis*, we sampled AMPs from 81 individuals belonging to six species. Analysis of the activity of their AMPs against *B. dendrobatidis* indicated that they were effective, but no more effective than the AMPs of stream-breeding species. This indicated that the factor or factors that provide the almost perfect protection from *B. dendrobatidis* infection that microhylids experience in the field must be something other than AMPs. If the protection arises from microenvironment use, it should be lost when animals are maintained in isolated laboratory environments under constant temperature and humidity conditions that favour the growth and development of *B. dendrobatidis*. If it arises from symbiotic skin microbes, it might be reduced under laboratory conditions because the environment is more constant, environmental sources of colonising microbes are absent, and bathing frogs during exposure experiments could reduce or remove some skin microbes.

We tested this hypothesis by carrying out a laboratory infection experiment. Adult male microhylids (*Cophixalus ornatus*), and hylids (*L. wilcoxii*) were housed in similar containers in a laboratory at JCU. *Litoria wilcoxii* served as known susceptible controls. Infection experiments involved bathing all experimental and control individuals in *B. dendrobatidis* culture solution, which either contained zoospores harvested from laboratory cultures for experimental animals or was sterile for controls.

We carried out a series of experimental exposures using increasing concentrations of zoospores. Exposure to concentrations of 10,000 zoospores/mL produced infections in both species. All exposed *Cophixalus ornatus* became infected. They reached maximum infection intensity rapidly (7-28 days post-exposure). Maximum intensity was relatively low, with no infected animals returning more than 78 zoospore equivalents from a standard swab sample, and only one individual *C. ornatus* remained infected 35 days after exposure. This experiment demonstrated that when maintained in environmental conditions that reduce skin microbiota and favour the growth of *B. dendrobatidis*, microhylids are not constitutively immune to infection by *B. dendrobatidis*. However, it also demonstrates that even under those conditions, they are capable of rapidly and effectively combating the infection, not allowing it to progress to high intensity and

eventually clearing it. Further work is needed to determine the mechanisms underlying the very low susceptibility of microhylids to *B. dendrobatidis*. This should focus on their skin microbiota, which may have been reduced in effectiveness by the experimental conditions but increased in effectiveness as frogs were left undisturbed, eventually clearing their infections, and on their adaptive immune defences, which is the only remaining system if AMPs, microenvironmental effects, and skin microbes are eliminated.

Results addressing Objective 3

We found that the development of infections on frogs in nature follows a pattern consistent with the hypothesis that they typically become infected by contact of their posterior abdomen or feet with substrates (possibly including other frogs) contaminated by *B. dendrobatidis* zoospores. As infection intensity increases, other body regions become more infected. The dorsal surface usually becomes infected last and infections on the dorsal surface typically do not reach high intensities. This result is important for the collection of diagnostic samples by swabbing; it suggests that, until more is understood, swab samples should be collected from body parts in an order that minimises the chance of moving zoospores from more heavily infected body regions to less heavily infected ones. Our suggested swabbing patterns is dorsal surface, then feet, then thighs and posterior abdomen (Project 6.6). These results have been published as North, S., and Alford, R. A. 2008. Diseases of Aquatic Organisms 81:177-188. to ensure that they are accessible to the larger research and management communities.

In heavy infections, we found that frogs dying of chytridiomycosis lose control of their plasma electrolytes, then develop cardiac arrhythmia and ultimately heart failure. The results are consistent with a disturbance to the sodium-potassium ionic balance of plasma caused by damage to the skin, particularly the ventral skin, simply causing heart failure (Projects 6.4 and 6.5). This is consistent with other work we have been involved in, which show that the symptoms that characterise the disease chytridiomycosis occur when the concentration of *B. dendrobatidis* thalli in the skin reach a critical threshold level. These results are elaborated on in the detailed report on Project 6.

Supporting research/technique development and testing

The work we carried out to directly address the objectives of Tender 43-04 required the development and refinement of many techniques that will now be available for further work in this field. We tested the efficacy of various reagents for denaturing the DNA of *B. dendrobatidis*, and developed criteria for the use of sodium hypochlorite; this is essential to ensure that samples taken for diagnostic purposes and epidemiological research are not cross-contaminated (Project 4.1). We examined the effects of sampling techniques for detection of the DNA of *B. dendrobatidis* using real time PCR. Our results showed that the standard swabbing technique used to detect the pathogen is highly efficient and sensitive, and that it is possible to detect it in environmental samples via water filtration (Project 4.2). Unexpectedly, we found that handling tadpoles with certain types of gloves can cause mortality (Project 4.3); those results have been published (Cashins et al., 2008. Herpetological Review 39:298-301). We examined how *B. dendrobatidis* adapts to culture. Many pathogens alter their biology in response to long-term maintenance in cultures. It is often thought that long-term culture leads to the evolution of decreased virulence. We found that cultured *B. dendrobatidis* can adapt to thermal environments between 12 and 23°C, and that rather than decreasing virulence, it is maintained or even increased in long-term culture (Projects 6.2 and 6.3). These results highlight the importance of cryopreserving isolates as they are acquired, so that they can be studied

Summary and recommendations

In summary, our work shows that, in addition to the previously known effects of macroenvironment (populations at elevations below 300-400 m were not seriously affected by initial outbreaks of chytridiomycosis, while populations above those elevations were, and prevalence of *B. dendrobatidis* infections fluctuates seasonally) both behaviour and microenvironment use are important. Species that are more likely to aggregate in retreat sites and/or use relatively cool, moist retreat sites are likely to be more vulnerable to the disease, due both to increased transmission rates and increased rates of intraindividual reinfection. These effects may be exacerbated by droughts, which would force more individuals into less space. Species that occupy warmer, drier diurnal retreats are less vulnerable to infection and to the development of lethal chytridiomycosis. Microenvironmental effects may explain the precipitous, synchronous nature of the outbreaks that caused declines in the 1990s, and could occur again in populations now coexisting with the pathogen. Droughts may increase transmission rates, and prolonged periods of cloudy, moist weather might prevent frogs from experiencing elevated body temperatures and drier skins, allowing pathogen populations to accumulate on infected individuals exponentially and drastically increasing concentrations of infective zoospores in the environment and thus rates of transmission to uninfected individuals. Our results indicate that epidemic outbreaks may occur if the apparently stable host-pathogen relationships that *B. dendrobatidis* has reached with many remaining species are disrupted by climate change or even weather fluctuations. This indicates that ongoing monitoring of apparently stable populations is needed. Our results also strongly indicate that populations on the periphery of species' ranges may be important for conservation, since they experience environmental conditions very different from populations in the core range. If core range populations are attacked by a pathogen such as *B. dendrobatidis*, they may collapse, leaving only peripheral populations. This highlights the need for full, proper surveys to document species' actual ranges, instead of relying on environmental models based on the properties of core populations.

Our laboratory data on microenvironment selection indicate that frogs do not alter their diurnal behaviour in response to infection by *B. dendrobatidis*, but that if given a choice among thermal and humidity environments in captivity, many species will choose environments that decrease the probability of *B. dendrobatidis* infections building up to cause chytridiomycosis. Laboratory experiments aimed at evaluating the vulnerability of species to the disease must present animals with a range of thermal and humidity environments to choose from, or their results will not be meaningful for populations in the field. The enclosures we designed are simple to construct and maintain, and could serve as a standard for this purpose. Our laboratory data also indicate that some species may be able to avoid water containing *B. dendrobatidis* zoospores, but only after entering it, and therefore exposing themselves to infection. However, by avoiding infected water, these species may decrease the probability of infections building up to cause chytridiomycosis. Our data suggest that the innate immune defences, in the form of antimicrobial peptides, of the two species we examined (*L. genimaculata* and *L. rheocola*) have responded to natural selection exerted by chytridiomycosis. The proportions of frogs of both species that secreted peptides effective against *B. dendrobatidis* were greater in high elevation populations that have been subjected to strong selection. This suggests that selective breeding for resistance, or translocation of frogs from populations that have developed resistance naturally, may be a means of increasing the resistance of frogs in vulnerable populations.

Our data on tadpoles indicate that they do serve as intraspecific reservoir hosts. Some proportion of tadpoles are always infected in the many rainforest species that have an annual reproductive cycle, with tadpoles persisting in the stream all year. This may help to explain the greater impact of chytridiomycosis on stream-breeding species, which tend to have long larval periods; pond breeders lack this reservoir host. It may be impractical to eliminate *B. dendrobatidis* from environments in which overwintering larvae are present, as it would need to be removed from both terrestrial and aquatic habitats. We also showed that gloves used to handle tadpoles should be carefully assessed for potential toxic effects before they are used to handle large numbers of individuals.

Our results on pathogenesis indicate that high-intensity infections disrupt the normal functioning of frog skin, leading to ionic imbalance and eventual death through heart failure. Preliminary experiments showed that it may be possible to treat frogs, even in late, symptomatic stages of infections, by providing balanced electrolytes in combination with antifungal agents. At least in the species we studied, low intensity infections occur mostly on the ventral surfaces, particularly the posterior abdomen and thighs, and to a lesser extent on the feet. We suggest that routine swabbing to collect samples for diagnostic PCR analysis should work from areas that become infected later towards those that become infected earlier, to avoid spreading infections on individuals.

We found that the prevalence of infection by *B. dendrobatidis* in frogs of the family Microhylidae is extremely low. This may in part be due to their fully terrestrial life history, which should reduce exposure to infective zoospores. It is not due to extremely effective innate immune defences in the form of antimicrobial peptides, since in laboratory experiments microhylids became infected at the same exposure levels as nonmicrohylids.

However, microhylids cleared infections more rapidly and completely, indicating that some mechanism other than AMPs provides increased resistance. Future research should examine the skin microbiota and inducible immune system of microhylids.

We suggest that there is a need for substantial future research on the microbial assemblage inhabiting frog skin and its interactions with *B. dendrobatidis*. This field was unknown at the time RFT 43/2004 was called and the research program reported on here was developed. Results from North American systems indicate that amphibian skin harbours many microbes that help control the growth and reproduction of *B. dendrobatidis*, that natural populations that show greater resistance to chytridiomycosis harbour more of these species, and that artificial supplementation with “beneficial” bacteria can reduce the severity of chytridiomycosis. Further development of these ideas could produce probiotic means of managing the impact of *B. dendrobatidis* on Australian frog populations, but was beyond the scope of the work we tendered for.

Publications arising from research funded in whole or in part by Tender 43-04

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