6.3 b Chytrid Fungus Aug 2009 R Alford

The host-pathogen biology of the amphibian chytrid skin fungus, *Batrachochytrium dendrobatidis*, and its implications for work proposed to be carried out at Ella Bay, Queensland

21 January 2010

Professor Ross A. Alford, School of Marine and Tropical Biology, James Cook University, Townsville, Queensland 4810, Australia

Email ross.alford@jcu.edu.au

Summary

The amphibian chytrid fungus appears to have been introduced into southeastern Queensland in the 1970s. It rapidly dispersed up the east coast, and must have done so by natural means, since it reached a great many remote sites in the Wet Tropics more or less simultaneously. It caused declines and disappearances of populations of eight species in the Wet Tropics at all sites above 400 m elevation, but despite intensive monitoring has never been shown to cause declines or disappearances at sites below 400m, despite being endemic at all such sites in the Wet Tropics that have been adequately sampled. It occurs at multiple sites within 20 km of the Ella Bay site, and extensive sampling undertaken at multiple locations on the Ella Bay site revealed that the fungus is present there. Since the fungus is already present, and all available evidence indicates that all of the *B. dendrobatidis* in Queensland belongs to a very recently dispersed clone, there is no reason to institute measures to quarantine the site. However, any handling of frogs that may be necessary should be undertaken using proper hygiene procedures to avoid transmitting the infection among individuals.

Background: the amphibian chytrid fungus and Queensland frog declines

It has been more than 20 years since the problem of amphibian declines in Australia was first recognized (Czechura 1990, Richards et al. 1993a, Alford 1999, McDonald 1999). Declines in Queensland followed a clear temporo-spatial sequence (Figure 1): initial declines occurred in the Conondale Ranges of southeastern Queensland, starting in 1979, with the last frogs observed in 1981, followed by declines in Eungella National Park in central Queensland in 1985, then most of the Wet Tropics, possibly starting near the centre of the region in about 1989 but occurring throughout most of the region in 1991, and finally in the northernmost Wet Tropics in 1993 (Czechura 1990, Richards et al. 1993a, McDonald 1994, Laurance et al. 1996, McDonald 1999). Richards et al. (1993a) showed that many species had declined to local extinction over the southern Wet Tropics by the summer of 1991-92. The pattern of declines is summarized in Figure 1.

Laurance et al (1996) were the first to suggest that this pattern might have been caused by the spread of a novel introduced pathogen, leading to a series of epidemic outbreaks in naïve and highly susceptible hosts. The agent that caused the declines was identified by Berger et al (1998) as a chytrid fungus, which was later described (Longcore et al. 1999) as the amphibian chytrid skin fungus, *Batrachochytrium dendrobatidis* (*Bd*). Heavy infections by this pathogen produce the disease chytridiomycosis, which can (but does not always) lead to rapid mortality of frogs belonging to susceptible species.

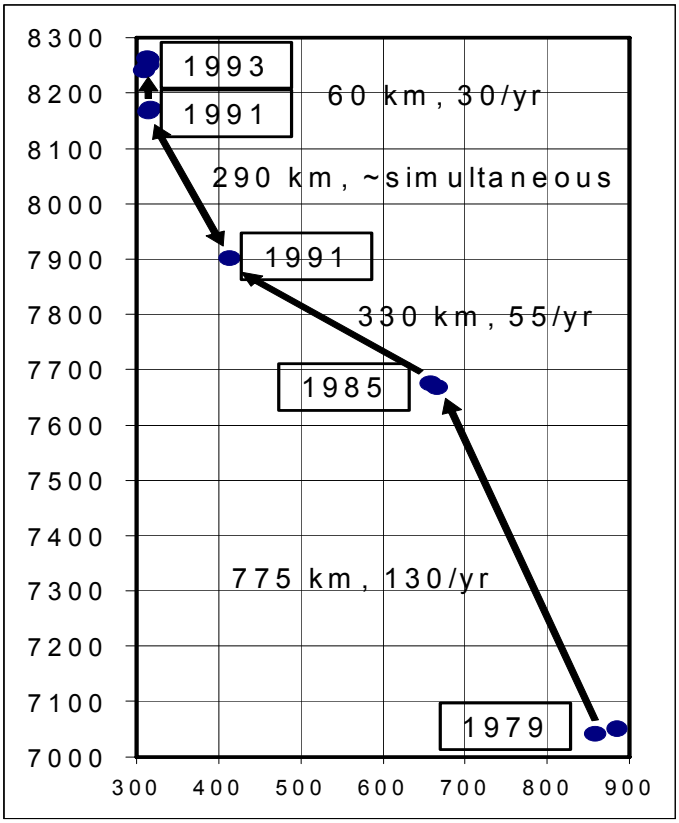

Figure 1. Temporospatial pattern of frog declines in Queensland, 1979-1993. Coordinates follow the Australian Map Grid. Distances and km/year refer to distances between decline events and the rate at which a causative agent would have travelled between them, except for the southern Wet Tropics, where declines happened more or less simultaneously over a 290 km long region.

Chytridiomycosis is an unusual disease in several respects. It is unusual in that it can severely effect many species across most of an entire class of vertebrates (Daszak 1999). On amphibians, the thalli of *B. dendrobatidis* live only in the epidermis, which becomes infected by contact with waterborne flagellated zoospores, and propagate only by producing zoosporangia that release zoospores to the external environment. The symptoms of chytridiomycosis appear only in frogs with relatively heavy infections (Berger et al. 2005, Carey et al. 2006), so the development of chytridiomycosis requires multiple generations of successful colonization and recolonisation of the host by propagules. If the population of the fungus on the frog does not reach critical levels, the infection remains asymptomatic and does not appear to injure the host (Berger et al. 2005, Carey et al. 2006, Voyles and R 2007).

The very broad host range of *B. dendrobatidis*, including larvae and adults of many species that are resistant to chytridiomycosis as well as of many species that are vulnerable to the disease, may explain its unusual ability to drive populations of many species to extinction, since it can persist in less affected reservoir hosts (de Castro and Bolker 2005, Woodhams and Alford 2005). It may also persist outside amphibians; Johnson and Speare (2005) showed that *B. dendrobatidis* can survive for extended periods in the laboratory on a variety of potential environmental substrates, and Lips et al. (2006) found *B. dendrobatidis* DNA on one of nine haphazardly-chosen stream boulders. Most authorities now agree that *B. dendrobatidis* probably has recently spread globally, most likely facilitated by human trade in amphibians (e.g., (Skerratt 2007)). It may have reached Australia by that mechanism, however within Australia,

it must have dispersed via presently unknown natural agencies. The fact that it apparently (Figure 1) reached all locations, including highly remote sites, in the southern Wet Tropics within 1-2 years argues that it is an extremely efficient disperser. A Ph.D. student at James Cook University, Mr. Scott Cashins, has a found that it can live on the exoskeletons of aquatic insects (S. Cashins, pers comm.), suggesting that insects may be a primary route of dispersal.

In epidemic outbreaks and when it is present as an endemic, vulnerability to chytridiomycosis caused by *B. dendrobatidis* varies widely among species. Initial epidemic outbreaks often drive populations of some species to local extinction, cause others to decline but not to extinction, and leave others apparently unaffected (Richards et al. 1993a, Lips 1999, Fellers et al. 2001, Bell et al. 2004, Bosch and Martinez-Solano 2006, Lips et al. 2006). McDonald and Alford (1999) showed that in Queensland, species more tightly associated with streams were more likely to suffer declines. This pattern has repeatedly emerged in subsequent analyses of tropical rainforest amphibians (Williams and Hero 1998, Hero et al. 2005).

The susceptibility of amphibians to chytridiomycosis is strongly affected by environmental conditions. In the Australian Wet Tropics, it has never caused population declines, even in highly susceptible species, at elevations below 300 to 400 metres (Richards et al. 1993a, McDonald 1994, McDonald 1999, McDonald et al. 2005), although it is widely distributed among low-elevation frog populations in the region (McDonald et al. 2005, Woodhams and Alford 2005, Rowley and Alford 2007b). Even at higher elevations, the timing of declines appears to be controlled in part by the environment; for example Richards et al. (1993b) documented declines at Kirrama, Queensland in a highly susceptible species (*Litoria rheocola*) approximately one year after the first known date of occurrence of *B. dendrobatidis* in that area (Berger 1999), indicating that the population crash occurred well after the first appearance of the pathogen. The prevalence of *B. dendrobatidis* in Wet Tropics frogs now fluctuates seasonally in a manner consistent with those elevational effects (McDonald et al. 2005, Woodhams and Alford 2005); it is higher in the cooler, dryer winter months and lower in the warm, wet summer months. Similar seasonal and elevational effects have been reported in other areas (Ron 2005, Longcore et al. 2007). These effects are probably caused by temperature effects on the growth rates of pathogen populations on hosts. Piotrowski (2004) examined the growth rates of *B. dendrobatidis* populations *in vitro* and showed that the pathogen grew between 4 and 25^{o}C and reproduced most rapidly between 17 and 25^{o}C. Temperatures above 30^{o}C killed the fungus.

Studies at the individual level provide some insight into the possible mechanisms of the effects of weather and climate. Woodhams et al. (2003) subjected experimentally infected *Litoria chloris* to a variety of thermal regimes and found that 16 hours of exposure to temperatures of 37^{o}C cured all individuals of infection, while 16 hours at 8^{o}C caused infections to progress more slowly than they did in frogs housed at a constant 20^{o}C. Rowley (2006, Rowley and Alford 2007b) tracked frogs of several sympatric species that were affected to different degrees by epidemic outbreaks of chytridiomycosis in the Australian Wet Tropics, and found they used the environment in ways that exposed them to very different moisture and temperature microenvironments. Both across species and among individuals within species, frogs that attained body temperatures above 25^{o}C had lower prevalences of infection by *B.*

dendrobatidis. The rank order of species with respect to how frequently body temperatures reached this level was the same as the rank order with respect to how severely populations were affected by chytridiomycosis in the late 1980s and early 1990s. Rowley and Alford (2007a) demonstrated that the behavior patterns of tracked frogs were also likely to affect the probability of transmission of *B. dendrobatidis*; frogs of more vulnerable species encountered potentially infective water and conspecifics more often than did individuals of less vulnerable species.

Taken together, individual-level studies suggest that in the field, infections on individuals of many species may be maintained at relatively low prevalence and intensity by various combinations of dry environmental conditions, which inhibit the release of zoospores and thus reduce the rate of reproduction of the fungus, and higher body temperatures, which greatly slow the growth rate of the fungus, kill some thalli as they approach the region of 28-30^{o}C, and may clear infections at temperatures above 30^{o}C. These temperature effects are highly consistent with the pattern seen in the Australian Wet Tropics, where susceptible frog species co-occur with the pathogen at low elevations with no apparent effects on their populations.

In addition to environmental effects, immune function could obviously affect vulnerability to *B. dendrobatidis* and other diseases at the individual, population, and species levels. Most studies of the details of the pathology of chytridiomycosis have noted that it appears that the adaptive and cellular immune systems of amphibians do not show strong responses to the pathogen, even in advanced stages of the disease (Pessier 1999, Berger et al. 2005). Amphibians also possess innate immune defenses, in the form of antimicrobial peptides (AMPs) that are secreted by the granular glands onto the skin surface. Woodhams and his co-workers (Woodhams et al. 2006a, Woodhams et al. 2006b, Woodhams et al. 2007a) have demonstrated strong correlational evidence that vulnerability of Wet Tropics frogs to population declines caused by *B. dendrobatidis* is related to the effectiveness of their AMPs against the fungus. Another factor that can affect vulnerability to chytridiomycosis is interactions between *B. dendrobatidis* and other microbes that inhabit amphibian skin. The skin of many amphibians supports a complex microbial assemblage, with which the zoospores of *B. dendrobatidis* must interact during the infection process (Belden 2007, Culp et al. 2007). Changes in the composition of this assemblage are likely to alter these species interactions, with some assemblages potentially excluding the pathogen while others may be readily invaded by it (Belden 2007). Harris et al. (2006) demonstrated that several genera of bacteria commonly isolated from salamanders of two species inhibited the growth of *B. dendrobatidis* in culture. Three bacterial metabolites have been found to occur on the skin of *P. cinereus* at concentrations sufficient to completely inhibit the growth of *B. dendrobatidis in vitro* (R. N. Harris, *Pers. comm.*). Woodhams et al. (2007b) found that a significantly greater proportion of individuals of the threatened species *Rana muscosa* carried bacteria with activity against *B. dendrobatidis* in a population that had coexisted with the pathogen for six years than in a population that was declining due to chytridiomycosis. Understanding the complex ecology of the microbiota of amphibian skin is at a very early stage, but may prove very useful in understanding the interactions between amphibians, *B. dendrobatidis*, and other pathogens and how these are modified by environmental factors (Woodhams 2007).

Distribution and ecology of *B. dendrobatidis* in the lowland Wet Tropics, with particular reference to the Ella Bay site

At the date of this report, *B. dendrobatidis* has been found at all sites in the Wet Tropics at which adequate sampling has been carried out. Speare et al. (2005) carried out an extensive survey of its occurrence in the Wet Tropics, concentrating on upland areas but including a few sites at elevations of ca. 200m or less. They found *B. dendrobatidis* at all sites for which an adequate sample size was obtained; their results are illustrated in Figure 2, which combines their results with data collected more recently at a series of lowland sites by members of my research group. It shows that *B. dendrobatidis* occurs at a large number of relatively low elevation sites, including many (Bell's Peak, Tully Powerlink site, Mena Creek sites 1 and 2) in blocks of rainforest that are not directly linked to the larger forest blocks on the more inland ranges. Many low elevation sites have been monitored, with variable intensity, over many years. No *B. dendrobatidis*-associated population declines have been reported at any of these sites.

The data presented in Figure 2 indicate that *B. dendrobatidis* occurs at many locations near the Ella Bay site. Many lines of reasoning making the fact that we found the fungus during our surveys at Ella Bay (see below) unremarkable: 1) the pathogen is clearly highly dispersive, see Figure 1 for an approximation of large scale patterns in Queensland since its first appearance; 2) the pathogen has been found at all low elevation sites in the Wet Tropics at which adequate sampling effort has been made, including sites in blocks of forest isolated from the main blocks on the inland ranges; 3) the Mena Creek and Frenchman's Creek sites at which *B. dendrobatidis* is known to occur are within 20 km of the Ella Bay site; 4) the Frenchman's Creek site is separated by less than 1.5km from forest that is contiguous with the Ella Bay site; 5) Bell's Peak is in a block of forest continuous with the forest at the Ella Bay site.

Figure 2. Low elevation sites in the southern Wet Tropics at which *B. dendrobatidis* was known to occur prior to sampling at Ella Bay. All sites are at elevations of 200m or less, with the exception of Bell's Peak in the Malvern Thompson Range (ca. 900m), which is in the same forest block as Ella Bay.

At low elevation sites the prevalence of *B. dendrobatidis* shows strong seasonal fluctuations. Figure 3 illustrates seasonal patterns found at upland and lowland sites (lowland sites were Frenchman Creek and Kirrama Bridge 1, see Figure 2, plus Ethel Creek, Mount Spec). Lowland prevalences were always lower than upland prevalences, and were zero at some sites during the summer months. These falls in prevalence coincide with periods during which environmental temperatures are largely outside the optimum temperature range for the growth of *B. dendrobatidis*.

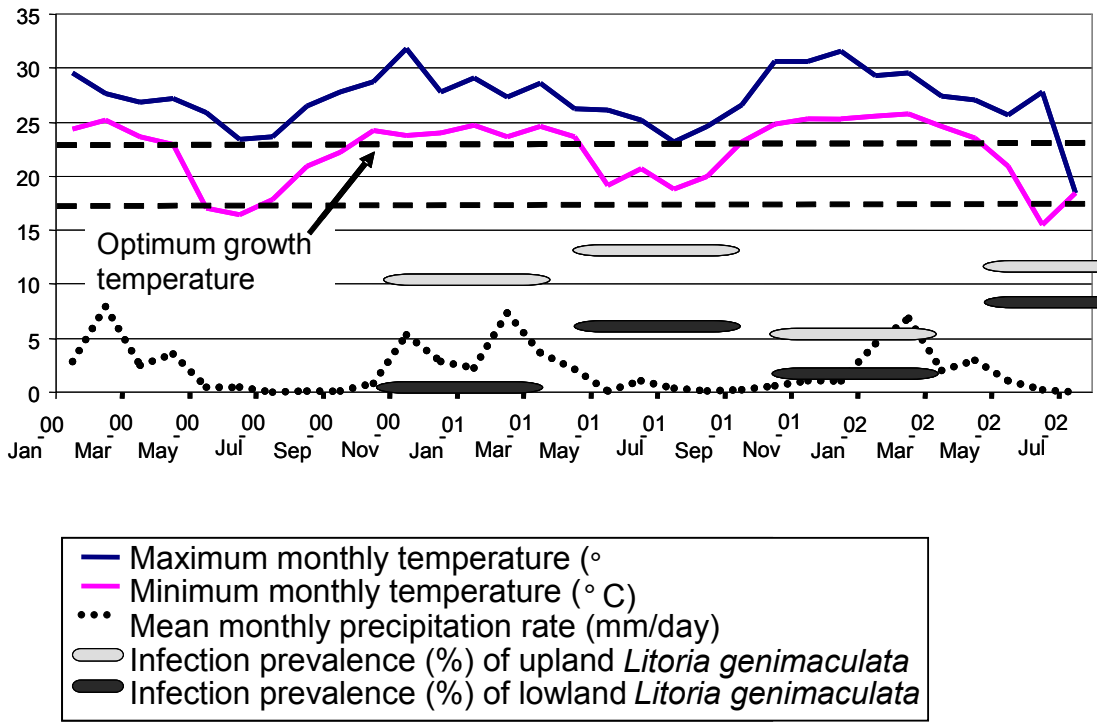

Figure 3. Prevalence of *B. dendrobatidis* infections detected in *Litoria genimaculata* using histological examination at upland and lowland sites by (Woodhams and Alford 2005). Because histology was used, true prevalences were probably underestimated several-fold (Hyatt et al. 2007).

Recent sampling by our group at the two Mena Creek sites and the Tully Powerlink site illustrated in Figure 2 confirms that this trend occurs at lowland sites. Three sampling trips were undertaken in 2007, three in 2008, and one in May 2009. All were undertaken with appropriate permits. The 2007 and 2008 trips visited all three sites, while the 2009 visited only one Mena Creek site. We observed strict quarantine precautions (NSW National Parks and Wildlife Service 2001) to ensure that samples were not cross-contaminated and that we did not transport *B. dendrobatidis* into or between sites. We sampled along a total of 800 m of creek at each site, over three or four nights per sampling trip. In 2008, for which detailed results appear in Table 1, each site was visited four nights per site in April and three nights per site in each of June and August. In 2008 we found frogs of a total of 12 species (Table 1), including many species that are not rainforest endemics. Three of the species encountered are classified as rare or endangered under Queensland legislation; other threatened species that could be found in the area , *Litoria nannotis,* which is classified as Endangered, and *Cophixalus infacetus*, which is classified as Rare, were not encountered in our surveys.

During these surveys, we sampled all frogs for possible infection by *B. dendrobatidis* by swabbing individuals using a standard technique (North and Alford 2008). Samples were analysed using diagnostic PCR by Pisces Molecular Laboratories, Colorado, USA. Results for prevalence of infection by *B. dendrobatidis* in the most commonly encountered species, *Litoria rheocola*, appear in Figure 4. Infected frogs were encountered on our first sampling trip in the winter of 2007, with infection

prevalence of approximately 45%. Prevalence in the spring, summer, and autumn of 2007-08 was zero, however the infection reappeared in frogs in winter of 2008, again in about 40% of the 91 frogs sampled. No trips were undertaken until late Autumn of 2009, when the infection was found at a prevalence of about 10%. This is highly significant, since it confirms the trend previously detected of strong seasonal fluctuations in infection prevalence at lowland sites, with prevalences at or approaching zero over the warmer summer period and increasing substantially in winter. For this reason, we scheduled our survey at the Ella Bay site for winter, to maximize the chance of detecting the presence of the fungus.

Table 1. Species and numbers of frogs encountered at Tully Powerlink and Mena Creek 1 and 2 sites along 800 m of creek on three sampling trips in 2008.

Species	Status (Queensland)	Rainforest endemic?	Number counted on trip		
			April	June	August
Cophixalus ornatus	Common	Yes	3		
Litoria bicolor	Common	No		3	1
Litoria caerulea	Common	No	1		
litoria genimaculata	Rare	Yes	10	36	23
Litoria gracilenta	Common	No		3	4
litoria infrafrenata	Common	No	3	2	2
Litoria "lesueuri"	Common	No	12	3	7
Litoria rheocola	Endangered	Yes	222	89	71
Litoria rothi	Common	No	1		
Litora xanthomera	Common	Yes	1		
Nyctimystes dayi	Endangered	Yes			1
Rana daemeli	Common	Yes	4		2

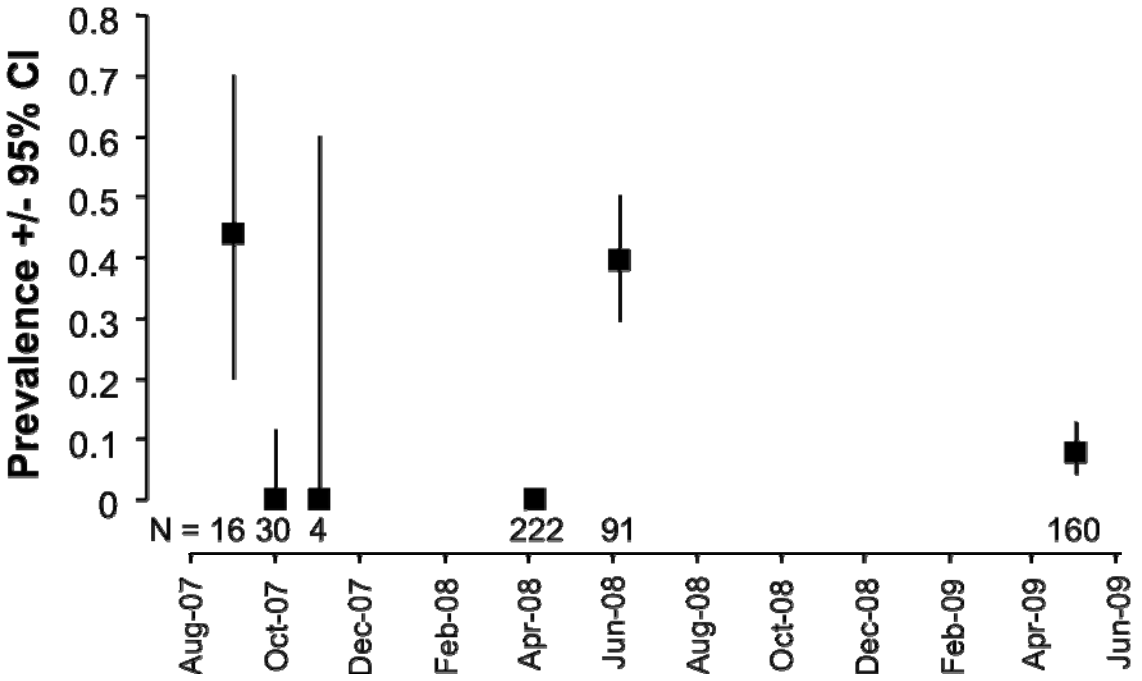

Figure 4. Prevalence of infections by *B. dendrobatidis* in *Litoria rheocola* at Mena Creek. The annual pattern of fluctuation of prevalences in frog populations is evident, with prevalence declining greatly in summer and increasing in autumn to a peak in winter.

In addition to performing broad surveys for infection, at the Mena Creek sites we tracked individual *Litoria rheocola* for short periods during June and August of 2008. We took diagnostic PCR samples using swabbing. We found that the infection status of individuals changed quickly, with animals both acquiring and losing infections over a period of a few days. More than half of the frogs that were infected at the start of tracking episodes lost their infections during tracking. We selected *L. rheocola* for tracking because this species is highly susceptible to chytridiomycosis at high elevation sites; all known populations above 400m declined to local extinction during outbreaks in the early 1990s, while populations at lower elevations persisted although the pathogen was present. Our data are the first to document that individuals can entirely lose their infections at low elevation sites, adding support to the observation that despite relatively high prevalences, *B. dendrobatidis* does not affect low elevation frogs at the population level, and individuals can recover from infections with no apparent lasting negative effects.

Table 2. Changes in infection status observed in tracked *L. rheocola* at Mena Creek sites during June and August 2008. + indicates animal was infected, - indicates uninfected.

Infection status		Number of individuals
Start	End	
-	-	7
-	+	2
+	-	5
+	+	3

Surveys at the Ella Bay site

We surveyed frogs at the Ella Bay site for infection by the amphibian chytrid fungus during August 2009. Locations at which samples were taken appear in Figure 5. Sampling and frog handling protocols used were identical to those used in our standard surveys, outlined above. Diagnostic quantitative PCR reactions to detect the presence of *B. dendrobatidis* DNA were carried out in the laboratory of Professor Andrew Storfer at Washington State University in the USA.

Figure 5. Aerial view of the Ella Bay site, with locations at which frogs were swabbed for detection of infection by *B. dendrobatidis* indicated (Sites 1-4, plus house, stable, and creek next to stable).

In total, swab samples were taken from 42 adult frogs belonging to 7 species during the surveys, which took place on the evenings of 26 and 27 August 2009. These results are summarized in Table 3. Three swabs returned definite positive results for the presence of *B. dendrobatidis*: one *Litoria wilcoxii* swabbed at site 1, one *L. rheocola* swabbed at site 1, and one *Rana daemeli* swabbed at the "creek next to stable" site. The quantitative PCR analyses for all three of these frogs returned positive results for the presence of *B. dendrobatidis* DNA in all of three replicate subsamples. The PCR analysis for the *L.rheocola* individual was run a second time to ensure that the positive result was correct, and again returned positive for the presence

of *B. dendrobatidis* DNA in all three replicate subsamples. This is the most sensitive and specific test for the presence of the amphibian chytrid (Hyatt et al. 2007), and the three positive results establish conclusively that the fungus is present at the Ella Bay site. The fact that infected frogs were present at both Site 1 and the "creek next to stable" site confirms the presence of *B. dendrobatidis* in both major branches of the creek system that crosses the Ella Bay site. The relatively low prevalence of infection is not surprising, given that the samples were taken in late August, when prevalences in lowland sites are expected to be decreasing as spring approaches (Figure 4). It is in line with the prevalence of approximately 10% measured in autumn at Mena Creek (Figure 4).

Table 3. Species and numbers of individuals swabbed for detection of infection by *B. dendrobatidis* using quantitative PCR.

Species	Number swabbed	Number positive
L. rubella	14	0
L. gemiculata	2	0
L. rheocola	4	1
B. marinus	12	0
L. wilcoxi (complex)	7	1
C. ornatus	1	0
R. daemeli	2	1

Recommendations for the Ella Bay site

B. dendrobatidis is present at the Ella Bay site, and at surrounding sites in the lowland Wet Tropics. At nearby sites its prevalence cycles seasonally, reaching at least 40% each winter in a frog species, *Litoria rheocola*, that has suffered declines due to epidemic outbreaks of chytridiomycosis at upland sites but has not suffered any known declines at lowland sites. All available evidence evidence indicates strongly that *B.* dendrobatidis has recently invaded the entire Wet Tropics region, and therefore the fungus found at the Ella Bay site will not be genetically differentiated from *B. dendrobatidis* occurring at other sites in the Wet Tropics. There is thus no need for quarantine procedures. The only precautions that should be needed with respect to *B. dendrobatidis* are with respect to handling frogs. Individuals should be handled and housed separately using sterile bags, gloves and containers if handling them is necessary, as could happen should individuals need to be relocated.

Literature Cited

Alford, R. A. 1999. Ecology - Resource use, competition, and predation. Pages 240-278 *in* R. McDiarmid, Altig, R, editor. Tadpoles: the biology of anuran larvae. University of Chicago Press, Chicago, IL.

Belden, L., Harris, RN. 2007. Infectious diseases in wildlife: the community ecology context. Frontiers in Ecology and the Environment **5**:533-539.

Bell, B. D., S. Carver, N. J. Mitchell, and S. Pledger. 2004. The recent decline of a New Zealand endemic: how and why did populations of Archey's frog Leiopelma archeyi crash over 1996-2001? Biological Conservation **120**:189-199.

Berger, L., R. Speare, P. Daszak, D. Green, A. Cunningham, C. Goggin, R. Slocombe, M. Ragan, A. Hyatt, K. McDonald, H. Hines, K. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences; USA **95**:9031-9036.

Berger, L., R. Speare, and L. F. Skerratt. 2005. Distribution of Batrachochytrium dendrobatidis and pathology in the skin of green tree frogs Litoria caerulea with severe chytridiomycosis. Diseases of Aquatic Organisms **68**:65-70.

Berger, L., Speare, R, Hyatt, A. 1999. Chytrid fungi and amphibian declines: Overview, implications and future directions. Pages 23-33 *in* A. Campbell, editor. Declines and Disappearances of Australian Frogs. Environment Australia, Canberra.

Bosch, J., and I. Martinez-Solano. 2006. Chytrid fungus infection related to unusual mortalities of Salamandra salamandra and Bufo bufo in the Penalara Natural Park, Spain. Oryx **40**:84-89.

Carey, C., J. E. Bruzgul, L. J. Livo, M. L. Walling, K. A. Kuehl, B. F. Dixon, A. P. Pessier, R. A. Alford, and K. B. Rogers. 2006. Experimental exposures of boreal toads (Bufo boreas) to a pathogenic chytrid fungus (Batrachochytrium dendrobatidis). EcoHealth **3**:5-21.

Culp, C. E., J. O. Falkinham, and L. K. Belden. 2007. Identification of the natural bacterial microflora on the skin of eastern newts, bullfrog tadpoles and redback salamanders. Herpetologica **63**:66-71.

Czechura, G., Ingram, G. 1990. Taudactylus diurnus and the case of the disappearing frogs. Memoirs of the Queensland Museum **29**:361-365.

Daszak, P., Berger, L, Cunningham, AA, Hyatt, AD, Green, DE, Speare, R. 1999. Emerging Infectious Diseases and Amphibian Population Declines. Emerging Infectious Diseases **5**:735-748.

de Castro, F., and B. Bolker. 2005. Mechanisms of disease-induced extinction. Ecology Letters **8**:117-126.

Fellers, G. M., D. E. Green, and J. E. Longcore. 2001. Oral chytridiomycosis in the mountain yellow-legged frog (Rana muscosa). Copeia **4**:945-953.

Harris, R. N., T. Y. James, A. Lauer, M. A. Simon, and A. Patel. 2006. Amphibian pathogen Batrachochytrium dendrobatidis is inhibited by the cutaneous bacteria of Amphibian species. EcoHealth **3**:53-56.

Hero, J. M., S. E. Williams, and W. E. Magnusson. 2005. Ecological traits of declining amphibians in upland areas of eastern Australia. Journal of Zoology **267**:221-232.

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. Diseases of Aquatic Organisms **73**:175-192.

Johnson, M. L., and R. Speare. 2005. Possible modes of dissemination of the amphibian chytrid Batrachochytrium dendrobatidis in the environment. Diseases of Aquatic Organisms **65**:181-186.

Laurance, W. F., K. R. McDonald, and R. Speare. 1996. Epidemic disease and the catastrophic decline of Australian rain forest frogs. Conservation Biology **10**:406-413.

Lips, K. R. 1999. Mass mortality and population declines of anurans at an upland site in western Panama. Conservation Biology **13**:117-125.

Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proceedings of the National Academy of Sciences of the United States of America **103**:3165-3170.

Longcore, J. E., A. P. Pessier, and D. K. Nichols. 1999. Batrachochytrium dendrobatidis gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia **91**:219-227.

Longcore, J. R., J. E. Longcore, A. P. Pessier, and W. A. Halteman. 2007. Chytridiomycosis widespread in anurans of northeastern United States. Journal of Wildlife Management **71**:435-444.

McDonald, K. 1994. Declining frog populations in the wet tropics. Queensland Department of Environment and Heritage Report **NOVOL**:00-00.

McDonald, K., Alford, RA. 1999. A review of Declining Frogs in Northern Queensland. Pages 14-22 *in* A. Campbell, editor. Declines and Disappearances of Australian Frogs. Environment Australia, Canberra.

McDonald, K. R., D. Mendez, R. Muller, A. B. Freeman, and R. Speare. 2005. Decline in the prevalence of chytridiomycosis in frog populations in North Queensland, Australia. Pacific conservation biology **11**:114-120.

New South Wales National Parks and Wildlife Service. 2001. Hygiene protocol for the control of disease in frogs. Circular Number 6. NSW NPWS, Hurstville, NSW.

North, S., Alford, RA. 2008. Infection intensity and sampling locality affect Batrachochytrium dendrobatidis distribution among body regions on green-eyed tree frogs Litoria genimaculata. Diseases of Aquatic Organisms **81**:177-188.

Pessier, A., Nichols, DK, Longcore, JE, Fuller, MS. 1999. Cutaneous chytridiomycosis in poison dart frogs (Dendrobates spp.) and White's tree frogs (Litoria caerulea). Journal of Veterinary Diagnostic Investigation **11**:194-199.

Piotrowski, J. S., S. L. Annis, and J. E. Longcore. 2004. Physiology of Batrachochytrium dendrobatidis, a chytrid pathogen of amphibians. Mycologia **96**:9-15.

Richards, S., K. McDonald, and R. Alford. 1993a. Declines in populations of Australia's endemic tropical rainforest frogs. Pacific conservation biology **1**:66-77.

Richards, S. J., K. R. McDonald, and R. A. Alford. 1993b. Declines in populations of Australia's endemic tropical rainforest frogs. Pacific conservation biology **1**:66-77.

Ron, S. R. 2005. Predicting the distribution of the amphibian pathogen Batrachochytrium dendrobatidis in the New World. Biotropica **37**:209-221.

Rowley, J. 2006. Why does chytridiomycosis drive some frog populations to extinction and not others? The effects of interspecific variation in host behaviour. Ph.D. James Cook University, Townsville, Queensland, Australia.

Rowley, J., Alford, RA. 2007a. Behaviour of Australian rainforest stream frogs may affect the transmission of chytridiomycosis. Diseases of Aquatic Organisms **77**:1-9.

Rowley, J. J. L., Alford, R. A. 2007b. Movement patterns and habitat use of rainforest stream frogs in northern Queensland, Australia: implications for extinction vulnerability. Wildlife Research **34**:371-378.

Skerratt, L., Berger, L, Speare, R, Cashins,S, McDonald, KR, Phillott, AD, Hines, HB, Kenyon, N. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth **4**:125-134.

Speare, R., Skerratt, LF, Berger, L, Hines, H, Hyatt, A, Mendez, D, McDonald, K, Hero, J-M, Marantelli, G, Muller, R, Alford, R, Woods, R. 2005. Final report to Department of the Environment and Heritage on RFT63/2003: A project that designs and trials a pilot survey to map the distirbution on chytridiomycosis (caused by the amphibian chytrid fungus) in Australian frogs.

Voyles, J., Berger, L, Young, S, Speare, R, Webb,, and W. R, J, Rudd, D, Campbell, R, Skerratt, LF. 2007. Electrolyte depletion and osmotic imbalance in amphibians with chytridiomycosis. Diseases of Aquatic Organisms **77**:113-118.

Williams, S. E., and J. M. Hero. 1998. Rainforest frogs of the Australian Wet Tropics: guild classification and the ecological similarity of declining species. Proceedings of the Royal Society of London Series B-Biological Sciences **265**:597-602.

Woodhams, D., Rollins-Smith, LA, Alford, RA, Simon, MA, Harris, RN. 2007. Innate immune defenses of amphibian skin: antimicrobial peptides and more. Animal Conservation **10**:425-429.

Woodhams, D. C., and R. A. Alford. 2005. Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. Conservation Biology **19**:1449-1459.

Woodhams, D. C., R. A. Alford, and G. Marantelli. 2003. Emerging disease of amphibians cured by elevated body temperature. Diseases of Aquatic Organisms **55**:65-67.

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

Woodhams, D. C., L. A. Rollins-Smith, C. Carey, L. Reinert, M. J. Tyler, and R. A. Alford. 2006a. Population trends associated with skin peptide defenses against chytridiomycosis in Australian frogs. Oecologia **146**:531-540.

Woodhams, D. C., J. Voyles, K. R. Lips, C. Carey, and L. A. Rollins-Smith. 2006b. Predicted disease susceptibility in a panamanian amphibian assemblage based on skin peptide defenses. Journal of Wildlife Diseases **42**:207-218.

Woodhams, D. C., V. T. Vredenburg, M. A. Simon, D. Billheimer, B. Shakhtour, Y. Shyr, C. J. Briggs, L. A. Rollins-Smith, and R. N. Harris. 2007b. Symbiotic bacteria contribute to innate immune defenses of the threatened mountain yellow-legged frog, Rana muscosa. Biological Conservation **138**:390-398.