

Inconclusiveness of Chytridiomycosis as the Agent in Widespread Frog Declines

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Abstract: *Although there is considerable evidence to support the hypothesis that the chytrid fungus *Batrachochytrium dendrobatidis* is the primary agent responsible for widespread declines in amphibian populations, particularly rainforest frog populations in Australia and Central America, I argue the case has not yet been made conclusively. Few specimens were collected at the time of population declines, so it may never be possible to conclusively determine their cause. It remains unclear whether the pathogen is novel where declines have occurred. Although it is not necessary that the infection be novel for it to be implicated in declines, if a preexisting pathogen has only recently caused extinctions, cofactors must be important. Whether the pattern of outbreaks represents a "wave" of extinctions is unclear, but if it does, the rate of spread in Australia is implausibly high for a waterborne pathogen, given the most likely estimates of epidemiological parameters. Although *B. dendrobatidis* is an amphibian pathogen according to Koch's postulates, the postulates are neither necessary nor sufficient criteria to identify a pathogen. The following key pieces of information are necessary to better understand the impact of this fungus on frog communities: better knowledge of the means and rate of transmission under field conditions, prevalence of infection among frog populations, as distinct from morbid individuals, and the effect of the fungus on frogs in the wild. It is crucial to determine whether there are strains of the fungus with differing pathogenicity to particular frog species and whether host-pathogen coevolution has occurred or is occurring. Recently developed diagnostic tools bring into reach the possibility of addressing these questions and thus developing appropriate strategies to manage frog communities that may be affected by this fungus.*

Key Words: chytrid fungus, epidemiological model, rainforest frogs

Falta de Datos Concluyentes de la Quitridiomycosis como Agente de las Declinaciones Globales de Ranas

Resumen: *Aunque existe evidencia considerable para sustentar la hipótesis de que el quitridiomyceto *Batrachochytrium dendrobatidis* es el principal agente responsable de las declinaciones globales de poblaciones de anfibios, particularmente las poblaciones de ranas de bosques lluviosos en Australia y América Central, argumento que no se puede concluir sobre el caso. Pocos especímenes fueron recolectados durante las declinaciones poblacionales, de manera que probablemente nunca se puedan determinar sus causas concluyentemente. No es claro si el patógeno es nuevo donde han ocurrido las declinaciones: Aunque no es necesario que la infección sea nueva para ser involucrada en las declinaciones, si un patógeno preexistente solo ha causado extinciones recientemente, los cofactores deben ser importantes. No es claro si el patrón de epidemias representa una "oleada" de extinciones, pero si lo hace, la tasa de dispersión en Australia es inverosímilmente alta para un patógeno transportado por agua, en función de las estimaciones más probables de parámetros epidemiológicos. Aunque *B. dendrobatidis* es un patógeno anfibio de acuerdo con los postulados de Koch, estos criterios no son necesarios ni suficientes para identificar a un patógeno. Las siguientes piezas de información clave son necesarias para un mejor entendimiento del impacto de este hongo sobre comunidades de ranas: mejor conocimiento de los medios y tasas de transmisión en condiciones de campo; prevalencia de la infección en poblaciones de ranas, a diferencia de individuos mórbidos; y el efecto del hongo sobre ranas silvestres. Es crucial determinar si hay cepas del hongo con diferente patogenicidad para determinadas especies de ranas y si ha ocurrido o esta ocurriendo coevolución huésped-patógeno. Herramientas de diagnóstico desarrolladas*

recientemente ponen al alcance la posibilidad de abordar estas preguntas y por lo tanto desarrollar estrategias adecuadas para manejar comunidades de ranas que puedan estar afectadas por este hongo.

Palabras Clave: hongo quitridio, modelo epidemiológico, ranas de bosque lluvioso

Introduction

Over the last half century, populations of many amphibians worldwide have declined markedly (Houlahan et al. 2000). In particular, populations of frogs have crashed in apparently pristine rainforests in Australia (Campbell 1999) and Central America (Lips 1999; Young et al. 2001). A number of hypotheses have been proposed to account for these declines (Alford & Richards 1999; Blaustein & Kiesecker 2002; Collins & Storer 2003). The hypothesis I focus on here is that epidemic disease is responsible. This suggestion was first put forward by Laurance et al. (1996) to explain the Australian declines, although Blaustein et al. (1994) proposed earlier that the fungus *Saprolegnia* was implicated in amphibian declines in the U.S. Pacific Northwest. Laurance et al. presented several lines of circumstantial evidence suggesting a pathogen was responsible for declines, although they were unable to identify a putative etiological agent. Their hypothesis was criticized on a number of grounds (Alford & Stephens 1997; Hero & Gillespie 1997) and responded to by Laurance et al. 1997.

Since then, a novel chytrid fungus, *Batrachochytrium dendrobatidis*, which is pathogenic to a variety of frog species, has been identified (Berger et al. 1998; Longcore et al. 1999) and proposed as the agent causing declines of rainforest frogs in both Australia and Central America (Berger et al. 1998; Daszak et al. 1999, 2003). Ranaviruses have also been suggested as causing population decline in amphibia, particularly salamanders (Daszak et al. 1999). Here, however, I concentrate on the role of *B. dendrobatidis* in frog declines, with an emphasis on Australian rainforest frogs.

There is unequivocal evidence that the fungus is pathogenic to adult frogs of some species (Berger et al. 1998; Longcore et al. 1999). What is less clear is whether it is the primary agent responsible for the decline or extinction of rainforest frogs in Australia and Central America. I used simple epidemic models to evaluate this hypothesis and to identify the research directions necessary to establish whether the fungus is a major contributor to frog declines.

Biology of *Batrachochytrium dendrobatidis*

Batrachochytrium dendrobatidis is a chytrid fungus that causes epidermal infection in a wide variety of frog species. It was identified following histological examination of dead and moribund frogs from Australia and North

and Central America (Berger et al. 1998; Longcore et al. 1999). To date, it has been identified on at least 46 anuran species in Australia, 24 species in the Americas, 3 species in Africa, and 5 species in Europe (Speare & Berger 2004) and has also been found recently on wild tiger salamanders (Davidson et al. 2003). It is the first chytrid known to infect vertebrate hosts. In anurans, it attacks keratinized tissue, which is present mainly in adults. In tadpoles, it does infect the keratinized mouthparts but does not appear to be pathogenic (Pessier et al. 1999).

Berger et al. (1998) demonstrated the pathogenicity of the fungus by infecting six great barred frogs (*Mixophyes fasciolatus*, an Australian rainforest frog) with an aqueous suspension of *B. dendrobatidis* sporangia obtained from a naturally infected frog of the same species, keeping four frogs as untreated controls, with a further four controls exposed to the sporangia suspension after it had been passed through a 45- μ m filter. All six infected frogs became moribund within 10–18 days after exposure, whereas the eight controls were unaffected. Similarly, to fulfill Koch's postulates, Longcore et al. (1999) infected two juvenile blue poison dart frogs (*Dendrobates azureus*) with cultured *B. dendrobatidis*. The infected frogs died and the fungus was reisolated from one of the dead frogs. There is also very strong evidence that the fungus has been responsible for large-scale mortalities in several captive populations of frogs (e.g., Parker et al. 2002).

Whether *B. dendrobatidis* can survive in natural conditions without amphibians is uncertain. It can develop in the laboratory for at least one generation on dead frog skin, and the fact that it can be cultured on keratin in the laboratory suggests it may be capable of living saprophytically (Longcore et al. 1999). Zoospores placed in sterilized lake water remained viable for up to 7 weeks at 23° C (Johnson & Speare 2003). The ability of the organism to grow on or survive in sterile media under laboratory conditions does not, however, necessarily imply it will survive in the field in competition with the wide range of saprophytic organisms in any natural freshwater environment.

Why *Batrachochytrium* May Not Be Responsible for Frog Extinctions

Although it has been stated that *B. dendrobatidis* is the cause of amphibian mass deaths in both Australia and Central America (Daszak et al. 1999), the available evidence

does not yet show unequivocally that the fungus is responsible for wide-scale population declines and extinctions in amphibia.

First, it is not clear that this is a novel infection in areas where it has been reported as being associated with amphibian declines. Some recent genetic evidence is consistent with a recent common origin of many strains worldwide (Morehouse et al. 2003) but is not conclusive. The “Out of Africa” hypothesis is an intriguing recent suggestion (Daszak et al. 2003; Weldon et al. 2004). The fungus has been found in several frog species in Africa. A record from a museum specimen of *Xenopus laevis* from 1938 is the earliest known occurrence of *B. dendrobatidis* worldwide and there is no evidence of an increase in prevalence in archived *X. laevis* since that time (overall mean prevalence was 0.026). The fungus does not seem to cause mortalities in *X. laevis*. The hypothesis is that the fungus originated in Africa but has spread recently to the rest of the world by trade in amphibia, particularly *X. laevis*.

The pathogen need not be a novel infection for it to be responsible for declines, but if it has been endemic in frog populations and is only now causing declines, some other factor would need to be the ultimate causal agent of the declines. For example, Carey et al. (1999) suggest that environmental stressors may be compromising the immune response of amphibians.

Berger et al. (1998) found that *B. dendrobatidis* was present on a high proportion of moribund frogs collected during mortality events, but it was not present in archived samples. Moribund frogs collected following mass mortalities in Big Tableland, Queensland, in 1993–1994 (four species) and in Fortuna, Panama, in 1997 (six species) had *B. dendrobatidis* present at high prevalence (Berger et al. 1998). However, their reported sample sizes were quite small (10/15 frogs infected in Queensland; 19/19 infected in Panama). Retrospective analysis of archived toe clips collected at least 2 years before declines in both Central America and Australia failed to find any evidence of chytrid infection (Berger et al. 1998). Similarly, Fellers et al. (2001) found high frequencies of mouthpart deformities characteristic of *B. dendrobatidis* infection in *Rana muscosa* tadpoles collected in the last decade, but no such deformities in museum specimens from earlier decades.

These pre- and postdecline data sets are not directly comparable. The moribund frogs were sampled because they were sick, whereas the archived toe clips represent a sample that was probably approximately random with respect to disease status. Both sets of samples could conceivably have come from populations with the same underlying mean prevalence of the pathogen, particularly if its distribution is spatially or temporally heterogeneous. For example, assuming a homogeneous distribution of infection, given 0 infected frogs out of 42 (the toeclip data from archived Australian frogs in Berger et al.) it is possible

to reject, at $p = 0.05$, the null hypothesis that the prevalence of infection is > 0.07 , but the data are consistent with the pathogen being present at any prevalence less than this, such as the prevalence of 0.026 recorded from archived *X. laevis* in Africa (Weldon et al. 2004). If prevalence is heterogeneous in space or time, as is certainly the case (Berger et al. 2004; Retallick et al. 2004), the data would be consistent with even higher mean prevalence. Further, the histological method used to diagnose infection may return false negatives (Berger et al. 1999).

One of the arguments proposed by Laurance et al. (1996) supporting the theory that an epidemic disease was responsible for declines in rainforest frogs in Australia is that there was a south to north “extinction wave.” When they published their paper, no causative agent for infections had been proposed, but Daszak et al. (1999) have reiterated that the pattern of amphibian declines associated with chytridiomycosis in Australia and Central America is consistent with the pattern expected as a virulent pathogen disperses through a naïve population. A pathogen epidemic is certainly a likely cause of a spatially propagating pulse of mortality in a population. Recent examples include the herpes epidemic among pilchards along the Australian coastline (Jones et al. 1997) or phocine distemper virus in seals in the North Sea (Swinton et al. 1998). In principle, climatic or other abiotic factors can cause mortality to spread spatially. Coral bleaching is an example (Berkelmans et al. 2004). It is hard, however, to conceive of a climatic change over a 15-year period that may have been responsible for the declines observed in Australian frogs, given the latitudinal and elevational range of the declines.

The available evidence for Australia does not unequivocally show a “wave” of extinctions among rainforest frogs moving from south to north (Alford & Stephens 1997). There are three areas of rainforest patches, each separated from the other areas by several hundred kilometers of drier sclerophyll forest and woodland. Frog declines occurred in the southernmost area first, in the middle area second, and in the northernmost area last. Within each area, declines were roughly synchronous. To infer a steady south–north progression from these data is essentially basing a regression on three data points. Aside from this statistical issue, how the pathogen moves through areas that are not rainforest and whether a rate of spread of 100km/year for a waterborne pathogen of amphibians is plausible remain open questions.

Laboratory evidence demonstrates clearly that the fungus is a fatal pathogen of frogs, that it can be transmitted in water, and that it fulfils Koch’s postulates (Berger et al. 1998; Daszak et al. 1999; Longcore et al. 1999). Although Koch’s postulates are a powerful classical means to identify an organism as a pathogen, they have their limitations (Sutter 1996) and are neither necessary nor sufficient conditions to infer that an organism is a pathogen in all situations. For example, whether transmission of

B. dendrobatidis occurs at levels sufficient to cause lethal infection when infective stages are at concentrations that typically might be found in natural water bodies has yet to be demonstrated clearly because collecting and identifying zoospores in natural water bodies is not possible currently. The great barred frog, in which Berger et al. (1998) demonstrated transmission and lethality of *B. dendrobatidis*, is not one of the species that has declined in the wild (Campbell 1999), although captive colonies have been affected by fungus outbreaks (Berger et al. 1998).

An apparent challenge to the hypothesis that epidemic spread of *B. dendrobatidis* is the causative agent of frog declines and extinctions in Australia is that the species has an extremely broad host range, but only some of the affected species have declined. The existence of reservoir hosts on which the pathogen has little impact, however, increases the likelihood that the pathogen may produce extinctions. Some species (*Litoria nannotis*, *L. rheocola*, and *Nyctimystes dayi*) have declined at high elevations, but populations remain stable at elevations < 400 m (McDonald & Alford 1999). If *B. dendrobatidis* is indeed the cause of the decline of these species at high elevation, there must be some environmental mediation of the impact of the pathogen. Temperature is the most obvious and well-documented environmental influence on *B. dendrobatidis*. In culture, the fungus grows best at about 23° C, and can be killed by exposure to temperatures above 30° C (Longcore et al. 1999). Housing the Australian frog *Litoria chloris* at 37° C for < 16 hours clears the frogs of *B. dendrobatidis* infection (Woodhams et al. 2003). Whether other environmental or behavioral factors such as the microhabitat occupied by the host are important to the impact of the pathogen is unknown.

Predictions of Single-Host, Single-Pathogen Models

Simple mathematical models can be valuable in evaluating the potential effect on a population of this, or any other pathogen, provided the limitations implicit in the assumptions underlying the model are recognized. One important result is that the relationship between disease pathogenicity, pathogen prevalence, and the impact on the host population is somewhat counterintuitive. Very pathogenic microparasites have a relatively small impact on the population density of their hosts and are present at low prevalence, simply because the disease kills the hosts before the pathogen can infect another host (Anderson 1979). Conversely, pathogens that cause very mild disease are likely to be present in most hosts and have little effect on the host population density. Figure 1 shows that even the prevalence of infection in morbid hosts declines monotonically with pathogenicity. All animals must die of something, and if a putative pathogen is present in most of the animals in the population, most of those that die carry it too. Of course, if pathogenicity is high, infected hosts die at a greater rate than others, but this is not

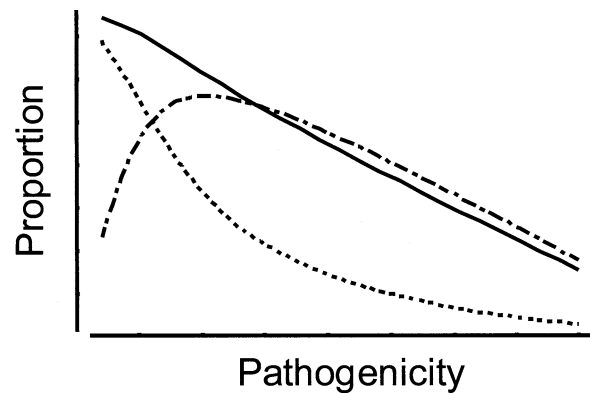


Figure 1. Pathogenicity of a microparasite (arbitrary units) versus the proportion by which the host population is depressed below its disease-free equilibrium (unevenly dashed line), prevalence in morbid hosts (solid line), and prevalence in all hosts (evenly dashed line) (after McCallum & Dobson 1995).

enough to compensate for the decline with pathogenicity of prevalence in the general population. If prevalence of a microparasite is much higher in morbid hosts than in the general population, this shows that infection is associated with morbidity, but the relationship may not be causative.

These simple models also suggest that a pathogen alone cannot drive its sole host to extinction. The rate at which a naïve host acquires infection per unit of time is known as the force of infection. This quantity is crucial in determining the dynamics of a host-parasite interaction. In general, the force of infection is likely to be a function of the density or population size of infected stages, infected hosts, and susceptible hosts and vectors (if they are necessary for disease transmission). It may also depend on the environment and the spatial distribution of host classes. In most conventional host-pathogen models (dating back to Hamer [1906] and Kermack & McKendrick [1927]), the force of infection is determined by the “mass action” assumption, which is that the force of infection is βI , where I is the population density of infected hosts and β is a constant. As the host density declines to zero, the force of infection likewise tends to zero, and the pathogen dies out. Assuming mass action, all pathogens thus have a threshold host density N_T below which the pathogen cannot persist. In the absence of Allee effects (e.g., Courchamp et al. 1999) or stochasticity, the pathogen will die out before it can drive its host to extinction.

These are useful conclusions, but they need to be qualified. They apply to a single-host, single-microparasite interaction, describe the situation at equilibrium, and apply to an interaction in which the death of the host results in the death of the parasites infecting it. Finally, the mass-action assumption has begun to be questioned by a number of authors, and the weight of evidence is increasingly

that it is an inadequate descriptor of the infection process in many host-pathogen interactions (McCallum et al. 2001).

Each of the assumptions (single-host species, equilibrium, and death of pathogens following host death) will not apply strictly to the *B. dendrobatidis* and frog interaction. Whether transmission follows mass action cannot be determined without experimental studies of transmission in natural or near-natural conditions.

Reservoir Hosts

Almost all pathogens that have produced extinctions infect a reservoir host, in addition to the affected species (Gog et al. 2002). A reservoir host is one in which the pathogen is relatively benign relative to the pathogen's impact on the endangered species. This means the pathogen occurs at high prevalence in the reservoir (Fig. 1) and is usually enzootic (present at a reasonably constant level). Consequently the force of infection in the endangered species remains high, even as the population of the endangered species declines to extinction.

The most obvious potential reservoir species for *B. dendrobatidis* are other anuran species. The fungus affects a wide variety of frog species but extinctions and declines have occurred in only a few. If other frog species are acting as reservoirs, we can make the following testable predictions. First, although the fungus may not be entirely benign in reservoir nondeclining species, it should be less pathogenic in these species in the conditions under which they normally live than in species that have declined. Second, where species that have declined still exist, *B. dendrobatidis* should occur at lower prevalence in the endangered species than in sympatric reservoir species. Third, chytrid-infected reservoirs should still exist at sites where endangered species have either declined or disappeared.

If other anurans act as reservoirs for *B. dendrobatidis* infection, this provides a means by which the fungus may have dispersed between rainforest patches in eastern Australia. The question remains, however, as to whether observed rate of spread is plausible for a waterborne pathogen. Tadpoles of the affected species may also act as reservoirs (Daszak et al. 1999; Rachowicz & Vredenburg 2004). It appears the fungus can infect the keratinized mouthparts of tadpoles without affecting their viability. Tadpoles have apparently persisted in places where adult frogs have disappeared, although more work on chytrid infection on tadpoles is needed (Daszak et al. 2003). Little theoretical work is available to predict the effect on host-pathogen population dynamics of one life-history stage acting as a reservoir for infection. Without modeling this explicitly, one might expect tadpole reservoirs to produce behavior analogous to that generated by long-lived infectious stages. In general, these tend to destabilize host-parasite interactions, potentially leading to cy-

cles and host extinction (Anderson & May 1980); thus, one possibility is that intraspecific reservoirs might generate limit cycle behavior, causing periodic outbreaks of a pathogen in the host population.

B. dendrobatidis may exist saprophytically, surviving and reproducing on keratin in the environment (Longcore et al. 1999). If the fungus can persist, reproduce, and multiply on environmental keratin indefinitely, then the potential to maintain a high force of infection to any endangered frogs that might be present is obvious. If, however, the fungus persists only for a limited period (perhaps because it may be an inferior competitor with other saprophytic organisms), then the impact on population dynamics should be similar to that of a long-lived infective stage.

Several published models of two-host, one-pathogen interactions can be used to understand the potential effect of reservoir hosts on endangered species (e.g., Freeland 1983; Price et al. 1988; Grosholz 1992). A shared pathogen acts essentially as an agent of competition, with results analogous to standard Lotka-Volterra models. Two hosts regulated by a common pathogen may coexist if transmission within species exceeds transmission between species. If transmission occurs at the same rate within and between species, then the species that supports the higher prevalence of pathogen infection if present alone competitively replaces the other (Holt & Pickering 1985). These conclusions are complicated, however, if there are free-living infective stages or if factors other than the pathogen regulate the population density of the hosts (Begon & Bowers 1994). They also may not apply in a nonhomogeneous environment.

Plausibility of the Rate of Spread

An "infection wave" traveling at about 100 km/year seems extremely fast for a waterborne pathogen. The simplest possible model of spatial spread is a diffusion model, in which particles move in random directions that are unaffected by local particle distribution. In this model, the rate of spread C , which has units of distance per unit time, is given by the following relationship (van den Bosch et al. 1992):

$$C = \sqrt{2rs}, \quad (1)$$

where r is the intrinsic rate of increase of the dispersing organism and s is the diffusion coefficient. Although the qualitative prediction of Eq. 1 that the distance dispersed should be a linear function of time is often quite accurate, the equation is useful only if one can estimate r and s . One key point evident from Eq. 1 is that the rate of spread depends on the intrinsic rate of increase of the population in question and the rate at which individuals move.

The diffusion coefficient s is an estimate of the mean distance moved, in random directions, per unit time. If a pathogen is transmitted by contact, the coefficient will be

approximately the same for a pathogen as it is for its host. One way to gain a plausible estimate for s is to suppose that the cane toad (*Bufo marinus*) is a vector for *B. dendrobatidis*. Cane toads can be infected by *B. dendrobatidis* (Berger et al. 1998) and are common, with a continuous distribution, throughout the region where the "infection wave" is hypothesized to have occurred (although not in the rainforest stream microhabitats occupied by most of the declining species). Since their introduction into Queensland, toads have spread northward at a rate of approximately 25 km/year (Lampo & DeLeo 1998). Intrinsic rates of increase are difficult to estimate (McCallum 2000), but r for the cane toad is about 2.86/year (Lampo & DeLeo 1998). Combining these estimates of C and r for the cane toad, s can be estimated at about 110 km²/year. Under the assumptions of the diffusion model, this rate of movement applies to toads at any point in their range, not just at the current limit of their distribution.

For *B. dendrobatidis* to move 100 km/year with cane toads as vectors, the rate of increase of the pathogen would need to be 16 times that of cane toads: $r > 45.8$. Unfortunately, there are no data that would permit this rate to be directly estimated in natural populations of cane toads, or any other anuran. Rates of increase of pathogens are usually described by R_0 , which is the number of secondary infections produced, per primary infection, when the pathogen is first introduced into a naïve host population. The intrinsic rate of increase r is related to R_0 by

$$r \approx \frac{\ln(R_0)}{\mu_0}, \quad (2)$$

where μ_0 is the mean age of reproduction, which for a pathogen is the mean time from initial infection to secondary infection. In culture at 20–23° C, zoospores are first produced after 4–5 days, or 0.0123 years (Longcore et al. 1999), which is an absolute lower limit to μ_0 . In tadpoles of yellow-legged frogs (*Rana muscosa*) at 13° C, sporangia (which potentially can release zoospores) are first detected 3 weeks after the tadpoles are exposed, and infection is detected in postmetamorphic frogs at 18 days (Rachowicz & Vredenburg 2004). These results suggest that μ_0 may be approximately 20 days (0.049 years). No data are available to estimate R_0 , which is likely to be a function of the environment, host species, and host density and depends on the properties of the pathogen itself. If the generation time μ_0 is about 20 days, then R_0 needs to be > 12 to generate the observed rate of spread, which requires chytridiomycosis to be as contagious as the most contagious human diseases such as measles, which has an R_0 of about 15 in cities (Anderson & May 1991). If μ_0 is at its lowest possible value of 5 days, then R_0 need only be about 1.8.

Data on the transmission dynamics of *B. dendrobatidis* within frog populations are needed before reliable conclusions about the rate of possible spread of the pathogen can be drawn. If spatial spread via anurans is rejected as

implausible, it is necessary to hypothesize flying vectors such as birds or insects, or human intervention, to explain the observed rate of spread.

Metapopulation Models

Frogs do not live in the homogeneous environments implicitly assumed in simple epidemic models; rather, they live in many discrete patches. Although several species have declined and disappeared simultaneously in some places (Laurance et al. 1996), in other places some species have persisted while others declined to extinction (Campbell 1999). Numerous authors (e.g., Storfer 2003) have noted that amphibians often exist as metapopulations, with dynamically varying patterns of occupancy across a network of patches. Populations in a given patch are more likely to decline than increase in a given year (Alford & Richards 1999), which means monitoring programs may falsely conclude that a species is in trouble because populations are declining in most years. A further problem that may be less well recognized is that monitoring programs are usually established around extant populations and not in currently unoccupied patches (Skelly et al. 2003). This means the monitoring program detects the patch extinction component of metapopulation dynamics but not the colonization component.

Few existing host-pathogen models deal simultaneously with spatial heterogeneity and reservoirs. McCallum and Dobson (2002) constructed a very simple, stylized metapopulation model that shows that a host that is highly susceptible to a pathogen may persist in association with a reservoir species in a metapopulation, provided the colonization rate of the susceptible species is sufficiently high. Although some species of Australian rainforest frogs have recolonized sites from which they initially disappeared (McDonald & Alford 1999), at this point there is little information from which to obtain comparative patch colonization rates for putative reservoir frog species and susceptible species.

Discussion

Current Status of the Chytrid Epidemic Hypothesis

Whether *B. dendrobatidis* has been the primary causative agent of rainforest frog declines in Australia remains an open question. Where extinctions were rapid, and museum specimens from the time of decline are not available, it will never be possible to attribute the declines unequivocally to chytridiomycosis or to any other cause. There are two extreme positions concerning the role of *B. dendrobatidis* in frog declines. One possibility is that the pathogen is a novel infection in Australia, the Americas, and Europe and that the pulse of declines we are currently experiencing is the result of exposure of

susceptible frog species to the pathogen for the first time. At the other extreme is the possibility that the fungus is a ubiquitous saprophyte in almost all freshwater environments worldwide, and it is only when frogs are exposed to certain environmental stressors or cofactors that the fungus causes (or is associated with) overt disease.

In between these extremes lies a range of possibilities, in which there is some role for environmental cofactors and stressors increasing the susceptibility of certain frog species to epidemics of the disease, and a role for various human activities to increase the movement of strains of the fungus to which local frog populations are not adapted. At present, there is insufficient evidence to reject any hypothesis on this continuum unequivocally, but the available data do make certain possibilities unlikely, and we can identify critical experiments and studies that will enable us to restrict the possibilities further.

A Research Agenda for Frog Chytridiomycosis

In cases where there were declines, but not extinctions, it is possible to investigate the prevalence and dynamics of chytridiomycosis in the remaining frog communities, and where declines are still occurring, it may be possible to investigate the dynamics of the host–pathogen interaction at the time of decline. Such investigations will help us understand whether the fungus has been involved in the decline of rainforest frogs and help identify management strategies for preventing further declines or assisting populations to recover.

Three levels of epidemiological information are needed. First, there is little reliable published data on the prevalence of infection in rainforest frog populations as distinct from morbid individuals. If a pathogen is having a continuing detrimental effect on a particular population, then the prevalence in morbid individuals should be higher than that in the general population. However high the prevalence is in morbid individuals, if it is the same in the general population, the putative pathogen is unlikely to be having a detrimental effect on the host population. Information on prevalence is required for species that have declined and those that have remained stable. It is needed with both spatial and temporal resolution. If *B. dendrobatidis* is an agent of decline, the prediction is that its prevalence should be higher in those species that have not declined than in species that have declined. In particular, if the fungus has spread between rainforest patches, it should be detectable in frog species occupying areas between patches. Some data are beginning to be collected on the prevalence of *B. dendrobatidis* in frog populations (Retallick et al. 2004). Recently developed nondestructive diagnostic tests that permit repeated testing of the same individuals in the field (Nowak 2003; A. D. Hyatt, personal communication) should improve the data situation considerably.

Second, there is a need for estimation of basic epidemiological parameters, including the development time of infection, pathogenicity to frogs, and transmission dynamics of the pathogen. These need to be determined as a function of infective dose, pathogen strain, frog species, and temperature. An obvious prediction, which remains untested, is that the pathogenicity of the fungus is temperature dependent in those species that have declined at elevations above 400 m but not at lower elevations, including *L. rheocola*, *L. nannotis*, and *N. dayi* (McDonald & Alford 1999). There is clear evidence that *B. dendrobatidis* grows better in vitro at 23° C than at higher temperatures, but what happens in vivo is less clear (Daszak et al. 2003). It is critical that such studies be undertaken in the field as well as in the laboratory.

One important aspect of the pathogen's transmission dynamics that needs to be determined is the extent to which frogs reinfect themselves under field conditions. Laboratory studies on disease progression, in which frogs are housed in small containers, should not be expected to produce results that are informative about self-reinfection in the field. The zoospores are released from the sporangium onto the surface of the epidermis or into the water in which the frog is living (Piotrowski et al. 2004). Whether zoospores immediately reinfect the host or whether they are dispersed into the water column may depend on host behavior and microhabitat. Mesocosm experiments with pathogens are ethically controversial but are essential to understand transmission.

Third, information is needed on the force of infection (the rate at which uninfected frogs acquire infection). Potentially, this can be obtained by introducing uninfected "sentinel" frogs into the environment and then removing them after a fixed amount of time to determine the proportion that have acquired infection. Age-specific prevalence data can also be used to estimate the force of infection, provided infection can be assumed to be age-independent and the recovery rate is known (McCallum 2000). For such information to be useful in understanding the dynamics of infection (in particular, for estimating R_0), it needs to be associated with estimates of the prevalence of the pathogen in the environment, either on frogs of all species infected or of zoospores.

The extent to which *B. dendrobatidis* can persist in aquatic environments in the absence of amphibians is a critical question. Freshwater environments contain a wide variety of fungi and bacteria, which strongly compete with each other for access to growth substrates (Yuen et al. 1999). Such competition will also occur on anuran skin, where antimicrobial peptides will modify its outcomes (Rollins-Smith et al. 2002; Apponyi et al. 2004). If the fungus persists in natural environments primarily as a saprophyte, then understanding disease emergence may be largely a question in saprophyte community ecology, to which the dynamics on frogs are of secondary importance.

Finally, we need to be alert to the likelihood that there are multiple strains of *B. dendrobatidis*, and that pathogenicity to particular frog species and under particular temperature regimes may vary between these strains. Co-evolution of hosts and pathogens following the introduction of a pathogen to a novel population may be extremely rapid (within a few years, in the case of myxomatosis and rabbits [Fenner & Fantini 1999]), which means any such strain divergence would be unlikely to be detected with the neutral markers commonly employed in studies of molecular evolution.

Implications for Management

If epidemic disease is responsible for frog declines, the most obvious management action is to attempt to limit further spread of the pathogen. This might be done by restricting movement of amphibia by humans and by ensuring that humans who come into contact with potentially infected amphibia (especially researchers) employ strict disinfection procedures. Guidelines for this have been established in Australia (Speare 2001). If *B. dendrobatidis* proves to be endemic in most areas in which it might potentially become established, such procedures will not help but are a sensible precautionary step.

In the same vein, some authors suggest (e.g., Hess 1996) that wildlife corridors need to be considered with caution where disease poses a threat to a spatially subdivided population. McCallum and Dobson (2002), however, show that a host metapopulation can persist with high connectivity, even in the worst-case scenario of a lethal disease maintained by a reservoir host, whereas too little connectivity will always lead to host extinction. The fear of a pathogen threat should not, therefore, necessarily prevent establishing or maintaining corridors between isolated populations. Nevertheless, maintaining or creating high connectivity may be a problem if the endangered species disperses more slowly than a reservoir host.

It is unlikely that a means for treating amphibian populations for chytridiomycosis in the field will be developed in the near future, so manipulative methods for controlling the pathogen should concentrate on captive populations and the development of appropriate strategies for reintroductions. In some cases, it has proved possible to treat captive frogs for chytridiomycosis, either with elevated temperatures (Woodhams et al. 2003) or by chemotherapy (Parker et al. 2002). Further research is necessary to identify appropriate strategies for reintroduction of captive-reared animals to areas from which they have disappeared. If it is the case that *B. dendrobatidis* is endemic in most environments and disease outbreaks are the result of changes in cofactors, then the crucial problem is to identify and alleviate those cofactors, after which reintroduction would then be possible. Management of a threat that causes population decline

requires first identifying the agent and then neutralizing it (Caughley 1994). Otherwise, reintroduction is futile.

If *B. dendrobatidis* is the primary cause of declines, with limited influence of cofactors, reintroductions will likewise fail unless the pathogen disappears from or can be removed from the environment or the reintroduced animals can be made resistant to infection. What little evidence there is suggests that the fungus persists in frog populations or the environment after decline, so simple reintroductions will probably fail. One exciting area of hope is the possibility that there may be evolution of either resistance in frogs or decreased virulence in the pathogen following initial declines (Retallick et al. 2004). If this is the case, then selection for resistance in captive populations before reintroduction may be the best long-term prospect for the recovery of populations affected by *B. dendrobatidis*.

Acknowledgments

I am grateful to A. Blaustein and D. Skelly for commenting on an earlier version of this manuscript. This research was supported by the Australian Research Council.

Literature Cited

- Alford, R. A., and S. J. Richards. 1999. Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics* 30:133–165.
- Alford, R. A., and S. J. Stephens. 1997. Lack of evidence for epidemic disease as an agent in the catastrophic decline of Australian rain forest frogs. *Conservation Biology* 11:1026–1029.
- Anderson, R. M. 1979. Parasite pathogenicity and the depression of host population equilibria. *Nature* 279:150–152.
- Anderson, R. M., and R. M. May. 1980. Infectious diseases and population cycles of forest insects. *Science* 210:658–661.
- Anderson, R. M., and R. M. May 1991. *Infectious diseases of humans*. Oxford University Press, Oxford, United Kingdom.
- Apponyi, M. A., T. L. Pukala, C. S. Brinkworth, V. M. Maselli, J. H. Bowie, M. J. Tyler, G. W. Booker, J. C. Wallace, J. A. Carver, and F. Separovic. 2004. Host-defence peptides of Australian anurans: structure, mechanism of action and evolutionary significance. *Peptides* 25:1035–1054.
- Begon, M., and R. G. Bowers. 1994. Host-host-pathogen models and microbial pest control: the effect of host self regulation. *Journal of Theoretical Biology* 169:275–287.
- Berger, L. et al. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Science U. S. A.* 95:9031–9036.
- Berger, L. et al. 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Australian Veterinary Journal* 82:434–439.
- Berger, L., R. Speare, and A. Kent. 1999. Diagnosis of chytridiomycosis in amphibians by histologic examination. James Cook University, Townsville, Australia. Available from <http://www.jcu.edu.au/school/phtm/PHTM/frogs/histo/chhisto.htm> (accessed November 2004).
- Berkelmans, R., G. De'ath, S. Kininmonth, and W. J. Skirving. 2004. A comparison of the 1998 and 2002 coral bleaching events on the

- Great Barrier Reef: spatial correlation, patterns, and predictions. *Coral Reefs* **23**:74–83.
- Blaustein, A. R., and J. M. Kiesecker. 2002. Complexity in conservation: lessons from the global decline of amphibian populations. *Ecology Letters* **5**:597–608.
- Blaustein, A. R., D. G. Hokit, R. K. O'Hara, and R. A. Holt. 1994. Pathogenic fungus contributes to amphibian losses in the Pacific Northwest. *Biological Conservation* **67**:251–254.
- Campbell, A., editor. 1999. Declines and disappearances of Australian frogs. Environment Australia, Canberra.
- Carey, C., N. Cohen, and L. Rollins-Smith. 1999. Amphibian declines: an immunological perspective. *Developmental and Comparative Immunology* **23**:459–472.
- Caughley, G. 1994. Directions in conservation biology. *Journal of Animal Ecology* **63**:215–244.
- Collins, J. P., and A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. *Diversity and Distributions* **9**:89–98.
- Courchamp, F., T. Clutton-Brock, and B. Grenfell. 1999. Inverse density dependence and the Allee effect. *Trends in Ecology & Evolution* **14**:405–410.
- Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2003. Infectious disease and amphibian population declines. *Diversity and Distributions* **9**:141–150.
- Daszak, P., L. Berger, A. A. Cunningham, A. D. Hyatt, D. E. Green, and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases* **5**:735–748.
- Davidson, E. W., M. Parris, J. P. Collins, J. E. Longcore, A. P. Pessier, and J. Brunner. 2003. Pathogenicity and transmission of chytridiomycosis in tiger salamanders (*Ambystoma tigrinum*). *Copeia* **2003**:601–607.
- Fellers, G. M., D. E. Green, and J. E. Longcore. 2001. Oral chytridiomycosis in the mountain yellow-legged frog (*Rana muscosa*). *Copeia* **2001**:945–953.
- Fenner, F., and B. Fantini. 1999. Biological control of vertebrate pests: the history of myxomatosis; an experiment in evolution. CABI Publishing, Wallingford, Oxon, United Kingdom.
- Freeland, W. J. 1983. Parasites and the coexistence of animal host species. *The American Naturalist* **121**:223–236.
- Gog, J., R. Woodroffe, and J. Swinton. 2002. Disease in endangered metapopulations: the importance of alternative hosts. *Proceedings of the Royal Society of London Series B: Biological Sciences* **269**:671–676.
- Grosholz, E. D. 1992. Interactions of intraspecific, interspecific, and apparent competition with host-pathogen population dynamics. *Ecology* **73**:507–514.
- Hamer, W. H. 1906. Epidemic disease in England—the evidence of variability and the persistence of type. *The Lancet* **1**:733–739.
- Hero, J. M., and G. R. Gillespie. 1997. Epidemic disease and amphibian declines in Australia. *Conservation Biology* **11**:1023–1025.
- Hess, G. 1996. Disease in metapopulation models: implications for conservation. *Ecology* **77**:1617–1632.
- Holt, R. D., and J. Pickering. 1985. Infectious disease and species coexistence: a model of the Lotka-Volterra form. *The American Naturalist* **126**:196–211.
- Houlahan, J. E., C. S. Findlay, B. R. Schmidt, A. H. Meyer, and S. L. Kuzmin. 2000. Quantitative evidence for global amphibian population declines. *Nature* **404**:752–755.
- Johnson, M. L., and R. Speare. 2003. Survival of *Batrachochytrium dendrobatidis* in water: Quarantine and disease control implications. *Emerging Infectious Diseases* **9**:922–925.
- Jones, J. B., A. D. Hyatt, P. M. Hine, R. J. Whittington, D. A. Griffin, and N. J. Bax. 1997. Special topic review: Australasian pilchard mortalities. *World Journal of Microbiology and Biotechnology* **13**:383–392.
- Kermack, W. O., and A. G. McKendrick. 1927. A contribution to the mathematical theory of epidemics. *Proceedings of the Royal Society of London Series B: Biological Sciences* **115**:700–721.
- Lampo, M., and G. A. DeLeo. 1998. The invasion ecology of the toad *Bufo marinus*: from South America to Australia. *Ecological Applications* **8**:388–396.
- Laurance, W. F., K. R. McDonald, and R. Speare. 1996. Epidemic disease and the catastrophic decline of Australian rain forest frogs. *Conservation Biology* **10**:1–9.
- Laurance, W. F., K. R. McDonald, and R. Speare. 1997. In defense of the epidemic disease hypothesis. *Conservation Biology* **11**:1030–1034.
- Lips, K. R. 1999. Mass mortality and population declines of anurans at an upland site in western Panama. *Conservation Biology* **13**:117–125.
- 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.
- McCallum, H. I., and A. P. Dobson. 1995. Detecting disease and parasite threats to endangered species and ecosystems. *Trends in Ecology and Evolution* **10**:190–194.
- McCallum, H. 2000. Population parameters: estimation for ecological models. Blackwell Science, Oxford, United Kingdom.
- McCallum, H., and A. Dobson. 2002. Disease, habitat fragmentation and conservation. *Proceedings of the Royal Society of London Series B: Biological Sciences* **269**:2041–2049.
- McCallum, H., N. D. Barlow, and J. Hone. 2001. How should transmission be modelled? *Trends in Ecology & Evolution* **16**:295–300.
- McDonald, K., and R. Alford. 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.
- Morehouse, E. A., T. Y. James, A. R. D. Ganley, R. Vilgalys, L. Berger, P. J. Murphy, and J. E. Longcore. 2003. Multilocus sequence typing suggests that the chytrid pathogen of amphibians is a recently emerged clone. *Molecular Ecology* **12**:395–403.
- Nowak, R. 2003. Ecology—tests improve odds in frog fungus fight. *New Scientist* **177**:18–18.
- Parker, J. M., I. Mikaelian, N. Hahn, and H. E. Diggs. 2002. Clinical diagnosis and treatment of epidermal chytridiomycosis in African clawed frogs (*Xenopus tropicalis*). *Comparative Medicine* **52**:265–268.
- Pessier, A. P., D. K. Nichols, J. E. Longcore, and M. S. Fuller. 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.
- Price, P. W., M. Westoby, and B. Rice. 1988. Parasite-mediated competition: some predictions and tests. *The American Naturalist* **131**:544–555.
- Rachowicz, L. J., and V. T. Vredenburg. 2004. Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. *Diseases of Aquatic Organisms* **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.
- Rollins-Smith, L. A., J. K. Doersam, J. E. Longcore, S. K. Taylor, J. C. Shamblyn, C. Carey, and M. A. Zasloff. 2002. Antimicrobial peptide defenses against pathogens associated with global amphibian declines. *Developmental and Comparative Immunology* **26**:63–72.
- Skelly, D. K., K. L. Yurewicz, E. E. Werner, and R. A. Relyea. 2003. Estimating decline and distributional change in amphibians. *Conservation Biology* **17**:744–751.
- Speare, R. 2001. Recommendations from workshop in getting the jump on amphibian disease. Attachment 5. Pages 131–147 in R. Speare and Steering Committee of Getting the Jump on Amphibian Disease. Developing management strategies to control amphibian diseases: decreasing the risks due to communicable diseases. School of Public Health and Tropical Medicine, James Cook University, Townsville, Australia.

- Speare, R., and L. Berger. 2004. Chytridiomycosis in amphibians in Australia. James Cook University, Townsville, Australia. Available from <http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyspec.htm> (accessed November 2004).
- Storfer, A. 2003. Amphibian declines: future directions. *Diversity and Distributions* **9**:151-163.
- Sutter, M. C. 1996. Assigning causation in disease: beyond Koch's postulates. *Perspectives in Biology and Medicine* **39**:581-592.
- Swinton, J., J. Harwood, B. T. Grenfell, and C. A. Gilligan. 1998. Persistence thresholds for phocine distemper virus infection in harbour seal *Phoca vitulina* metapopulations. *Journal of Animal Ecology* **67**:54-68.
- van den Bosch, F., F. R. Hengeveld, and J. A. J. Metz. 1992. Analysing the velocity of animal range expansion. *Journal of Biogeography* **19**:135-150.
- Weldon, C., L. H. du Preez, A. D. Hyatt, R. Muller, and R. Speare. 2004. Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases* **10**:2100-2105.
- 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.
- Young, B. E., et al. 2001. Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* **15**:1213-1223.
- Yuen, T. K., K. D. Hyde, and I. J. Hodgkiss. 1999. Interspecific interactions among tropical and subtropical freshwater fungi. *Microbial Ecology* **37**:257-262.

