

Impact and Dynamics of Disease in Species Threatened by the Amphibian Chytrid Fungus, *Batrachochytrium dendrobatidis*

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Abstract: Estimating disease-associated mortality and transmission processes is difficult in free-ranging wildlife but important for understanding disease impacts and dynamics and for informing management decisions. In a capture-mark-recapture study, we used a PCR-based diagnostic test in combination with multistate models to provide the first estimates of disease-associated mortality and detection, infection, and recovery rates for frogs endemically infected with the chytrid fungus *Batrachochytrium dendrobatidis* (Bd), which causes the pandemic amphibian disease chytridiomycosis. We found that endemic chytridiomycosis was associated with a substantial reduction (approximately 38%) in apparent monthly survival of the threatened rainforest treefrog *Litoria pearsoniana* despite a long period of coexistence (approximately 30 years); detection rate was not influenced by disease status; improved recovery and reduced infection rates correlated with decreased prevalence, which occurred when temperatures increased; and incorporating changes in individuals' infection status through time with multistate models increased effect size and support (98.6% vs. 71% of total support) for the presence of disease-associated mortality when compared with a Cormack-Jolly-Seber model in which infection status was restricted to the time of first capture. Our results indicate that amphibian populations can face significant ongoing pressure from chytridiomycosis long after epidemics associated with initial Bd invasions subside, an important consideration for the long-term conservation of many amphibian species worldwide. Our findings also improve confidence in estimates of disease prevalence in wild amphibians and provide a general framework for estimating parameters in epidemiological models for chytridiomycosis, an important step toward better understanding and management of this disease.

Keywords: amphibian declines, *Batrachochytrium dendrobatidis*, chytridiomycosis, endemic, epidemiology, mark-recapture, survival, transmission

Impacto y Dinámica de la Enfermedad en Especies Amenazadas por el Hongo Quitridio Anfibio *Batrachochytrium dendrobatidis*

Resumen: La estimación de la mortalidad asociada a enfermedades y los procesos de transmisión es difícil en vida silvestre libre pero importante para entender los impactos y la dinámica de las enfermedades y para información para decisiones de manejo. En un estudio de captura-marca-recaptura, utilizamos una prueba diagnóstica basada en PCR en combinación con modelos multiestado para proporcionar las primeras estimaciones de mortalidad asociada con enfermedades y de tasas de detección, infección y recuperación de ranas infectadas endémicamente por el hongo quitridio *Batrachochytrium dendrobatidis* (Bd), que provoca la quitridiomycosis, enfermedad pandémica de anfibios. Encontramos que la quitridiomycosis se asoció con una reducción sustancial (~38%) en la supervivencia mensual aparente de la rana arborícola amenazada *Litoria pearsoniana* no obstante un largo período de coexistencia (~30 años); la tasa de detección no fue

influida por el estatus de la enfermedad; una mejor recuperación y la reducción en las tasas de infección se correlacionaron con una disminución en la prevalencia, lo que ocurrió cuando las temperaturas incrementaron y la incorporación de cambios en el estatus de infección de individuos en el tiempo con los modelos multiestado incrementaron el efecto del tamaño y soporte (98.6% vs. 71% de soporte total) para la presencia de mortalidad asociada a enfermedades cuando comparamos con el modelo Cormack-Jolly-Seber en el que se restringió el estatus de la infección para el tiempo de la primera captura. Nuestros resultados indican que las poblaciones de anfibios pueden enfrentar la presión significativa actual de la quitridiomycosis mucho después de que aminoren las epidemias asociadas con invasiones iniciales de Bd, una consideración importante para conservación a largo plazo de muchas especies de anfibios en el mundo. Nuestros hallazgos también mejoran la confianza en estimaciones de la prevalencia de enfermedades en anfibios silvestres y proporcionan un marco general para la estimación de parámetros en modelos epidemiológicos para quitridiomycosis, un paso importante hacia un mejor entendimiento y manejo de esta enfermedad.

Palabras Clave: *Batrachochytrium dendrobatidis*, declinaciones de anfibios, endémico, epidemiología, marca-recaptura, sobrevivencia, transmisión

Introduction

Amphibians have declined globally. Around one-third of the world's approximately 6000 species are now considered threatened, and up to 122 species may be extinct (Stuart et al. 2004). Among many threatening processes, the disease chytridiomycosis has been implicated in many of the most rapid and recent declines (Stuart et al. 2004; Lips et al. 2006). The fungal pathogen that causes chytridiomycosis, *Batrachochytrium dendrobatidis* Longcore et al. (1999) (hereafter Bd), has been found in 233 species on five continents (Africa, the Americas, Europe, Australasia) (Olson & Ronnenberg 2008). Now formally recognized as an international notifiable disease (obligatory reporting of detection of Bd to the World Organisation for Animal Health) (World Organisation for Animal Health 2008), chytridiomycosis is thought to be the most significant disease affecting biodiversity of vertebrates (Skerratt et al. 2007) and is therefore a problem of central importance in applied conservation biology.

Current evidence suggests that Bd originated in Africa and was repeatedly introduced as a novel pathogen to new geographic locations (Morehouse et al. 2003; Weldon et al. 2004; Fisher & Garner 2007). Unlike most wildlife pathogens, Bd appears capable of driving populations to extinction because it has a broad host range, infects both larval and adult amphibians, and may exist in the environment for at least several weeks without a host. Thus, it can persist independent of a particular species undergoing epidemic declines (Cleaveland et al. 2002). Management of the disease at present is limited to restricting the spread of Bd, with conventional mitigation strategies (e.g., treatment or culling) unlikely to be effective at broad spatial or temporal scales (Department of the Environment & Heritage 2006). For many affected species, long-term survival in the wild (assuming survival through initial or repeated epidemics) will thus depend on evolutionary processes as the pathogen becomes endemic.

Some populations and species have indeed persisted after decline and possibly have begun to recover, despite coexistence with Bd for long periods (e.g., Retallick et al. 2004; McDonald et al. 2005; Rodríguez-Contreras et al. 2008). Such cases suggest that a shift in the host-pathogen relationship favoring host survival may occur (McCallum 2005), although the mechanisms remain unresolved. Understanding the properties of the host-parasite relationship in endemic situations is thus critical for informing management decisions and the long-term conservation of many amphibian species.

In Australia the oldest Bd record is from a museum frog specimen collected in southeast Queensland in 1978 (Speare 2006), which coincides with frog declines in a number of species and two species' extinctions in the region (Berger et al. 1998; Hines et al. 1999). There is no evidence that climatic anomalies caused these declines, either directly or as suggested by current models of climate-linked disease outbreaks (Pounds et al. 2006; Laurance 2008). The invasion of Bd is thought to be the cause of these and other subsequent extinctions elsewhere in Australia (Skerratt et al. 2007, but see Alford et al. 2007 for additional information). Frog assemblages in southeast Queensland thus provide an ideal opportunity to test the extent to which Bd affects endemically infected frog populations and to investigate the dynamics of these Bd infections.

As with all wildlife diseases, quantifying the impacts and dynamics of chytridiomycosis in the wild is difficult. One approach that shows considerable promise for studies of disease in free-ranging wildlife is the use of capture-mark-recapture (CMR) methods (Williams et al. 2002). Underpinning CMR theory is the estimation of detection probability (the probability of detecting an individual in a sample if it is alive and present in the sampling area). Accounting for variation in detection probability is necessary for estimating a range of demographic parameters, such as survival. In wildlife disease studies, one major consideration is the extent to which infections alter

detection probability, which may confound a number of basic epidemiological parameters of interest (Jennelle et al. 2007). For example, where an infection reduces detection rate (e.g., by reducing activity), disease prevalence in a sample could significantly underestimate prevalence in the population and result in spurious inferences. Despite this source of considerable potential bias, detection rate has been rarely considered in studies of wildlife disease (Jennelle et al. 2007).

In another application CMR models can be used directly to model disease-associated mortality and estimate epidemiological parameters, such as infection and recovery rates (Oli et al. 2006). Multistate CMR models provide state-dependent maximum likelihood estimates of survival and detection rates and estimate the transition rates between discrete states (e.g., infected or uninfected) (Williams et al. 2002). Rarely used in studies of infectious disease (Oli et al. 2006), multistate models have been used recently to investigate the effects and dynamics of *Mycoplasma* infections in House Finches (*Carpodacus mexicanus*) (Faustino et al. 2004) and facial tumor disease in Tasmanian devils (*Sarcophilus harrisi*) (Lachish et al. 2007). Multistate models require that the infection status of individuals is determined each time they are encountered. Until recently, they could not be used in Bd research because diagnosis was made via histology of toe clips taken at the time of first capture (e.g., Retallick et al. 2004) and resampling was limited for ethical reasons. With the development of a nondestructive, real-time PCR test for Bd with high sensitivity and specificity (Hyatt et al. 2007), individuals can now be tested repeatedly over time.

We used multistate mark-recapture models in combination with real-time PCR diagnostic testing to address several questions about the effects and dynamics of endemic Bd infections. First, we detected and estimated disease-associated mortality. Second, we investigated whether detection rate is likely to confound prevalence estimates from samples. Third, we generated the first estimates of state-transition probabilities for any frog population (analogous to infection and recovery rates).

Methods

Mark-Recapture Study Site and Species

We conducted a mark-recapture study along a mid-elevation section of Peter's Creek ($26^{\circ}40'47''$ S $152^{\circ}36'27''$ E; 489 m asl) located on the Conondale Range in southeast Queensland, Australia (Fig. 1). The region is subtropical with warm, wet summers (mean maximum temperature approximately 27°C ; mean rainfall approximately 780 mm) and cooler, dryer winters (mean maximum temperature approximately 19.5°C ; mean rainfall approximately 250 mm) (Australian Bureau of Meteorology records for Maleny; 425 m asl).

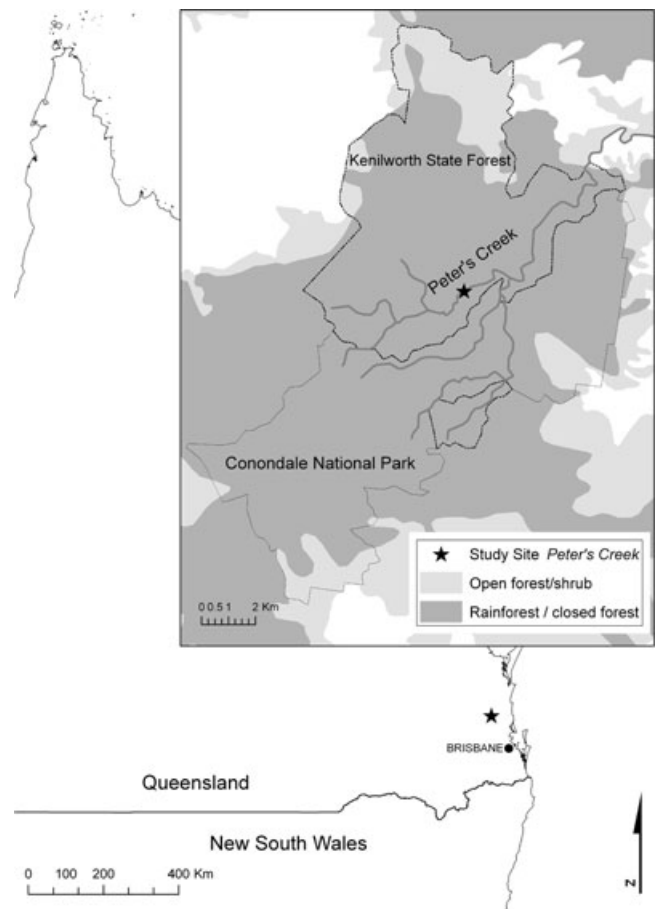


Figure 1. Location of study site within Queensland, Australia. Inset shows precise location of the study site on Peter's Creek ($26^{\circ}40'47''$ S $152^{\circ}36'27''$ E; 489 m asl) and the predominant vegetation types of the surrounding area.

We sampled six species encountered at Peter's Creek, although we considered only individuals of the cascade treefrog (*Litoria pearsoniana*) because of sparse recapture histories for other species. *L. pearsoniana* is a small, stream-breeding treefrog restricted to forested areas of southeast Queensland and northern New South Wales, Australia (Parris 2001). *L. pearsoniana* is listed as vulnerable under the Queensland Nature Conservation (Wildlife) Regulation 2006 and near threatened and "in significant decline. . . in part due to chytridiomycosis" by the IUCN (Hero et al. 2004).

Sampling

We surveyed a 120-m transect of Peter's Creek every month between August 2006 and March 2007 (the active season for this species). Each visit consisted of three consecutive nights of sampling. Frogs were captured in plastic freezer bags, weighed, and removed. A fresh pair of plastic gloves was worn to process each frog. Frogs

were swabbed (10 strokes on each of the feet, thighs, and hands, and on the left, center, and right ventral surfaces of the body) with a sterile swab (MW 100-100, Medical Wire and Equipment, Bath). Swabs were stored in the field on ice and later at room temperature (Hyatt et al. 2007). After swabbing frogs were measured (snout-urostyle length [SUL]) and inspected for clinical signs of infection (erythema of ventral surfaces and digits). Last, frogs were uniquely marked by toe clipping with disinfected fine-tipped scissors following the code of Hero (1989) and released at the point of capture.

We analyzed swabs in the laboratory for the presence of Bd with the TaqMan real-time PCR protocol (Boyle et al. 2004) and included an internal positive control to signal amplification inhibition (Hyatt et al. 2007). Hyatt et al. (2007) detail the properties of the test, including sensitivity, specificity, limitations, and comparisons with other methods.

Effects of Infections on Survival

MODELING OVERVIEW

We considered data only from adult male *L. pearsoniana* ($n = 134$) in our CMR analyses. No females were caught more than once, likely because of their tendency to visit the stream only briefly to breed. Juveniles were not suitable for marking and were excluded.

We constructed CMR models in program MARK (White & Burnham 1999) to estimate the apparent survival and detection rate of frogs and to test hypotheses deemed relevant for explaining patterns in the data set. Multistate models are an extension of conventional Cormack-Jolly-Seber (CJS) models, which, in addition to estimating state-dependent survival and detection rates, make use of the repeat-infection testing of individuals to model transitions between disease states (representing infection and recovery rates). A "recovery" was thus defined as a state transition between sampling periods from a positive test result to a negative test result and vice versa for an "infection." We also developed a conventional CJS open-population model in which frogs were restricted to being Bd-positive or -negative at first capture (see Supporting Information).

GOODNESS OF FIT AND MODEL SELECTION

As analogues of the CJS model assumptions, multistate models assume that marked individuals in state r at time i behave in the same way (i.e., have the same probability of survival to time $i+1$ and the same probability of recapture and of moving to state s). We used the program U-CARE (Choquet et al. 2005) to assess the fit of the data to the most general Jolly MoVe-JMV) model and to estimate the variance-inflation factor (\hat{C}). In addition, the probability of changing states from time i to $i+1$ depends only on the

state at time i and not on any previous states. At present, there are no published data that suggest a violation of this assumption (Speare 2006). Unpublished laboratory experiments, however, suggest that reinfection duration may be shortened after treatment in some species, but the duration and generality of such an effect is unknown (R.S., unpublished data). If this effect applies to wild, self-cured frogs, modeling state transitions as a higher-order Markov process may be required. In addition, marks cannot be lost or missed and sampling periods must be instantaneous. Toe-clip codes are permanent, every effort was made to minimize errors in marking or reading, and our trapping periods were short (3 days) compared with the interval between trips (30 days).

Multistate models also assume that state is accurately determined each time an individual is observed. Although negative and positive results in all three wells of a triplicate PCR test are generally regarded as providing a well-supported absence or presence of Bd, there remains uncertainty about the interpretation of equivocal results (one or two wells positive), and both false negatives and false positives remain a possibility. We adopted all recommended sampling and laboratory protocols to minimize and detect false positives (contamination) and retested a minority of samples that returned equivocal results (Hyatt et al. 2007). Hyatt et al. (2007) consider that equivocal results most likely reflect low levels of Bd present on the sample, which are characteristic of very light infections. In our analyses frogs were thus considered to test either positive (one to three wells positive for Bd in a triplicate PCR) or negative (zero wells positive) in an effort to minimize false-negative errors. Although this was at the expense of potentially committing more false-positive errors, we believed it was important to include frogs with even very light Bd loads in the positive class so as to gain the most conservative estimate of disease-associated mortality (light infections should have little immediate impact on survival).

Model ranking was based on small-sample-size-corrected Akaike's information criterion (AICc) (Anderson & Burnham 2002). The best model has an AICc score of zero, whereas models with AICc scores >2 are considered substantially less well supported than the best model. To account for model-selection uncertainty, robust parameter estimates were generated after weighted model averaging across relevant models in the candidate set (Burnham & Anderson 2002). Where cited, the effect size of disease (on the logit scale) was model averaged from relevant models with AICc scores <4 (Burnham & Anderson 2002).

CMR-MODEL FACTORS AND PARAMETERS

The primary factor of interest was individual infection status (disease). We examined time dependence (i.e., monthly variation) in parameter estimates and several

covariate surrogates for time selected a priori for their potential to influence disease dynamics or frog behavior. These included apparent disease prevalence (the proportion of positive frogs in each sampling period) and mean temperature between visits (averaged hourly readings from two Thermochron i-button 1922L data loggers [Dallas Semiconductor, Dallas, Texas] housed in the shade 20 cm above ground level). As an alternative to time dependence in detection rate, we included models that allowed detection rates to vary as a function of the mean temperature across the days of sampling (sampling temperature—obtained as above). Other covariates (e.g., rainfall, number of rain days and dry days, humidity) were also considered but subsequently omitted after preliminary investigations indicated inferior fit to the data.

We used a 3-step process to estimate parameters. In step 1, we modeled transition rate as either time dependent or as a function of the covariates prevalence or mean temperature. In step 2, we used the best transition model to model survival and detection rates. The candidate set thus included time-dependent and covariate-constrained models representing interactive, additive, group, and constant model combinations (varying survival and detection-rate factors and with fully parameterized transition rate). When the best model was found, transition rate was reduced sequentially in a third step to test the terms and the interaction separately (Cooch & White 2001). (See Supporting Information for details of the CJS model.)

MOVEMENT

In CMR analyses survival is confounded with permanent emigration. Differences in survival estimates between disease states could therefore remain a direct result of differences in emigration rates if, for example, disease influences host behavior. To investigate this possibility, we used the measurable movement of individual recaptured frogs within the study area (movements among stream sections) as an index of likely emigration rate. We assumed animals that move more within the study area are also more likely to emigrate entirely from the study area, although our actual survival estimates will remain confounded.

RETURN RATES

Return rate (the proportion of marked animals recaptured) has been used in previous studies to test for an effect of Bd on host survival (Kriger & Hero 2006). This method has two limitations: survival is confounded with detection rate and detection rate may itself be state dependent (Cooch & White 2001; Jennelle et al. 2007). In estimating detection rate, our CMR analyses provided an opportunity to test the validity of using return rate as a surrogate of survival rate.

Table 1. Return rates of adult *L. pearsoniana* at Peter's Creek grouped by the status of their infection with *Batrachochytrium dendrobatidis* at first capture.

| Infection status* | Not recaptured | Recaptured | Total | Recaptured (%) |
|-------------------|----------------|------------|-------|----------------|
| Neg | 43 | 32 | 75 | 42.7 |
| Pos | 21 | 4 | 25 | 16 |
| Total | 64 | 36 | 100 | 36 |

*Positive (pos) for Bd, one to three wells positive in triplicate real-time PCR; negative (neg) for Bd, zero wells positive in triplicate real-time PCR.

In this test we compared return rates of adult *L. pearsoniana* categorized as positive or negative for Bd at the time of first capture (Table 1). Males and females were included but animals that had no opportunity of recapture were excluded (final $n = 100$). For comparison with previous studies and for broad geographic coverage, we extended the analysis to include data currently available in the literature in a single logistic model. Data were extracted from three studies and five frog populations (Fig. 3). Return-rate analyses were conducted in R (Version 2.2.0) (R Development Core Team 2006).

Results

Sample and Infection Pattern

We made 453 captures of 252 uniquely marked adult frogs at Peter's Creek during monthly visits between 14 September 2006 and 15 February 2007. No frogs were encountered in August 2006 or March 2007. *L. pearsoniana* accounted for 59.9% of 334 PCR tests and 80% of all positive results for the season. Three other species also returned positive PCR results (*L. chloris*, *L. wilcoxi*, and *Adelotus brevis*).

Infections in *L. pearsoniana* were acinical; the presence of erythema (8.1% of all tested animals) did not predict infection status ($\chi^2 = 0.183$, $df = 1$, $p = 0.6686$). There was no difference in SUL between Bd positive and negative *L. pearsoniana* ($t = -0.086$, $df = 55$, $p = 0.465$) or a difference in body condition (analysis of covariance log-transformed SUL and mass; $F_{1,151} = 0.006$, $p = 0.946$).

When monthly data were pooled into seasons (spring, September, October, November; summer, December, January, February), there was a substantial effect of season on infection prevalence among captured individuals ($\chi^2 = 9.732$, $df = 1$, $p = 0.002$), with frogs caught in spring (mean prevalence = 38.0%, 95% CI = 27.3–49.6%) being 2.74 times more likely (odds ratio 95% CI = 1.37–5.55; Fisher's exact test) to be infected than in summer (mean prevalence = 18.2%, 95% CI = 11.8–26.2%). In a logistic model, decreasing prevalence was significantly related to increases in mean ambient temperature in the

Table 2. Transitions between disease states as indicated by real-time PCR diagnostic tests for *Batrachochytrium dendrobatidis* (see Methods) in wild-caught and recaptured male *L. pearsoniana*.

| Transition type* | Total | Spring | Summer |
|------------------|-------|--------|--------|
| Pos-neg | 4 | 1 | 3 |
| Neg-pos | 12 | 6 | 6 |
| Neg-neg | 32 | 11 | 21 |
| Pos-pos | 4 | 2 | 2 |
| All transitions | 52 | 20 | 32 |

* Positive (pos) for *Bd*, one to three wells positive in triplicate real-time PCR; negative (neg), for *Bd*, zero wells positive in triplicate real-time PCR.

30 days prior to sampling ($\Delta dev = 11.940$, $df = 4$, $p = 0.006$; quasi-binomial; dispersion parameter 1.58).

CMR Modeling

MULTISTATE MODELS

We observed 52 state transitions from 42 frogs (Table 2). Two frogs remained positive for 2 months and a third remained positive over 3 months. Negative frogs were routinely captured after the same and greater time periods ($n = 16$ frogs, range 3–6 months). No frogs made a back transition (i.e., negative to positive to negative or positive to negative to positive).

There was no evidence of overdispersion or lack of fit of the most general JMV model ($\hat{c} = 0.943$). In step 1,

the covariate prevalence had marginally greater support (57%) than mean temperature (support approximately 43%) for affecting transition rate, although on the basis of the AICc scores, these models were indistinguishable ($\Delta AICc = 0.577$, Table 3). We thus proceeded in modeling survival and detection rates with prevalence-dependent transition parameters, because we favored the clear biological link between prevalence and disease dynamics while acknowledging that temperature may be the mechanistic driver.

The best three models ($AICc < 2$) in the final candidate set all had survival varying additively with disease and mean temperature and transition rates varying with the interaction of disease and prevalence (Table 3). Models indicating an effect of disease on survival held 98.6% of the support in the candidate set. The mean difference in survival estimates between states across months was approximately 38% (model-averaged effect size on the logit scale = 1.99, 95% CI = 0.57–3.42) (Fig. 2a). There was uncertainty as to whether detection rate was constant or varied with disease or sampling temperature, such that model averaging indicated that detection rate was essentially constant (Fig. 2b). Model-averaged transition rates indicated that infections and recoveries were negatively correlated and were strongly associated with disease prevalence (Fig. 2c). Results of the CJS models were similar, although the effect of disease on survival was considerably more uncertain and effect size was reduced compared with the multistate results (Supporting Information).

Table 3. Summary results of multistate model ranking used to generate robust estimates of monthly survival, detection, and transition rates through model averaging for male *L. pearsoniana*.*

| Model structure | | | AICc | QAICc | W | K | Deviance |
|-----------------|-----------|------------|---------|--------|-------|----|----------|
| survival | detection | transition | | | | | |
| dis+Mtemp | constant | dis*prev | 293.524 | 0.000 | 0.258 | 8 | 57.692 |
| dis+Mtemp | temp | dis*prev | 294.288 | 0.764 | 0.176 | 9 | 56.179 |
| dis+Mtemp | dis | dis*prev | 295.352 | 1.828 | 0.103 | 9 | 57.243 |
| dis*Mtemp | constant | dis*prev | 295.533 | 2.009 | 0.094 | 9 | 57.423 |
| dis*Mtemp | temp | dis*prev | 295.779 | 2.255 | 0.083 | 10 | 55.359 |
| dis+Mtemp | dis+temp | dis*prev | 296.595 | 3.071 | 0.055 | 10 | 56.175 |
| dis+Mtemp | constant | dis+prev | 296.839 | 3.315 | 0.049 | 7 | 63.249 |
| dis+Mtemp | constant | prev | 297.339 | 3.815 | 0.038 | 6 | 65.960 |
| dis*Mtemp | dis | dis*prev | 297.431 | 3.907 | 0.037 | 10 | 57.011 |
| ... | | | | | | | |
| dis*Mtemp | dis*temp | dis*prev | 299.980 | 6.456 | 0.010 | 12 | 54.833 |
| ... | | | | | | | |
| dis*time | dis*time | dis*prev | 323.625 | 30.101 | 0.000 | 24 | 46.775 |
| dis*time | dis*time | dis*temp | 324.202 | 30.678 | 0.000 | 24 | 47.352 |
| dis*time | dis*time | dis*time | 338.899 | 45.375 | 0.000 | 30 | 43.673 |

*The best three models ($\Delta AICc < 2$), the following six models ($\Delta AICc < 4$), and the covariate-constrained model are shown. The general model is shown in the bottom row. (See Supporting Information for full model set.) Abbreviations: dis, individual disease status; Mtemp, mean temperature in the recapture interval; prev, population disease prevalence; time, time dependence (i.e., different in each month); constant, constant across months and states; $\Delta AICc$, difference in AICc between the current and best model; W, the AICc weight; K, number of parameters in the model; deviance, model deviance. The AIC score is corrected for small sample size (AICc).

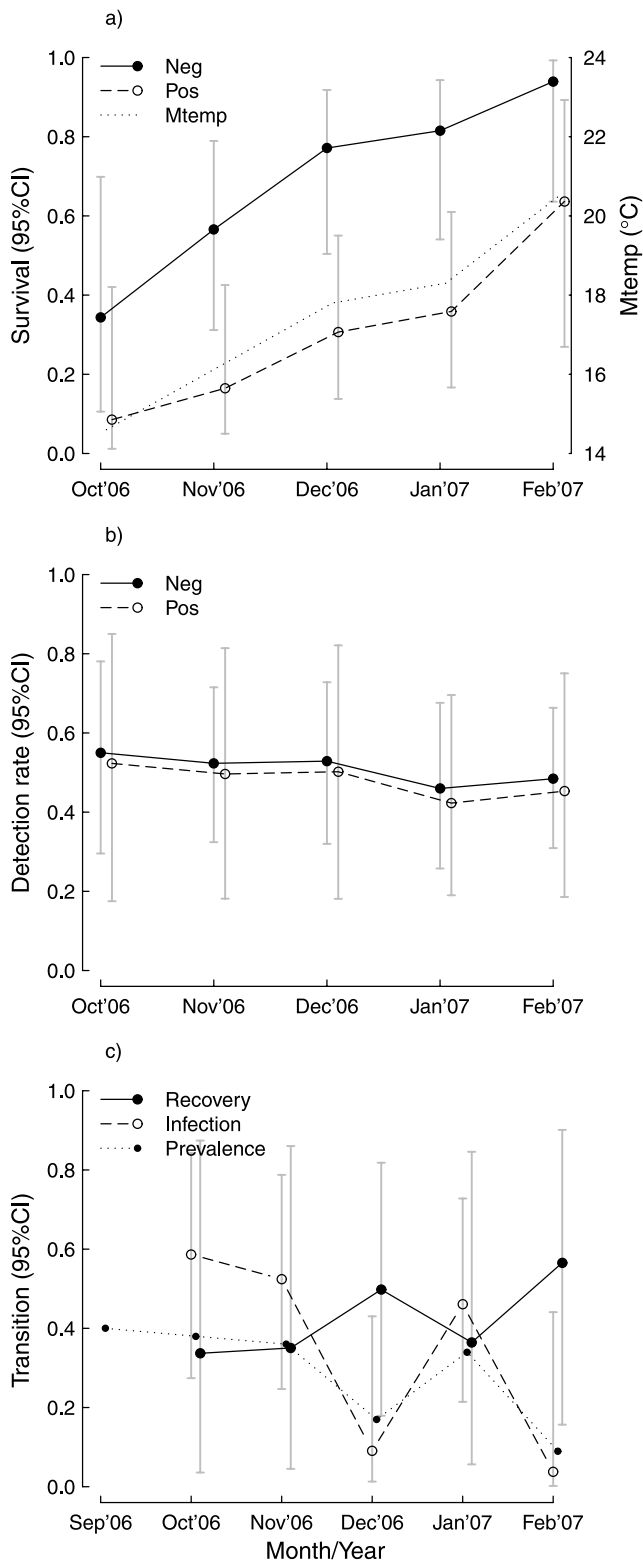


Figure 2. Model-averaged estimates of (a) survival, (b) detection rate, and (c) transition rates from the multistate capture-mark-recapture model for *L. pearsoniana* males at Peter's Creek (neg, frogs testing negative for *Batrachochytrium dendrobatidis* [Bd]; pos, frogs testing positive for Bd; Mtemp, mean

RETURN RATE

Consistent with our CMR analyses, uninfected *L. pearsoniana* at Peter's Creek were 3.86 times more likely to be recaptured at least once after marking than infected frogs (odds ratio 95% CI = 1.14–16.98, $p = 0.017$; Fisher's exact test). In the extended analysis, both study population and disease status were significant terms affecting return rate, but the interaction term was not significant (species $\Delta dev = 44.310$, $df = 4$, $p < 0.0001$, status $\Delta dev = 15.368$, $df = 1$, $p < 0.0001$). Odds ratios indicated uninfected frogs were consistently more likely to be recaptured than infected frogs (Fig. 3).

MOVEMENT

Recaptured frogs exhibited high site fidelity within the 120-m transect. Fifty-nine percent of 39 frogs with location data were recaptured in the same section of stream (i.e., they moved <10 m) between sampling sessions, and 82% were recaptured in the same or adjacent section (i.e., moved <20 m). Infection status was unrelated to movements among stream sections between sampling sessions ($\chi^2 = 0.0995$, $df = 1$, $p = 0.753$).

Discussion

Transmission is a key process for understanding host-pathogen dynamics, modeling the effects of disease on populations, and evaluating management strategies (Oli et al. 2006), but it is difficult to quantify in the field (McCallum et al. 2001). Rachowicz and Briggs (2007) used field enclosures and laboratory experiments to quantify the transmission rate of Bd in tadpoles (the rate at which infected hosts infect uninfected hosts) at a number of host densities, but no studies have yet been published for wild adult frogs, likely due to the difficulty of conducting appropriate field experiments.

In practice the easiest transmission parameter to estimate in wild animals is the force of infection, which is the rate at which susceptible hosts acquire infections. The transition rate from the negative to the positive class as modeled here (infection rate) with multistate CMR models is directly related to the force of infection (Heisey

temperature in the recapture interval; recovery, transition rate from positive for Bd to negative for Bd [i.e., recovery rate]; infection, transition rate from negative for Bd to positive for Bd [i.e., infection rate/force of infection]; prevalence, population disease prevalence estimate; error bars, 95% confidence intervals around the estimate). Table 3 contains contributing models and their weights.

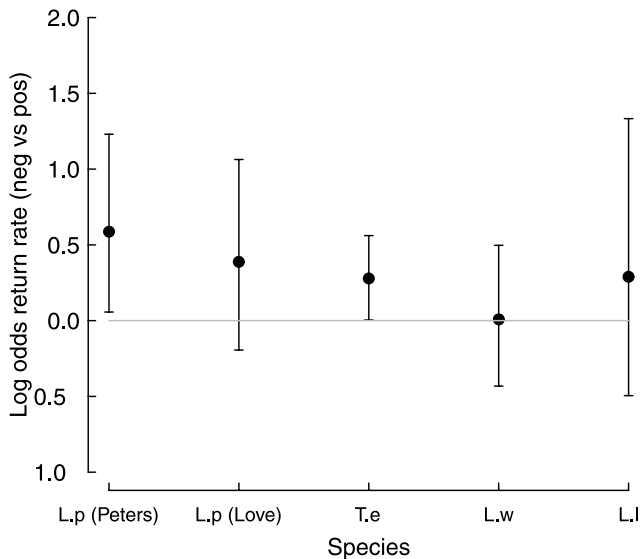


Figure 3. Log odds of return rates for frogs that were positive for *Batrachochytrium dendrobatidis* (*Bd*) versus negative for *Bd* in two populations of *L. pearsoniana* (*L.p* [Peters] and *L.p* [Love]) and one population each of *Taudactylus eungellensis* (*T.e*) at Eungella central coastal Queensland (Retallick et al. 2004); *Litoria wilcoxii* (*L.w*) in Numinbah Valley southeast Queensland (Kriger & Hero 2006); and *L. lesueuri* (*L.l*) at Eungella (Retallick et al. 2004). Log odds above zero indicate negative frogs were more likely to be recaptured (i.e., return to the sample) than infected frogs. Error bars are 95% confidence intervals around the estimate. Range of odds ratios for five populations 1.02–3.86 (Fisher's exact test). See Table 1 for data used to obtain odds ratio for *L.p* (Peters).

et al. 2006) and of direct use in epidemiological modeling applications (Oli et al. 2006). We found that infection rate was strongly and positively associated with disease prevalence. This is, of course, as would be expected with an infectious disease, but would not be the case if transmission was driven by a constant level of infection in an unidentified reservoir. Our prevalence variable was only an estimate of the actual prevalence in the population at the time of sampling. Such “error in variables” is likely to cause a bias toward accepting a null hypothesis (Carroll et al. 1995); thus, the estimate of the effect is likely to be conservative. We have provided the first estimates of the two-way transition probabilities between infection states, a key insight into the transmission dynamics of endemic chytridiomycosis in free-ranging adult frogs.

Another important advantage of using CMR models in general is the ability to model detection rate. Previous studies of *Bd*, and most other infectious diseases (Jennelle et al. 2007), have implicitly assumed that detection rate does not depend on infection status, but few have tested

this assumption. In our study the detection rate of frogs was not obviously dependent on infection status. With respect to sampling bias, this means that prevalence of *Bd* within our samples can be regarded as an unbiased estimate of prevalence among the population of male *L. pearsoniana*.

DISEASE IMPACTS

Our mark–recapture model provided strong support for an association between endemic *Bd* infections and reduced host survival. Nevertheless, few dead or moribund frogs were observed and we were unable to use clinical signs to reliably detect infection, indicating that *Bd* can be insidious where it is endemic. This is not surprising given that experimental chytridiomycosis in susceptible species, including *Litoria*, is typically acinical apart from during a very short terminal phase of 2–3 days in fatal cases compared with the incubation period of 18–76 days (Berger et al. 2004). The likelihood of an adult frog being infected was unrelated to frog size, body condition, or movement, so we have little reason to suspect that the association between *Bd* and reduced survival was an artifact of predisposition or differential emigration. Although we have no evidence as to whether *Bd* has changed in pathogenicity or virulence since its likely introduction to southeast Queensland, this result is consistent with the hypothesis that chytridiomycosis has at least contributed to *L. pearsoniana* declines in the past. More critically, the pathogen is clearly capable of causing significant ongoing mortality despite long periods of coexistence (at least 30 years).

Our results contrast with those of Retallick et al. (2004), who found no clear evidence that endemic *Bd* infections were associated with reduced survival in the endangered rainforest frog *Taudactylus eungellensis*. Although such a difference may reflect true differences in the host–pathogen relationship, it may equally arise from the methodological differences between the studies. The PCR-based diagnostic method we used not only provides a more accurate picture of disease prevalence in the population by being more sensitive but it also allows infection status to be determined each time an individual is captured. This allowed the use of the multistate framework, which can account for the processes of infectious diseases far more realistically. In our case the choice of framework had a substantial effect on survival estimates, model selection, and interpretation of results. The survival difference between infected and uninfected *L. pearsoniana* was greater (approximately 38% vs. approximately 16%) and more confidently supported (98.6% vs. 71% of total support) in the multistate model that incorporated transitions between disease states than in the CJS model that did not.

Consistent with our CMR analyses, uninfected *L. pearsoniana* at Peter's Creek were nearly four times more

likely to be recaptured after marking (i.e., return to the sample) than infected frogs, a result evident across a number of other species and populations endemically infected with Bd in Queensland. With no evidence that detection rate may confound such a comparison, return rate appears a useful surrogate for survival and could prove a rapid first-pass assessment of whether Bd infections are likely to be negatively affecting an infected population. Furthermore, this result considerably improves our confidence that a negative impact of endemic Bd infections is not likely to be restricted to our CMR study site but is broadly apparent within Queensland frog populations. Detection rate could, however, still vary and interact with other factors not included in our study, such as sex, site, and age. Given the risk of underestimation of effect size (due to lost information about transitions) and potential confounding from factors such as detection rate, we encourage researchers to use the more robust CMR methods where possible. The potential ramifications of spurious results misinforming management decisions could be large for amphibian conservation.

DISEASE DYNAMICS

Our results have implications for interpreting mechanisms of amphibian decline associated with chytridiomycosis. Seasonal and elevational variation in occurrence of Bd infections and amphibian mortalities has long implicated climatic conditions as a major factor governing the effects of Bd, with cooler periods and locations being associated with higher prevalence and greater mortality (Berger et al. 2004; Woodhams & Alford 2005; Kriger & Hero 2007). Laboratory cultures confirm temperature-dependent growth: Bd grows slowly at low temperatures, optimally at 17–25 °C, stops at 28 °C, and dies at 30 °C (Piotrowski et al. 2004). Laboratory experiments show that virulence decreases at high temperatures (approximately 27+ °C) and host survival time may increase at low temperatures (approximately 8 °C) (Woodhams et al. 2003; Berger et al. 2004). Nevertheless, temperature does not influence survival time of boreal toads at intermediate temperatures (12–23 °C) (Carey et al. 2006).

At our Peter's Creek study site, Bd prevalence was inversely related to increasing mean temperatures as spring progressed into summer, but the effect of infection on the log odds of survival of infected individuals remained relatively constant over the range of mean temperatures experienced during the season (12–23 °C). Coupled with evidence that capture probability is not strongly related to infection status, this suggests that prevalence in sampled frogs may indeed prove an appropriate indicator of the impact of chytridiomycosis at the population level. When reduced background survival coincides with periods of high disease prevalence, extinction risk will reach a maximum. At Peter's Creek frogs were nearly three times more likely to be infected in spring when survival

of both infected and uninfected frogs was low than in summer when survival of both classes improved.

This raises a question central to the dynamics of this infectious disease: What mechanisms contribute to consistent seasonal changes in disease prevalence? The hypothesis that increased temperatures simply limit the growth of Bd in the environment or on hosts cannot in itself result in decreased prevalence. For that to occur, infected frogs must be lost from the population via death or recovery, while the proportion of uninfected frogs must simultaneously increase via decreased infection rate. Our CMR results are consistent with this process: frogs suffered significant reductions in survival when infected and were more likely to recover and less likely to become infected at times when prevalence also decreased. In practice, proliferation, transmission, and prevalence are epidemiologically related; the causal link is likely to be temperature and its effect on Bd growth. This also explains why our multistate model indicated only slightly less support for temperature-dependent transition rate than prevalence-dependent transition rate (exemplifying the general problem of choosing correlated variables in model-selection procedures). Further investigations of these parameters in wild amphibians will be central to the development of epidemiological and population models for endemic chytridiomycosis (Briggs et al. 2005; Oli et al. 2006).

We have shown that endemic Bd infections are associated with substantial reductions in the survival of the threatened rainforest frog, *L. pearsoniana*, despite a long period of coexistence (approximately 30 years). Many amphibian populations may therefore face significant ongoing pressure from chytridiomycosis long after epidemics associated with initial Bd invasions subside. Further data are required to test the generality of this result, but it is clear that the capacity for endemic Bd infections to regulate populations and increase extinction risk is a theme requiring future research attention. Given the difficulty of managing Bd infections in wild amphibian populations, our results reemphasize the importance of adopting enforceable protocols for preventing the further spread of Bd and that complacency following species' survival through epidemic invasion is not warranted.

Acknowledgments

We thank S. Lachish and D. Pavlacky for help with the CMR modeling, K. Kriger, A. Phillott, and P. Symonds for essential sampling insight, and H. Hines, E. Meyer, and J. Clarke for their help in the design of the project. Fieldwork was conducted with the impeccable assistance of B. Barth, D. Buchholz, S. Murray, and C. Sanderson. We also thank R. Wilson. The manuscript was vastly improved by comments provided by S. Lachish, H. Hines,

and two anonymous reviewers. Major funding for the project was supplied by Australian Research Council Discovery Grant DP0451402 to H.M., R.S., A. Hyatt, and P. Daszak. K.M. was supported by an Australian Postgraduate Award, an Australian Biosecurity CRC professional development award, and a Wildlife Preservation Society of Australia student research award. Funding by the Australian Government Department of the Environment and Heritage (tender 42/2004 on the epidemiology of chytridiomycosis) contributed to the cost of diagnostic testing, and we thank R. Campbell for PCR analyses. This study was approved under University of Queensland Animal Ethics permit SIB/144/06/ARC and Queensland Parks and Wildlife permits (WITK037994406, WISP03806206, TWB/21/2006, TWB/34/2006).

Supporting Information

Details of the conventional Cormack–Jolly–Seber model (Appendix S1) and a full table of results of multistate model ranking used to generate robust estimates of monthly survival, detection, and transition rates through model averaging for male *L. pearsoniana* (Appendix S2) are available as part of the on-line article. The author is responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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