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Endemicity of chytridiomycosis features pathogen over-dispersion

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Summary

1. Pathogens can be critical drivers of the abundance and distribution of wild animal populations. The presence of an over-dispersed pathogen load distribution between hosts (where few hosts harbor heavy parasite burdens and light infections are common) can have an important stabilizing effect on host-pathogen dynamics where infection intensity determines pathogenicity. This may potentially lead to endemicity of an introduced pathogen rather than extirpation of the host and/or pathogen.
2. Over-dispersed pathogen load distributions have rarely been considered in wild animal populations as an important component of the infection dynamics of microparasites such as bacteria, viruses, protozoa and fungi.
3. Here we examined the abundance, distribution and transmission of the model fungal pathogen *Batrachochytrium dendrobatidis* (Bd, cause of amphibian chytridiomycosis) between wild-caught *Litoria rheocola* (common mist frogs) to investigate the effects of an over-dispersed pathogen load distribution on the host population in the wild. We quantified host survival, infection incidence and recovery probabilities relative to infectious burden, and compared the results of models where pathogen over-dispersion either was or was not considered an important feature of host-pathogen dynamics.
4. We found the distribution of Bd load between hosts to be highly over-dispersed. We found that host survival was related to infection burden, and that accounting for pathogen over-dispersion allowed us to better understand infection dynamics and their implications for disease control. In addition, we found that the pattern of host infections and recoveries varied markedly with season whereby (i) infections established more in winter, consistent with temperature dependent effects on fungal growth, and (ii) recoveries (loss of infection) occurred frequently in the field throughout the year but were less likely in winter.
5. Our results suggest that pathogen over-dispersion is an important feature of endemic chytridiomycosis, and that intensity of infection determines disease impact. These findings have important implications for our

understanding of chytridiomycosis dynamics and the application of management strategies for disease mitigation. We recommend quantifying individual infectious burdens rather than infection state where possible in microparasitic diseases.

Key-words

Aggregation, amphibian declines, frog, macroparasite, mark-recapture, microparasite, multi-state, pathogen distribution, recovery, transition

Introduction

Pathogens are increasingly being identified as important drivers of the abundance and distribution of wild animal populations (Altizer, Harvell & Friedle 2003; Grogan *et al.* 2014; Voyles *et al.* 2014). The complex host-pathogen dynamics that drive these systems have classically been explained within the micro- and macro-parasite epidemiological paradigm (the host is the relevant unit of study for microparasitic infections while the individual parasite is the relevant unit of study for macroparasitic infections). Within this paradigm, pathogens are categorised according to a number of characteristics common to each group, such as the apparent degree of pathogen load over-dispersion seen between hosts in macro- but not micro-parasites (Hudson *et al.* 2002; Wilson *et al.* 2002). Microparasites typically include viruses, bacteria, protozoa and fungi, whereas macroparasites include larger organisms such as helminths and arthropods. In this study we investigate and discuss the presence and importance of pathogen over-dispersion as a key feature of endemic chytridiomycosis which has classically been considered a microparasitic disease in terms of population effects.

Several recently emerged infectious pathogens (such as the fungus *Batrachochytrium dendrobatidis* [Longcore, Pessier & Nichols 1999] in amphibians and Hendra virus in bats; Wang *et al.* 1998) appear to defy clear categorisation within the aforementioned paradigm as their dynamics fail to follow typical patterns (Briggs, Knapp & Vredenburg 2010; Murray *et al.* 2013; Plowright *et al.* 2015). Furthermore, traditional mitigation strategies based on the above paradigm are proving to be poor tools for the control of these diseases in wild populations (for example, ‘vaccination’ by prior

exposure was unsuccessful in providing practical levels of protective immunity in the study by Cashins *et al.* (2013)). An improved understanding of the dynamics of these diseases is thus essential for managing them *in situ*, and will have broad applicability to emerging infectious diseases in general. For example, recognizing the occurrence of environmentally-associated seasonal peaks in chytridiomycosis severity (Phillott *et al.* 2013) led to the development of management strategies to address key environmental factors and vulnerable life stages (using techniques such as head-starting amphibians, translocations to habitats unfavourable to disease, and habitat modification; for a review of strategies currently under development for mitigating Bd *in situ*, see Scheele *et al.* (2014)).

Batrachochytrium dendrobatidis (hereafter Bd), the cause of the amphibian fungal skin disease chytridiomycosis has had a devastating impact upon amphibian populations around the world (through range contractions, population declines and extirpations, and species extinctions; Skerratt *et al.* (2007)). In the past, single-celled fungal pathogens like Bd have been typically considered as microparasites both taxonomically and for the purposes of modeling their disease dynamics (Anderson & May 1981). However, Bd infections demonstrate a number of features more common to larger parasites such as helminths and arthropods (Hudson & Dobson 1998; Briggs, Knapp & Vredenburg 2010).

Bd has a short life cycle within a single host involving two forms (infectious zoospore and reproductive sporangium; Berger *et al.* 2005a). In contrast to typical microparasites it appears to suppress an effective adaptive immune response in hosts (Rosenblum *et al.* 2012; Cashins *et al.* 2013; Fites *et al.* 2013). While it is able to multiply asexually at a moderate rate on individual hosts, duration of infection can be long, and pathogenicity relies on high infectious burdens, a feature typical of macroparasites (although severity may differ among individuals, populations and species, with numerous intrinsic determinants of host susceptibility; Voyles *et al.* 2009; Vredenburg *et al.* 2010). Infectious burden also appears to be strongly dependent on external factors affecting the life cycle of the pathogen, such as temperature and moisture (Voyles *et al.* 2012), similar to macroparasitic diseases, and hence population infections often display highly seasonal dynamics and

spatiotemporal distribution patterns consistent with environmental determinants (Murray *et al.* 2013; Phillott *et al.* 2013).

Pathogen over-dispersion, another feature well recognised as common to macroparasites, occurs with chytridiomycosis (Skerratt *et al.* 2011), but its underlying causes and effects on disease dynamics have not been investigated. Pathogen over-dispersion (otherwise known as parasite aggregation, pathogen aggregation, or pathogen distribution heterogeneity) describes a distribution of infectious organisms amongst hosts whereby most infected individuals have low infectious burdens, while very few hosts have high burdens. Over-dispersion is an important characteristic for understanding the dynamics of macroparasite diseases (Hudson & Dobson 1998), and it implies that the infection intensity pattern (described by the intensity-frequency curve) among hosts within a population tends to be highly positively skewed - thus infectious organisms are spatially aggregated among hosts (Wilson *et al.* 2002).

Whilst it has been known that infectious burden is important in determining the effects of chytridiomycosis on individuals (Voyles *et al.* 2009) the effects of the distribution of infection burden, particularly over-dispersion, within populations has largely been ignored (Briggs, Knapp & Vredenburg 2010; Vredenburg *et al.* 2010; Skerratt *et al.* 2011). Over-dispersion likely provides one explanation for why some species and populations persist with chytridiomycosis while others in previous studies that do not exhibit over-dispersion have been driven to extinction (Lips *et al.* 2006; Skerratt *et al.* 2007; Briggs, Knapp & Vredenburg 2010; Vredenburg *et al.* 2010). It may also help to explain the inability to detect a difference in survival probability between two disease states (infected and uninfected) in the multi-state mark-recapture study by Briggs, Knapp and Vredenburg (2010), because the effects of a small proportion of highly infected frogs may be unobserved when grouped with low infection results.

Chytridiomycosis provides a unique opportunity to examine the phenomenon of pathogen over-dispersion with a microparasite (Hudson & Dobson 1998; Skerratt *et al.* 2011). Unlike typical microparasitic infections, the epidermal localization of chytridiomycosis and the use of real time PCR enable the non-invasive and relative quantification of burdens among hosts (Hyatt *et al.* 2007). Given pathogen over-

dispersion is likely a key feature of endemic chytridiomycosis, and infection intensity affects both survival and infection transmission probabilities, examining its effects on disease dynamics could have important implications for our understanding of the disease ecology of microparasites.

We used the multi-state mark-recapture framework to investigate transmission and recovery dynamics of endemic chytridiomycosis in *Litoria rheocola* (the common mist frog; Liem 1974) in tropical north Queensland, Australia, as a function of individual-level infection status, population-level apparent prevalence, and environmental covariates. We chose this study species and tropical stream system as it represents hundreds of similar species and systems that have survived introduction of chytridiomycosis and now exist with endemic disease in Australia and the Americas (Berger *et al.* 1998; Lips *et al.* 2006; Skerratt *et al.* 2007; Murray *et al.* 2009; Skerratt, Speare & Berger 2011; Phillott *et al.* 2013). The study aimed to firstly, characterize the presence of Bd pathogen over-dispersion in the context of a wild population of endemically Bd infected amphibians, and secondly, to investigate infection and recovery state transition dynamics throughout seasons and years. In particular, we wanted to determine whether defining infection as a binary variable (two infection states: uninfected and infected) or tertiary variable (three states: uninfected, and two discrete levels of infectious load which takes into account pathogen over-dispersion) affects our understanding of infection dynamics. Here we demonstrate that an over-dispersed distribution of a microparasite within the host population plays an important role in defining disease dynamics.

Materials and methods

Species, site and sampling

We collected mark-recapture encounter data (via toe-tip marks) for adult male common mist frogs (*Litoria rheocola*) from a 150 m stream transect in lowland tropical rainforest of Tully Gorge National Park (145° 38' E 17°46' S, 130 m above sea level), Queensland, Australia over 22 trips between November 2005 and October 2007 (see Phillott *et al.* 2013 for further details of field work at this site). Bd is

suspected to have arrived at this site around 1989. Although annual survival rates are low (12%) there is high recruitment (91%) and the population appears stable (Phillott *et al.* 2013). *L. rheocola* is an obligate stream-breeder, and the breeding season for this population occurs from May to August (coinciding with the dry winter season; Bureau of Meteorology 2008) however adult males maintain calling territories at the stream throughout the year (Hodgkison & Hero 2002; Phillott *et al.* 2013). Individual frogs were skin-swabbed at every capture (maintaining strict hygiene, and following standard protocols; Phillott *et al.* 2010; Phillott *et al.* 2013), and swabs were analyzed for the presence of Bd DNA via quantitative PCR (qPCR; one well, one zoospore equivalent [*zse*] considered positive; Hyatt *et al.* 2007; Skerratt *et al.* 2011).

Multi-state mark-recapture modeling

We applied the information theoretic approach using Akaike's Information Criterion (IT-AIC, following the steps outlined in Phillott *et al.* 2013) to explore state-specific endemic chytridiomycosis infection dynamics using the multi-state mark-recapture (MSMR) framework (see Appendix S1 for more details). We hence performed two- and three-state multi-state modeling with program MARK (version 6.0; White, Kendall & Barker 2006) to elucidate the individual-level effect of chytridiomycosis infection on survival probability in the field by assigning frogs to an infection state at each capture via qPCR results. We particularly wanted to determine the probabilities for infection and recovery transitions, in order to understand the nature of infection dynamics *in situ* (Murray *et al.* 2009; Cooch *et al.* 2012).

We investigated the best predictors (several, due to model uncertainty) from the Cormack-Jolly-Seber (CJS) analysis for existing survival and recapture parameters for this dataset (Phillott *et al.* 2013) in the new context of state transition probabilities. Hence we investigated survival as a function of infection status (γ), apparent trip prevalence (π), mean daily maximum temperature (°C) for the 28 days preceding each trip (λ), and a cyclical seasonal linear trend variable (τ, where autumn is considered equivalent to spring). Recapture probability was investigated as a function of infection status (γ), mean daily relative humidity (%) at maximum temperature for the 28 days preceding each trip (ε), mean daily radiation (MJ/m^2) over 28 days preceding each trip (ζ), and capture effort (in days per trip δ). Weather variables were obtained from the

SILO climate database which provides spatially interpolated values from regional meteorological stations (Jeffrey *et al.* 2001; Bureau of Meteorology 2008).

We defined infection status (γ) as a time-varying individual covariate categorized into either two or three states on the basis of infection intensity (*zse*) at each capture. In the two-state analysis, A = Bd negative (uninfected) and B = Bd positive (infected). In the three-state analysis, Bd load was discretized into groups: A = Bd negative, B = 1-4 *zse* "low", C > 4 *zse* "high". This low-burden group of hosts is the most poorly defined in terms of disease processes; individuals may be newly infected, recovering, resistant, their burdens may represent background contamination, or they may contain unaccounted sampling or laboratory error (McClintock *et al.* 2010). The chosen threshold between infection states (4 *zse*) allowed us to separately model the transmission dynamics of this low-burden group and eliminated potential confounding from the high-infected host group. In addition, multi-state analysis methods have high data requirements, and this threshold permitted Bd positive results to be split evenly between states B (low intensity) and C (higher intensity) providing sufficient power for analysis (66 samples $zse \leq 4$; 64 samples $zse > 4$; Fig. 1). We acknowledge that by artificially discretizing the continuous variable *zse* into low and high categories of intensity of infection there is some loss of information and some potential misclassification of infection levels close to the cutoff value (although the diagnostic accuracy of the quantitative PCR at James Cook University is very high, sensitivity 73% and specificity approximating 100%; Hyatt *et al.* 2007; Skerratt *et al.* 2011). Regardless, the results remain interpretable in terms of the effects of comparative levels of infection. The sample size was not sufficient for categorization into additional levels of infection intensity such as a moderate group, and although it would have been ideal to perform sensitivity analyses for determining this threshold, unfortunately these were not possible due to data sparseness. Indeed, varying the cut-off value by a single zoospore in either direction rendered the data unanalysable due to failure of numerical convergence of the majority of models tested.

The state transition parameter ψ_i^{rs} defines the probability that an individual in state *r* at time *i* will be in state *s* at time $i + 1$. Importantly where there are more than two states, this includes the probability of transitions from each state in the MSMR Jolly-Movement Model (JMV; Lebreton *et al.* 2009), including the probability of remaining

in the same state ψ_i^{rr}, and the outgoing probabilities for each state must sum to one (Fig. 1). States in this study represent discrete infection conditions (defined by *zse* infection intensities) in which the marked individual may potentially be encountered, conditional on being in that state and alive. Following the results in Phillott *et al.* (2013), and to incorporate both individual and population-level effects, we hypothesized that state transition probabilities are influenced by infection status (γ), apparent trip prevalence (π) and seasonal environmental covariates such as temperature (λ). As an example of how these effects might influence the transitions between states, recoveries should be associated with increased ambient temperature to reduce Bd growth (Voyles *et al.* 2012) and promote host thermoregulatory immunomodulation (Richards-Zawacki 2010). Similarly, recoveries should also be associated with reduced prevalence as they require an absence of re-infection.

We applied goodness of fit tests and applied the most conservative estimate of $\hat{c}$ = 1.110 and 1.097 for the two and three-state analyses respectively (Appendix S1). Candidate model sets for two and three-state analyses were constructed separately *a priori* using a restricted form of the all subsets approach, and tested systematically (Appendix S1; Lukacs, Burnham & Anderson 2010; Hegyi & Garamszegi 2011; Doherty, White & Burnham 2012). We constructed models using the intercept design matrix coding format and the logistic (logit) link function. Where numerical convergence was suspect, we employed the alternate optimization routine from within MARK, and assessed each model individually for estimable parameter count, adjusting as necessary (Lebreton *et al.* 2009; Cooch *et al.* 2012). We used $QAIC_c$ to rank model parsimony (Burnham & Anderson 2002), model averaging to reduce selection bias (Lukacs, Burnham & Anderson 2010), and we estimated monthly parameter probabilities ($1\ month = \frac{365}{12} \approx 30.42\ days$), reporting unconditional 95% confidence intervals (95% CI; Burnham & Anderson 2002). Akaike weights were used to determine relative variable importance from entire candidate model sets (Doherty, White & Burnham 2012), and we report evidence ratios and model averaged effect sizes where appropriate for comparisons between states (Burnham & Anderson 2002). Model averaged effect sizes were based on model averaged real parameter estimates and confidence intervals were unbounded on the real probability scale using the delta method for difference between two variances with the model

averaged variance-covariance matrix. Raw encounter history and predictor variable data together with model averaged parameter estimates for the three-state analysis are available in Appendix S3. We additionally performed a discrete time simulation for a population of adult frogs employing the model-averaged trip-based parameter estimates from the three-state multi-state mark-recapture analysis over the study period to demonstrate the impact of estimated state transition and survival parameters on actual population numbers. Detailed methods and results from this simulation are available in Appendix S1 and S2.

Results

Infection pattern summary

We made 424 captures of 243 uniquely marked adult male *L. rheocola* frogs throughout the two year study period (109 frogs were caught more than once). Forty-seven frogs (43% of those caught more than once) changed infection state at least once (became infected or recovered), and 13 frogs (28% of those caught more than twice) changed state two or more times (although only three of these, 23%, were re-infected after recovery). State transitions were approximately even with 28 infection and 34 recovery transitions. Two frogs gained and lost infection several times. The highest infection intensity recorded prior to recovery was 123 *zse*. Apparent Bd infection prevalence for the whole study period was 130/421 = 0.3088 (binomial 95% CI 0.2650 to 0.3553 by Clopper-Pearson method, assuming statistical independence). The intensity-frequency histogram for qPCR swab results for the whole study period was highly positively skewed (Fig. 2; 291 records for Bd negative and 21 high *zse* records were truncated for visualization; N = 421, range 0 to 4028 *zse*). The variance to mean ratio of infectious organisms per host (s^2/m) was 2227.47 (very much higher than one, indicative of pathogen over-dispersion). To further examine evidence of over-dispersion, the Weibull distribution ($\alpha = 0.46901$, $\beta = 15.259$) and negative binomial distribution were fit to the population-level apparent infection prevalence data (Fig. 2), and the corrected moment estimate of *k* (of the negative binomial distribution) was 0.0069, indicating a high degree of pathogen over-dispersion (Wilson *et al.* 2002). Testing the most parsimonious zero-inflated negative binomial

model (with season and year as covariates) against the standard negative binomial model indicated that there was no significant difference between the models (AIC-corrected Vuong z-statistic = 0.531, p = 0.298), and thus zero inflation (excessive 'uninfected' samples) is unlikely to have contributed to the observed over-dispersion (Zuur *et al*. 2009; see Appendix S2 for further details).

Multi-state mark-recapture results

Model averaged parameter estimates using individual time-varying infection intensity results revealed marked seasonality in survival and transition probabilities in both analyses (for example in the three state analysis, survival probability varied seasonally from 0.48 in winter to 0.79 in summer for frogs in the high burden state). Monthly model averaged estimates for state-dependent survival, recapture and state transition probabilities are reported with unconditional 95% confidence intervals in Figs 3 and 4 for two- and three-state multi-state analyses, respectively (see Appendix S2 for ranked tables of model results). Although the two and three-state MSMR analyses cannot be directly compared with AIC due to their differing candidate model sets, the three state MSMR analysis better captured the over-dispersion of infectious burden because it treated relatively highly infected individuals independently to low and uninfected individuals. In contrast, the two state analysis grouped all infected individuals together regardless of infectious burden. While survival differed between infected and uninfected frogs in the two-state analysis, apparent survival probability estimates for the infected group were higher than those for the uninfected group, except during one winter trip session. Confidence intervals for the infected group were considerably wider, however, and overlapped those for the uninfected group for all trip sessions (Fig. 3a). In comparison, when the infected group was separated into two infection categories (group B with 1-4 *zse*, group C > 4 *zse*) in the three-state analysis, frogs with differing levels of infectious burden had differing survival probabilities (frogs with > 4 *zse* had consistently lower survival; Fig. 4a). While recapture probabilities were relatively stable throughout the study period in both analyses, in the two-state analysis both uninfected and infected frogs had similar recapture probabilities (Fig. 3b), whereas in the three-state analysis the low-burden group had low recaptures compared with the high-burden group (although confidence margins were wide in the three-state analysis; Fig. 4b, see Appendix S2 for further details).

Parameter estimates revealed marked seasonality in state transition probabilities between infection states. In the two-state analysis, frogs were much more likely to become infected in winter (correlating with prevalence), while there was a moderate reduction in the probability for recovery transitions during this period in the infected group (Fig. 3c). The three-state analysis further highlighted these trends with some exceptions despite overlapping confidence margins (transitions constituting the gain of or increase in infectious burden shown in Fig. 4c; reduction of infectious load or loss of infection transitions shown in Fig. 4d). The highest probability for infection transitions occurred during winter from the uninfected (group A) to low-burden frogs (group B). Recovery transition (loss of infection) probabilities were seasonal, peaking during summer and autumn, and were similar between both high and low-burden groups. Stationary transition probabilities (shown in Fig. S1, Appendix S2) were derived from the aforementioned model-averaged transition probabilities and probability theory which states that the sum of the probabilities of leaving each state must equal one. Hence throughout most of the year, among those surviving a sampling interval, frogs were most likely to either remain in the uninfected state, or return to that state through infection recovery (Fig. S1, Appendix S2). Low-burden frogs (group B) were observed to increase their infectious load (to group C) at a relatively low and stable rate throughout the study (Figs 4c, S1b, Appendix S2). Hypothetical population dynamics (including variation in total population size) based on these transition and survival probabilities are exemplified in a series of three population dynamics simulation models illustrated in Fig. 5.

Despite model selection uncertainty, the most parsimonious models in both analyses modeled apparent survival and state transition probabilities as a function of a multiplicative interaction between individual-level infection state and population-level infection prevalence (the models $S(\gamma \times \pi)\rho(\delta)\psi(\gamma \times \pi)$ and $S(\gamma \times \pi)\rho(\gamma + \zeta)\psi(6\gamma \times \pi)$, with 9.1% and 20.4% support for two- and three-state analyses, respectively). Ranked relative predictor variable importance (reporting only those >0.1) for the two-state analysis were prevalence (π = 0.6228) and temperature (λ = 0.3400) for survival; days (δ = 0.36301), radiation (ζ = 0.29044) and relative humidity (ε = 0.24491) for recapture; and prevalence (π = 0.9254) for transition. For the three-state analysis these were prevalence (π = 0.8700) and temperature (λ =

0.1298) for survival; radiation (ζ = 0.5109), relative humidity (ε = 0.2632) and days (δ = 0.1674) for recapture; and prevalence (π = 0.9264) for transition.

The model averaged effect size as a mean across trips for the survival difference between infected and uninfected groups in the two-state analysis was 0.1070, with the infected group demonstrating higher apparent survival overall (95% CI -0.0577 to 0.2717). Similarly, the model averaged effect sizes for survival in the three-state analysis were as follows: B-A 0.1184 (95% CI -0.0448 to 0.2815), B-C 0.2610 (95% CI -0.1573 to 0.6794) and A-C 0.1427 (95% CI -0.2086 to 0.4940). While there was limited support for an effect of individual infection status on apparent survival in the two-state analysis (the evidence ratio comparing most parsimonious models with and without γ was 2.8222), there was correspondingly strong support in the three-state analysis (evidence ratio 695.20), and strong support in both analyses for an effect of infection status on state transition probability (evidence ratios > 918.90 and 319.36 for the two- and three-state analyses, respectively; Lukacs *et al.* 2007).

Discussion

Pathogen over-dispersion and survival probabilities

We found marked aggregation of Bd within our endemically infected wild amphibian population, as demonstrated by a highly over-dispersed intensity-frequency distribution curve (Fig. 2). Thus, while most infected individuals had low burdens, a few hosts had high burdens. Categorizing infectious burdens into low or high groups based on qPCR swab results allowed us to partially resolve paradoxical results from our two-state analysis which were similar to those reported by Briggs, Knapp and Vredenburg (2010). The model averaged estimates from our two-state analysis incongruously revealed a lower apparent survival probability for uninfected frogs compared with infected frogs, although confidence intervals for the infected state were wide (Fig. 3a). After taking pathogen over-dispersion into account, apparent survival probability of infected frogs fell to either side of the uninfected group, with high-burden frogs having the lowest survival estimates (Fig. 4a). The reason for a difference between the two and three state analyses is the high degree of pathogen over-dispersion and its differential effects; approximately half the infected frogs were classed in the low-burden group (Fig. 2). In addition, infection intensity was found to

be seasonally associated with survival as well as transmission and recovery probabilities. Our results are consistent with previous field work showing over-dispersion (Skerratt *et al.* 2011), and linking reduced survival with higher Bd infection intensities (Murray *et al.* 2009), and also demonstrates that quantifying infectious burdens is key to understanding the ecology of chytridiomycosis.

We used the MSMR framework to provide dynamic estimates of first-order Markov infection state transition probabilities and state-dependent survival estimates from field data whilst accounting for imperfect detection (Murray *et al.* 2009; Cooch *et al.* 2012). Compared with the single-state Cormack-Jolly-Seber model (Phillott *et al.* 2013), the MSMR framework permits reassessment of individual disease status at each capture, which is essential for examining individual-level infection dynamics and survival probabilities in a system where infection status fluctuates. Most disease studies utilizing MSMR analyses to date have categorized individuals on the basis of their infection status (uninfected versus infected states; Murray *et al.* 2009; Briggs, Knapp & Vredenburg 2010). This binary definition in the presence of pathogen over-dispersion can greatly diminish our understanding of survival and state transition probabilities, and here we demonstrate the importance of this effect through comparisons of two and three state analyses.

Transition probabilities – infection and recovery

In our study, frogs gained and lost infection frequently, consistent with previous field data on mountain yellow-legged frogs (*Rana muscosa* and *R. sierrae*) in temperate USA (Briggs, Knapp & Vredenburg 2010), and some individuals demonstrated numerous state transitions. Comparing two- and three-state analyses helped resolve the nature and magnitude of transition probabilities between disease states (Figs 3c, 4c and 4d). As expected from previous studies on the temperature dependence of Bd (Voyles *et al.* 2012), we found that frogs were most likely to become infected during winter months (June to August in the southern hemisphere), with the transition to a low infectious burden (1-4 *zse*) being most probable (Fig. S1a, Appendix S2). Alternatively, recovery from both low and high infectious burdens was equally probable and high throughout most of the year, dropping moderately during winter (Figs S1b and S1c, Appendix S2).

A relatively long incubation period (roughly 3-8 weeks between exposure and clinical signs; Berger *et al.* 2005b; Voyles *et al.* 2009) in an environmentally responsive pathogen means more chance for pathogen-adverse environmental conditions (such as temperature spikes) to favour host recovery and survival. Thus, recovery transitions may be favoured over infection transitions throughout most of the year in areas with higher temperatures such as at low elevation tropical regions. A long incubation period also artificially increases point prevalence measures and decreases mortality measures in comparison with pathogens that have short incubation periods. This is also likely to lead to a highly over-dispersed intensity-frequency distribution because most of the infected population is in the subclinical phase of the disease at any point in time (in endemically infected populations, unlike propagating epidemics which can rapidly lead to widespread mortality). Re-infection transitions were comparatively uncommon, however (only three of the 13 frogs that were observed to change state two or more times), possibly suggesting adaptive immunity may occur in the field. However, the third simulation scenario (Fig. 5c) assumed no effect of adaptive immunity and resulted in population dynamics consistent with our expectations and dynamics observed in the wild.

Uninfected frogs versus those with low infectious burdens

Finding that uninfected frogs had lower apparent survival probabilities than those in the low-burden state (Fig. 4a) was unexpected. The difference in apparent survival between these infection states was small to moderate (11% for two-state analysis, 12-26% for three-state analysis). Perhaps this survival discrepancy, and part of the cause for the high level of pathogen over-dispersion, is due to the low infection group representing a greater proportion of more resistant individuals. Under laboratory conditions conducive to disease progression, infections occur only at low levels for about a week post-exposure (Hyatt *et al.* 2007) suggesting low infections in susceptible wild individuals would only be maintained if conditions for the disease were suboptimal or if individual frogs were relatively resistant. In comparison, the uninfected group would contain susceptible individuals that eventually become exposed and die from the disease but are not re-caught prior to death. Similarly, the lower winter survival probabilities in the low burden and uninfected frogs compared with other seasons (given that pathogenicity relies on high infectious burdens, and that these burdens are driven by weather; Voyles *et al.* 2009; Murray *et al.* 2013) is likely

due to frogs increasing their intensity of infection and dying without being detected in the high burden state.

Alternatively, the above discrepancy may be due to emigration confounding in capture-mark-recapture studies (Murray *et al.* 2010; Schmidt 2010). For example, differential permanent emigration rates between the two states may lead to different apparent survival probabilities. We have no *a priori* reason to suspect higher emigration in uninfected frogs or in those with high zoospore burdens compared with those having low burdens (Roznik *et al.* 2015). Rather, frogs appeared to maintain calling territories on the stream year-round suggesting site fidelity (Phillott *et al.* 2013). Tracking studies comparing movements of uninfected and infected frogs could be used to resolve potential confounding due to emigration if it appears to be an issue (Roznik & Alford 2015).

Implications of pathogen over-dispersion

Implications of pathogen over-dispersion for the management of endemic chytridiomycosis can be separated into two categories; those that affect the way we study, model and report this disease; and those that affect actual disease dynamics. In the first instance, we have demonstrated empirically that failing to account for different levels of infectious burden between hosts can lead to biases in understanding of population dynamics (for example, through mark-recapture state categorizations, or ecological modeling). In addition, we highlight that the commonly reported measure of disease abundance, population infection prevalence, should not be used to compare populations with differing levels of pathogen over-dispersion. In the second instance, pathogen over-dispersion impacts population dynamics where infectious burden affects 1) pathogenicity, 2) the rate of production of the infectious stage released to the environment, or 3) the degree of host resistance or immunity (May & Anderson 1979). The first two conditions occur in chytridiomycosis, based on this study and past work (Hyatt *et al.* 2007; Murray *et al.* 2009).

The specific effects of pathogen over-dispersion on populations will likely depend on the degree and predominant causes of the observed over-dispersion, and elucidating these may assist with predicting long-term population outcomes and hence management approaches. There are three main potential causes of observed over-dispersion including 1) heterogeneous exposure, 2) variable multiplication within the

host, and 3) sampling artifact (Hudson & Dobson 1998). In the context of endemic chytridiomycosis the first two are likely to be most important. To rule out the third option, we fitted and compared mixture models and a standard negative binomial model to the infection intensity data. We demonstrated that over-dispersion is an important component of the variance structure of the system and that it could not be explained by zero-inflation, which largely rules out sampling artifact as a major contributor. Our results demonstrate that environmental variation (seasonality) was a significant predictor of the underlying infection process (observed in both the mark-recapture analyses and the count model component in the zero-inflated negative binomial model, Appendix S2). This result is consistent with previous studies (Murray *et al.* 2013). Variable multiplication in the host due to environmental seasonality is likely to be an important cause of over-dispersion in endemic chytridiomycosis (Berger *et al.* 2009). This is also likely to lead to seasonal heterogeneity in exposure as zoospores can either disperse or re-infect the same host.

In our study, prevalence was ranked with highest predictor variable importance in the three state analysis for both survival and transition probabilities, despite a strong correlation with temperature, suggesting that a host component also plays an important role in determining the distribution of infectious burdens. We were unfortunately unable to examine host immunity/resistance as a direct covariate in either the mark-recapture analyses or the mixture models for over-dispersion as there is currently insufficient knowledge of resistance markers to chytridiomycosis in amphibians (although this is an active area of research).

Two major reasons Bd infection dynamics differ from the classic microparasite paradigm are 1) the relative absence of effective adaptive immunity to Bd (Cashins *et al.* 2013; Fites *et al.* 2013; McMahon *et al.* 2014) and the prominence of variation in host susceptibility to infection (Berger *et al.* 2005b; Tobler & Schmidt 2010), and 2) the ectothermic nature of amphibian hosts leading to variations in pathogen growth due to thermal suitability (Murray *et al.* 2013). The former may be associated suppression and evasion of the host immune system, as well as host physiological and behavioural characteristics (Tobler & Schmidt 2010; Savage & Zamudio 2011). Species that have been driven to extinction by chytridiomycosis appear to be highly susceptible, have little variation in host susceptibility (Carey *et al.* 2006; Skerratt *et*

al. 2007; Berger *et al.* 2009; Bataille *et al.* 2013), and have occurred in areas that were highly favourable for the pathogen (Murray *et al.* 2011). Thus, it is important to identify the predominant cause of pathogen over-dispersion as this may provide an indication of potential long-term persistence of the population.

Conclusions

In conclusion, we have shown that pathogen over-dispersion is an important feature of a microparasitic disease, in this case endemic chytridiomycosis. Overlooking non-uniform pathogen distributions in microparasitic diseases may lead to paradoxical interpretations of disease dynamics. We also show that Bd infections occur seasonally and that recoveries are common and likely important for population persistence. Future management of endemic chytridiomycosis might focus on environmental manipulation to favor host recoveries (Scheele *et al.* 2014). Understanding the main causes of pathogen over-dispersion will indicate whether other disease control interventions should be targeted predominantly towards 1) assisting the longer-term evolution of resistance within the population via selection techniques, 2) reducing exposure and transmission of infection between hosts, 3) bolstering population size through approaches directed at habitat conservation, or 4) minimizing other threatening processes. We recommend quantifying individual infectious burdens rather than infection state where possible in microparasitic diseases.

Data Accessibility

Raw encounter history and predictor variable data, together with three-state model averaged parameter estimates are available in Appendix S3 online.

Acknowledgements

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Figure legends

Figure 1. Example schematic illustrating state transition probabilities (ψ) and survival probabilities (S) for the respective infection states at capture session six (drawn from the three-state multi-state analysis). The notation ψ^{rs} indicates the monthly state transition probability from state *r* to state *s* from time (capture session) *i* to *i+1*, and S^t represents survival probability from time *i* to time *i+1*, for individuals in state *t*. Circle sizes are representative of the relative expected population size (from the simulation), and arrow line thicknesses represent the relative magnitude of the respective probabilities.

Figure 2. Intensity-frequency histogram showing highly over-dispersed distribution of infectious organisms between individual hosts (highly positively skewed), together with the fitted Weibull and negative binomial distributions. N = 421; 291 Bd negative records and 21 high zoospore records were truncated for visualization; original data range 0 to 4028.

Figure 3. Model averaged estimates for monthly (a) survival probability, (b) recapture probability, and (c) state transition probability with unconditional 95% confidence intervals from the two-state multi-state analysis for male adult *L. rheocola* at Tully.

Figure 4. Model averaged estimates for monthly (a) survival probability, (b) recapture probability, (c) infection transition probabilities, and (d) recovery transition probabilities with unconditional 95% confidence intervals from the three-state multi-state analysis for male adult *L. rheocola* at Tully. States are defined as: state A = Bd negative (uninfected), state B = 1-4 *zse*, state C >4 *zse*.

Figure 5. Outcomes from the demographic simulation without recruitment (a); with recruitment to force population stability (b); with recruitment as model-averaged estimates from Phillott *et al.* (2013) (c).

Figure 1.

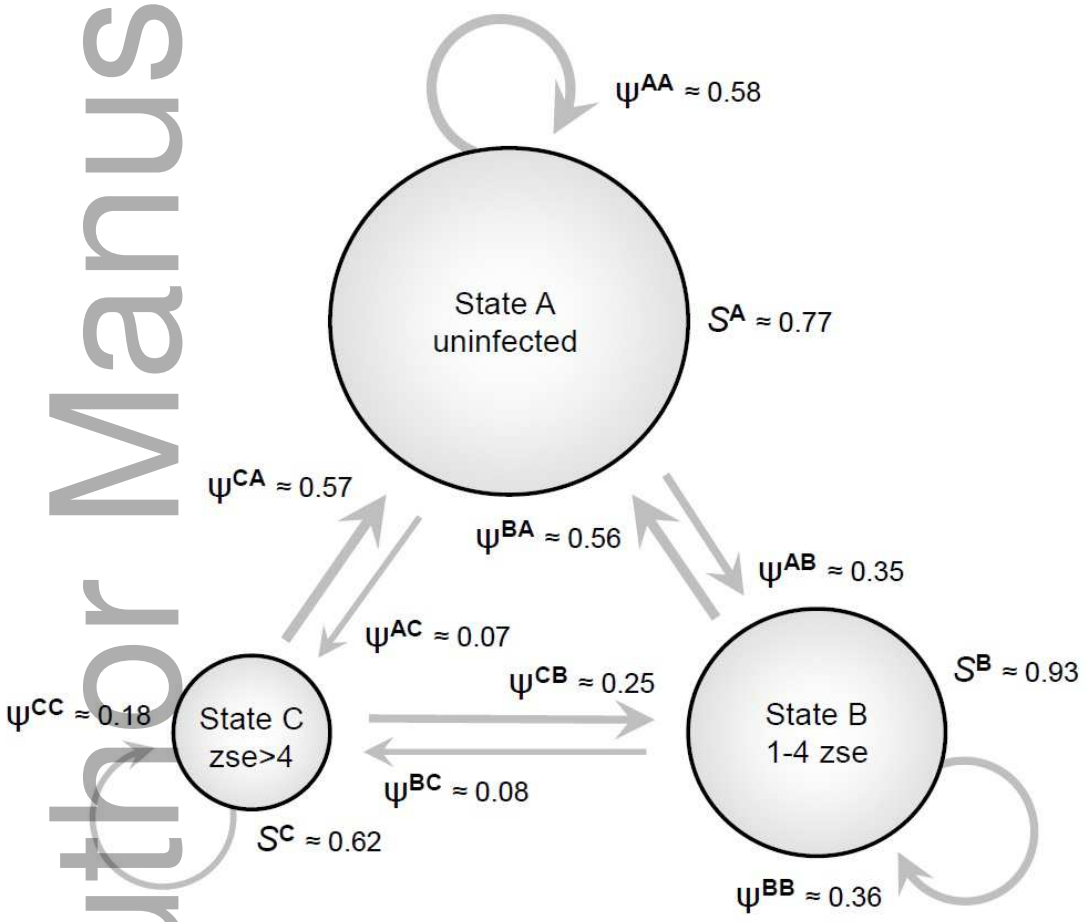


Figure 2.

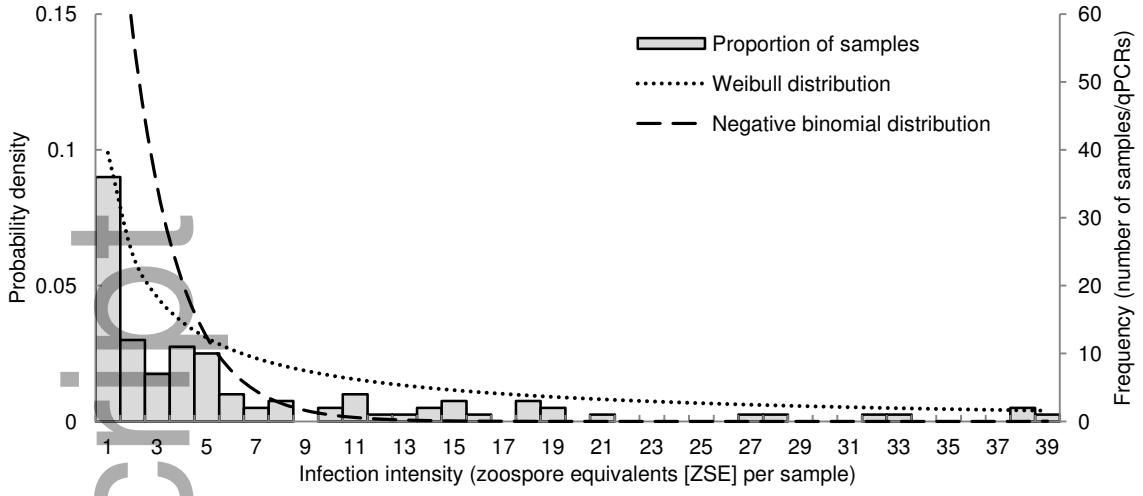
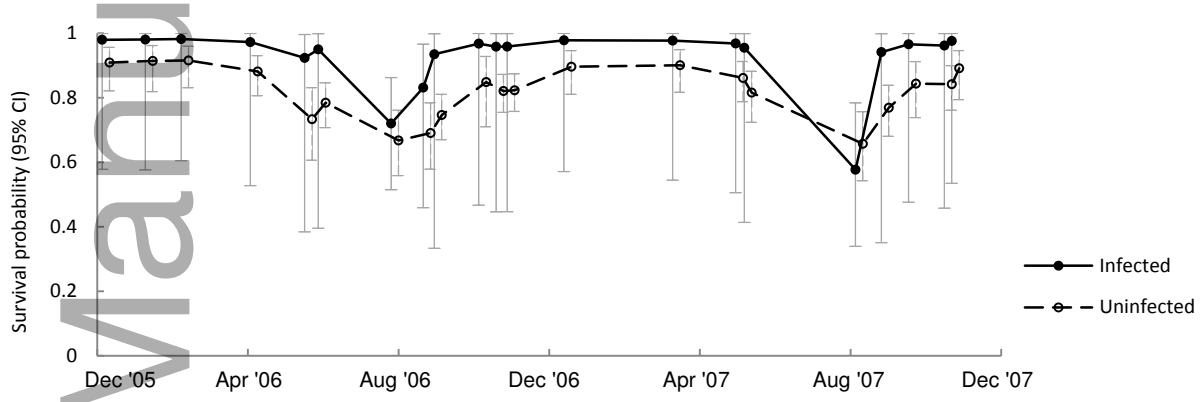


Figure 3.

(a)



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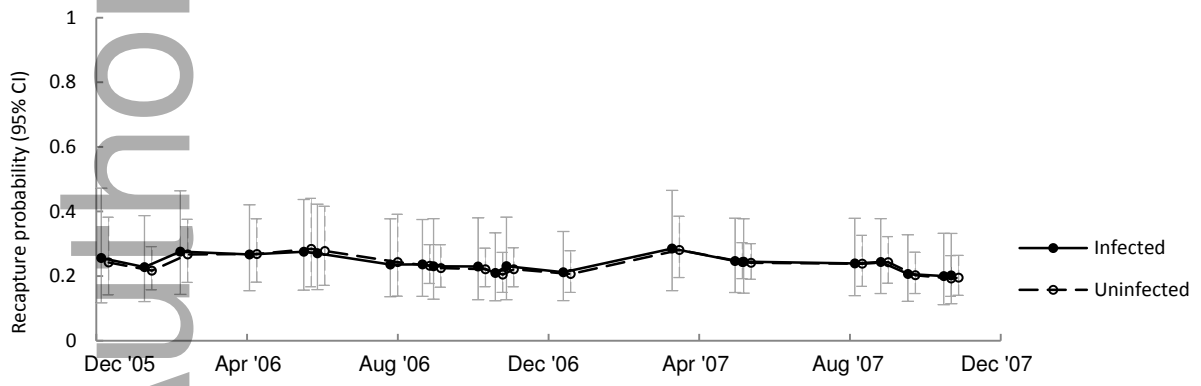

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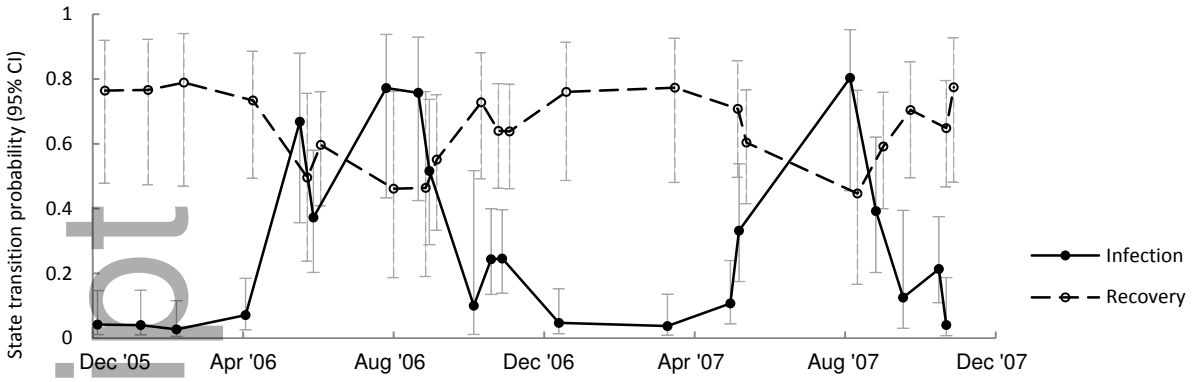
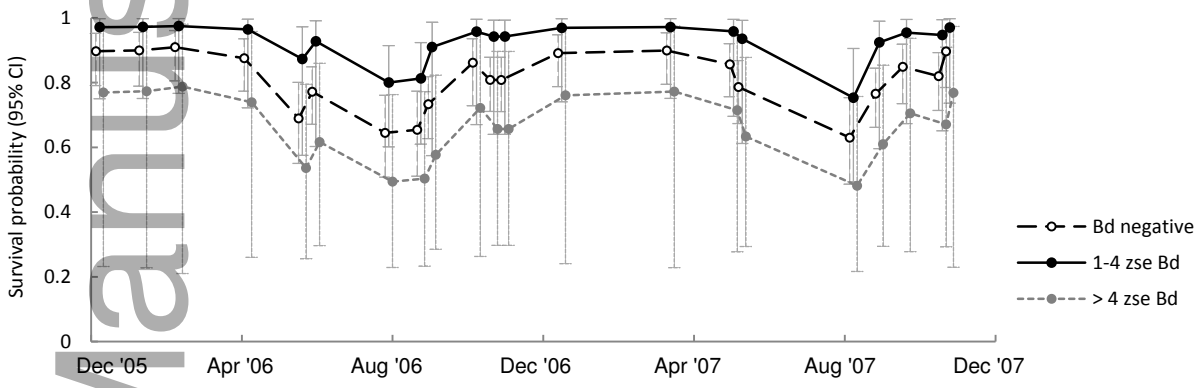


Figure 4.

(a)



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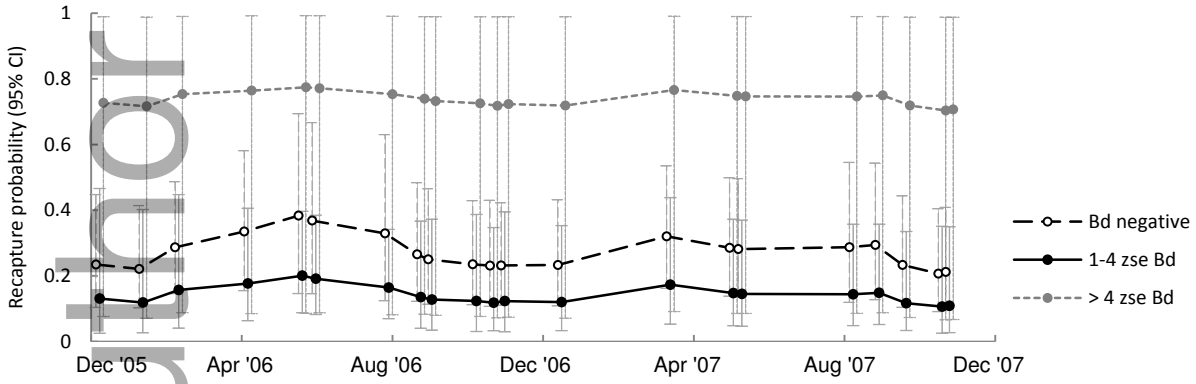

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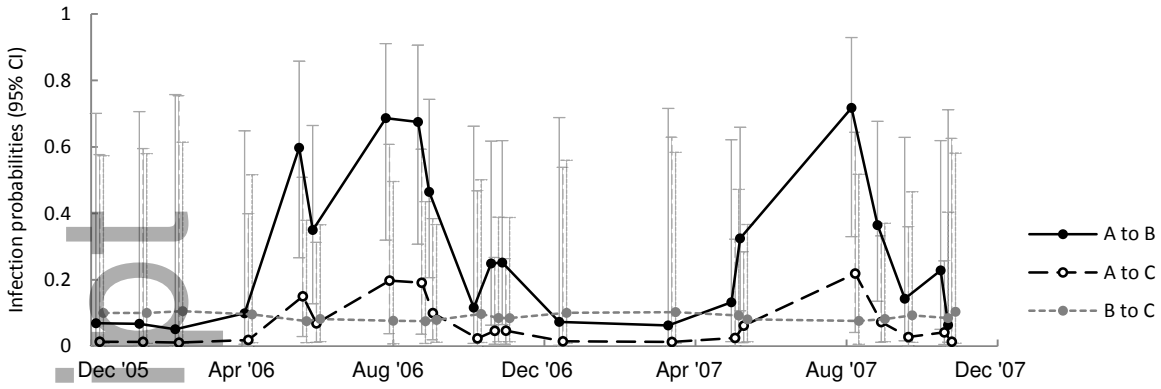

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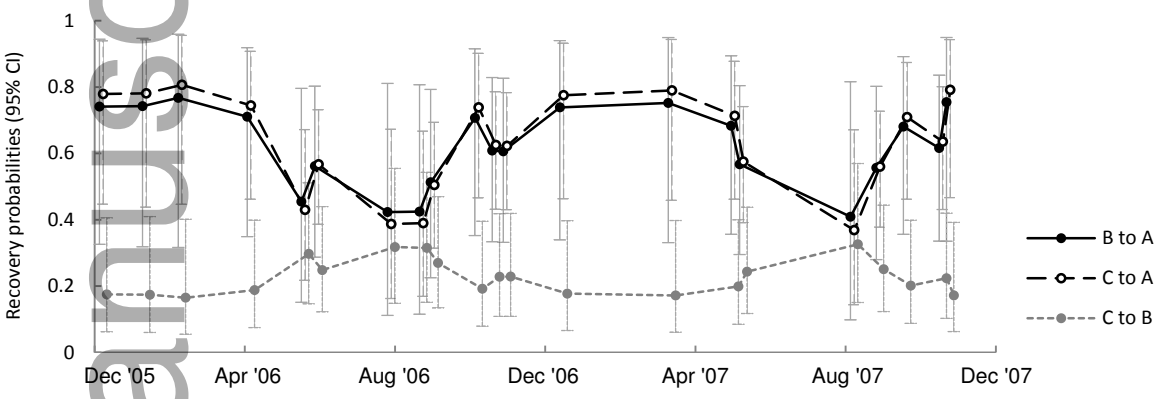

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871 **Figure 5.**

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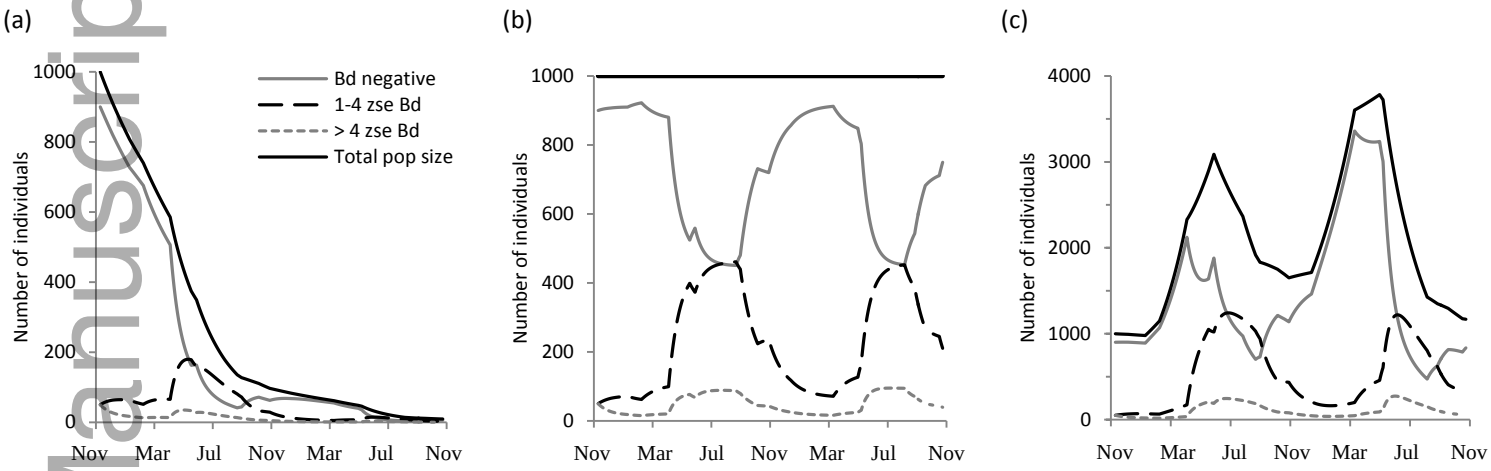

Supporting Information

The following Supporting Information is available for this article online:

Appendix S1. Portable document file (PDF) containing description of parameters, predictor variables, construction of candidate model sets, and population dynamics simulation methods.

Appendix S2. Portable document file (PDF) containing tables of results (Tables S1-S6), population dynamics simulation results, transition probabilities description, and results figure (Fig. S1).

Appendix S3. Excel spreadsheet containing raw encounter history and predictor variable data, together with three-state model-averaged parameter estimates.

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