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Grogan, LF

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# Endemicity of chytridiomycosis is driven by pathogen over-dispersion

Laura F. Grogan[*a b], Andrea. D. Phillott [a c], Benjamin C. Scheele [a d], Lee Berger [a], Scott D. Cashins [a], Sara C. Bell [a e], Robert Puschendorf [f], and Lee F. Skerratt [a]

[a] One Health Research Group, School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, Townsville, Australia

[b] Griffith Wildlife Disease Ecology Group, Environmental Futures Research Institute, School of Environment, Griffith University, Nathan, Australia

[c] Biological Sciences, Asian University for Women, Chittagong, Bangladesh

[d] Fenner School of Environment and Society, College of Medicine, Biology and Environment, Australian National University, Canberra, Australia

[e] Australian Institute of Marine Science, Townsville, Australia

[f] School of Biological Sciences, Plymouth University, Plymouth, United Kingdom

[*] Corresponding author: l.grogan@griffith.edu.au

## 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, where 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. Microparasites typically include viruses, bacteria, protozoa and fungi, whereas macroparasites include larger organisms such as helminths and arthropods.

However, 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 this 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. 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.

*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

91 extirpations, and species extinctions). In the past, single-celled fungal pathogens like
92 Bd have been typically considered as microparasites both taxonomically and for the
93 purposes of modeling their disease dynamics (Anderson & May 1981). However, Bd
94 infections demonstrate a number of features more common to larger parasites such as
95 helminths and arthropods (Hudson & Dobson 1998; Briggs, Knapp & Vredenburg
96 2010).
97
98 Bd has a short life cycle within a single host involving two forms (infectious zoospore
99 and reproductive sporangium; Berger *et al.* 2005a). In contrast to typical
100 microparasites it appears to suppress an effective adaptive immune response in hosts
101 (Rosenblum *et al.* 2012; Cashins *et al.* 2013; Fites *et al.* 2013). While it is able to
102 multiply asexually at a moderate rate on individual hosts, duration of infection can be
103 long, and pathogenicity relies on high infectious burdens, a feature typical of
104 macroparasites (Voyles *et al.* 2009; Vredenburg *et al.* 2010). Infectious burden also
105 appears to be strongly dependent on external factors affecting the life cycle of the
106 pathogen, such as temperature and moisture (Voyles *et al.* 2012), similar to
107 macroparasitic diseases, and hence population infections often display highly seasonal
108 dynamics and spatiotemporal distribution patterns consistent with environmental
109 determinants (Murray *et al.* 2013; Phillott *et al.* 2013).
110
111 Pathogen over-dispersion, another feature well recognised as common to
112 macroparasites, occurs with chytridiomycosis (Skerratt *et al.* 2011), but its effects on
113 disease dynamics have not been investigated. Over-dispersion likely provides one
114 explanation for why some species and populations persist with chytridiomycosis
115 while others that do not exhibit over-dispersion have been driven to extinction (Lips *et*

116 *al.* 2006; Skerratt *et al.* 2007; Briggs, Knapp & Vredenburg 2010; Vredenburg *et al.*
117 2010). It may also help to explain the inability to detect a difference in survival
118 probability between two disease states (infected and uninfected) in the multi-state
119 mark-recapture study by Briggs, Knapp and Vredenburg (2010), because the effects of
120 a small proportion of highly infected frogs may be unobserved when grouped with
121 low infection results.
122
123 Pathogen over-dispersion (otherwise known as parasite aggregation, pathogen
124 aggregation, or pathogen distribution heterogeneity) describes a distribution of
125 infectious organisms amongst hosts whereby most infected individuals have low
126 infectious burdens, while very few hosts have high burdens. Over-dispersion is an
127 important characteristic for understanding the dynamics of macroparasite diseases
128 (Hudson & Dobson 1998), and it implies that the infection intensity pattern (described
129 by the intensity-frequency curve) among hosts within a population tends to be highly
130 positively skewed - thus infectious organisms are both spatially and temporally
131 aggregated among hosts (Wilson *et al.* 2002).
132
133 Chytridiomycosis provides a unique opportunity to examine the phenomenon of
134 pathogen over-dispersion with a microparasite (Hudson & Dobson 1998; Skerratt *et*
135 *al.* 2011). Unlike typical microparasitic infections, the epidermal localization of
136 chytridiomycosis and the use of real time PCR enable the non-invasive and relative
137 quantification of burdens among hosts (Hyatt *et al.* 2007). Given pathogen over-
138 dispersion is a feature of endemic chytridiomycosis, and infection intensity affects
139 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

Multi-state mark-recapture analysis (MSMR; Lebreton *et al.* 2009) has recently emerged as a unified framework for capture-mark-recapture field studies (CMR; Lebreton *et al.* 1992). In this framework the chance of encountering an individual on a particular occasion is a product of its probability of recapture (ρ), conditional on its probability of surviving the interval (*S*), and its probability of making one of a number

188 of defined transitions between states (ψ). The state concept enables investigation of
189 time-varying individual categorical variables, such as site, breeding status or disease
190 status (see Lebreton *et al.* 2009 for review and synthesis), and has thus expanded
191 CMR studies to the investigation of individual-level disease dynamics (Cooch *et al.*
192 2012). The advantage of CMR for studying wildlife disease is that it accounts for
193 imperfect detection, compared with traditional epidemiological cohort near-census
194 follow-up. Multi-state modeling is increasingly being used for the study of disease in
195 wild animals (see for example Senar & Conroy 2004; Conn & Cooch 2009; Rossi *et*
196 *al.* 2011). Multi-state mark-recapture has been applied to the study of
197 chytridiomycosis in several ecological systems to date (Murray *et al.* 2009; Briggs,
198 Knapp & Vredenburg 2010), and provides less confounded parameter estimates
199 (Jennelle *et al.* 2007) than the previously used measure of 'return rate' (Kriger & Hero
200 2006).
201
202 We applied the information theoretic approach (IT-AIC, following the steps outlined
203 in Phillott *et al.* 2013) to explore state-specific endemic chytridiomycosis infection
204 dynamics using the MSMR framework. We hence performed two- and three-state
205 multi-state modeling with program MARK (version 6.0; White, Kendall & Barker
206 2006) to elucidate the individual-level effect of chytridiomycosis infection on survival
207 probability in the field by assigning frogs to an infection state at each capture via
208 qPCR results. We particularly wanted to determine the probabilities for infection and
209 recovery transitions, in order to understand the nature of infection dynamics *in situ*
210 (Murray *et al.* 2009; Cooch *et al.* 2012).
211

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 ($\gamma$), apparent trip prevalence ($\pi$), mean daily maximum temperature (°C) for the 28 days preceding each trip ($\lambda$), and a cyclical seasonal linear trend variable ($\tau$, where autumn is considered equivalent to spring). Recapture probability was investigated as a function of infection status ($\gamma$), mean daily relative humidity (%) at maximum temperature for the 28 days preceding each trip ($\varepsilon$), mean daily radiation ($MJ/m^2$) over 28 days preceding each trip ($\zeta$), and capture effort (in days per trip $\delta$). 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 ($\gamma$) 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 repeatability of the quantitative PCR at James Cook University is very high; Hyatt *et al.* 2007). However, 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.

The state transition parameter $\psi_i^{rs}$ 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 $\psi_i^{rr}$, 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 ($\gamma$), apparent trip prevalence ($\pi$) and seasonal environmental covariates such as temperature ($\lambda$). 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

261 immunomodulation (Richards-Zawacki 2010). Similarly, recoveries should also be
262 associated with reduced prevalence as they require an absence of re-infection.
263
264 We applied the bootstrap and median $\hat{c}$ goodness of fit tests with the general model
265 $S(\gamma)\rho(\gamma)\psi(\gamma)$ (further details on goodness of fit testing and modeling assumptions can
266 be found in Appendix S1). Bootstrapping yielded p = 0.61 ($\hat{c}$ = 1.028), and median $\hat{c}$
267 yielded $\hat{c}$ = 1.110 (95% CI 0.925 - 1.295; 1000 simulations) for the two-state multi-
268 state dataset, hence the most conservative estimate of $\hat{c}$ = 1.110 was used. Similarly
269 for the three-state analysis, bootstrapping yielded p = 0.64 ($\hat{c}$ = 1.026), and median $\hat{c}$
270 gave $\hat{c}$ = 1.097 (95% CI 0.944 - 1.250; 1000 simulations), hence $\hat{c}$ = 1.097 was
271 employed. Candidate model sets for two and three-state analyses were constructed
272 separately *a priori* using a restricted form of the all subsets approach, and tested
273 systematically (Appendix S1; Lukacs, Burnham & Anderson 2010; Hegyi &
274 Garamszegi 2011; Doherty, White & Burnham 2012). We constructed models using
275 the intercept design matrix coding format and the logistic (logit) link function. Where
276 numerical convergence was suspect, we employed the alternate optimization routine
277 from within MARK, and assessed each model individually for estimable parameter
278 count, adjusting as necessary (Lebreton *et al.* 2009; Cooch *et al.* 2012). We used
279 $QAIC_c$ to rank model parsimony (Burnham & Anderson 2002), model averaging to
280 reduce selection bias (Lukacs, Burnham & Anderson 2010), and we estimated
281 monthly parameter probabilities ($1\ month = \frac{365}{12} \approx 30.42\ days$), reporting
282 unconditional 95% confidence intervals (95% CI; Burnham & Anderson 2002).
283 Akaike weights were used to determine relative variable importance from entire
284 candidate model sets (Doherty, White & Burnham 2012), and we report evidence
285 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). The Weibull distribution ($\alpha$ = 0.46901, $\beta$ = 15.259) and negative binomial distribution were fit to the 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).

## Multi-state mark-recapture results

Model averaged parameter estimates revealed marked seasonality in survival and transition probabilities in both analyses (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). While survival differed between infected and uninfected frogs in the two-state analysis, apparent survival probability estimates for the infected group were incongruously 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).

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. S2, 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. S2, 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, S2b, 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 S1 (Appendix S2).

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 ($\pi = 0.6228$) and temperature ($\lambda = 0.3400$) for survival; days ($\delta = 0.36301$), radiation ($\zeta = 0.29044$) and relative humidity ($\varepsilon = 0.24491$) for recapture; and prevalence ($\pi = 0.9254$) for transition. For

374 the three-state analysis these were prevalence ($\pi$ = 0.8700) and temperature ($\lambda$ =
375 0.1298) for survival; radiation ($\zeta$ = 0.5109), relative humidity ($\varepsilon$ = 0.2632) and days ($\delta$
376 = 0.1674) for recapture; and prevalence ($\pi$ = 0.9264) for transition.
377
378 The model averaged effect size as a mean across trips for the survival difference
379 between infected and uninfected groups in the two-state analysis was 0.1070, with the
380 infected group demonstrating higher apparent survival overall (95% CI -0.0577 to
381 0.2717). Similarly, the model averaged effect sizes for survival in the three-state
382 analysis were as follows: B-A 0.1184 (95% CI -0.0448 to 0.2815), B-C 0.2610 (95%
383 CI -0.1573 to 0.6794) and A-C 0.1427 (95% CI -0.2086 to 0.4940). While there was
384 limited support for an effect of individual infection status on apparent survival in the
385 two-state analysis (the evidence ratio comparing most parsimonious models with and
386 without $\gamma$ was 2.8222), there was correspondingly strong support in the three-state
387 analysis (evidence ratio 695.20), and strong support in both analyses for an effect of
388 infection status on state transition probability (evidence ratios > 918.90 and 319.36 for
389 the two- and three-state analyses, respectively; Lukacs *et al.* 2007).
390
391

392 # Discussion

393

394 **Pathogen over-dispersion and survival probabilities**

395 We found marked aggregation of Bd within our endemically infected wild amphibian
396 population, as demonstrated by a highly over-dispersed intensity-frequency
397 distribution curve (Fig. 2). Thus, while most infected individuals had low burdens, a
398 few hosts had high burdens. Categorizing infectious burdens into low or high groups
399 based on qPCR swab results allowed us to partially resolve paradoxical results from
400 our two-state analysis which were similar to those reported by Briggs, Knapp and
401 Vredenburg (2010). The model averaged estimates from our two-state analysis
402 revealed a lower apparent survival probability for uninfected frogs compared with
403 infected frogs, although confidence intervals for the infected state were wide (Fig. 3a).
404 After taking pathogen over-dispersion into account, apparent survival probability of
405 infected frogs fell to either side of the uninfected group, with high-burden frogs
406 having the lowest survival estimates (Fig. 4a). The reason for a difference between the
407 two and three state analyses is the high degree of pathogen over-dispersion and its
408 differential effects; approximately half the infected frogs were classed in the low-
409 burden group (Fig. 2). In addition, infection intensity was found to be seasonally
410 associated with survival as well as transmission and recovery probabilities. Our
411 results are consistent with previous field work showing over-dispersion (Skerratt *et al.*
412 2011), and linking reduced survival with higher Bd infection intensities (Murray *et al.*
413 2009), and also demonstrates that quantifying infectious burdens is key to
414 understanding the ecology of chytridiomycosis.

415

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. S2a, Appendix S2). Alternatively, recovery from both low and high infectious burdens was equally

441 probable and high throughout most of the year, dropping moderately during winter
442 (Figs S2b and S2c, Appendix S2).
443
444 A relatively long incubation period (roughly 3-8 weeks between exposure and clinical
445 signs; Berger *et al.* 2005b; Voyles *et al.* 2009) in an environmentally responsive
446 pathogen means more chance for pathogen-adverse environmental conditions (such as
447 temperature spikes) to favour host recovery and survival. Thus, recovery transitions
448 may be favoured over infection transitions throughout most of the year in areas with
449 higher temperatures such as at low elevation tropical regions. A long incubation
450 period also artificially inflates point prevalence measures and deflates mortality
451 measures compared with pathogens that have short incubation periods. This is also
452 likely to lead to a highly over-dispersed intensity-frequency distribution because most
453 of the infected population is in the subclinical phase of the disease at any point in time
454 (in endemically infected populations, unlike propagating epidemics which can rapidly
455 lead to widespread mortality). Re-infection transitions were comparatively
456 uncommon, however (only three of the 13 frogs that were observed to change state
457 two or more times), possibly suggesting adaptive immunity may occur in the field.
458 However, the third simulation scenario (Fig. S1c, Appendix S2) assumed no effect of
459 adaptive immunity and resulted in population dynamics consistent with our
460 expectations and dynamics observed in the wild.
461
462 **Uninfected frogs versus those with low infectious burdens**
463 Finding that uninfected frogs had lower apparent survival probabilities than those in
464 the low-burden state (Fig. 4a) was unexpected. The difference in apparent survival
465 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 errors 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, is particularly susceptible to errors in interpretation when 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 chytridiomycosis, we focus on the second cause which is likely to be generally the most important given what we know of the disease, although exceptions may occur

(Berger *et al.* 2009). Variation in pathogen replication on the host within a population is caused by differences in host susceptibility (Berger *et al.* 2005b; Tobler & Schmidt 2010) and temperature suitability for pathogen growth (Murray *et al.* 2013). The former may be associated with past exposure history, and physiological and behavioural characteristics. There is some evidence for each of these, although their specific importance remains uncharacterized (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

541 through approaches directed at habitat conservation, or 4) minimizing other
542 threatening processes. We recommend quantifying individual infectious burdens
543 rather than infection state where possible in microparasitic diseases.
544

## 545 Data Accessibility

546
547 Raw encounter history and predictor variable data, together with three-state model
548 averaged parameter estimates are available in Appendix S3 online.
549

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551
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561

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## Figure legends

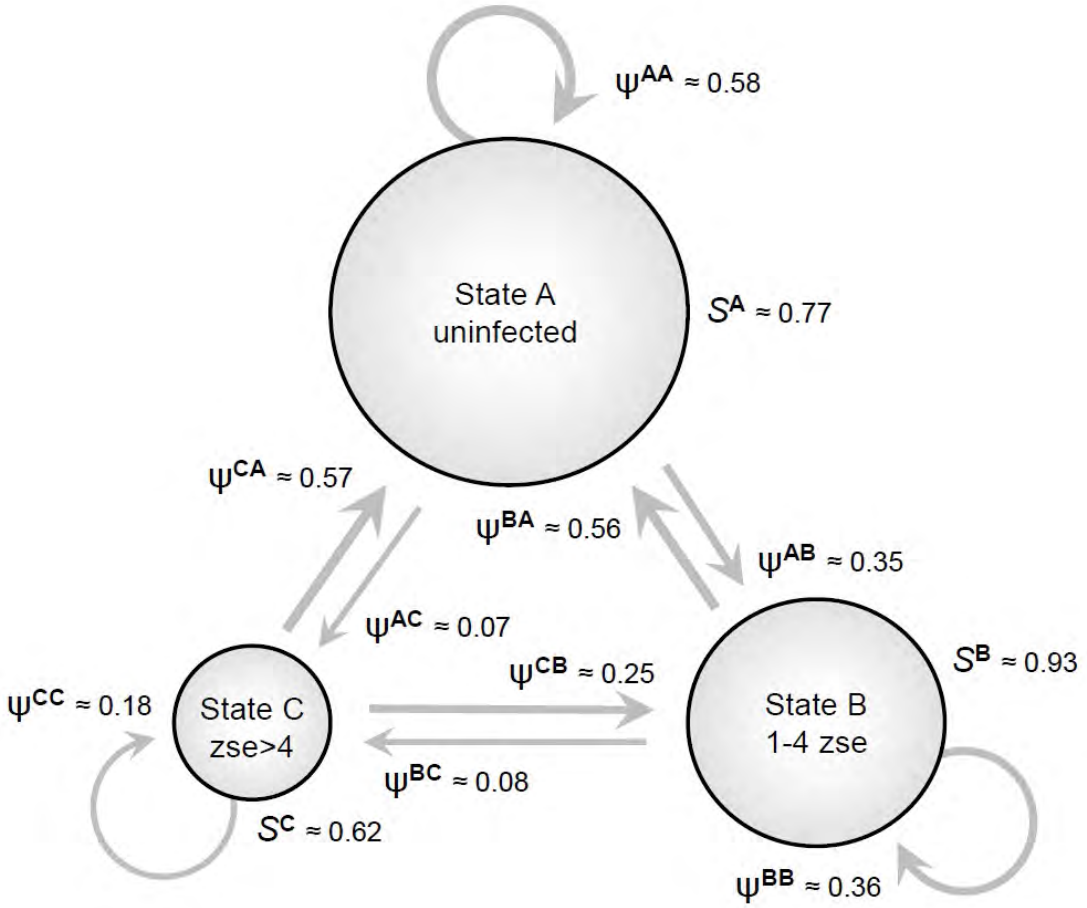
**Figure 1.** Example schematic illustrating state transition probabilities ($\psi$) and survival probabilities (S) for the respective infection states at capture session six (drawn from the three-state multi-state analysis). The notation $\psi^{rs}$ indicates the monthly state transition probability 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*.

804 **Figure 1.**

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807 **Figure 2.**

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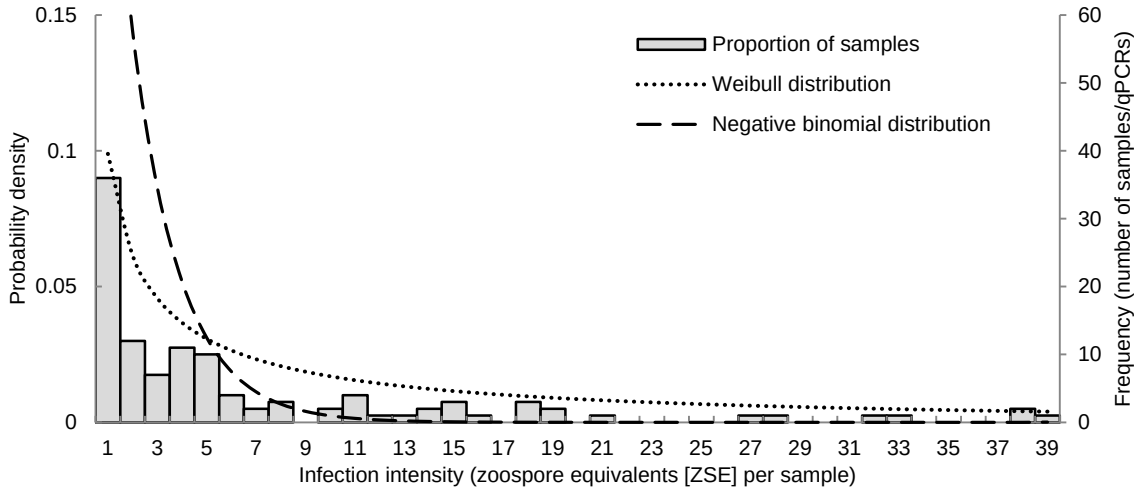


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**Figure 3.**

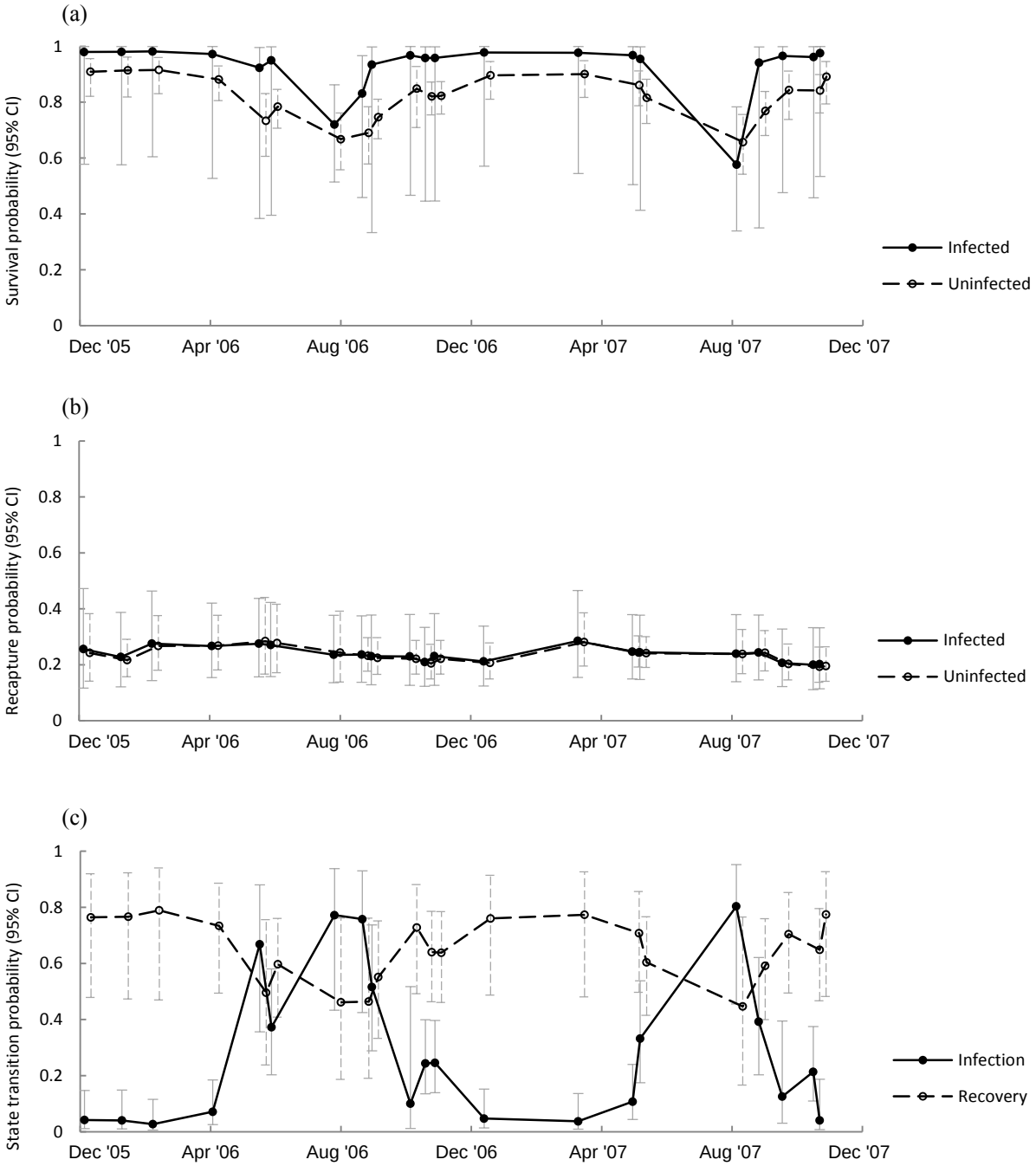
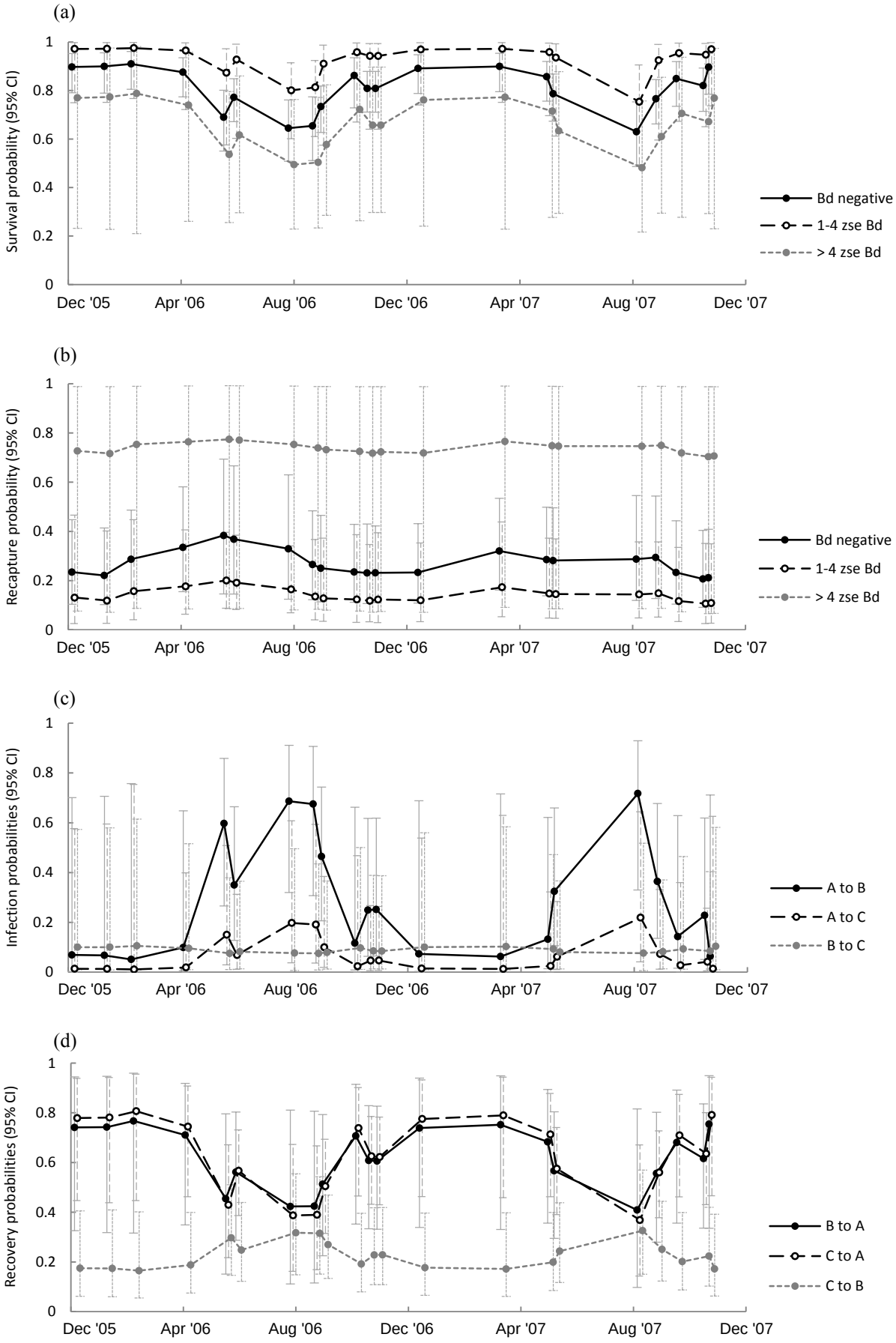

(a)
Survival probability (95% CI)
1
0.8
0.6
0.4
0.2
0
Dec '05
Apr '06
Aug '06
Dec '06
Apr '07
Aug '07
Dec '07
Infected
Uninfected
(b)
Recapture probability (95% CI)
1
0.8
0.6
0.4
0.2
0
Dec '05
Apr '06
Aug '06
Dec '06
Apr '07
Aug '07
Dec '07
Infected
Uninfected
(c)
State transition probability (95% CI)
1
0.8
0.6
0.4
0.2
0
Dec '05
Apr '06
Aug '06
Dec '06
Apr '07
Aug '07
Dec '07
Infection
Recovery

**Figure 4.**


(a)
Survival probability (95% CI)
1
0.8
0.6
0.4
0.2
0
Dec '05
Apr '06
Aug '06
Dec '06
Apr '07
Aug '07
Dec '07
Bd negative
1-4 zse Bd
> 4 zse Bd
(b)
Recapture probability (95% CI)
Bd negative
1-4 zse Bd
> 4 zse Bd
(c)
Infection probabilities (95% CI)
A to B
A to C
B to C
(d)
Recovery probabilities (95% CI)
B to A
C to A
C to B

## 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 figures (Figs S1, S2).

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