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Population-level influence of a recurring disease on a long-lived wildlife host

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Despite a heightened interest regarding the role of infectious diseases in wildlife conservation, few studies have explicitly addressed the impacts of chronic, persistent diseases on long-term host population dynamics. Using mycoplasmal upper respiratory tract disease (URTD) within natural gopher tortoise *Gopherus polyphemus* populations as a model system, we investigated the influence of chronic recurring disease epizootics on host population dynamics and persistence using matrix population models and Markov chain models for temporally autocorrelated environments. By treating epizootics as a form of environmental stochasticity, we evaluated host population dynamics across varying levels of outbreak duration (ρ), outbreak recurrence (f), and disease-induced mortality (μ). Baseline results indicated a declining growth rate (λ) for populations under unexposed or enzootic conditions ($\lambda_{Enzootic} = 0.903$, 95% CI: 0.765–1.04), and a median time to quasi-extinction of 29 years (range: 28–30 years). Under recurring epizootics, stochastic growth rates overlapped with baseline growth rates, and ranged between 0.838–0.902. Median quasi-extinction times under recurring epizootics also overlapped for most scenarios with those of baseline conditions, and ranged between 18–29 years, with both metrics decreasing as a function of f and μ. Overall, baseline (enzootic) conditions had a greater impact on λ than epizootic conditions, and demographic vital rates were proportionately more influential on λ than disease- or outbreak-associated parameters. Lower-level elasticities revealed that, among disease- and outbreak-associated parameters, increases in μ, force of infection (ϕ), and f negatively influenced λ. The impact of disease on host population dynamics depended primarily on how often a population underwent an epizootic state, rather than how long the epizootic persisted within the exposed population. The modeling framework presented in this paper could be widely applied to a range of wildlife disease systems in which hosts suffer from persistent recurring diseases.

Much attention has been directed towards the role of pathogens within the field of conservation biology (May 1988, Scott 1988, Cleveland et al. 2002, Lafferty and Gerber 2002, Ostfeld et al. 2002, Tompkins et al. 2002, Smith et al. 2006, Begon et al. 2009). While several studies have addressed the effects of wildlife disease on demographic parameters such as survival and fecundity, few have assessed how diseases may impact the long-term dynamics and persistence of host populations (but see Doak et al. 1994, Albon et al. 1997, Brook and Kikkawa 1998, Haydon et al. 2002, Gerber et al. 2005). Additionally, assessments of the risk of disease outbreak have primarily focused on pathogens that cause high mortality (Cleveland et al. 2002). However, very little work has addressed the effects of chronic endemic infections on threatened host populations (but see Cross et al. 2009). Consequently, the effects of endemic or persistent pathogens have remained less well-understood (Cleveland et al. 2002). Despite this dearth of focus on chronic infections, research suggests that pathogens that elicit a higher prevalence of subclinical, rather than clinical, infections are more likely to regulate long-term host population dynamics than pathogens that produce higher proportions of overt clinical disease among infected hosts (Boots et al. 2003). Moreover, pathogens associated with high mortality rates are more likely to fade out from a host population earlier than less fatal pathogens, and therefore, have a less pronounced effect on long-term dynamics of host populations (Claessen and deRoos 1995). Because chronic, or persistent, infections could potentially have greater long-term impacts than acute infections on host population dynamics, their assessment within wildlife populations of conservation concern warrants attention.

The dynamics of chronic disease in host populations are heavily influenced by the duration of host morbidity and infectiousness, and the rate of disease recrudescence. For the purpose of this study we define disease chronicity as the duration of morbidity, which corresponds to the amount of time an infected individual experiences active and overt clinical disease. For chronic diseases, tissue damage and/or altered host function resulting from this clinical disease state can be

considered lifelong. Clinical disease is usually accompanied by host infectiousness, in which adequate numbers of infectious microbes are shed to transmit infection to susceptible contacts (Casadevall and Pirofski 2002). Transition from an active (infectious) clinical disease state to a clinically silent and asymptomatic carrier state occurs after the infectious period, in which the pathogen is sequestered within hosts in the form of a latent infection. Recrudescence of active clinical disease from a latent subclinical state can be triggered by several factors, such as: 1) exposure to other infectious individuals, which stimulates a rapid memory immune response and possibly more severe clinical disease, 2) superinfection with a secondary pathogen (Casadevall and Pirofski 2003), or 3) physiological stress caused by environmental or anthropogenic factors (Padgett and Glaser 2003).

We can apply these general concepts of chronic disease natural history from the individual-level to the level of host populations. For example, population-level recrudescence would manifest itself as a recurring epizootic. The rate of epizootic recurrence or re-emergence is then defined as the rate at which a critical threshold of individuals within an exposed population becomes clinically ill, and either spreads infection or elicits reactivation of chronically infected individuals from a latent/subclinical state to a clinical disease state. In disease ecology terms, this would represent conditions under which the basic reproductive number (R_0) of a pathogen is greater than one, and infection (and subsequent disease) propagates through a population (Swinton et al. 2002). Recurring epizootics occur in many wildlife populations, and are often associated with seasonal environmental factors (Altizer et al. 2006). Consistent with the definition of infectiousness/morbidity duration at the individual-level, outbreak duration (at the population-level) can be defined as the epizootic time span, or the number of consecutive years in which a disease outbreak directly affects demographic parameters such as survival, fecundity, or growth. Although lifelong effects of chronic disease can have significant repercussions on individuals, we focus our efforts here on modeling disease processes at the population-level, and therefore explicitly account for outbreak frequency and duration rather than individual patterns of infectiousness and morbidity.

From the perspective of population dynamics, outbreak duration and recurrence could be represented using a Markov chain model. Under this framework, the emergence and re-emergence of an outbreak within a population at a specific time is probabilistically dependent on: 1) the temporal autocorrelation term ρ, which represents the length of outbreak duration, and 2) the overall frequency term f, which defines the overall frequency of outbreak recurrence. This approach has been used to study the effects of fire and hurricane disturbance on the population dynamics of plant species (Pascarella and Horvitz 1998, Caswell 2001). Under temporally autocorrelated environments, vital rates (i.e. survival, fecundity, and/or growth) display a memory of past conditions, which can have a profound impact on population dynamics (Tuljapurkar and Haridas 2006). In other words, repeated consecutive exposure to adverse environmental conditions is expected to produce different results than repeated sporadic exposure in relation to long-term population dynamics. By modeling disease outbreaks as a form of environmental stochasticity in which the probability of outbreak occurrence is temporally autocorrelated, the effects of outbreak duration and recurrence on the long-term dynamics and persistence of wildlife populations can be assessed.

In this study, we use mycoplasmal upper respiratory tract disease (URTD) in natural gopher tortoise *Gopherus polyphemus* populations as a model system to assess the impacts of outbreak duration and recurrence on the long-term dynamics and persistence of threatened host populations. Little is known regarding the dynamics of URTD within, and its subsequent impacts on, gopher tortoise populations. Our goal was to assess the potential effects of disease on the long-term growth and persistence of gopher tortoise populations at varying levels of outbreak duration, recurrence, and disease-induced mortality. Findings from this study will help quantify the potential threat of URTD on the dynamics and persistence of gopher tortoise populations, and also provide a framework for understanding population-level influences of chronic and persistent diseases in other wildlife systems.

Methods

Study system

Mycoplasmal respiratory infections in virtually all hosts are generally chronic and clinically silent, with low mortality but nearly 100% morbidity (Simecka et al. 1992, Minion 2002). Overt clinical signs are observed in early infections, and may be exacerbated in periods of stress or as the individuals get older. However, pathogenic mycoplasmal species can often cause gross and histological lesions, primarily in the respiratory tract, even when overt clinical signs are absent (Simecka et al. 1992).

Mycoplasma agassizii was first defined as an etiologic agent of URTD in free-ranging desert tortoises *Gopherus agassizii* in Nevada, USA (Jacobson et al. 1991, Brown et al. 1994). Although there have been many speculations linking mycoplasmal upper respiratory tract disease (URTD) to die-off events (Gates et al. 2002, Seigel et al. 2003), little is known about the effects of this chronic disease on the long-term dynamics of gopher tortoise populations (Holder et al. 2007). Clinical signs, when present, are often expressed intermittently (Brown 2002), making observations of clinically ill animals in the field a difficult task (Wendland 2007). Tortoises spend most of their time in burrows. Consequently, direct observations are quite limited. Given the intermittent expression of clinical signs (even in experimentally infected tortoises that can be observed frequently), it is not surprising that tortoises encountered in the field may often appear to be healthy. However, wild tortoises that were not exhibiting clinical signs did maintain subclinical chronic infections in which substantive tissue damage is present (Jacobson et al. 1991, McLaughlin et al. 2000). *Mycoplasma agassizii*, like most mycoplasmal infections, causes chronic infections in which infected individuals transition between clinical and subclinical disease states. Therefore, the duration of clinical disease and the rate of disease recrudescence are of major epizootiological importance when attempting to elucidate how a chronic disease could alter the long-term dynamics of wildlife populations.

Model formulation

Recrudescence rate

We assumed that the rate of individual recrudescence was equivalent to the force of infection (ϕ), and therefore that subclinically infected individuals experienced recurring bouts of clinical disease at the same rate that previously unexposed susceptible individuals became clinically infected. This is likely a conservative assumption as experimental exposure of gopher tortoises with subclinical disease resulted in a rapid increase in clinical signs as well as increased shedding (McLaughlin 1997), similar to what has been observed in *M. gallisepticum* infection in songbirds (Sydenstricker et al. 2005).

Demographic effects of the disease

We assumed that only the reproductive adults would suffer significant effects of disease in the form of disease-induced mortality. This assumption is supported by a study showing strong stage-specific seroprevalence, whereby only a marginal percentage of pre-reproductive individuals tested positive for *M. agassizii* exposure (Wendland et al. 2010). We therefore assumed that disease outbreaks (or epizootics) did not influence the survival of pre-reproductive tortoises. Also, preliminary analyses revealed no evidence that *M. agassizii* exposure influenced reproductive parameters (i.e. clutch size and gravidity) over a four-year period (White 2009); therefore, we assumed that the disease had no effect on reproductive parameters. Perhaps the most pressing concern regarding URTD in tortoise populations involves its speculated role in tortoise die-off events (Berry 1997, Seigel et al. 2003), so we focused on quantifying potential impacts of recurring mortality events on long-term host population dynamics by limiting the effects of URTD to depressed survival.

Disease-induced mortality (μ)

We assumed that disease-induced mortality (μ) acts only on clinically ill individuals, and that the mortality of subclinically infected individuals during enzootic disease states is not significantly different from the baseline mortality rates of uninfected reproductive adults. We also made the conservative assumption that disease-induced mortality acted randomly on clinically ill individuals (i.e. individuals actively presenting clinical signs of disease) across all scenarios of epizootic duration and recurrence. Virulence and pathogenicity were assumed to remain constant over time, and neither the duration nor frequency of epizootics affected the rate at which individuals died from clinical disease. In other words, disease-induced mortality was assumed to be independent of epizootic duration and recurrence frequency, and at each time step the survival rate of clinically infected individuals was reduced by a constant proportion μ ($0 \leq \mu \leq 1$).

Modeling disease outbreak

Disease outbreaks were modeled as a form of environmental stochasticity in which populations were subjected to enzootic or epizootic conditions according to probabilities defined by a Markov Chain model (Caswell 2001, Tuljapurkar and Haridas 2006). The enzootic state was defined as the baseline condition of a population in which the incidence of clinical disease is negligible, and disease-induced mortality does not occur. We defined an epizootic state as a condition in which a threshold level of clinically ill and infectious individuals is surpassed, and an increased incidence of clinical disease is associated with an increase in mortality due to disease (μ). Under epizootic conditions subclinical individuals with latent infection recrudesce to a clinical disease state at a rate ϕ. Under enzootic conditions following epizootic events, previously infected individuals within a population may remain subclinically infected, and consequently serve to maintain seroprevalence at generally constant levels through time. When the epizootic occurs, adult survival was reduced by a proportion μ ($0 \leq \mu \leq 1$).

In addition to addressing the population-level effects of epizootics (i.e. recurring mortality from disease), we also assessed the impacts of epizootic recurrence and duration on long-term host population dynamics. Epizootic recurrence was defined as the overall frequency (f) with which epizootic conditions occurred within a population over time. Epizootic duration (ρ) was defined by an autocorrelation term, which represented the duration of time a population experienced epizootic conditions. For example, under scenarios of acute epizootics (i.e. short duration), a host population would experience recurring outbreaks that would quickly subside. In other words, epizootic states would seldom occur across consecutive years, but would act on a population for only short periods of time. Under scenarios in which duration was chronic (i.e. long duration), populations would suffer from epizootic conditions for long spans of time.

Parameter estimation

To parameterize a stage-structured population projection matrix with three demographic stages (hatchling, pre-reproductive juvenile, and reproductive adult) and two disease states (uninfected/subclinically infected, and clinically infected), the following parameters were needed: 1) stage-specific annual survival probabilities (σ_H, σ_{PR}, σ_R), 2) annual growth rate of pre-reproductive individuals to the reproductive stage (γ), 3) annual fecundity rate (m), and 4) force of infection (ϕ). Methods used for estimating these parameters are described in detail in Supplementary material Appendix A1; a brief overview follows.

Stage-specific survival probabilities (σ_H, σ_{PR}, σ_R)

Annual hatchling survival probability was defined as the summary estimate of a meta-analysis of five gopher tortoise hatchling survival studies (Perez-Heydrich 2010). Survival probabilities for pre-reproductive juvenile and reproductive adult survival rates were estimated using a capture-mark-recapture analysis of four years of data collected from three populations that had previously been described as URTD-free (Wendland 2007).

Pre-reproductive growth probability (γ)

The probability that pre-reproductive tortoises survive and grow to become reproductive adults was inestimable from the four-year mark–recapture data due to the slow-growing nature of this long-lived species. Therefore, we estimated γ using the fixed stage duration method described by Caswell (2001).

Fecundity (m)

The annual fecundity was estimated as the product of one-half times clutch size (cs), proportion of females gravid (pg), nesting success probability (ns) and hatching success rate (hs) (i.e. $m = (cs \times pg \times ns \times hs)/2$). Mean clutch size and proportion of females gravid were obtained from radiographs of adult females sampled from the all study populations prior to oviposition. Nest and hatch success were defined as the summary estimates of meta-analyses conducted using findings from published studies (Perez-Heydrich 2010).

Force of infection (ø)

The force of infection represents the per-capita annual rate at which susceptible individuals become infected (Swinton et al. 2002). Ozgul et al. (2009a, b) estimated annual force of infection for *M. agassizii* from a four-year CMR study of gopher tortoise populations with high ($\geq 25\%$) and low ($< 25\%$) seroprevalence. We used their estimate of force of infection under high seroprevalence conditions ($\phi = 0.22 \pm$ SE 0.04) to quantify the transition from an uninfected (or subclinically infected) state to a clinically infected state among reproductive adult tortoises.

Construction of population projection and Markovian transition matrices

We used matrix population models with four stages (hatchling, pre-reproductive juvenile, uninfected/subclinically infected reproductive adult, and clinically infected reproductive adult) to describe demography of gopher tortoises, and two-state Markovian transition matrices to model the transition of enzootic population conditions to epizootic states (Fig. 1). Population projection matrices were parameterized using the demographic rates presented in Table 1, and followed the form:

$$A = \begin{pmatrix} 0 & 0 & m\sigma_R & m\sigma_R(1-\mu) \\ \sigma_H & \sigma_P(1-\gamma) & 0 & 0 \\ 0 & \sigma_P\gamma & \sigma_R(1-\phi) & 0 \\ 0 & 0 & \phi\sigma_R & \sigma_R(1-\mu) \end{pmatrix}$$

Under enzootic conditions $\phi = \mu = 0$, and the population projection matrix reduced to three demographic stages. Separate population projection matrices were constructed to represent each type of epizootic state defined according to varying levels of disease-induced mortality (μ). Disease-induced mortality was defined as the proportional increase in mortality (or proportional reduction in survival) associated with URTD. When a population was subjected to epizootic conditions, the survival of adults with clinical disease was reduced by a proportion μ. Thus, the survival of adults with active clinical disease was given by $\sigma_R(1 - \mu)$, to account for this proportional reduction in survival. Population projection matrices for epizootic and enzootic states differed from each other only in their values for μ, whereby $\mu = 0$ for the enzootic state, and 0.01, 0.05, 0.10, 0.20 and 0.30, for the different epizootic states.

Nine Markovian transition matrices (P_i) were generated to describe acute, intermediate, and chronic epizootic conditions under low, intermediate, and high frequencies of recurrence. The autocorrelation term ρ was used to quantify the chronicity (or duration) of epizootic states (i.e. $\rho_{acute} = -0.10$, $\rho_{intermediate} = 0.40$, $\rho_{chronic} = 0.73$). When $\rho < 0$, enzootic and epizootic states tend to alternate so that when a population experiences an outbreak, it transitions quickly from an epizootic state to an enzootic state; however, when $\rho > 0$, long sequences of enzootic and epizootic states are generated so that the transitions between enzootic and epizootic states are prolonged (Caswell 2001). The outbreak frequencies (f) used to parameterize the Markovian transition matrices (i.e. $f_{low} = 0.1$, $f_{intermediate} = 0.2$, $f_{high} = 0.3$) represented how often populations experienced recurring epizootics. Because the true recrudescence rate of URTD is unknown, we used a range of values of f to explore how epizootic recurrence may affect population dynamics. Using all possible combinations of ρ and f, values of p and q were calculated for the Markovian transition matrices using the following equations adapted from Caswell (2001):

$$p = 1 - \rho - (1 - f)(1 - \rho)$$
$$q = (1 - f)(1 - \rho)$$

One notable difference between our parameterization and Caswell's is that we substituted the long-term frequency of state 1 (f), with the long-term frequency of state 2 (1 – f) so that f would represent the long-term frequency of outbreaks rather than the long-term frequency of normal years (Fig. 1). The resulting Markovian transition matrix was then defined as

$$P = \begin{pmatrix} 1-p & 1-q \\ p & q \end{pmatrix}$$

Stochastic population growth rate (λ_S)

In order to address the potential impacts of recurring epizootics on long-term population growth, we compared the stochastic population growth rate (λ_S) across different outbreak scenarios to the population growth rate under baseline, or enzootic, conditions ($\lambda_{Enzootic}$). Fifty-four different stochastic growth rates, each corresponding to a different combination of endemic-epizootic state grouping and Markov chain model (Fig. 2), were calculated using Tuljapurkar's approximation for autocorrelated environments (Tuljapurkar and Haridas 2006). Using this approach $\log \lambda_S \approx \log \lambda_0 - W_1 + W_2$, where λ_0 is the dominant eigenvalue of the average matrix, W_1 is the effect of interannual variability on λ_S, and W_2 is the effect due to autocorrelation. Using this formulation, we assessed the relative effect of temporal autocorrelation (W_2) over the effects of interannual variability (W_1) in order to address the impact of epizootic duration on host population dynamics. For details regarding the numerical computation of the terms W_1 and W_2 please refer to Tuljapurkar and Haridas (2006). The variance of λ_s (σ^2) was estimated under the assumption of asymptotic lognormality of the weighted sum of stage-specific abundances (Caswell 2001). Ninety-five percent confidence intervals were then generated as $exp(\log \lambda_S \pm 1.96 \sqrt{\sigma^2})$. The deterministic asymptotic growth rate of population under enzootic conditions ($\lambda_{Enzootic}$) was generated as the dominant eigenvalue of

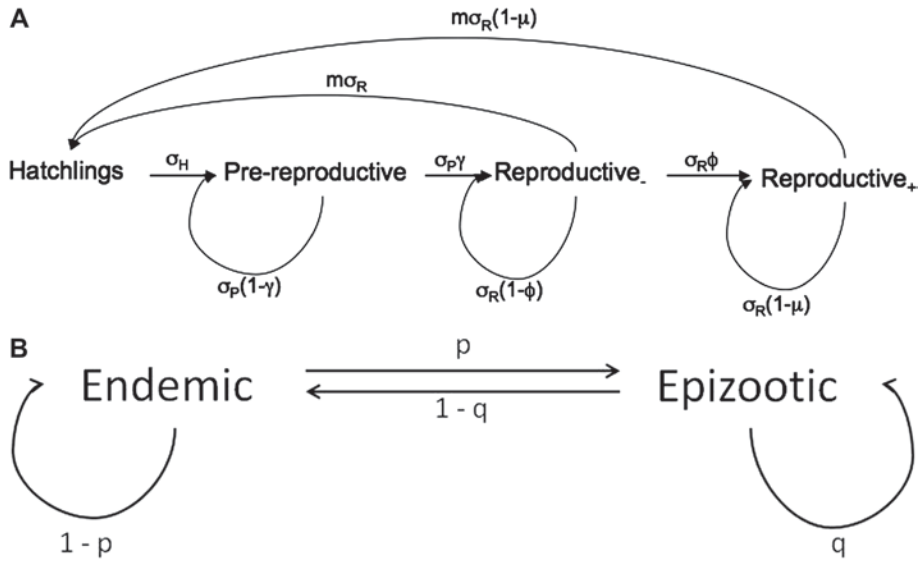

Figure 1. Conceptual models of (A) demographic processes, and (B) Markovian transitions for epizootic processes. (A) Transition rates along the loops are defined according to parameters listed in Table 1. (B) The Markov chain model parameters p and q are defined according to varying levels of epizootic duration and overall recrudescence.

the baseline population projection matrix. Standard errors for the deterministic $\lambda_{Enzootic}$ were calculated through series approximation using the sensitivities of $\lambda_{Enzootic}$ to lower-level demographic parameters (Caswell 2001).

Elasticity analysis

In order to quantify the overall importance of model parameters (i.e. demographic vital rates, disease- and outbreak-associated parameters) to the long-term growth rate of exposed populations, we employed elasticity analyses (de Kroon et al. 2000). We calculated elasticity of λ to matrix elements, as well as to lower-level vital rates using the chain rule for the baseline population projection matrix (population experiencing endemic rather than epizootic conditions) using standard methods (Caswell 2001). For each of 54 disease scenarios we also calculated stochastic elasticities using a simulation approach (50 000 time steps) as described by Caswell (2001). Additionally, we calculated lower-level elasticities of long-term population growth to demographic vital rates, and disease- and outbreak-associated parameters (i.e. ϕ, μ, f and ρ) using the vec permutation approach developed for matrix metapopulation models (Hunter and Caswell 2005, Ozgul et al. 2009b). Using this framework, we defined 'patches' as the endemic and epizootic population states, and the transition between these patches as the Markov chain model described above (details in Supplementary material Appendix A2).

Estimation of quasi-extinction parameters

The final metric used to investigate the population-level effects of recurring epizootics was persistence time, defined here as the length of time for a population to fall below a predefined quasi-extinction threshold. Quasi-extinction parameters for epizootic scenarios were estimated through stochastic simulation (Caswell 2001, Morris and Doak 2002). An initial population size of 500 tortoises was used to assess quasi-extinction times. The quasi-extinction threshold was set to 10% of the initial population size, or 50 individuals. Censuses across Florida have shown that only 31% of conservation lands harbor gopher tortoise populations with ≥500 tortoises (Miller 2001), and that most populations contain between 100 and 500 individuals (Smith et al. 2006). For this reason, an initial size of 500 tortoises was considered appropriate for our analyses. The initial population size was then multiplied by the stable stage distribution from the baseline population projection matrix in order to obtain an initial stage-structured population vector. For each simulation, a sequence of environments corresponding to a specific Markov chain model was generated and used to assign a projection matrix at each time step.

Population size with stage structure was then projected over time for each simulation, and the proportion of simulations that reached the quasi-extinction threshold at or before a given time step provided the cumulative probability distribution of quasi-extinction times (Morris and Doak 2002). Median quasi-extinction time (T_{q50}) was defined as the number of years a population would persist before the probability of quasi-extinction surpassed 0.50.

There were uncertainties associated with estimates of demographic and disease parameters, as quantified by sampling variances. Such parametric uncertainties would undoubtedly introduce uncertainties in estimates of quasi-extinction parameters. We used a parametric bootstrapping approach

Table 1. Estimates of demographic parameters used for the parameterization of a baseline population projection matrix. Please refer to the Supplementary material Appendix A1 for more details on the estimation of σ_P, σ_R, γ and m.

Parameter	Symbol	Estimate	Variance
Hatchling survival	σ_H	0.13	0.0014
Fecundity	m	0.50	0.0874
Pre-reproductive survival	σ_P	0.39	0.12
Pre-reproductive growth	γ	0.001614	0.000076
Reproductive survival	σ_R	0.90	0.0059
Force of infection	ϕ	0.22	0.0016

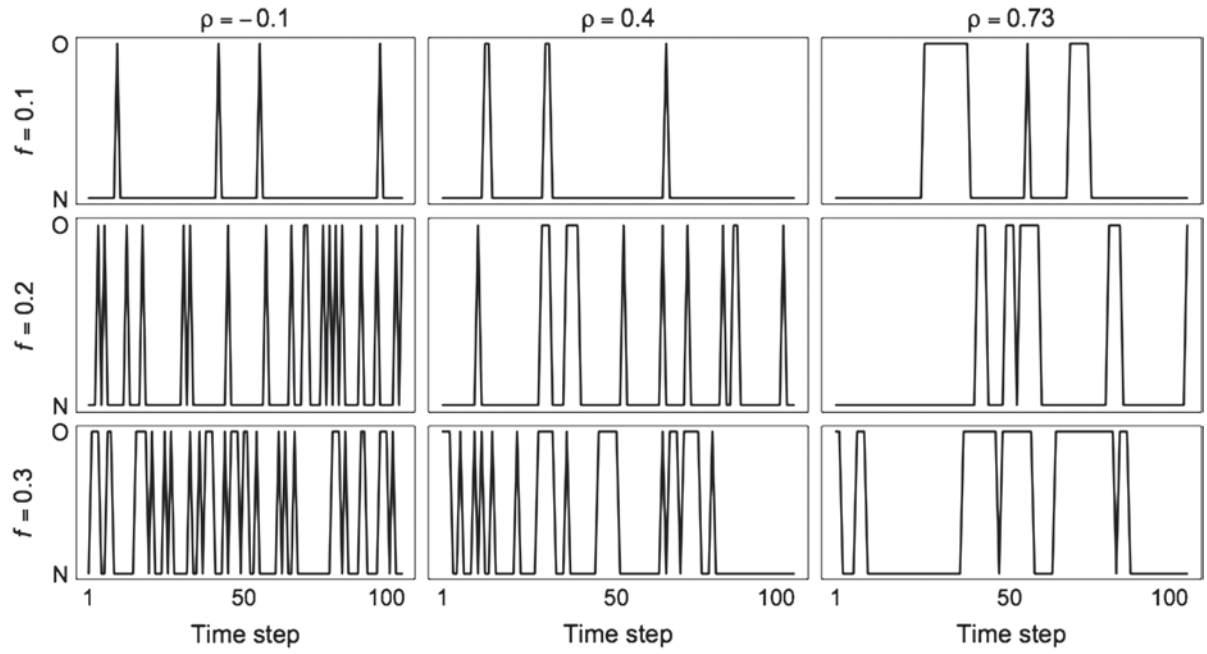

Figure 2. Scenarios of recurring epizootics simulated from two-state Markov chain models. Low, intermediate, and high frequencies of recurrence were simulated in conjunction with short, intermediate, and long scenarios of epizootic duration for 50 000 time steps; however, only the first 100 are displayed here. The two population states, endemic (N) and epizootic (O), are labeled along the y-axis.

to quantify the effects of parametric uncertainties on quasi-extinction parameters. For each of 5000 simulation runs, we sampled demographic and disease parameters from specified distributions and estimated probability of quasi-extinction ($\Pr\{T_q \leq 50\}$) and median extinction time (T_q50) (details in Supplementary material Appendix A3). We repeated this process 500 times, such that we obtained 500 estimates of quasi-extinction parameters. A 95% confidence interval (CI) was then calculated for each disease outbreak scenario using a percentile method. Quasi-extinction parameters under the enzootic condition were estimated similarly by repeating 5000 simulations of 50 time steps 500 times, except that population states remained constant through time.

Results

Baseline model

Under enzootic conditions and in the absence of clinical disease, the tortoise population was projected to decline by approximately 9.7% each year ($\lambda_{Enzootic} = 0.903$, 95% CI: 0.765–1.04). The survival of reproductive adults was proportionately the most influential parameter on the long-term population dynamics, both in terms of matrix entry as well as lower-level elasticities ($e(\sigma_R) = 0.780$), followed by fecundity ($e(m) = 0.000085$). The probability that the disease-free baseline population goes extinct within 50 years was 0.740 (95% CI: 0.727–0.752), and the corresponding median time to quasi-extinction was 29 years (95% CI: 28–30).

Outbreak model

Under scenarios of recurring epizootics, long-term population growth rates overlapped with those of the baseline population (under enzootic conditions). Stochastic growth rates (λ_S) calculated using Tuljapurkar's small noise approximation ranged between 0.838–0.902, and decreased as a function f (epizootic recurrence) and μ (disease-induced mortality; Fig. 3). The λ_S's were inversely related to f and μ, but increased marginally with an increase in ρ (outbreak duration). The interaction of f and μ reduced λ_S by as much as 7% (Fig. 3).

Stochastic elasticities calculated using the simulation approach and deterministic elasticities obtained from the vec-permutation approach displayed near-perfect correlation ($r > 0.993$) across all 54 disease scenarios. These results indicated that elasticities obtained using matrix metapopulation models represented the overall stochastic elasticity patterns well. Because both approaches provided similar results, we focused on elasticities obtained using the vec-permutation approach for subsequent discussion on elasticities.

Demographic parameters under endemic conditions made a higher proportional contribution to λ ($\sum e(B_{endemic})$: 0.702–0.968) than those under epizootic conditions ($\sum e(B_{epizootic})$: 0.032–0.298) across all outbreak scenarios. Overall, elasticity of λ to entries of the demographic block-diagonal matrix **B** (Supplementary material Appendix A2) indicated that the survival of reproductive adults (σ_R) had the largest proportional influence on λ compared to other demographic variables. Elasticity of λ to elements of the dispersal (i.e. Markovian outbreak transition) matrix **M** (Supplementary material Appendix A2) indicated that, among entries of matrix **M**, the probability of remaining under endemic conditions had the greatest proportional impact on λ across all outbreak scenarios (range of elasticity values: 0.472–0.955).

Lower-level elasticities revealed the same trends as above, and indicated that demographic parameters were proportionately more influential on λ than disease- or outbreak-associated parameters. Among demographic parameters, survival of reproductive adults had the largest lower-level elasticity across all outbreak scenarios ($e(\sigma_R) > 0.998$). Among

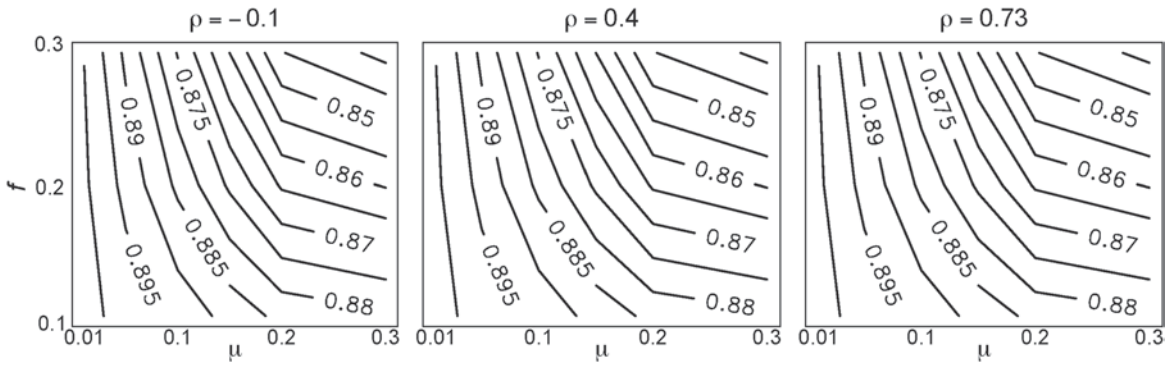

Figure 3. Contour plots of stochastic growth rates (λ_s), calculated using Tuljapurkar's small noise approximation for autocorrelated environments, as a function of disease-induced mortality (μ) and epizootic recurrence (f) at the three levels of eipzootic duration (ρ) considered in our study.

disease- and outbreak-associated parameters, disease-induced mortality had the largest overall proportional impact on λ (e(μ): –0.188 to –0.0099), followed by outbreak recurrence frequency (e(f): –0.0642 to –0.000979; Fig. 4), under epizootic scenarios in which $\mu \leq 0.20$. However, under epizootic scenarios in which $\mu > 0.20$, force of infection had the largest overall proportional impact on λ (e(ϕ):–0.233 to –0.202) among disease- and outbreak-associated parameters, followed by outbreak recurrence frequency (e(f): –0.0713 to –0.0145; Fig. 4). Lower-level elasticities for disease-associated parameters indicated that increases in f, μ and ϕ would result in declines in λ, whereas increases in ρ would increase λ (Fig. 4). Additionally, with increases in μ, elasticities of λ to ϕ became more negative, indicating a greater negative effect on λ, whereas those for μ became less negative and quickly approached 0 when $\mu > 0.20$. In other words, as disease-induced mortality increased, the negative influence of the force of infection on λ became more pronounced while that of disease-induced mortality declined.

The probability of quasi-extinction within a 50-year time period ranged between 0.746 and 0.980 across all scenarios of recurring epizootics. Under most scenarios of short or intermediate epizootic duration, probability of quasi-extinction within 50 years was significantly greater than that under enzootic conditions, and increased with epizootic frequency and disease-induced mortality (Fig. 5). The confidence interval for $Pr\{Tq \leq 50\}$ became wider as values of ρ increased, suggesting a greater effect of parametric uncertainty on estimates of quasi-extinction parameters in strongly autocorrelated environments characterized by longer epizootic duration. Median quasi-extinction times ranged between 18–29 years across outbreak scenarios, and were also inversely related to f and μ. Estimates of λ_S and median quasi-extinction time (T_{q50}) for all values of ρ, f, and μ are presented in Table A3 and A4 (Supplementary material Appendix A4), respectively.

Varying the duration of epizootics (ρ) had little effect on λ_S. The effect of autocorrelation on λ_s (W_2) was very small relative to the effect of interannual variability, or fluctuations in endemic/epizootic population states under Tuljapurkar's small noise approximation for autocorrelated environments (W_1; Fig. 6). At most, W_2 was only about 0.0058 times the magnitude of W_1 across all scenarios, indicating a negligible effect of temporal autocorrelation of endemic/epizootic population conditions on long-term population growth.

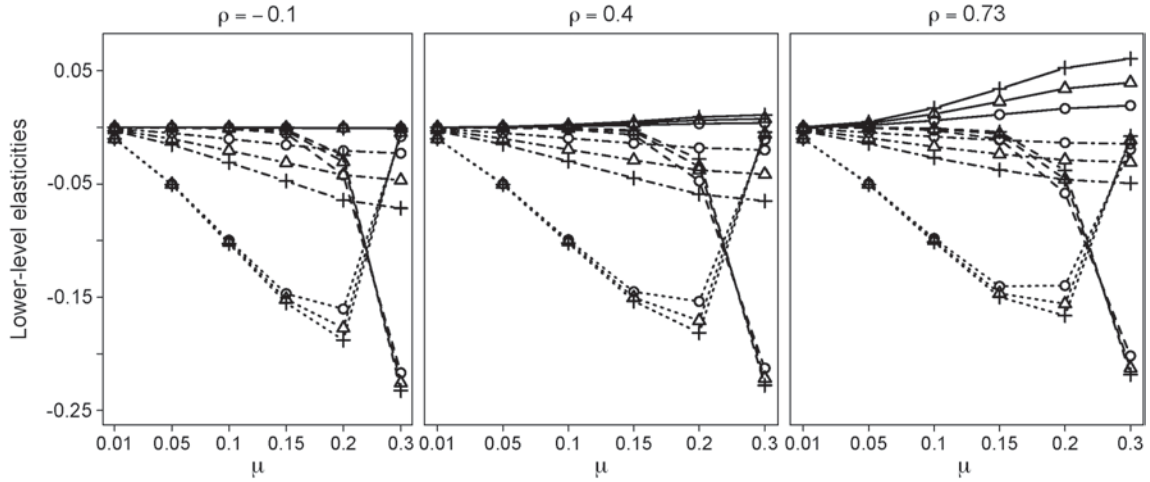

Figure 4. Elasticity of λ_s to changes in outbreak- and disease-associated parameters (f, μ, ρ and ϕ) calculated using the vec-permutation matrix approach. The three levels of *f* are represented through different symbols (circle: f = 0.1, triangle: f = 0.2, cross: f = 0.3), and the four disease-associated parameters are represented by different line types (solid: ρ, dot-dash: f, dashed: ϕ, dotted: μ).

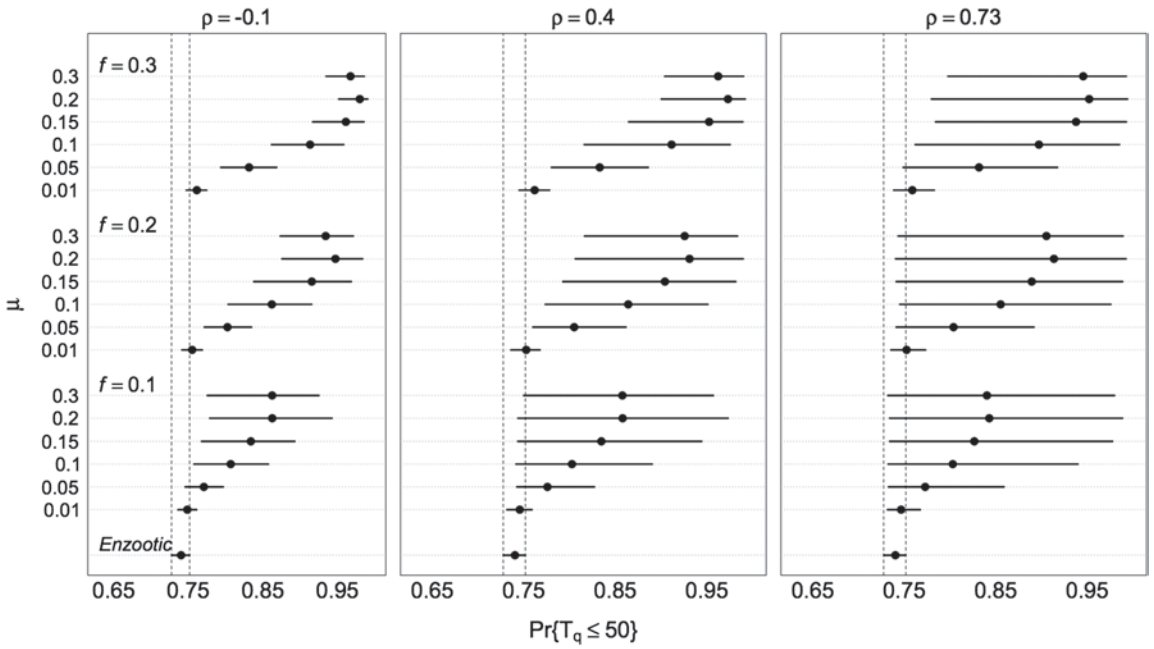

Figure 5. Ninety-five percent confidence limits of probability of quasi-extinction by 50 years ($Pr(T_q \leq 50)$ as a function of disease-induced mortality (μ) and epizootic recurrence (f) at the three levels of epizootic duration (ρ) assessed. Confidence limits were calculated from 500 runs of 5000 simulations of vital rates over a 50 year period. Vertical, dashed lines represent the 95% confidence limits of mean probability of quasi-extinction obtained for a baseline population under enzootic conditions.

Discussion

Disease can pose an indirect threat to the viability of wildlife populations by reducing survival and reproductive rates, and genetic diversity of hosts and contributing to environmental and demographic stochasticity within fragmented or small host populations (de Castro and Bolker 2005). Unfortunately, logistical difficulties and cryptic disease processes often make the study of wildlife diseases within natural populations a complicated venture, and the population-level consequences of chronic diseases remain poorly understood. Proper management strategies are difficult to formulate and implement based on field studies alone, and models of population and disease dynamics can be useful as heuristic and predictive tools. In this study, we were particularly interested in quantifying the effect of recurrent epizootics on the long-term host population dynamics and persistence. Recurrent epizootics have been commonly observed as seasonal phenomena associated with changes in host social behavior, immune function, and birth and death pulses within wildlife populations (Altizer et al. 2006). In house finches, for example, recurring outbreaks of mycoplasmal conjunctivitis have been linked to seasonal changes in host aggregation patterns and reproduction (Altizer et al. 2004). In field voles, seasonal patterns of cowpox virus were associated with the recruitment of susceptible hosts through birth pulses (Begon et al. 2009). Cyclical increases in parasite infestation within red grouse populations were associated with compromised immune defenses of males during the mating season (Mougeot et al. 2006). In these examples, epizootics are fairly predictable from seasonal host attributes. In other cases, however, mechanisms for disease recurrence are largely unknown, and models can be used to understand the effects of recurring epizootics on host population dynamics.

The framework we present in this paper allows for the incorporation of chronic and recurring disease dynamics into an assessment of host population viability in a relatively simple and straightforward manner. The potential

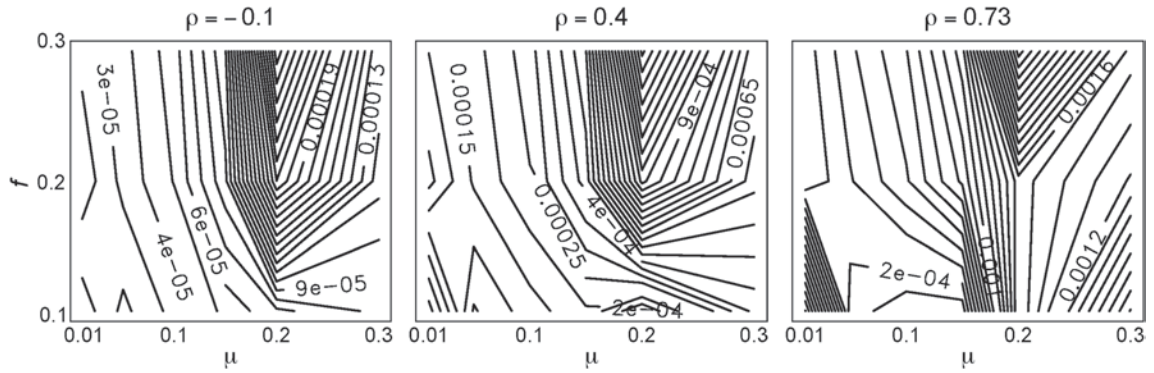

Figure 6. Relative effects of autocorrelation (W_2) over interannual variability (W_1) obtained through Tuljapurkar's small noise approximation.

effects of chronic and recurring epizootics on host wildlife populations were modeled as a form of environmental stochasticity. However, for recurring epizootics the common assumption of independent and identically distributed environmental states fail to properly describe the non-random transitions between endemic and epizootic states experienced by exposed populations. By imposing an autocorrelation structure to potential outbreak states through time with the use of a Markov chain, we were able to determine how factors such as epizootic duration and recurrence could influence the long-term growth and persistence of host populations exposed to chronic and endemic infections. Additionally, we demonstrated how matrix metapopulation models (Hunter and Caswell 2005, Ozgul et al. 2009b) can adequately quantify the proportional influence of disease- and outbreak-associated parameters on long-term growth rate of populations transitioning between endemic and epizootic conditions.

The results of our case study indicated that even under unexposed and/or endemic conditions gopher tortoise populations were at substantial risk of local quasi-extinction. When subjected to recurring epizootics, annual population growth rate declined by up to 16%, and median persistence time declined by up to 11 years. Increases in the force of infection, outbreak frequency, and disease-induced mortality all had negative effects on the long-term growth and persistence of exposed populations, whereas outbreak duration had negligible impacts.

According to the Markov chain model used to describe the transitions between epizootic and endemic conditions, long time spans of outbreak conditions were coupled with long time spans of endemic conditions. Therefore, while populations experienced longer outbreak periods, they also had more time to recover from these outbreaks. With increases in epizootic frequency, however, these recovery periods were shortened. Thus, the long-term growth and persistence of populations under the threat of frequent epizootics was compromised due to reduced recovery periods between outbreaks. In a natural setting, the frequency of outbreak recurrence is likely to increase through the exposure of subclinical or uninfected tortoises to clinically diseased animals that are actively shedding mycoplasma, as might occur through relocation events. At the individual level, normal biological parameters and behaviors may contribute to recrudescence. For example, as males become reproductively active, increased conspecific aggression might lead to both increased stress and increased exposure risks. Time of exposure might also be an inherent risk factor, especially with respect to individual nutritional status or the stage of female reproductive cycle at the time of exposure. Recrudescence may also be triggered by secondary infections or extrinsic stress factors, such as those associated with adverse environmental events (i.e. drought, hurricanes, or habitat degradation). Although mechanisms driving epizootic recurrence frequency are ill-defined for URTD, we found this parameter to have a strong effect on long-term host population dynamics.

We also assessed how population growth rate (λ) was influenced by demographic, disease- and outbreak-associated parameters using a matrix metapopulation modeling framework (Hunter and Caswell 2005, Ozgul et al. 2009b). Through elasticity analyses we found that population dynamics under enzootic conditions (i.e. baseline values of demographic parameters) had the greatest impact on the long-term growth rate of a population. Regardless of the level of disease-induced mortality, outbreak duration, or outbreak recurrence frequency, it was the baseline (or enzootic) condition of an exposed population that was most influential to the long-term population growth rate. Overall, we also found that demographic parameters (i.e. survival, fecundity and growth) were proportionately more influential on λ than disease- and outbreak-associated parameters. The survival of reproductive adults proved to be the most important lower-level parameter to λ, consistent with life history theory and previous analyses of turtle population dynamics (Crouse et al. 1987, Crowder et al. 1994, Doak et al. 1994, Heppell 1998). The relatively low importance of disease-related parameters compared to host demographic parameters may be a consequence of declining growth rates ($\lambda < 1.0$) at baseline conditions, and our assumption that the disease only influenced survival of reproductive females. Disease-related processes could likely have a greater impact on population dynamics in situations where multiple vital rates are negatively influenced by the disease. Among disease- and outbreak-associated parameters, the proportional influence of disease-induced mortality was surpassed by that of force of infection when disease-induced mortality was high ($\mu = 0.30$). Theory suggests that a population threshold exists below which disease transmission would not be sustainable and the disease would ultimately disappear (Anderson 1991). For example, when infectious contacts with susceptible hosts declined, phocine distemper virus faded out from a population of North Sea harbor seals (Swinton et al. 1998). The proportional influence of disease-associated parameters on long-term population growth, therefore, is contingent on disease persistence. In our study, at the threshold point in which more than 20% of clinically infected individuals die from disease, the relative effect of disease-induced mortality on the long-term population growth rate decreased and was surpassed by the force of infection.

Of the Markovian parameters driving outbreak dynamics, epizootic frequency adversely affected long-term population dynamics, whereas epizootic duration had little impact. In other words, the impact of disease depended primarily on how often a population underwent an epizootic state, rather than how long the epizootic persisted within the exposed population. Moreover, although large temporal autocorrelation effects (W_2) were expected for weakly damped populations (Tuljapurkar and Haridas 2006), such as our study population, W_2 remained small relative to the effect of temporal variability (W_1) in our study. The findings from this study, therefore, agree with those reported by Fieberg and Ellner (2001), who argue that stochastic growth rates are generally not substantially affected by the temporal autocorrelation of environmental states.

Evidence for URTD-associated mortality is based primarily on anecdotal evidence for associations between die-off events and seroprevalence and necropsies of moribund animals from populations undergoing disease outbreaks (Jacobson et al. 1995, Berry 1997, Schumacher et al. 1997, Homer et al. 1998, Brown et al. 1999, Seigel et al. 2003), and no direct link has been established between URTD infection and tortoise mortality (Sandmeier et al. 2009).

However, such a link has always been notoriously difficult to confirm in wildlife disease systems (Plowright et al. 2008) and may be particularly challenging when evaluating the effects of chronic disease in long-lived species. Moreover, the specific mechanisms driving URTD recrudescence in latently infected gopher tortoises remain unknown. Given these challenges, our study served as a baseline description of potential outcomes arising from different scenarios of disease dynamics, and also provided a framework for modeling the population-level influence of URTD in a gopher tortoise population.

In many disease systems, the force of infection can be density- or frequency-dependent (Wilcox and Elderd 2003, Lloyd-Smith et al. 2005). Although a previous study found no evidence that population density influenced the force of infection in our study system (Ozgul et al. 2009a, p. 793), we explored this possibility using empirically estimated parameters and functional form of density-dependent effect on the force of infection (Supplementary material Appendix A5). Modeling force of infection as a density-dependent process had no substantial impact on estimates of quasi-extinction parameters. Moreover, theoretical and empirical studies addressing how environmental (e.g. hurricanes, drought) and anthropogenic factors (e.g. unauthorized relocations) can affect disease recrudescence at the individual level would improve our understanding of the overall expected frequency of epizootic recurrence within natural populations. Such analyses could help develop a more refined mechanistic understanding of how recurring diseases can influence the dynamics and persistence of host populations (Altizer et al. 2006).

When little is known regarding the disease ecology of a host wildlife system, modeling exercises like the one presented in this paper can provide a baseline understanding of outcomes arising from potential scenarios of disease dynamics, and suggest future research strategies to help guide management actions. For example, the finding that demographic parameters under enzootic conditions had a higher influence on λ than those under epizootic conditions indicates that strategies aimed at improving demographic parameters, particularly survival rates, would promote the long-term growth and persistence of gopher tortoise populations regardless of disease emergence. Therefore, in most cases, specific management strategies need not be specific to outbreak conditions.

McCoy et al. (2007) surveyed ~ 60 gopher tortoise populations in Florida before the emergence of URTD. They subsequently resampled 10 of these populations, and found that some populations with relatively high percentages of seropositive individuals had not declined, and that some populations with low percentage of seropositive individual had experienced population declines. Because some populations where URTD was absent or occurred in low frequency, experienced population declines, they questioned the causal role of URTD in tortoise population declines. Consistent with these observations, we found that vital rates estimated from the relatively URTD-free populations indicated declining populations, and that there was no evidence to suggest that URTD was the only or the primary cause of gopher tortoise population declines. Loss and degradation of habitat might have played a more significant role in tortoise population declines; consequently, protection and appropriate management of tortoise habitat would be the most effective strategy to manage gopher tortoise populations in either the presence or absence of URTD (McCoy et al. 2006, 2007). Given the relative importance of epizootic frequency on estimates of quasi-extinction parameters, educational programs that inform the public about the risks associated with the unauthorized release of tortoises into established gopher tortoise populations are likely to be effective management actions to mitigate pathogen introductions and spread.

Long-term surveillance will continue to be instrumental in measuring disease impacts on host demographic parameters such as survival and fecundity. In this study, we assumed that only mortality was affected by *M. agassizii* infection, and did not consider the impacts that long-term, chronic morbidity might have on other population parameters. This assumption stemmed mainly from the widespread speculation of URTD as a driving force in documented mortality events (Jacobson et al. 1995, Berry 1997, Schumacher et al. 1997, Homer et al. 1998, Brown et al. 1999, Seigel et al. 2003). In this paper, we focused on modeling the impacts of these speculated die-off events on long-term population dynamics. In fact, most mycoplasmal respiratory infections exhibit a high morbidity, low mortality profile and affect weight gain, ability to survive, and in some cases, reproduction (Simecka et al. 1992, Minion 2002). For instance, based on preliminary analyses that revealed no significant effect of URTD on reproductive parameters (White 2009), we assumed a negligible effect of disease on fecundity. In desert tortoises, females with clinical signs of URTD have shown reduced reproduction, but regained normal reproductive function when provided food and water in captivity or when clinical signs were absent (Rostal et al. 2001). Therefore, although we made the conservative assumption that disease had no effect on fecundity for our heuristic purposes, this assumption may be inaccurate in certain stages of disease outbreak, in different tortoise populations, for different habitats, or for different tortoise species. Although mortality is the most severe consequence of infectious diseases, it is often the most rare event; morbidity with substantive tissue damage is far more common but also more difficult to quantify (Wobeser 2006). For example, the presence of antibody to *M. agassizii* is highly correlated with histological lesions and destruction of the normal respiratory architecture (Jacobson et al. 1991, McLaughlin et al. 2000), but the impacts of this level of tissue damage on critical biological functions are unknown.

Using mycoplasmal URTD within natural gopher tortoise populations as a model wildlife disease system, we demonstrated how population-level impacts of chronic recurring disease outbreaks on host wildlife populations can be evaluated using matrix population models and Markov chain models for temporally autocorrelated environments. The relative ease of implementation and straightforward interpretation of results of matrix population models (Caswell 2001) have resulted in their extensive use for guiding management decisions for wildlife populations. The approach we presented in this paper could be widely applied to a range of wildlife disease systems in which hosts suffer from persistent recurring diseases such as 1) those caused by multi-host pathogens, 2) pathogens that can maintain themselves subclinically within hosts but cause intermittent bouts of recrudescing clinical disease, or 3) pathogens that can persist in the environment and cause re-infection of hosts. Clearly, further empirical

and modeling studies on the long-term impacts of chronic diseases in natural populations are needed, especially in light of the increasing threat of disease in already imperiled host wildlife populations.

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References

Albon, S. D. et al. 1997. The role of parasites in the dynamics of a reindeer population. – Proc. R. Soc. B 269: 1625–1632.

Altizer, S. et al. 2004. Seasonal dynamics of mycoplasmal conjunctivitis in eastern North American house finches. – J. Anim. Ecol. 73: 309–322.

Altizer, S. et al. 2006. Seasonality and the dynamics of infectious diseases. – Ecol. Lett. 9: 467–84.

Anderson, R. M. 1991. Discussion: the Kermack-McKendrick epidemic threshold theorem. – Bull. Math. Biol. 53: 3–32.

Begon, M. et al. 2009. Seasonal host dynamics drive the timing of recurrent epidemics in a wildlife population. – Proc. R. Soc. B 276: 1603–1610.

Berry, K. H. 1997. Demographic consequences of disease in two desert tortoise populations in California, USA. Conservation, restoration and management of tortoises and turtles - an international conference. – N. Y. Turtle and Tortoise Society (< http://nytts.org/proceedings/berry1.htm >).

Boots, M. et al. 2003. The population dynamical implications of covert infections in host–microparasite interactions. – J. Anim. Ecol. 72: 1064–1072.

Brook, B. W. and Kikkawa, J. 1998. Examining threats faced by island birds: a population viability analysis on the Capricorn silvereye using longterm data. – J. Appl. Ecol. 35: 491–503.

Brown, D. R. 2002. Mycoplasmosis and immunity of fish and reptiles. – Front. Biosci. 7: 1338–1346.

Brown, M. B. et al. 1994. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. – Infection Immunity 62: 4580–4586.

Brown, M. B. et al. 1999. Upper respiratory tract disease in the gopher tortoise is caused by *Mycoplasma agassizii*. – J. Clin. Microbiol. 37: 2262–2269.

Casadevall, A. and Pirofski, L. 2002. What is a pathogen? – Ann. Med. 34: 2–4.

Casadevall, A. and Pirofski, L. 2003. Microbial virulence results from the interaction between host and microorganism. – Trends Microbiol. 11: 157–158.

Caswell, H. 2001. Matrix population models. – Sinauer Ass.

Claessen, D. and deRoos, A. M. 1995. Evolution of virulence in a host–pathogen system with local pathogen transmission. – Oikos 74: 401–413.

Cleveland, S. et al. 2002. The role of pathogens in biological conservation. – In: Hudson, P. J. et al. (eds), The ecology of wildlife diseases. Oxford Univ. Press, pp. 139–150.

Cross, P. C. et al. 2009. Disease, predation and demography: assessing the impacts of bovine tuberculosis on African buffalo by monitoring at individual and population levels. – J. App. Ecol. 46: 467–475.

Crouse, D. T. et al. 1987. A stage-based population model for loggerhead sea turtles and implications for conservation. – Ecology 68: 1412–1423.

Crowder, L. B. et al. 1994. Predicting the impact of turtle excluder devices on loggerhead sea turtle populations. – Ecol. Appl. 4: 437–445.

de Castro, F. and Bolker, B. 2005. Mechanisms of disease-induced extinction. – Ecol. Lett. 8: 117–126.

de Kroon, H. et al. 2000. Elasticities: a review of methods and model limitations. – Ecology 81: 607–618.

Doak, D. et al. 1994. Modeling population viability for the desert tortoise in the western Mojave Desert. – Ecol. Appl. 4: 446–460.

Fieberg J. and Ellner, S. P. 2001. Stochastic matrix models for conservation and management: a comparative review of methods. – Ecol. Lett. 4: 244–266.

Gates, C. A. et al. 2002. Characterization of a gopher tortoise mortality event in west-central Florida. – Fla Sci. 65: 159–184.

Gerber, L. R. et al. 2005. Exposing extinction risk analysis to pathogens: is disease just another form of density dependence? – Ecol. Appl. 15: 1402–1414.

Haydon, D. T. et al. 2002. Integrating epidemiology into population viability analysis: managing the risk posed by rabies and canine distemper to the Ethiopian wolf. – Conserv. Biol. 16: 1372–1385.

Heppell, S. S. 1998. Application of life-history theory and population model analysis to turtle conservation. – Copeia 1998: 367–375.

Holder, G. et al. 2007. Gopher tortoise management plan. – Fla Fish Wildlife Conserv. Commission.

Homer, B. L. et al. 1998. Pathology of diseases in wild desert tortoises from California. – J. Wildlife Dis. 34: 508–523.

Hunter, C. M. and Caswell, H. 2005. The use of the vec-permutation matrix in spatial matrix population models. – Ecol. Modell. 188: 15–21.

Jacobson, E. R. et al. 1991. Chronic upper respiratory-tract disease of free-ranging desert tortoises (*Xerobates agassizii*). – J. Wildlife Dis. 27: 296–316.

Jacobson, E. R. et al. 1995. Mycoplasmosis and the desert tortoise (*Gopherus agassizii)* in Las Vegas Valley, Nevada. – Chelonian Conserv. Biol. 1: 281–286.

Lafferty, K. D. and Gerber, L. R. 2002. Good medicine for conservation biology: the intersection of epidemiology and conservation theory. – Conserv. Biol. 16: 593–604.

Lloyd-Smith, J. O. et al. 2005. Should we expect population thresholds for wildlife disease? – Trends Ecol. Evol. 20: 511–519.

May, R. M. 1988. Conservation and disease. – Conserv. Biol. 2: 28–30.

McCoy, E. D. et al. 2006. Declines of the gopher tortoise on protected lands. – Biol. Conserv. 128: 120–127.

McCoy, E. D. et al. 2007. Conservation strategies and emergent diseases: the case of upper respiratory tract disease in the gopher tortoise. – Chelonian Conserv. Biol. 6: 170–176.

McLaughlin, G. S. 1997. Upper respiratory tract disease in gopher tortoises, *Gopherus polyphemus*: pathology, immune responses, transmission and implications for conservation and management. Wildlife Ecology and Conservation. – Univ. of Florida, p. 110.

McLaughlin, G. S. et al. 2000. Pathology of upper respiratory tract disease of gopher tortoises in Florida. – J. Wildlife Dis. 36: 272–283.

Miller, P. S. 2001. Preliminary population viability assessment for the gopher tortoise (*Gopherus polyphemus*) in Florida. PVA Workshop. – Fla Fish Wildlife Conserv. Commission.

Minion, F. C. 2002. Molecular pathogenesis of mycoplasma animal respiratory pathogens. – Front. Biosci. 7: 1410–1422.

Morris, W. F. and Doak, D. 2002. Quantitative conservation biology. – Sinauer Ass.

Mougeot, F. et al. 2006. Elevated spring testosterone increases parasite intensity in male red grouse. – Behav. Ecol. 17: 117–125.

Ostfeld, R. S. et al. 2002. Conservation medicine: the birth of another crisis discipline. – In: Aguirre, A. A. et al. (eds), Conservation medicine: ecological health in practice. Oxford Univ. Press, pp. 17–25.

Ozgul, A. et al. 2009a. Upper respiratory tract disease, force of infection and effects on survival of gopher tortoises. – Ecol. Appl. 19: 786–798.
Ozgul, A. et al. 2009b. Influence of local demography on asymptotic and transient dynamics of a yellow-bellied marmot metapopulation. – Am. Nat. 173: 517–530.
Padgett, D. A. and Glaser, R. 2003. How stress influences the immune response. – Trends Immunol. 24: 444–448.
Pascarella, J. B. and Horvitz, C. C. 1998. Hurricane disturbance and the population dynamics of a tropical understory shrub: megamatrix elasticity analysis. – Ecology 79: 547–563.
Perez-Heydrich, C. 2010. Ecological investigations of mycoplasmal upper respiratory tract disease in natural populations of threatened gopher tortoises: insights from population ecology, mathematical epidemiology, and behavioral ecology. – PhD thesis, Infectious Diseases and Pathology, College of Veterinary Medicine, Univ. of Florida.
Plowright, R. K. et al. 2008. Causal inference in disease ecology: investigating ecological drivers of disease emergence. – Front. Ecol. Environ. 6: 420–429.
Rostal, D. C. et al. 2001. Reproduction and URTD in the desert tortoise (*Gopherus agassizii*): an eight year follow-up. 26th Ann. Meeting Symp. of the Desert Tortoise Council. – (< www.deserttortoise.org/abstract/abstracts2OO1/2001abs34.html >).
Sandmeier, F. C. et al. 2009. Upper respiratory tract disease (URTD) as a threat to desert tortoise populations: a reevaluation. – Biol. Conserv. 142: 1255–1268.
Schumacher, I. M. et al. 1997. Relationship between clinical signs of upper respiratory tract disease and antibodies to *Mycoplasma agassizii* in desert tortoises from Nevada. – J. Wildlife Dis. 33: 261–266.
Scott, M. E. 1988. The impact of infection and disease on animal populations: implications for conservation biology. – Conserv. Biol. 2: 40–56.
Seigel, R. A. et al. 2003. Swine flu or 1918 pandemic? Upper respiratory tract disease and the sudden mortality of gopher tortoises (*Gopherus polyphemus*) on a protected habitat in Florida. – J. Herpetol. 37: 137–144.
Simecka, J. W. et al. 1992. Mycoplasma disease of animals. – In: Maniloff, J. et al. (eds), Mycoplasmas: molecular biology and pathogenesis. Am. Soc. Microbiol., pp. 391–415.
Smith, K. F. et al. 2006. Evidence for the role of infectious disease in species extinction and endangerment. – Conserv. Biol. 20: 1349–1357.
Swinton, J. et al. 1998. Persistence thresholds for phocine distemper virus infection in harbour seal *Phoca vitulina* metapopulations. – J. Anim. Ecol. 67: 54–68.
Swinton, J. et al. 2002. Microparasite transmission and persistence. – In: Hudson, P. J.et al. (eds), The ecology of wildlife diseases. Oxford Univ. Press, pp. 83–101.
Sydenstricker, K. V. et al. 2005. Re-exposure of captive house finches that recovered from *Mycoplasma gallisepticum* infection. – J. Wildlife Dis. 41: 326–333.
Tompkins, D. M. et al. 2002. Parasites and host population dynamics. – In: Hudson, P. J. et al. (eds), The ecology of wildlife diseases. Oxford Univ. Press, pp. 45–62.
Tuljapurkar, S. and Haridas, C. V. 2006. Temporal autocorrelation and stochastic population growth. – Ecol. Lett. 9: 324–334.
Wendland, L. D. 2007. Epidemiology of mycoplasmal upper respiratory tract disease in tortoises infectious diseases and pathology. – PhD thesis, Univ. of Florida, p. 178.
Wendland, L. D. et al. 2010. Social behavior drives the dynamics of respiratory disease in threatened tortoises. – Ecology 91: 1257–1262.
White, C. L. 2009. Gopher tortoise (*Gopherus polyphemus*) habitat selecton, health, and fecundity. – PhD thesis, Infectious diseases and pathology, Univ. of Florida, p. 151.
Wilcox, C. and Elderd, B. 2003. The effect of density-dependent catastrophes on population persistence time. – J. Appl. Ecol. 40: 859–871.
Wobeser, G. A. 2006. Essentials of disease in wild animals. – Blackwell.