DISSERTATION

PATHOGEN PERSISTENCE IN WILDLIFE POPULATIONS: CASE STUDIES OF PLAGUE IN PRAIRIE DOGS AND RABIES IN BATS

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY DYLAN GEORGE ENTITLED, PATHOGEN PERSISTENCE IN WILDLIFE POPULATIONS: CASE STUDIES OF PLAGUE IN PRAIRIE DOGS AND RABIES IN BATS, BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

PATHOGEN PERSISTENCE IN WILDLIFE POPULATIONS: CASE STUDIES OF PLAGUE IN PRAIRIE DOGS AND RABIES IN BATS

Disease ecology focuses, in part, on how pathogens persist within host wildlife populations. For my dissertation my colleagues and I investigated pathogen persistence mechanisms in two host-pathogen systems: *Yersinia pestis* (plague) in prairie dogs and rabies virus in bats.

Plague, caused by the bacterium *Yersinia pestis*, recently spread into the range of black-tailed prairie dogs (*Cynomys ludovicianus*) in North America, and has caused drastic and rapid reduction in local prairie dog populations which have generated a metapopulation dynamic for prairie dogs. We developed a stochastic patch occupancy model to determine if prairie dog populations could persist long-term given the effects of plague. Our model demonstrates that metapopulation dynamics can allow prairie dog persistence. Town extinction in this system is caused by plague. Thus, town extinction and plague colonization are two sides of the same coin, which allows to us to interpret plague dynamics implicit within the prairie dog metapopulation. Long-term metapopulation dynamics indicate plague persists within the system and does not require the involvement of additional reservoir hosts (i.e., other resistant rodent species).

Bats are a natural reservoir for rabies, and an increasing number of emerging zoonotic viruses. Little is known about mechanisms that generate unique seasonal

patterns and allow enzootic pathogen persistence in bat populations. We propose that life history characteristics unique to many bat species coupled with viral adaptations allow for rabies persistence. First, we developed a statistical model to investigate seasonal patterns of rabies cases in bats. Second, we used data from a five-year study of rabies in big brown bats (*Eptesicus fuscus*) to parameterize a dynamic disease model that elucidates pathogen persistence mechanisms. We show rabies persists in two distinct ways, (1) through effects on bat population viability, and (2) through effects on viral persistence within a viable bat population. Mortality rates vary across seasons, and low rates during hibernation allow long-term bat population viability. Within a viable bat population, viral persistence occurs because of a lengthy incubation period, enhanced by the metabolic effects of host torpor. The mechanisms we identify may be operating in a similar manner for other bat-borne diseases.

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CHAPTER 1

PATHOGEN PERSISTENCE IN WILDLIFE POPULATIONS: BASIC CONCEPTS

A fundamental question in disease ecology focuses on how pathogens persist within a host population. This chapter reviews basic concepts of pathogen persistence, and to do so I will refer to well-known analytical and simulation results for a directly transmitted, life-long immunizing pathogen such as measles in human populations. Also, I will briefly highlight how modeling wildlife pathogen systems has contributed to our basic understanding of pathogen persistence. This review will provide a framework from which to introduce complexities that are common in wildlife pathogens that I addressed in my research.

The basics of pathogen persistence typically are reduced to two concepts: threshold theory and fadeout theory (Swinton et al. 2001). Threshold theory consists of determining the basic reproductive number of the infection, the famous R_0 value, which defines whether or not an epidemic can occur within an entirely susceptible population. Fadeout theory focuses post-epidemic and considers whether a pathogen can be maintained in a population given it can invade and cause an epidemic.

To elucidate threshold theory, I will use a simple epidemic model represented by a system of ordinary differential equations (a model where the infection cannot persist in the population without external infectious input and the rate of new cases exceeds expectations of cases). In brief, the model tracks the disease dynamics of different disease classes within a population. Those disease classes include susceptible individuals, S, infectious individuals, I, and recovered or removed individuals, R. Thus, these types of models are typically referred to as SIR models. The model tracks how individuals move among disease classes from S to I to R. I will not go into the mathematics but the equations describe how individuals move among the different classes within a population for a pathogen resembling measles (directly transmitted, short infectious period, life-long immunity).

R_0 is a fundamental concept in population biology (Begon et al. 2006). In disease ecology, R_0 is defined as the average number of secondary infections produced when one infected individual is introduced into an entirely susceptible host population (Heesterbeek 2002). From this definition, we can see that R_0 is what is known as a threshold concept. If a pathogen is not able to infect at least one more host, then fewer and fewer hosts will be infected until the pathogen dies out within the population. Alternatively, if the pathogen can infect more than one host on average, then it will increase in the population. Thus, $R_0 = 1$ is a threshold. For $R_0 > 1$ the pathogen increases in the population, and $R_0 < 1$ the pathogen decreases in the population. This concept can be extended to populations where the entire population is not susceptible. In this case, the R_0 value is referred to as the effective reproductive number, R_{eff}, and is a discounted version of R_0 where R_{eff} equals sR_0, and s is the susceptible fraction of the population (Anderson and May 1991).

We begin by considering a directly transmitted pathogen in a host population that maintains constant size. Following Begon *et al* (2006), R_0 can be determined as a function of (i) how easily the pathogen is transmitted from individual to individual, or rather, the transmission coefficient, β, (ii) how long the infectious individuals remain infectious, L, and (iii) the number of susceptible individuals in the host population, S. Thus,

$$R_0 = S \beta L \qquad (1)$$

Note the direct relationship between S and R_0, such that as S decreases so does R_0.

Given the above definition of R_0, (1), we can translate our understanding of R_0 as a threshold concept to determine the optimal size of the susceptible population in which the pathogen can persist. This is known as the critical community size (CCS). So if, $R_0 = 1$ we can rearrange (1) as follows:

$$S_T = 1 / \beta L \qquad (2)$$

where S_T is the critical community size, or rather, the susceptible population size below which the pathogen cannot persist. Thus, if the population has fewer susceptible individuals than S_T, the pathogen will decline in the population because $R_0 < 1$. Alternatively, if the population has a greater number of susceptible individuals than S_T, the pathogen will increase in the population because $R_0 > 1$. It should be noted that if a pathogen is transmitted independently of the total population size, or rather, frequency dependent transmission occurs, then no population threshold exists (Anderson and May 1991).

This provides us with a means of determining how many individuals we need to vaccinate. If we are able to hold the number of susceptible individuals below S_T, then R_0

< 1 and the pathogen cannot persist. Thus, if the susceptible class within the population remains below S_T, then the entire population is protected from the effects of the pathogen. This is called herd immunity and is the basis of immunization programs.

For further clarity, we can derive an equation to determine the critical proportion of a population that should be removed from susceptible status in a population. If S_T is the threshold number of susceptible individuals and S_0 is the original number of susceptible individuals in a population, then the critical proportion would be:

$$p_c = 1 - S_T / S_0$$

where $S_T = 1 / \beta L$, from (2), and $S_0 = R_0 / \beta L$. So,

$$p_c = 1 - (1 / \beta L) / (R_0 / \beta L)$$

$$p_c = 1 - 1 / R_0 \qquad (3)$$

Again, p_c is the proportion of the population that needs to be removed from the susceptible class to minimize pathogen persistence.

Threshold theory was originally developed to explain concepts in human epidemiology (Anderson and May 1991), yet wildlife models were instrumental in establishing this conceptual framework. In particular, Anderson et al. (1981) developed a theoretical model to explore factors contributing to the persistence of fox rabies. A primary result from this modeling was a threshold density of foxes required to maintain rabies, a critical community size for foxes. This result was compared against the number of foxes killed per square kilometer (0.4 foxes per km^2) to demonstrate theoretically that rabies should be able to persist within European foxes. This well-known study provides a fundamental description of fox rabies persistence and has been the basis for many rabies models for foxes, raccoons, and African wildlife (Lloyd-Smith et al. in review).

Although $R_0 > 1$ is a necessary condition for pathogen persistence, it is not sufficient. For highly infectious pathogens with short infectious periods, we need to consider a subsequent concept of fadeout theory. Fadeout theory considers how a pathogen persists within a population after the initial pathogen introduction. Generally, a fadeout results in pathogen extinction because of random fluctuations in the number of infectious individuals. A fadeout can happen following an epidemic or from an endemic state. Epidemic fadeout occurs when there are few susceptible individuals in the population immediately following an epidemic such that sustained transmission is not possible (Lloyd-Smith et al. 2005). This can happen because random fluctuations, including demographic stochasticity, removes latent and infectious individuals and thereby the pathogen, or by a sustained period where $R_{eff} < 1$ (Anderson and May 1991; Lloyd-Smith et al. 2005). The trough of the epidemic curve brings the number of infectious individuals close to zero and random fluctuations in the population can remove the remaining individuals. The inter-epidemic periods present the greatest opportunity for pathogen fadeout. Alternatively, endemic fadeout occurs when infectious individuals, and the pathogen, are lost from the population randomly because of fluctuations about the equilibrium state of infectious individual (I^*) (Lloyd-Smith et al. 2005). Typically, populations that have a low number of infectious individuals at equilibrium are more prone to endemic fadeout.

Models representing wildlife populations have been useful in demonstrating the utility of fadeout theory. Swinton et al. (1998) used mathematical models and numerical simulations to explore persistence mechanisms in harbor seal populations in the North Atlantic during the 1980s. They calculated the critical community size for harbor seals in

the North Atlantic and determined that given the maximal reproductive success of harbor seals that the seal population would not be sufficiently large to maintain the pathogen. In addition, they found that the pathogen more than likely would not persist because the epidemic ended before sufficient numbers of susceptible seals could be replenished in the annual reproduction period. This study (Swinton et al. 1998) demonstrates a case where an epidemic in a wild population occurred, yet pathogen persistence did not. It provides a good example of epidemic fadeout in a wildlife system.

Threshold and fadeout theory are derived from human epidemiology (Bartlett 1960; Anderson and May 1991) and have proven exceptionally useful in developing fundamental concepts; however, their applicability to wildlife disease has been questioned (Lloyd-Smith et al. 2005), in part, because wildlife pathogens do not always behave like measles in human populations. In addition, early wildlife models did not fully explain pathogen persistence in wildlife. For example, deterministic models of fox rabies did an exceptional job of explaining rabies epidemics and spatially spreading waves of infections (e.g., Anderson et al. 1981; Murray et al. 1986; Murray 1987); however, they did not demonstrate the entire story of how fox rabies persisted between the epidemics (Dye et al. 1995). Because these models were based on systems of ordinary differential equations they produced fractions of foxes during the inter-epidemic periods that are not biologically realistic. Stochastic implementations of SIR models do not allow fractional foxes and thus demonstrate the difficulty of pathogen persistence more accurately; for example a model developed by Voigt et al. (1985) requires rabid fox immigration from other areas for rabies to persistence long-term (Dye et al. 1995).

Therefore, we must consider additional complexities to further understand pathogen persistence in some wildlife systems.

Although simple models are surprisingly robust and can inform baroque biological situations, it is well recognized that other mechanisms can strongly influence the persistence of pathogens (Grenfell and Dobson 1995; Hudson et al. 2001; Lloyd-Smith et al. 2005; Grenfell and Keeling 2007; Keeling and Rohani 2007). Biological complexities that can impact pathogen persistence include host-pathogen evolutionary dynamics, asymptomatic carrier states, reservoirs (alternate host, environmental, or vector), spatial structure, temporal forcing or seasonality, long incubation or latent periods, and host heterogeneities.

In my dissertation research, my colleagues and I were able to consider several of these additional biological complexities. A primary question of Chapter 1, the plague and prairie dog model, was to consider the necessity of alternate host reservoirs for the persistence of plague. In the classical view of plague persistence, the bacterium is maintained at low levels in an enzootic cycle including partially resistant rodent hosts, with occasional spillover to highly susceptible hosts like humans and prairie dogs (Poland and Barnes 1979; Poland et al. 1994; Gage and Kosoy 2005). However, the classical view is controversial because little direct evidence exists to support it (Cully and Williams 2001; Gage and Kosoy 2005; Salkeld and Stapp 2006; Salkeld and Stapp 2008). Using a metapopulation model of prairie dogs, we demonstrated that alternate host reservoirs are not necessary, and, that spatial structure, or rather, metapopulation structure, can allow long-term persistence of prairie dogs. Because plague causes the majority of local extinctions of prairie dog towns, we can also infer that plague persists

long-term as well. Thus, spatial structure in our system facilitates prairie dog and plague persistence. In Chapter 2, we considered causes of seasonality in rabies cases in bats in the United States generally and in Colorado specifically. We asked the question of what factors (life history or environmental) drive bat rabies virus seasonality. We developed a statistical model to compete hypothetical drivers of the seasonality of bat rabies cases, and found that seasonal changes in life history dynamics is the most straightforward explanation given the current data. Lastly, in Chapter 3, we continued investigating the seasonality and persistence mechanisms of bat rabies by developing a mathematical model to represent big brown bat (*Eptesicus fuscus*) biology in northern Colorado. Through sensitivity analysis, we show rabies persists in two distinct ways, (1) through effects on bat population viability, and (2) through effects on viral persistence within a viable bat population. Mortality rates vary across seasons and low rates during hibernation allows long-term bat population viability. Within a viable bat population, viral persistence occurs because of a lengthy incubation period, enhanced by the metabolic effects of host torpor that maintains the pathogen within adult bats until the birth pulse of new susceptibles amplifies the pathogen within the population. The inclusion of key bits of biological complexity allowed for a more complete story of pathogen persistence in these two systems.

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CHAPTER 2

METAPOPULATION DYNAMICS OF PRAIRIE DOGS: IMPLICATIONS FOR PRAIRIE DOG AND PLAGUE PERSISTENCE

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INTRODUCTION

Rapid changes in geographical distributions of pathogens are cause for concern in public health (Lederberg et al. 1992; Morens et al. 2004), domestic animal health (Ferguson et al. 1997; Tildesley et al. 2008), and wildlife conservation (Daszak et al. 2000; Leroy et al. 2004; Pedersen et al. 2007). Highly virulent pathogens, those that cause high mortality and quick death following infection (e.g., *Yersinia pestis*, Marburg virus, Ebola virus), can transform host populations from contiguous spatial structure to metapopulations, with local colonizations and extinctions of interconnected host sub-populations (Barnes 1993; Antolin et al. 2002). Plague, a vector-borne disease caused by the bacterium *Yersinia pestis*, was introduced into western North America in 1899 and spread into the range of the black-tailed prairie dog (*Cynomys ludovicianus*) on the Great Plains by the 1940s. Plague altered the landscape-level population dynamics of prairie dogs because of rapid and high mortality of infected prairie dogs (Hoogland 2006). This effect is illustrated by comparing prairie dog populations on either side of plague's

eastern boundary near the 100th meridian in the central Great Plains, where plague-free prairie dog towns are larger and more contiguous east of the boundary while towns subjected to plague to the west of the line are fragmented and smaller (Antolin et al. 2002). Several studies demonstrate that large towns have a higher probability of going extinct (Cully et al. 2000; Cully and Williams 2001; Stapp et al. 2004; Collinge et al. 2005; Savage 2007; Snall et al. 2008). The only known natural cause of such drastic and rapid reduction in local prairie dog population has been plague events (Barnes 1993). In fact, one would expect in the absence of plague, or unnatural population regulation, the larger the town the less likely it should go extinct. Thus, the presence of plague in this system has substantially altered population dynamics of prairie dogs. As such, plague-affected prairie dog populations function as classic metapopulations (Roach et al. 2001; Antolin et al. 2002; Stapp et al. 2004; Antolin et al. 2006; Snall et al. 2008) defined as groups of subpopulations each with independent population dynamics subject to local extinction and subsequent re-colonization (Hanski and Gaggiotti 2004). Similarly, in the Asian endemic range of plague in Kazakhstan, local extinctions caused by plague result in metapopulation dynamics in great gerbils (*Rhombomys opimus*) (Davis et al. 2007). In Colorado, the plague bacterium becomes undetectable in rodent hosts in times between epizootics (e.g. Salkeld and Stapp 2008). Whether and how plague and prairie dogs maintain long-term metapopulation dynamics remains an open question (Hoogland 2006).

Plague's recent introduction into North America directs attention away from evolutionary mechanisms for persistence (e.g., evolution of attenuated virulence or resistance to plague infection in prairie dogs). Three ecological mechanisms may explain

both sporadic outbreaks and persistence of plague: (1) the classical view of interacting epizootic and enzootic cycles, (2) local extinction of the pathogen with periodic long-distance transmission from different geographical areas, and (3) spatial structure of susceptible hosts facilitating maintenance of plague in a metapopulation setting. In the classical view, the bacterium is maintained at low levels in an enzootic cycle including partially resistant rodent hosts, with occasional spillover to highly susceptible hosts like humans and prairie dogs (Poland and Barnes 1979; Poland et al. 1994; Gage and Kosoy 2005). However, the classical view is controversial because little direct evidence exists to support it (Cully and Williams 2001; Gage and Kosoy 2005; Salkeld and Stapp 2006; Salkeld and Stapp 2008). The second mechanism, large-scale geographic spread from regions with partially resistant hosts, also seems unlikely given recent analyses of plague isolates from Colorado showing distinct genotypes localized to particular geographical areas, including those inhabited by prairie dogs (Lowell 2007). Here we focus on the third mechanism, that metapopulation dynamics of prairie dogs facilitate the persistence of plague without dependence on a partially resistant or enzootic host reservoir.

It is well known that metapopulation dynamics like those exhibited by prairie dogs can facilitate persistence of antagonistic interactions among species. Classic experimental work demonstrated that spatial structure can lead to persistent predator-prey dynamics by generating stable prey populations via spatial refuges that ultimately sustain the predator (Huffaker 1958). Numerous theoretical studies demonstrate the influence of host spatial structure, including metapopulations, on pathogen persistence (e.g., Anderson and May 1992; Hess 1996; Keeling and Gilligan 2000b; Keeling and Gilligan 2000a; Hess et al. 2001; Gog et al. 2002; McCallum and Dobson 2002; Keeling et al. 2004;

Keeling and Rohani 2008). Specifically, Hess (1996) suggested persistence of moderately virulent pathogens within host metapopulations can occur because patch colonization compensates for patch extinction and creates spatial refuges for hosts while maintaining sufficient pathogen transmission. The Hess (1996) model focuses on one host and one pathogen and thereby requires that hosts transmit their pathogens across the landscape. Similarly, a model by Keeling and Gilligan (2000a, 2000b) for plague persistence in black rats depends upon temporary enzootic persistence within spatially structured sub-populations because pathogen dispersal is coupled to host dispersal. Both McCallum and Dobson (2002) and Gog et al. (2002) extended Hess' results to include spillover of virulent pathogens from reservoir species which they argue is more common for wildlife systems. By incorporating reservoir species these two models decouple host dispersal from pathogen transmission across the landscape.

The specifics of the plague–prairie dog system deviate substantially from assumptions of previous theoretical work, and, therefore, may indicate a fundamentally different mechanism operates in prairie dog metapopulations. In contrast to moderately virulent pathogens (i.e., Hess 1996), plague causes nearly 100% mortality within infected prairie dog towns, but enzootic persistence within sub-populations of prairie dogs has not been observed. Thus, the required balance among local host extinction, colonization, and pathogen transmission among subpopulations described by these models is unlikely to exist in the Colorado prairie dog system. In particular, these models are unlikely to explain how pathogen transmission could occur in the face of the rapid extinction of host towns. We also lack conclusive evidence for multi-host dynamics in the prairie dog

system (Gage and Kosoy 2005) as required for the reservoir species models described by McCallum and Dobson (2002) and Gog et al. (2002).

Here we apply a stochastic patch occupancy model (SPOM) (Hanski 1994; Moilanen 1999; Hanski and Gaggiotti 2004; Moilanen 2004) to the prairie dog metapopulation. Our aim is to determine if and how prairie dog metapopulations as parameterized by the field data can generate persistent prairie dog populations for a long period of time. Subsequently, because plague is the primary cause of local prairie dog town extinctions we will be able to make inferences regarding plague movement on the landscape. In this approach, colonization of prairie dog towns is determined by prairie dog dispersal and conforms to traditional models of metapopulation dynamics (e.g., Hanski 1994). On the other hand, prairie dog town extinction is a function of among-town plague transmission, and our models test alternative hypotheses regarding prairie dog extinction, and hence, plague transmission at the landscape level. The probability of prairie dog town extinction may depend on the pathogen being transmitted widely about the landscape by unstructured spread. Extinction probabilities are typically expressed as a function of population size in metapopulation modeling (Hanski 1994; Moilanen 1999), and recent modeling work has successfully captured this general relationship for different prairie dog systems (Cully et al. 2000; Cully and Williams 2001; Roach et al. 2001; Stapp et al. 2004; Collinge et al. 2005; Wagner et al. 2006; Savage 2007; Snall et al. 2008). Alternatively, if prairie dogs themselves transmit plague to uninfected towns from infected towns, then extinction probabilities should be a function of connectivity to plague-infected towns. In this case, the metapopulation network would structure plague

spread. Comparing these hypotheses allows for inference of mechanisms of plague persistence.

We estimated model parameters for each hypothesis using twenty years of occupancy data of prairie dog towns in northern Colorado, compared our alternative hypotheses through model selection techniques, and inferred mechanisms of plague and prairie dog persistence based on the selected model. Finally, we validated the predictive ability of our selected model using an additional five years of occupancy data and projected our validated model into the future. Our modeling approach can be generally adapted to other spatially and temporally discrete disease systems.

METHODS

Study system

Prairie dogs are highly social ground dwelling rodents that form spatially discrete matrilineal social groups (coteries of 4-6 adult females, 1-2 adult males) in clustered burrows (Koford 1958; Roach et al. 2001). In black-tailed prairie dogs, plague causes mortality approaching 100%, spreads rapidly within towns during epizootics, and usually results in the extinction of entire towns within six to eight weeks (Barnes 1993; Cully and Williams 2001; Pauli et al. 2006; Webb et al. 2006). The highly social and colonial nature of prairie dogs likely facilitates mortality from plague as a result of individual contact and shared burrows (Hoogland 1995).

Our study site is located in short-grass steppe habitat on the Pawnee National Grassland and Central Plains Experimental Range in northern Colorado (Fig. 2.1). The site, administered by the United States Forest Service (USFS) and Agricultural Research

Service, represents approximately 80,000 ha of publicly owned land embedded within a checkerboard of lands under federal, state, and private ownership. It is divided into eastern and western units (Pawnee and Crow Valley, respectively) comprising different drainage systems separated by a 16-km wide strip of private land. The western unit has a greater proportion of contiguous federal grassland (46%) than the eastern unit (18%).

Prairie dog surveys and data: Area and occupancy of 79 prairie dog towns was determined yearly from 1981-2005 by the USFS and researchers with the Short-grass Steppe Long-term Ecological Research project. We divided the study area into two units (eastern Pawnee unit and western Crow Valley) that we refer to as the eastern and western metapopulations (38 and 41 towns respectively). Separation into two metapopulations is justified because *(i)* the units are separated by 16 km strip of private land, *(ii)* they are in two distinct drainages and previous analyses suggest prairie dogs disperse along drainages (Roach et al. 2001), *(iii)* there is good evidence for dispersal among towns within each metapopulation (Roach et al. 2001; Stapp et al. 2004), and *(iv)* genetic analyses (Lowell 2007) indicate that distinct *Y. pestis* genotypes circulate independently within the eastern and western PNG. Survey protocols and data issues have been detailed previously (Savage 2007).

Town Extinctions: Plague is the only known disease to cause town extinctions over a few months (Barnes 1993). The other main cause of town die-offs in the study area, poisoning, was officially stopped in the 1970s. Thus, towns were categorized as having gone extinct from plague in the first year a town was inactive after being previously mapped and active for one or more years. We are confident that large town extinctions were caused by plague. Since 2003 we have monitored towns before, during

and after plague epizootics, collected infect fleas by burrow swabs, and confirmed the presence of plague bacterium from fleas or dead prairie dogs on ten of the twelve towns experiencing extinction from 2003 to 2005 (courtesy of the Centers for Disease Control, Fort Collins, CO). We lack direct bacteriologic or serologic evidence of plague for extinctions prior to 2003, so a few extinctions of small towns could result from factors other than plague such as unauthorized poisoning, predation, or demographic stochasticity (Stapp et al. 2004). In short, the majority of town extinctions are attributable to plague events based on recent fieldwork and how plague decimates prairie dog towns within a few months after becoming infected.

Model

We modeled prairie dog population dynamics using a stochastic patch occupancy model (SPOM) (Hanski 1994; Moilanen 1999; Hanski and Gaggiotti 2004; Moilanen 2004). Use of discrete-time SPOM with an annual time step is justified because prairie dogs have a single birth pulse and discrete dispersal period each year (Hoogland 1995) and because town extinction occurs relatively quickly compared to the annual time step. Further, prairie dogs live in discrete patches (towns) that are either occupied or are re-colonized after local extinctions (Hoogland 1995). The discrete nature of the data readily allows parameter estimation and can be modified to reflect natural history of the plague-prairie dog system, including long-term dynamics. Major assumptions include that (1) all towns are available every year, (2) there is no creation or loss of towns, and (3) maximum town area is known and fixed.

A previous model of plague in prairie dogs (Snall et al. 2008) assumed an underlying metapopulation model with town area effects (unstructured extinction) where the metapopulation dynamic operates independently of plague. Our approach differs from this model because we assume that plague drives the underlying metapopulation dynamics, which is appropriate at least for our system, and because we test alternative hypotheses regarding town extinction and plague spread in this system.

Candidate model set and model selection

The SPOM approach depends upon estimating probabilities of colonization and extinction for each prairie dog town at each time step for each hypothesis. By characterizing town extinctions (Table 1), we compared the effects of plague transmission at the landscape level on spatial refuges for the hosts and plague persistence. Using these functional forms for colonization and extinction, we created likelihood functions, estimated parameters, and selected the most appropriate model given the data using Akaike Information Criteria (AIC, Moilanen 2000; Burnham and Anderson 2002).

The models include expressions for connectivity of towns to surrounding towns, colonization probability, and extinction probability for the i^{th} patch in the t^{th} time-step. Colonization probability was modeled similarly in all cases as an increasing function of connectivity to extant prairie dog towns.

Connectivity: Following Moilanen (1999), connectivity for the i^{th} patch at the t^{th} time is:

$$S(i,t) = \sum_j A_j \exp(-\alpha d_{ij}) p_j(t) \qquad (1)$$

where $i \neq j$, A_j is the maximum area of the j^{th} patch over the entire 25 years of the data set, $p_j(t)$ is the occupancy of the j^{th} patch at time t ($p_j(t)$ =1 if occupied and $p_j(t)$ =0 if unoccupied), $exp(-\alpha d_{ij})$ is the dispersal kernel, and d_{ij} is the drainage distance between the i^{th} and j^{th} patches calculated along contours of their respective watershed. Further, α can be interpreted as the inverse of the mean dispersal distance of the prairie dogs. The use of maximum area introduces some error into the model because there is a period of time when the area of a town grows following colonization. However, this error is unlikely to be large given that extinction happens over the course of weeks and populations experiencing multiple colonization events demonstrate exponential growth after re-colonization (unpublished results) suggesting that most of the time prairie dog subpopulations are close to zero or their maximum area.

Colonization probability: We characterized colonization probability as an increasing function of connectivity and incorporated an Allee effect within the form of this function as is appropriate for sexually reproducing species (Hanski 1994):

$$C(i,t) = f[S\ (i,t)] = \frac{S\ (i,t)^2}{S\ (i,t)^2 + y^2} \qquad (2)$$

where y is a parameter that reflects the colonizing ability of prairie dogs (higher y values indicate higher ability).

Extinction probability: We constructed unique equations to represent our hypotheses for prairie dog extinction probabilities and associated plague transmission.

(1) Unstructured extinction: Motivated by observed correlations between prairie dog town area and the probability of town extinction (Cully et al. 2000; Cully and Williams 2001; Roach et al. 2001; Stapp et al. 2004; Collinge et al. 2005; Wagner et al. 2006; Savage 2007; Snall et al. 2008), we used the maximum area of the prairie dog town

as a proxy for prairie dog population size (Hanski 1994; Moilanen 1999). As is typical in SPOM, the extinction probability was represented as a decreasing function of prairie dog town area:

$$E(i,t) = f[A(i)] = 1 - \exp\left[\frac{-e}{A(i)^x}\right] \quad (3)$$

where e is a constant and can be thought of as the plague colonization coefficient, and x scales extinction risk with town area, describing how quickly the probability of extinction decreases with increasing area (Hanski 1994). According to Hanski (1994), $x = 1$ is a threshold where $x > 1$ means extinction is unlikely for a wide range of town areas, but for $x < 1$ towns of all areas have some probability of extinction.

(2) Structured extinction: For this hypothesis, connectivity to recently plagued towns could explain extinction of prairie dog towns if infectious prairie dogs transmit plague by dispersing across the landscape. We used two different forms of extinction as a function of connectivity to plagued towns in order to avoid discriminating hypotheses during model selection with AIC solely on the basis of the number of parameters. The two functional forms were:

$$E(i,t) = f[S(i,t)] = 1 - \exp[-e_2 S_p(i,t)] \quad (4)$$

$$E(i,t) = f[S(i,t)] = \frac{S_p(i,t)^z}{S_p(i,t)^z + e_2^z} \quad (5)$$

where e_2 is the extinction parameter for prairie dog towns (or the transmission parameter for plague) and z is a parameter controlling the shape of the curve describing the probability of extinction (a parameter describing the transmission process for plague). It is not clear a priori that we can assume any particular value for z for extinction functions,

unlike the y parameter in the prairie dog colonization function. Thus, z was included as a parameter for the probability of extinction in (5).

Also, $S_p(i,t)$ is connectivity to recently plagued towns and is characterized as follows:

$$S_p(i,t) = \sum_j A_{pj} \exp(-\varepsilon d_{ij}) \pi_{pj}(t) \qquad (6)$$

where $i \neq j$, A_{pj} is the maximum area of the j^{th} plagued patch, $\pi_{pj}(t)$ is the occupancy of the j^{th} plagued patch at time $t-1$, and the dispersal kernel is the same form as that for the colonization function above but with ε as the parameter associated with mean transmission distance of plague.

Parameter estimation

We estimated model parameters separately for each metapopulation using the first twenty years of data, from 1981-2000, with the subsequent five years of data reserved for validation. We used a maximum likelihood approach (Moilanen 1999), coded in Matlab, to construct likelihoods of observed occupancy patterns

$$P[\boldsymbol{O(t+1)} \mid \boldsymbol{O(t)}] \times \ldots \times P[\boldsymbol{O(t+M)} \mid \boldsymbol{O(t+M-1)}] \qquad (7)$$

where $\boldsymbol{O(t)} = [O_i(t)]$ is the observed occupancy pattern at time t, and M is the number of years (20). Given the Markov condition and no missing data, each transition, $P[\boldsymbol{O(t+1)} \mid \boldsymbol{O(t)}]$, can be calculated independently as the following product:

$$P[O(t+1) \mid O(t)]$$

$$= \prod_{i=1}^{n} \begin{bmatrix} C(i,t) & \text{when} & O(i,t)=0 \text{ and } O(i,t+1)=1 \\ 1-C(i,t) & \text{when} & O(i,t)=0 \text{ and } O(i,t+1)=0 \\ E(i,t) & \text{when} & O(i,t)=1 \text{ and } O(i,t+1)=0 \\ 1-E(i,t) & \text{when} & O(i,t)=1 \text{ and } O(i,t+1)=1 \end{bmatrix} \quad (8)$$

where n is the number of towns in the metapopulation, $C(i,t)$ is the colonization probability, $E(i,t)$ is the extinction probability, and $O(i,t)$ is the occupancy of the i^{th} patch at the t^{th} time.

This maximum likelihood method can account for both quasi-equilibrium and non-equilibrium dynamics, including metapopulations that are growing, declining, or maintaining steady-state numbers of occupied towns. For the western metapopulation, we made a non-equilibrium assumption (Ovaskainen and Hanski 2001; Moilanen 2004) because of an increasing trend in the proportion of prairie dog towns occupied, likely resulting from cessation of prairie dog poisoning by the USFS in the 1970s (Fig. 2.2B). For the eastern metapopulation, we estimated parameters under both quasi-equilibrium and non-equilibrium assumptions because the occupancy pattern was inconsistent. Estimating parameters under the quasi-equilibrium assumption required using Monte Carlo approximation (for details see Moilanen 1999) to estimate the initial probability, $P[\boldsymbol{O(t_0)}]$. We estimated the parameters α, y, e, z, e_2, ε and x. We did not have reliable, independent estimates of dispersal distances for our specific system, necessitating estimation of α. In order to increase the opportunity for convergence we used non-linear regression (NLR), similar to that used by Hanski (1994) to determine initial parameter values from arbitrary parameter values. Subsequently, these NLR estimates were input as initial conditions for calculating final maximum likelihood estimates using (7). After

estimating our parameters for each of the models, we performed model selection using AIC.

Model simulation, validation and prediction

We simulated data from our selected model in two ways. First, we took a step-by-step approach and progressively predicted occupancy patterns at time $t+1$ from the observed occupancy patterns at time t. Second, we simulated the system over the entire 25-year data set by predicting occupancy patterns at time $t+1$ from the predicted occupancy pattern at each time t. We call this second type a full simulation.

Although AIC selects the best model among the set available, it does not quantify predictive ability of the selected model. We validated predictive ability of our selected model and the estimated parameter values as follows: *(i)* we compared parameter estimates to other independent estimates and biologically relevant values for our system, (*ii*) we evaluated the appropriateness of our model structure and parameter values by comparing the predictions of our step-by-step simulation to the last five years of observed field data, and *(iii)* we evaluated the model's predictive ability by comparing the predictions of our full simulations based on an initial condition to the last five years of observed field data.

Step-by-step simulations were used to validate the model structure for $C(i,t)$ and $E(i,t)$ by looking for inconsistencies between observed and simulated data over the $t+1$ time steps, in this case by comparing the overlap between empirical data and confidence intervals estimated from the simulations. In addition, we assessed consistency of model predictions from each year of the validation data (years 21-25) by receiver operator

characteristic (ROC) and area under the curve (AUC) statistics, a standard means of assessing models with binary responses (Hosmer and Lemeshow 2000).

We further validated the predictive ability of our model using the full simulations. Like the step-by-step simulations, we characterized variation of simulated predictions by confidence intervals, AUC statistics. For the full simulation we additionally used regression analysis of the model residuals using temporal covariates. To assess how well model predictions matched the observed data given metapopulation persistence, we removed simulated metapopulations that went extinct (Allen 2003). We also ran full simulations an additional 75 years in order to predict long-term average proportion of occupied towns. We evaluated the effect of initial conditions and the impact of unexplained stochastic factors on long-term predictions by conducting a sensitivity analysis comparing mean proportion of towns occupied to initial connectivity. For analyses of sensitivity to initial connectivity, we included all simulations regardless of metapopulation persistence. We used connectivity averaged across all towns for each time step of the first twenty years of the observed data as our initial mean connectivity and simulated the system 100 times to generate a quasi-equilibrium of the proportion of occupied towns. We averaged across the simulation runs to calculate a mean proportion of occupied patches for the respective initial mean connectivity.

RESULTS

Unstructured extinction (Model 1) best described the dynamics of the two metapopulations, based on AIC values and the highest Akaike weight (Table 1). Estimated parameters for the unstructured extinction model under the non-equilibrium assumptions are in Table 2. Parameter estimates for the eastern metapopulation under both equilibrium and non-equilibrium assumptions did not appreciably differ and produced quantitatively similar simulation results. Comparing parameter estimates to other studies partially validates that the unstructured extinction model captures the system. First, average dispersal distance of prairie dogs in our models ($1/\alpha$) are approximately 0.005 to 0.1 km per year (Table 2), which are within the maximum observed dispersal distance of an individual of 7 km per month (Garrett and Franklin 1988; Hoogland 1995). Second, our estimates of how town extinction scales with area, x, in both metapopulations (0.586 for eastern and 0.497 for western) indicate that large towns are prone to extinction. Estimates of $x < 1$ traditionally demonstrate that no patch area is impervious to extinction (Hanski 1994) or rather; large patches have a significant probability of extinction. Large towns have been found to have a substantial probability of extinction in other analyses of these metapopulations (Stapp et al. 2004; Savage 2007; Snall et al. 2008) and probability of infection in other analyses of black-tailed prairie dogs (Snall et al. 2008).

For both types of simulations (step-by-step and full) the empirical data fall within 95% confidence intervals of the output from simulation (Fig. 2.2) and provide compelling evidence that the empirical data could be considered a realization of the model simulation. AUC statistics range from 0.5 to 1.0 where a value of 0.5 indicates accuracy no better than chance and 1.0 for complete accuracy. As a general rule of thumb, models

with AUC values above 0.7 adequately predict binary response variables (Hosmer and Lemeshow 2000). AUC statistics for this model (Fig. 2.3) further demonstrate that the model adequately predicts the 2001-2005 observations for both simulation types (step-by-step and full), except for one year in the eastern metapopulation where the AUC values were below 0.7. Also, temporal trends were captured well by the model, since regressions of model residuals on time (time and time squared) demonstrated no significant pattern (western metapopulation: $R^2 = 0.051$, $F_{2,22} = 0.59$, p-value = 0.56; and eastern metapopulation: $R^2 = 0.14$, $F_{2,22} = 1.83$, p-value = 0.19). Lastly, initial conditions of simulations did not affect the predicted long-term average proportion of occupied patches (western: $F_{1,18} = 0.2116$, p-value=0.651, $R^2=0.001162$; eastern: $F_{1,18} = 0.04581$, p-value=0.833, $R^2=0.002538$) demonstrating our simulations generated robust results (Fig. 2.4).

CONCLUSIONS

We demonstrated that prairie dogs can persist in metapopulations where plague outbreaks drive local extinctions. Our results support the idea that plague persists in prairie dog populations because of host metapopulation structure rather than spillover events from partially resistant reservoir hosts. Some host species may be predisposed to long-term viability in metapopulations because of sociality like that seen in prairie dogs, which enforce a modular spatial structure (coteries within towns, towns abutting other towns) even when they are contiguously distributed across the landscape.

We explicitly tested alternative hypotheses about town extinction, and hence how plague is transmitted across the landscape, by relating the probability of extinction to

maximum town size (unstructured extinction) and connectivity to towns that had recently experienced plague outbreaks (structured extinction). The model with unstructured extinction had the most support based on model selection, but did not exclude prairie dogs as agents of plague transmission around the landscape. However, the structured extinction hypothesis assumes that plague spread is coupled with prairie dog colonization. Because the models accurately capture prairie dog colonization as a function of connectivity, rejection of the structured extinction models also argues against a significant role for prairie dog movement in plague spread. The lack of evidence for spillover hosts (Gage and Kosoy 2005; Salkeld and Stapp 2008) that maintain infections suggests that the agents spreading plague are not alternative, partially resistant hosts. Because plague is a vector-borne disease, species that move plague at the landscape level need not transport bacteria internally through infection (i.e., are not hosts) but rather could provide maintenance feeding for the flea vectors and transport bacteria externally via infected fleas. Plague-resistant carnivores (e.g., coyotes *Canis latrans* and swift fox *Vulpes velox*) and rodents (e.g., the northern grasshopper mouse, *Onychomys lecogaster*) are possible candidates that share flea species with prairie dogs and could move infected fleas among towns (Gage et al. 1994; Harrison et al. 2003; Salkeld and Stapp 2006; Salkeld et al. 2007). Thus, our findings suggest that, within metapopulations, highly virulent pathogens can persist within vectors transported on alternative hosts. By contrasting this study with previous work on multi-host dynamics, we suggest that pathogen reservoirs in general allow sufficient decoupling of host extinction and pathogen movement in a metapopulation context. Other such reservoirs could include alternative hosts (Cleaveland and Dye 1995; Gage and Kosoy 2005), alternative means of

vector dispersal for vector borne pathogens (Clement et al. 1986; Hendrickx et al. 2008), environmental reservoirs (Colwell 1996; Miller et al. 2004) and temporal refuges (Hosseini et al. 2004). The degree of virulence and decoupling of host dispersal that allows host-pathogen persistence generally merits further investigation.

Regardless of which species are involved in among-patch pathogen spread, we can characterize how plague moves across the landscape by considering underlying causes for the negative relationship between probability of extinction and maximum town area found in our model (Fig. 2.A1). We simulated plague transmission among prairie dog towns in three ways, using sampling procedures where towns were selected with probabilities that were: 1) equal for all towns, 2) proportional to their maximum area, or 3) proportional to their mean connectivity over time. We plotted how frequently towns were selected as a function of maximum area, given each method of assigning probabilities to towns (Fig. 2.A2). Comparing these histograms (Fig. 2.A2) to the histogram used to fit the extinction functions (Fig. 2.A1) demonstrates plague transmits to prairie dogs towns with equal probability (Fig. 2.A1 A & Fig. 2.A2 A). Two ways we simulated transmission, selection based on equal probabilities and mean connectivity, resulted in negative relationships between the frequency of selection (or probability of extinction) and maximum area (Fig. 2.A2 A, E). This occurs because small towns are most common in our data set. Small towns also show a large range of connectivity (Fig. 2.A2 F), and the negative relationship between frequency of extinction based on connectivity and maximum town area is thus an artifact of small towns occurring more often. This exercise suggests that at the landscape level, plague encounters towns of different sizes in proportion to their occurrence. Even small towns appear to be large

enough to be detected by animals moving vectors around the landscape (eastern metapopulation: minimum maximal area = 0.07 ha, mean maximal area = 32.7 ha; western metapopulation: minimum maximal area = 0.06 ha, mean maximal area = 35.0 ha). The relationship between the probability of extinction and town area varies in the literature (Cully et al. 2000; Cully and Williams 2001; Roach et al. 2001; Stapp et al. 2004; Collinge et al. 2005; Wagner et al. 2006; Savage 2007; Snall et al. 2008), and this exercise suggests that town sizes most prone to extinction are simply those that occur most commonly at particular sites.

Climate effects have been linked to extinction events in several previous studies (Stapp et al. 2004; Stenseth et al. 2006; Savage 2007; Snall et al. 2008), but the metapopulation framework we used in this study does not easily lend itself to incorporating sophisticated climate effects. Interestingly, the strong predictive ability of our selected models shows that the main trends in the proportion of towns occupied can be captured without incorporating climate effects. Undoubtedly, some of the variability in our model is due to climate effects, and further investigation into the impact of climate on landscape level plague patterns is warranted.

Understanding plague persistence and host metapopulation dynamics also has practical implications for prairie dog conservation biology. Prairie dogs are a species of conservation concern because they play a major role in ecosystems of the Great Plains and Intermountain West and are a main prey item of the endangered black-footed ferret (Kotliar et al. 1999; Antolin et al. 2002; Kotliar et al. 2006). Current prairie dog abundance has declined significantly within their historical range due to habitat loss from land conversion (e.g., grassland to agriculture), recreational shooting, intentional

poisoning, and plague. While several of these threats can and have been reduced on public land (e.g., poisoning), the threat of plague cannot be easily controlled, and it was thought there was the potential for extinction from plague. The United States Fish and Wildlife Service recently denied protective listing of black-tailed prairie dogs through the Endangered Species Act, in part, because they reasoned that smaller, isolated towns could provide some protection from plague (Manes 2006). Little or no evidence has been presented to support their assertion. Using this metapopulation model, we were able to project metapopulation dynamics into the future and partially address the controversial denial of protective listing for the black-tailed prairie dog by the USFW (Manes 2006). Our projections showed persistence over a 100 year time frame relevant to conservation biology and a quasi-equilibrium where approximately 20 to 60 percent (depending on the specifics of the site) of available towns could be occupied by prairie dogs (Fig. 2.2 C, D) given the effects of plague. These results demonstrate that smaller towns do not provide conservation protection in and of themselves because in our model both large and small towns have significant extinction risk due to plague (i.e., $x > 1$). It is the metapopulation context, where colonization is high enough to balance local extinction, which allows persistence of prairie dogs in the face of plague.

Most generally the plague-prairie dog system contributes to our overall understanding of pathogen persistence. Previous theoretical work has shown that host metapopulation structure can contribute to the persistence of moderately virulent pathogens (Hess 1996) or to the persistence of virulent pathogens in a multi-host context (Hess 1996; Gog et al. 2002; McCallum and Dobson 2002). In the plague-prairie dog system, we see that host metapopulation structure can lead to persistence of a highly

virulent pathogen within a single host system. However, alternative vector hosts may be a crucial element because it is unlikely that prairie dogs are predominantly responsible for plague transmission across the landscape. This mechanism highlights the importance of understanding vector dynamics when considering pathogen persistence.

Appendix 2.A: Fit of probability of extinction to empirical data and investigation of plague movement

See figure 2.A1 and 2.A2 with their corresponding legend

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Table 2.1. The candidate model set and results of model selection using AIC.

Model	# of parameters	Western metapopulation			Eastern metapopulation		
		AIC	Δ_i	w_i	AIC	Δ_i	w_i
Model 1: Unstructured plague spread $C(i,t)=\frac{S(i,t)^2}{S(i,t)^2+y^2}$ $E(i,t)=1-\exp\left[\frac{-e}{A(i)^x}\right]$	4	602.6	0	0.9999	599.7	0	1.0000
Model 2: Structured plague spread A $C(i,t)=\frac{S(i,t)^2}{S(i,t)^2+y^2}$ $E(i,t)=1-\exp[-e_2 S_p(i,t)]$	4	622.3	19.7	0.0001	846.3	246.6	0.0000
Model 3: Structured plague spread B $C(i,t)=\frac{S(i,t)^2}{S(i,t)^2+y^2}$ $E(i,t)=\frac{S_p(i,t)^z}{S_p(i,t)^z+e_2^{\,z}}$	5	624.9	22.3	0.0000	624.3	24.6	0.0000

Table 2.2. Parameter estimates for the selected model (unstructured plague) with non-equilibrium assumption.

Parameter	Western metapopulation	Eastern metapopulation
α	0.00677 m^{-1}	0.1743 m^{-1}
$1/\alpha$, mean dispersal distance	147.71 m	5.74 m
y, colonization coefficient	1288.423	105.591
e, extinction coefficient	0.445	1.214
x, area scaling coefficient	0.497	0.586

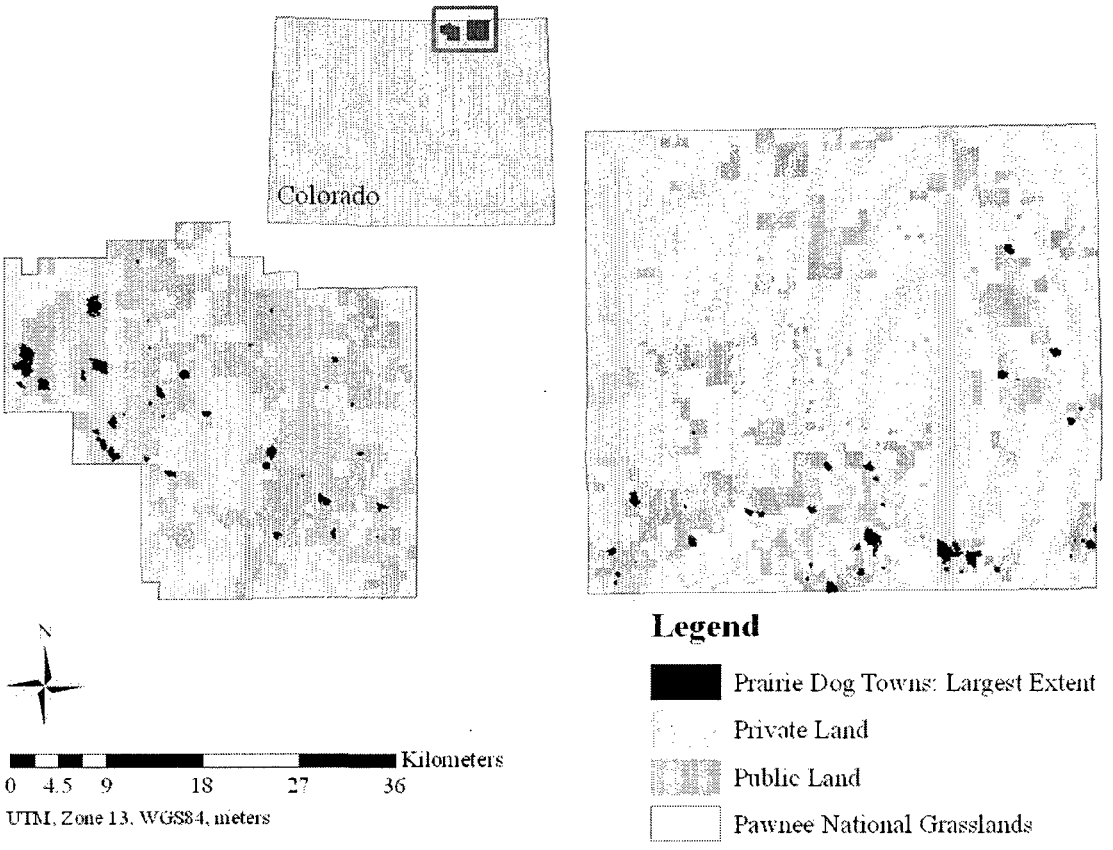


Figure 2.1. Metapopulation structure of prairie dog towns as demonstrated by the maximum area of towns on the Pawnee National Grassland and Central Plains Experimental Range in northern Colorado from 1981-2005.

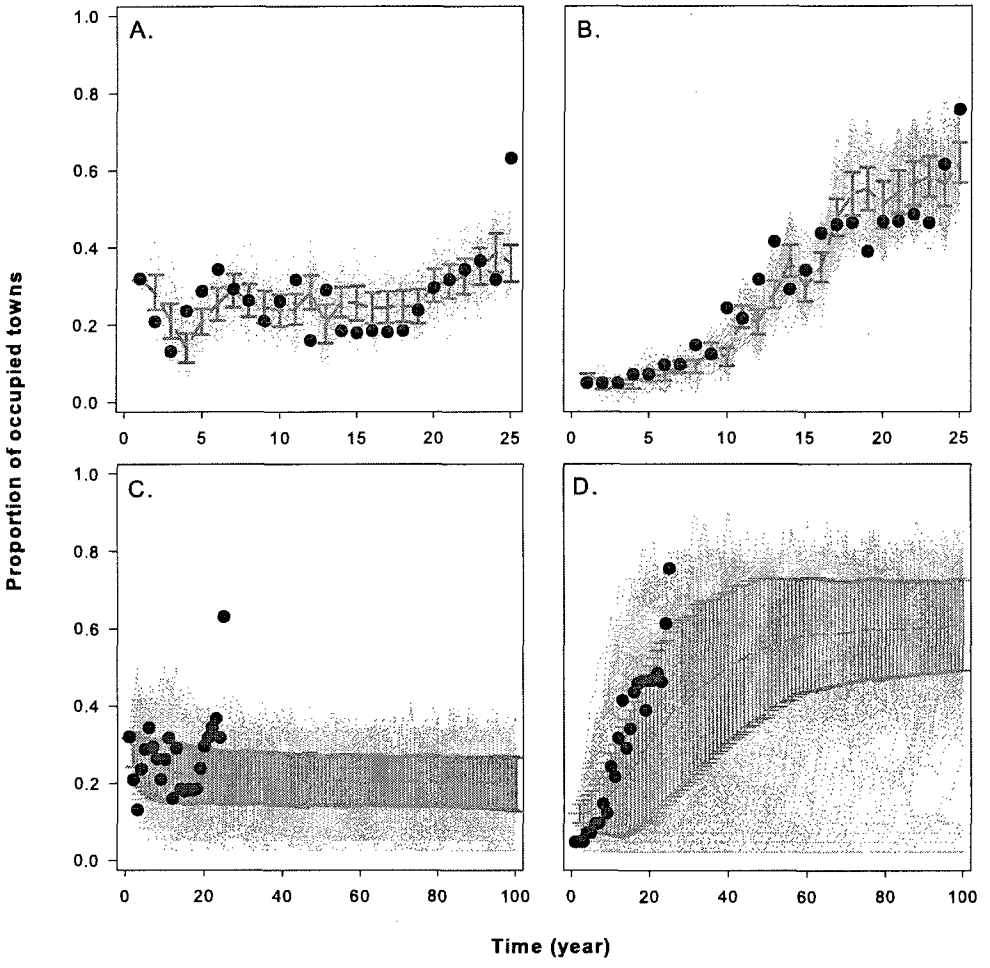


Figure 2.2. The proportion of occupied towns through time for each metapopulation (eastern and western) in northern Colorado. Light gray dotted lines represent output from simulation runs. Dark gray lines represent the mean of simulation runs with 95% confidence intervals. Black dots represent the empirical data. A. western metapopulation, step-by-step simulation. B. eastern metapopulation, step-by-step simulation. C. western metapopulation, full simulation. D. eastern metapopulation, full simulation.

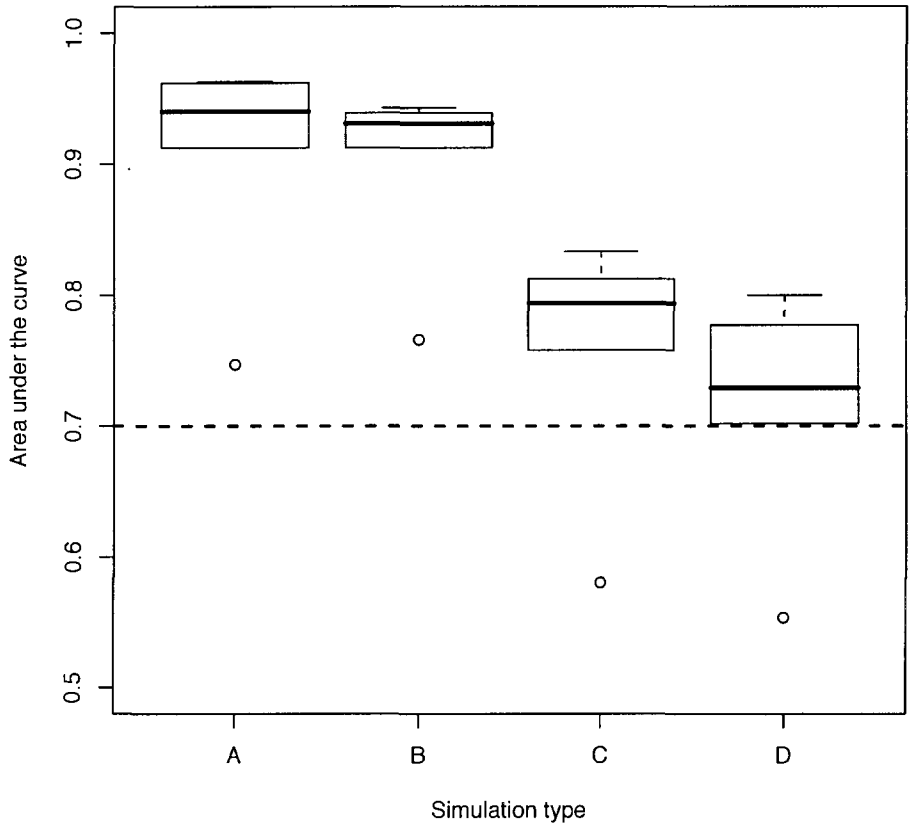


Figure 2.3. Dashed line shows the threshold value for adequate discrimination for area under the curve (AUC) values. Box and whisker plots (average is represented by the dark line, boxes represent first and third quartiles of the data, whiskers and open symbols represent the range of values) demonstrate the distribution of AUC values for each of the five years of independent empirical data used for model validation of each metapopulation and simulation type: A. western, step-by-step; B. western, full; C. eastern, step-by-step; D. eastern, full.

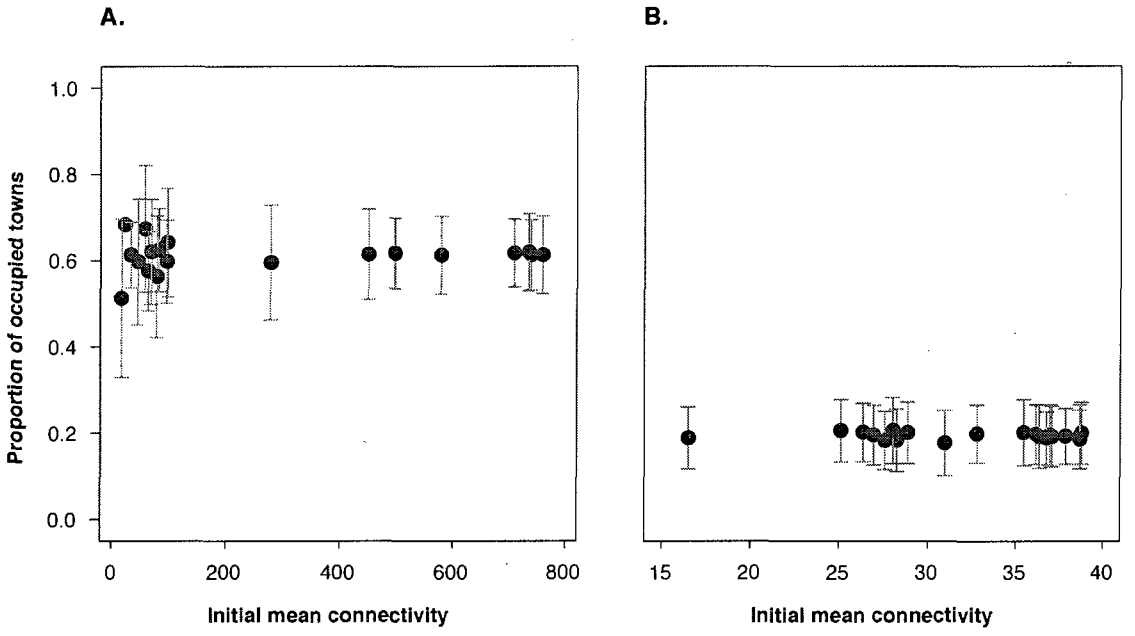


Figure 2.4. Sensitivity analysis demonstrating initial mean connectivity has little effect on mean proportion of occupied patches for A. western and B. eastern metapopulations. Black dots represent mean proportion of occupied towns and grey lines indicate one standard deviation of the mean.

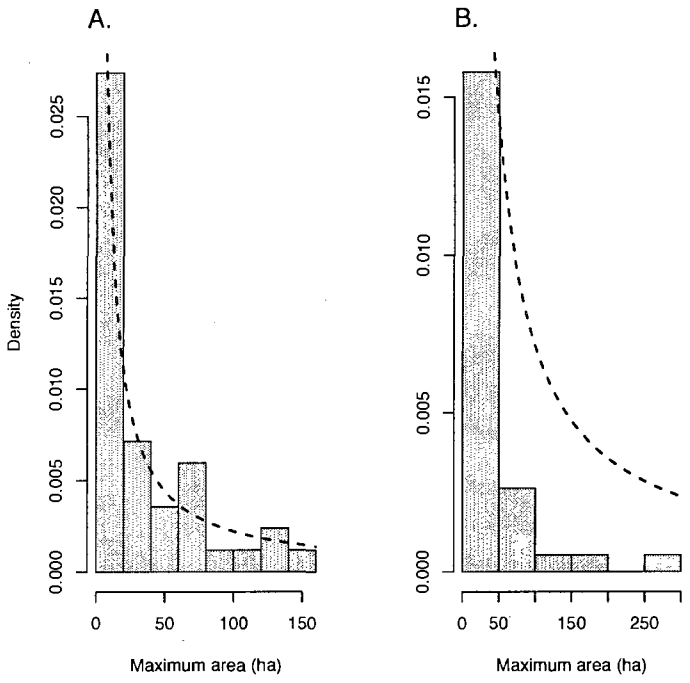


Figure 2.A1. The fit of the extinction function (dashed line) from Model 1, unstructured plague spread, to maximum prairie dog town area (histogram where bars are scaled to sum to one giving a density instead of a frequency) for each metapopulation (A. western and B. eastern).

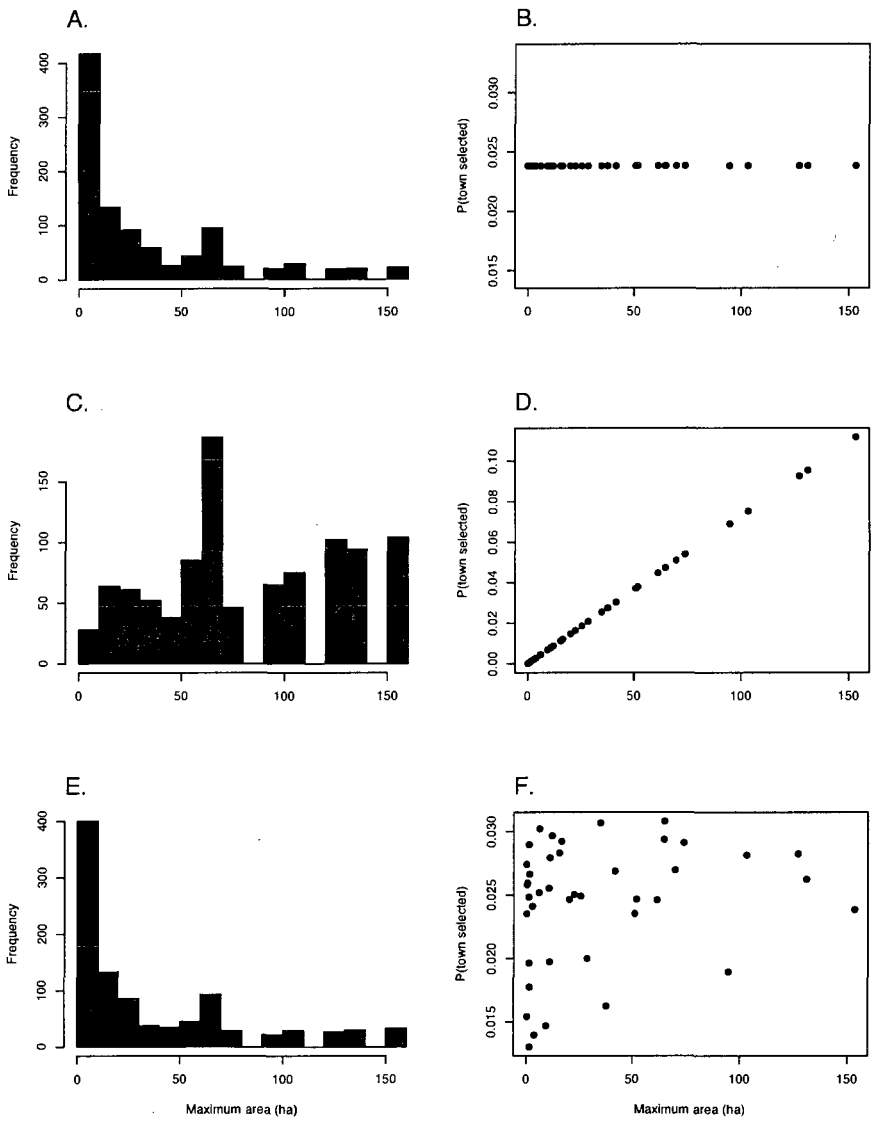


Figure 2.A2. Histograms for the western metapopulation across maximum town area of how frequently the sampling process selected towns where the probability of extinction was A) equal across all towns, C) proportional to maximum town area, E) proportional to average connectivity. Corresponding scatterplots of the probabilities that were used to select the different towns where the probability of extinction was B) equal across all towns, D) proportional to maximum town area, F) proportional to average connectivity. Patterns for the eastern metapopulation are equivalent to those shown.

CHAPTER 3

PATTERNS OF SEASONAL RABIES PREVALENCE IN BAT RABIES SAMPLES: THE IMPORTANCE OF HOST ECOLOGY

Dylan George, Colleen Webb, Jennifer Hoeting, Greg Ames, Michael Buhnerkempe, Tom O'Shea, Paul Cryan, Philip Riggs, Charles Rupprecht

INTRODUCTION

Mechanisms causing seasonal variation in the dynamics of human pathogens have been well explored (Anderson and May 1992; Keeling and Rohani 2007). Mechanisms generating seasonal patterns in pathogens circulating within wildlife populations, however, have been the subject of more recent investigations (Hosseini et al. 2004; Lass and Ebert 2006) and have the potential to provide better understanding of the increasing number of emerging zoonotic viruses (Daszak et al. 2000). Bat rabies dynamics exhibit a unique seasonal pattern in the number of cases in comparison to other wildlife reservoirs, indicating that different dynamics occur within bats (Fig. 3.1) (Blanton et al. 2007). Moreover, previous analyses have demonstrated higher prevalence of bat rabies virus in passive surveillance samples in the spring and especially autumn throughout the United States (Mondul et al. 2003). The strong association of bat rabies virus variants and

human deaths due to rabies in the United States (Blanton et al. 2007) heightens the value of understanding pathogen dynamics within this wildlife reservoir. This begs the question of why bats have unique seasonal patterns of pathogen dynamics, or rather, what factors drive bat rabies virus seasonality? A detailed understanding of the pattern is missing and, more importantly, the mechanisms that drive this pattern are poorly understood.

Pathogen seasonality can be affected by a combination of feedbacks among intrinsic factors such as host immunity and population biology (Anderson and May 1992; Hosseini et al. 2004) that vary temporally, and extrinsic environmental factors such as favorable climatic conditions at specific locations (Rogers et al. 2002). Several bat-specific mechanisms within each broad category (temporal and environmental) could influence the seasonality of rabies in bats including annual cycles in aggregation, reproduction, population level immunity, stress, food availability, and energetically acceptable climatic conditions. Temporal aspects of life history of temperate zone bats may help explain seasonal peaks of rabies in bats in the United States. For example, some bat species, such as big brown bats (*Eptesicus fuscus*), aggregate seasonally in maternity colonies of tens to hundreds of individuals for their annual birth pulse (Davis et al. 1968; Kurta et al. 1990). Seasonal aggregation and reproduction increases contact among individuals and thereby could facilitate pathogen transmission. Also, the influx of young, susceptible individuals into the bat population each summer would represent greater opportunity for sustained transmission within a population, similar to persistence dynamics within human populations (Anderson and May 1992).

Alternatively, climate may drive pathogen seasonality. Environmental covariates such as temperature and precipitation can impact temperate zone bat populations and dynamics of rabies in bats indirectly and directly. First, most temperate zone bats are insectivorous and their populations can be indirectly affected by environmental variables through impacts on their insect food sources, which are prone to variation in climate conditions (Kovats et al. 2001; Harvell et al. 2002). In addition, vector-borne diseases and those harbored in insectivorous wildlife reservoirs, are the most likely pathogens and parasites to be impacted by environmental factors (Altizer et al. 2006). Specifically, rain and low temperatures negatively influence insect numbers and activity (Williams 1961; Taylor 1963). Also, precipitation can increase bat confusion because of "chatter" while echolocating (Burles et al. 2009). It is reasonable to assume bat populations will be impacted negatively by conditions that do not favor insect populations. Also, host susceptibility could vary under different climatic conditions. Temperate zone bat species deal with seasonal variation in climate differently, but bats use two general strategies to cope with harsh climate: hibernation and migration. Regardless of which strategy is employed, the onset of harsher climate can bring increased stress for animals resulting in increased susceptibility to disease.

Second, environmental covariates can directly impact rabies in bats by affecting the rate of BRV activity, the transmission rate among bats, or host susceptibility. An intriguing feature of bat biology in the temperate zone is that many bats are facultative heterotherms, or rather; bat metabolism closely mirrors ambient temperatures during torpor (Speakman and Thomas 2003). Researchers have found a positive relationship between precipitation and ambient temperature with postnatal growth of temperate

insectivorous bats (Hoying and Kunz 1998; Hood et al. 2002). Experimental work examining the role of temperature in pathogenesis of bat rabies indicates that ambient temperatures controls disease progression in infected bats (Sadler and Enright 1959; Sulkin et al. 1960). Sadler and Enright (1959) found a positive relationship between ambient temperature and length of incubation period. Because rabies is a viral pathogen the metabolic rate of the host will determine the rate of viral activity such as viral replication and tissue tropism. The cooler the temperature and the more time in torpor, then the slower viral replication will be.

Precipitation can also impact bat rabies through effects on transmission rates. In summer bat activity, foraging and reproduction, can be negatively affected during periods of cool rainy weather (Grindal et al. 1992). During the summer, inclement weather has been shown to increase the frequency of use of torpor in pregnant and lactating females (Willis et al. 2006). If colonial bats spend more time in close proximity in the maternity roosts because of precipitation, then the greater the potential for transmission from bat to bat. Determining which factors most significantly influence the seasonality of rabies in bats will be an important step in predicting how the disease will react to climate change and impact human health and surveillance needs.

The purpose of this study is to explore how environmental drivers and temporal patterns in bat population biology might explain annual cycles of rabies prevalence in bats in the United States. We explore several hypotheses regarding bat rabies prevalence. In particular, we investigate environmental covariates hypothesized to impact rabies prevalence in bats (temperature, precipitation), and temporal variation in population biology (seasonal reproduction, aggregation, and immunity). Do environmental

covariates (temperature, precipitation) explain the seasonal pattern, or does the temporal variation in population biology? Thus, in this study we develop a general additive logistic model to explore the effects of biological, geographical and climatic factors and to discriminate among temporal or environmental mechanisms driving prevalence of rabies in bats. This information can be used to enhance surveillance and mitigation measures, thereby, minimizing the negative impact of this pathogen on public health.

METHODS

National analysis: We developed general additive models with binary responses to explore effects across multiple states of the United States and in more detail at a state level. For the national model we analyzed data that consist of results (positive or negative) from passive surveillance testing of bat samples in 37 states (Table 3.1) submitted to state diagnostic laboratories over a period of seven years (1996-2003). The samples were assessed by direct fluorescent antibody testing of brain tissue to detect rabies virus antigen. The data are reported by the states to the Centers of Disease Control and Prevention and include the test result; county; month and year of submission; and bat species (or genus). We categorized migratory status of each species of bat based on known life history information. A species was considered migratory if seasonal movements occur annually on a continental scale (i.e., hoary bats, *Lasiurus cinereus*; red bats, *L. borealis*; silver-haired bats, *L. noctivagans;* (Cryan 2003)) or on a broad regional scale (i.e., Mexican free-tailed bats *Tadarida brasiliensis mexicana* in the southwestern U.S.; Cockrum 1969). Non-migratory species were those that may make local

movements but generally do not migrate at such large scales (i.e., *Eptesicus fuscus*, big brown bats; *Myotis lucifugus*, little brown bats). Also, we excluded several states (California, South Carolina, Florida) because they did not record the number of negative submissions (Mondul et al. 2003), and we excluded some states (New Mexico, Texas and Pennsylvania) because these states have peculiarly high percentage of positive cases (Appendix 3.1) indicating different sampling, testing or reporting criteria in these states. Also, we assessed the periodicity of the cases using periodograms, a type of spectral analysis, and found an annual periodicity in bat rabies cases at the national and state levels (Appendix 3.2), so we aggregated data across years for our analysis.

We modified a previous analysis at the national scale (Mondul et al. 2003) that used a Mantel-Haenszel test to evaluate coarse patterns in the passive surveillance data among region of the United States, time of the year, and bat species group associated with virus variants more likely to cause human death (see Mondul et al. 2003 for details). The United States was divided into four quadrants (NE, NW, SE, SW). The NE included 14 states (CT, IL, IN, ME, MA, MI, NH, NJ, NY, OH, PA, RI, VT, WI), the NW included 12 states (ID, IA, KS, MN, MO, MT, NE, ND, OR, SD, WA, WY), the SE included 12 states (AL, DE, FL, GA, KY, MD, MS, NC, SC, TN, VA, WV), and the SW included 10 states (AZ, AR, CA, CO, LA, NV, NM, OK, TX, UT). Three months were included in each season: winter (December-February), spring (March-May), summer (June-August), and autumn (September-November). Two bat species groups were included and were designated based on the rabies virus variants. The first group included the variant most commonly associated with human deaths (*Lasiurus noctivagans,* silver-haired bats; *Tadarida brasiliensis mexicana,* Mexican free-tailed bats; and *Pipistrellus*

subflavus, eastern pipistrelle). The second group included all other bat species that were sampled. The prior analysis found the highest probability of bat samples being positive in the SW quadrant of the U.S. and in the autumn months. We extended their analysis using logistic regression to assure that we detected the same general patterns and to explore more detailed patterns across time. In addition, we included a month term evaluating how rabies prevalence changed each month across a year, and we considered how migratory and non-migratory species seasonal patterns differed.

We used general additive models because we expected complex patterns across time, and smooth functions minimize the number of covariates needed to explain these patterns and result in simpler models (Wood 2006). We let Y_{ij} be the binary infection status ($Y_i = 1$ for BRV positive bats and $Y_i = 0$ for BRV negative bats) for the individual bat $i = 1, \ldots, n$. Subsequently, we model the infection status of each bat as a Bernoulli random variable with parameter π_i:

$$Y_i \mid \pi_i \sim Bernoulli(\pi_i) \qquad (1)$$

The parameter π_i corresponds to the probability that the i^{th} bat is infected. We assume that all observations are conditionally independent. The infection probability parameter, π_i, is modeled as

$$\text{logit}(\pi_i) = \beta_0 + \mathbf{x}_i^T\beta + f_1(\mathrm{x}_{1i}) + f_2(\mathrm{x}_{2i}) + f_3(\mathrm{x}_{3i}, \mathrm{x}_{4i}) + \ldots \qquad (2)$$

where $\mathbf{x}_i^{\mathrm{T}}$ is the vector of covariates for i^{th} bat, β_0 is the intercept term, $\boldsymbol{\beta}$ is the parametric covariate vector, and f_j are the smooth functions of the covariates, x_k. Use of different $\boldsymbol{\beta}$ vectors and f_j smooth functions define different models.

To predict the probability of a sample being positive we derive the following equation from (2):

$$\pi_i = \frac{\exp(\beta_0 + \mathbf{x}_i^T\beta + f_1(\mathrm{x}_{1i}) + f_2(\mathrm{x}_{2i}) + f_3(\mathrm{x}_{3i},\mathrm{x}_{4i}) + \ldots)}{1 + \exp(\beta_0 + \mathbf{x}_i^T\beta + f_1(\mathrm{x}_{1i}) + f_2(\mathrm{x}_{2i}) + f_3(\mathrm{x}_{3i},\mathrm{x}_{4i}) + \ldots)} \quad (3)$$

Using (3) we are able to estimate prevalence for a particular month by averaging at the appropriate temporal or spatial scale.

State level analysis: We used a more restricted, but denser, set of the data, at the state level, to determine how environmental factors impact prevalence of rabies in bats. A finer-scaled analysis may be more appropriate to discriminate environmental signals because significant variation is introduced from differences in sampling across states, and this could distort signals from the environmental factors. For the state-level analysis we used the same data as the multi-state analysis but restricted it to the state of Colorado (CO) for several reasons. First, previous analysis demonstrated the risk of positive results in the submitted samples was highest in the southwest quadrant of the United States (Mondul et al. 2003) including CO. Data from CO contained a relatively large number of records representing many (31 out of 64) of the counties, including all of the most populated counties. Second, for logistic regression it can be difficult to discriminate patterns for data with high (~100%) or low proportions (~0%), so choosing a state like CO, with an intermediate average prevalence of rabies of approximately 20%, should provide as strong a signal in the data as possible. Lastly, recent research in CO on big brown bats has provided additional insight into pathogen dynamics (e.g., (Pape et al. 1999; Shankar et al. 2004; Shankar et al. 2005; Neubaum et al. 2006). Different from the national analysis, we did not include the limited data from the months of December through March (total of 16 case reports). This removal is justified, in part, because of bat

population biology, where these discarded data correspond to the times when non-migratory bats hibernate and migratory bats should be absent from Colorado. Thus, samples from the discarded months would be highly biased because any bat sample submitted would represent a significant deviation from normal behavior.

Environmental and geographical data: For the state analysis we considered the effect of different environmental and geographical covariates. Elevation and climatic data for Colorado were downloaded from the Northwest Alliance for Computational Science and Engineering Prism Data Explorer (http://prism.oregonstate.edu/). Variables extracted from this database included average monthly precipitation (ppt), average minimum monthly temperature (tmin), and average maximum temperature for the month (tmax). We included environmental variables for the month the sample was submitted and the two months previous to create a time-lagged variable for temperature and precipitation (e.g., tmax_1, tmax_2, tmax_3, ppt_1, ppt_2, ppt_3). Each county was assigned the spatial mean for elevation, precipitation, minimum and maximum temperature by nearest neighbor analysis of the raster cell nearest to the center of the county. Estimated county population data were downloaded from the U.S. Census Bureau (http://www.census.gov/popest/datasets.html). Each county population was matched to the year of sample. Human population of the county was included as a proxy for sampling effort.

State level model construction and selection: Similar to the national analysis, we used general additive models with binary responses to explore seasonal patterns in rabies prevalence in bats. In order to evaluate competing hypotheses regarding the drivers of seasonality in bat rabies, we developed a candidate model set following a dimension

reduction algorithm used in disease risk mapping (e.g., Winters et al 2008). To develop a candidate model set (Table 3.2), we choose covariates that were significantly associated with rabies prevalence in univariate tests of association (Wilcoxon's test; $p < 0.05$ – Appendix 3.3) but not strongly correlated with each other (Spearman rank correlation; $\rho_s < 0.8$). The temporal covariates consisted of month and any interactions with month. These covariates serve as a proxy for the temporal variation in life history of bats (birth pulse, hibernation, seasonal aggregation). The environmental covariates consisted of average maximum daily temperature for the month, average minimum daily temperature for the month, average precipitation for the month, and the average minimum, maximum temperature and precipitation from the previous two months. For both temporal and environmental models we included a minimal control for sampling effort (human population of the county). In addition, we considered several refining covariates to explain some variation associated with the environment and the bats because of the coarse resolution (county level) of our data including: elevation (average elevation of the county), migratory status of the species of bat (species effect), and spatial effects (effect of county). The combined covariates consisted of the union of the temporal and environmental covariates. This particular division of models allows for more explicit biological interpretation given the selection of a particular model. If a model from one of the different subsets is selected this indicates that those covariates are uniquely associated with seasonality in bat rabies (if a model from either temporal or environmental subset is selected), whereas a combination of covariates maybe responsible (if a model from the combined subset is selected). Alternatively, if a temporal model is selected then it could also mean that the spatial or temporal scale of environmental variables is inadequate

(Levin 1992; Pascual and Dobson 2005; Eisen and Eisen 2008). However, finer resolution data are currently unavailable.

Interpreting the selection of an environmental model is straightforward. The particular environmental factors associated with the model are strongly correlated with seasonal prevalence of rabies in U.S. bats. However, interpreting a temporal model requires an understanding of bat biology. Temperate zone species of bats have a strong annual cycle consisting most basically of a birth pulse in summer and over-winter hibernation or migration in autumn, depending on the species of bat. Because of this strong and consistent annual cycle in bat biology we can interpret a temporal model as representing this biological cycle. In essence, our default hypothesis consists of bat life history patterns explaining seasonal prevalence of rabies. The month covariate represents bat biology because seasonal rabies prevalence (Fig. 3.1) is correlated with seasonal life history events such as arousal from hibernation and annual birth pulses (Fig. 3.2).

We used Akaike Information Criteria (AIC) to select the most appropriate model given the CO data (Burnham and Anderson 2002). Statistical analyses were done using R (www.r-project.org), and results were considered significant when $p < 0.05$.

State analysis - model fit: We assessed model fit at the state level using several methods. First, we assessed each model in the candidate model set by the area under the curve (AUC) of a receiver operator characteristic (ROC) curve. The AUC statistic assesses each model's discriminatory ability (Hosmer and Lemeshow 2000). As a general rule of thumb, models with AUC values above 0.7 adequately discriminate binary response variables (Hosmer and Lemeshow 2000). Second, we compared observed and predicted monthly prevalence as a check to see that each model was capturing the

seasonal dynamics. We considered model residuals by county to determine which counties did not do well in estimating rabies prevalence. To accomplish this we calculated the county level prevalence for county j as the mean of the probability of infection for all bats in county j:

$$\pi_j = \frac{1}{n_j} \sum_{i=1}^{n_j} \pi_{ij} \qquad (4)$$

where n_j is the number of bats from county j. This provides the expected prevalence for the different counties. Similarly, we aggregated the fitted values for each county and calculated the difference between the observed and expected by county. This allows us to generate a map depicting the risk associated with the j^{th} county.

RESULTS

National results: The general additive models produced similar results to that of Mondul et al. (2003), providing confidence in use of this approach to discriminate other patterns (Table 3.3). The model exhibits significant variation of bat rabies prevalence across a year, and variation in different regions of the country with the SW quadrant having the highest probability of infected bats (Table 3.3). In particular, the national model describes bat rabies prevalence across a year in a way consistent with Mondul et al. (2003) such that there is a peak in prevalence in the spring and the autumn (Fig. 3.3A). At the national level migratory species have a significantly different seasonal pattern (Fig. 3.3B) than all bats, which are primarily non-migratory species (93%). Although not significantly different (Table 3.3), the prevalence of BRV in migratory species is higher than that of non-migratory species (Fig. 3.3). Specifically, there are prevalence peaks for

non-migratory species in the spring and autumn, whereas there are prevalence peaks for migratory species in the summer and in the winter.

State level - model selection: For the CO data, a temporal model (model 2) had the most support given the data; however, a combined model (model 7) also had substantial support (Table 3.2). The selection of a temporal and combined model indicates strong support for the temporal covariates overall. Additionally, after accounting for temporal covariates, an environmental covariate partially captures some of the seasonal variation in bat rabies prevalence in the samples submitted.

State level - model fit: For the state level analysis, area under the curve (AUC) goodness-of-fit statistics indicate a good fit for both of the selected CO models (Table 3.2). Predicted versus observed seasonality prevalence patterns (Fig. 3.4) were similar for both models and further demonstrate that each model well describes seasonal rabies dynamics in CO bats. Additionally, to assess the goodness of fit of the model to the data we considered residuals for each county, i.e. difference between the expected and observed prevalence from the samples (Fig. 3.5B). As we would expect, we found the models fit best for counties with the most records.

Description of selected models: For the CO data, rabies prevalence in bats significantly varies across the months of the year (Table 3.4) with high prevalence in the spring, April-May and autumn, September-October, and lower prevalence in the summer, July (Fig. 3.6A). Migratory species have a different seasonal signal with spring and autumnal peaks in prevalence occurring later than non-migratory species (Fig. 3.6B). The relatively large confidence intervals are most likely because of the limited number of samples for migratory species in CO (Fig. 3.6B). Also, there is a marginally significant

positive log-linear relationship with the county human population (Fig. 3.6C), which serves as a minimal control for sampling effort. The two different CO models have either a significant monotonically decreasing relationship with elevation (Fig. 3.6D), or a positive relationship with maximum daily temperature (Fig. 3.6E). Although maximum daily temperature and elevation are not strongly correlated, they are weakly correlated (Spearman rank correlation; ρ_s = -0.55; Appendix 3.4).

DISCUSSION

Temporal and environmental factors can drive seasonal patterns of rabies in bats. National trends in sampling indicate that rabies prevalence varies throughout the year and differs among migratory and non-migratory species (Fig. 3.3). To expand on these results, we developed a statistical model to explore biological, geographical and climatic factors and to discriminate among different hypothesized mechanisms driving bat RV prevalence within the passive surveillance samples. We found temporal factors best explain seasonality of rabies in bats, indicating life cycle patterns in bats drive rabies seasonality. However, there is also evidence that the environmental factor of average maximum daily temperature can influence rabies prevalence as well, most likely through a positive relationship between ambient temperature and rate of viral activity (Speakman and Thomas 2003).

The non-linear pattern of prevalence across months indicates that the annual biological cycles of bats most likely drive dynamics of rabies in bats in Colorado. The model that best fits the data contained covariates for a non-linear prevalence pattern across months of the year (Fig. 3.6A). The month parameter reflects our hypotheses that prevalence is driven by the life cycle of the bat. Non-migratory bats are the predominant

group of bats in the CO sample, 71% (Pape et al. 1999). Examining the peak in spring and autumn begins with considering the biological cycle for these species.

For non-migratory bats in Colorado, high prevalence in the spring is likely a function of adult bats emerging from hibernation. A proportion of these adults was infected the previous year, survived hibernation, and succumbed to rabies virus upon arousal from hibernation. Although there are few total cases in the early spring (Fig. 3.1), the proportion that are infected is relatively high and provides a potential persistence mechanism for BRV through hibernation and the spark for more infections later in the season. The lowest prevalence, with respect to the month parameters, occurs in July. This corresponds to a time in the life history of the big brown bat (the most abundant species) shortly after the birth pulse of new pups. At this time the pups are grown and flying, and appear to be susceptible to encounters with people regardless of infection status and thus are disproportionately submitted to public health departments. The spring and early summer birth pulse introduces a significant number of immunologically naïve juveniles into the population and lowers the prevalence because the number of negative cases increases faster than the number of rabid individuals. Additionally, maternal antibodies may protect newborns for a period of time minimizing the number positive cases in the mid-summer months. After the birth pulse, when the bats are still communally roosting with tens to hundreds of other bats, the potential for transmission remains high among newborn bats that are immunologically naïve resulting in a portion of the cohort becoming infected. Following the incubation period of the virus, infected young of the year begin succumbing to rabies in the autumn. The observed monthly prevalence roughly matches this hypothesis (Fig. 3.6A).

The interaction of migratory by month terms exhibited a significantly different pattern indicating that pathogen dynamics within migratory species behave differently than within non-migratory species. Migratory species exhibit a different seasonal rabies prevalence pattern with low prevalence in the spring and high prevalence in the autumn (Fig. 3.6B). In April, migratory tree bats are not found in as many states as in summer, and prior to migration they are not available for submissions to most health departments (Cryan 2003). They are also less likely to be encountered by people because they roost in trees, unlike the non-migratory species that roost in buildings where they are more likely to be encountered. The birthing grounds of most species of migratory tree bats range into Canada (Cryan 2003) and those individuals would not add to a mid-summer pulse of newborns in U.S. records. Tree bats begin migrating through the U.S. in late summer and early autumn (Cryan 2003). Both young of the year and adults are perhaps more likely to contact people as they move through inhabited areas and increase their detection probability. Furthermore, the young of the year would have ample time to incubate rabies virus if they were infected earlier in the summer resulting in higher autumn prevalence. However, at this time much of the pathogen dynamics of rabies in migratory bat species is speculative and requires more research.

In addition to life cycle differences, there are several other possible biological explanations for the significance of the migratory by month term. Different virus variants become adapted to different hosts (Rupprecht et al. 2002; Messenger et al. 2003; Mondul et al. 2003) and rabies variants associated with some migratory species (e.g., silver-haired bats, *Lasiurus noctivagans*) are known to have a greater infectivity (Mondul et al. 2003; Franka et al. 2006). Different host-pathogen associations most likely will dictate

different dynamics based on the different seasonal dynamics of migratory and non-migratory species. It is possible that some factor associated with migration makes infection by BRV more likely and at different times of the year. One possibility is that the physical stress of long distance travel weakens bats, rendering them more susceptible than bats that remain in Colorado and hibernate. These remain unanswered questions.

We were surprised to find that our environmental covariates did such a poor job of explaining BRV prevalence. Average maximum daily temperature explained some variation in BRV prevalence giving slight evidence for an environmental covariate (Fig. 3.6E). This finding is consistent with most of the colonies of non-migratory species preferring lower elevations because reproductive females of these species of bats prefer warmer temperatures (Weller et al. in press). Our finding that most environmental drivers considered do not significantly impact BRV seasonality indicates they are not as important but we also should consider the spatial resolution of our data. The county-level climate data are possibly at an inappropriate spatial resolution. For example, insect abundance and quality, the main food source for these bats, may be influenced less by county level climate than by finer scale microclimatic fluctuations. We are unable to address this concern with these data. Also, average county level environmental covariates are coarse predictor variables. The geographic scale of the environmental covariates needs to be assessed critically because BRV prevalence may be responding to climate variation at much finer geographic resolutions. However, these data do not allow such an evaluation. But this will be an issue for any similar study, (e.g., Winters et al. 2008) unless one has multiple levels with which to test the effect of scale (e.g., Farnsworth et al. 2006). Lastly, bats are highly mobile and some non-migratory species

make local movements between maternity and hibernation areas (Davis and Hitchcock 1965; Neubaum et al. 2006). Their mobility will minimize how well environmental covariates can predict BRV infection because the location of the environmental conditions where they were susceptible, exposed and infected could be different.

Several other biological reasons may explain why climatic factors do a poor job explaining seasonality of rabies in U.S. bats. First, the predominant species in the Colorado data is the big brown bat. Climatic drivers might not as directly affect this species or its food sources as other species because it has adapted to highly modified urbanizing ecosystems. Second, bats are nocturnal and their diurnal roost sites may buffer bats from climate extremes during the day. Third, big brown bat populations in urbanizing areas of Colorado most likely are increasing from pre-settlement times, which suggests these populations suffer minimal stress from competition. Thus, these populations could maintain sufficient energetic resources to cope effectively with climate fluctuations.

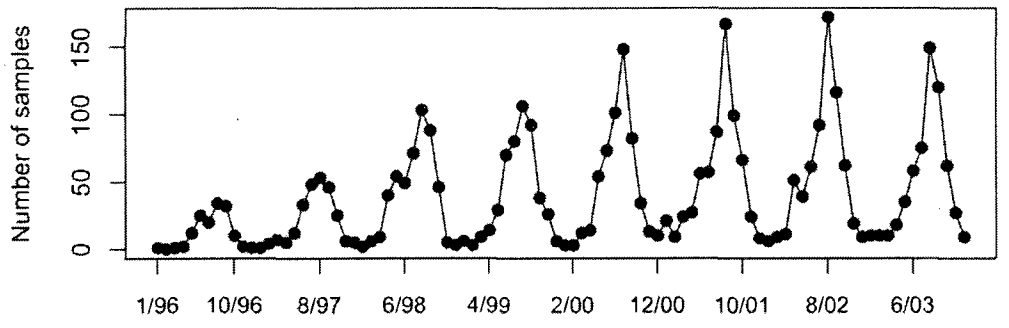
Using passive surveillance data for analysis of rabies prevalence in bats has several limitations. An additional limitation with these passive data is small sample size from some states and especially counties, along with any differences in county collection, testing and reporting. These small sample sizes make it difficult to estimate location-specific (particularly county-level) prevalence and can lead to poor model fit in these areas. In addition, heterogeneities within bat populations can affect pathogen dynamics. For example, sex bias in different disease dynamics (transmissibility, susceptibility, infectiousness, etc.) can play an important role in wildlife pathogen prevalence (Adler et al. 2008). Adult male and female bats have different physiological, distribution, and

aggregation traits (Weller et al. in press), and these coarse specimen data do not distinguish by sex. Also, these data do not distinguish adults from volant young-of-the-year, which can have different traits and importance to pathogen dynamics (Weller et al. in press).

This analysis proposes a hypothetical driver of BRV seasonality that needs to be confirmed with data appropriate for inference to wild populations. We do not assume that these data allow us to infer the complete story of seasonal BRV dynamics but they do provide intriguing ideas and highlight the need for more data and research in this area. Despite these limitations, these data have proven useful in describing trends in BRV prevalence in previous studies of bats (Mondul et al. 2003) and similar data have been used successfully in determining spatial patterns of raccoon rabies (Smith et al. 2002; Russell et al. 2005; Smith et al. 2005). Lastly, data addressing bat pathogen dynamics are rare and these are the best available for rabies in bats.

Overall, the model containing temporal covariates best explains the variation in prevalence given the passive surveillance data and candidate model set. Because the migratory by month and month variables were significant, this suggests that intrinsic temporal factors, such as the seasonal dynamics of bat population biology and immunity, are most important in driving seasonal BRV prevalence.

(a)



(b)

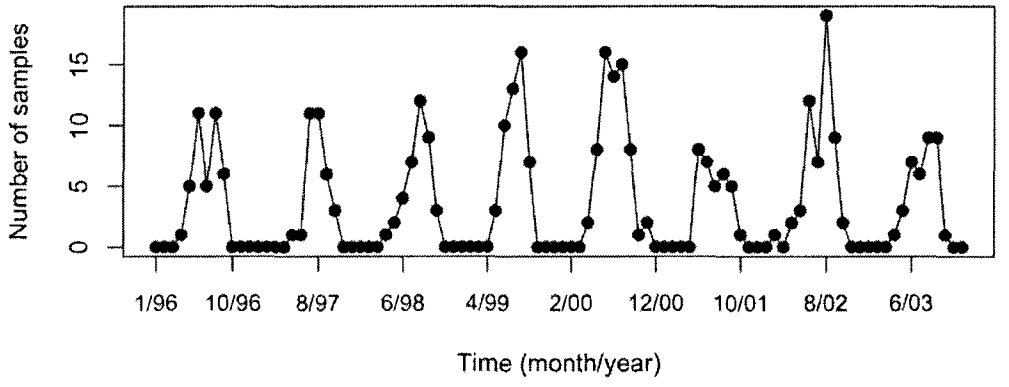


Figure 3.1. Time series of positive bat rabies samples by month from 1996-2003 in the (a) United States, and (b) Colorado.

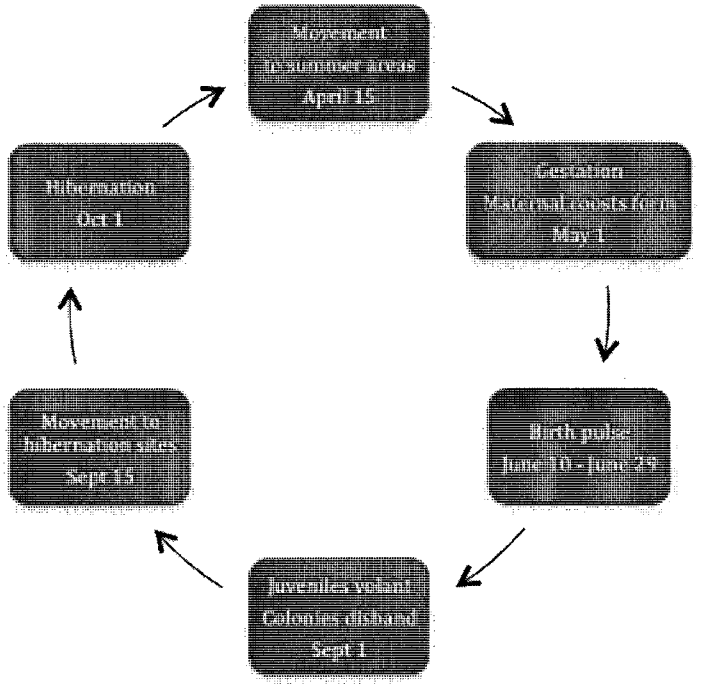


Figure 3.2. An example of a life history cycle for a non-migratory bat species (big brown bats in Colorado).

A. B.

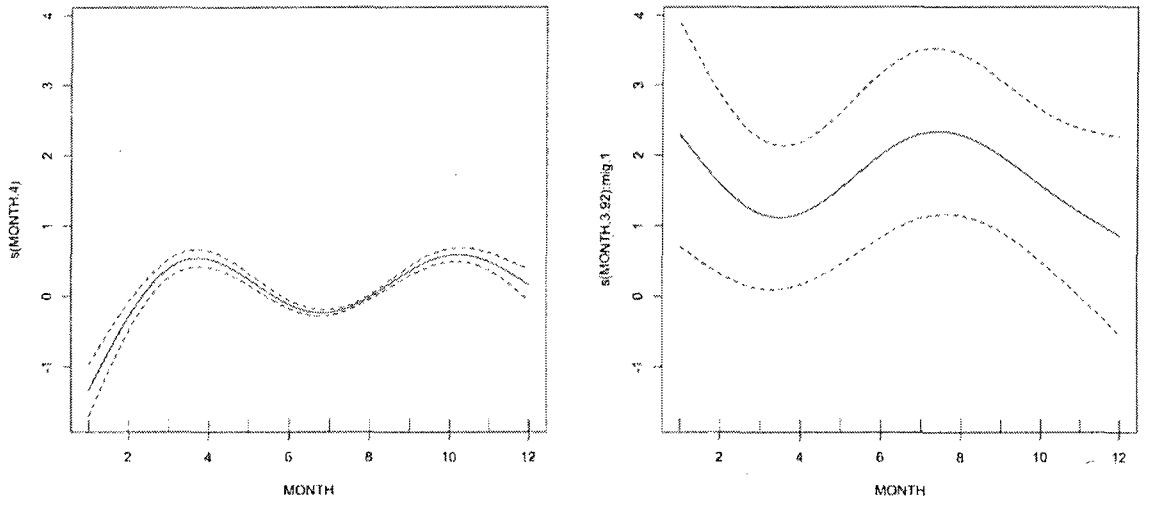


Figure 3.3. Model output for the national model demonstrating the effect for A. month and B. migratory species by month.

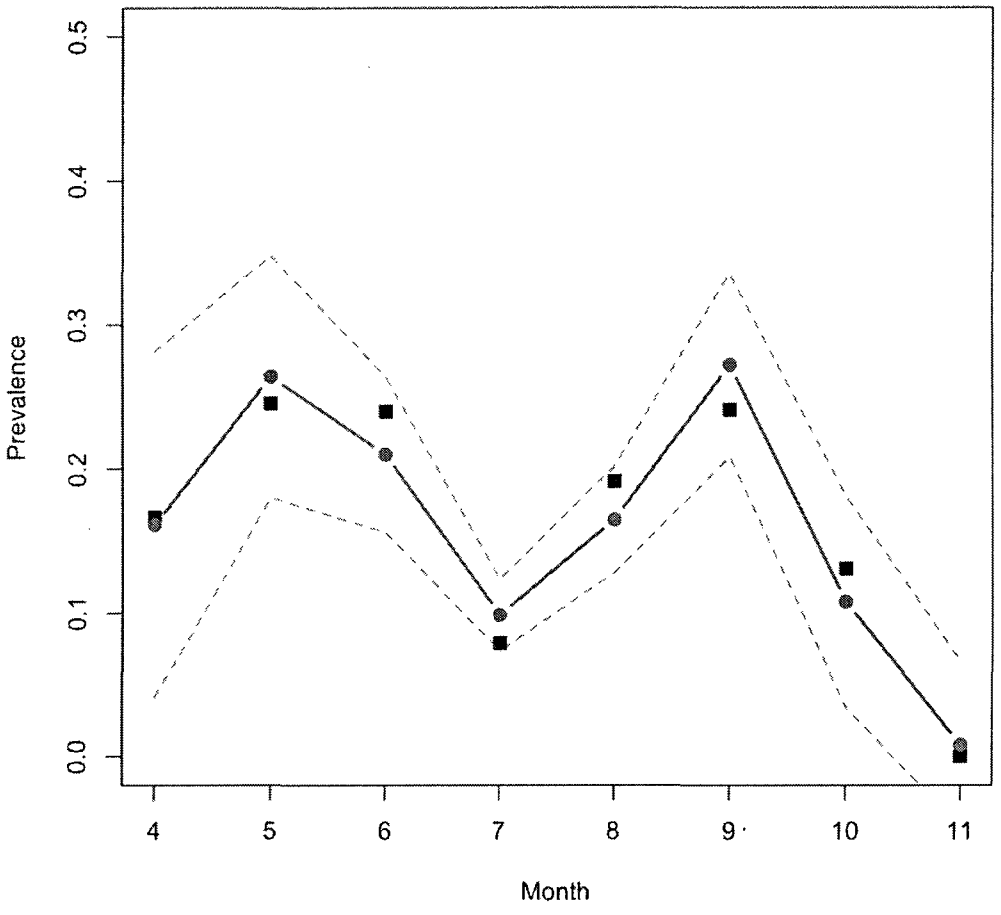
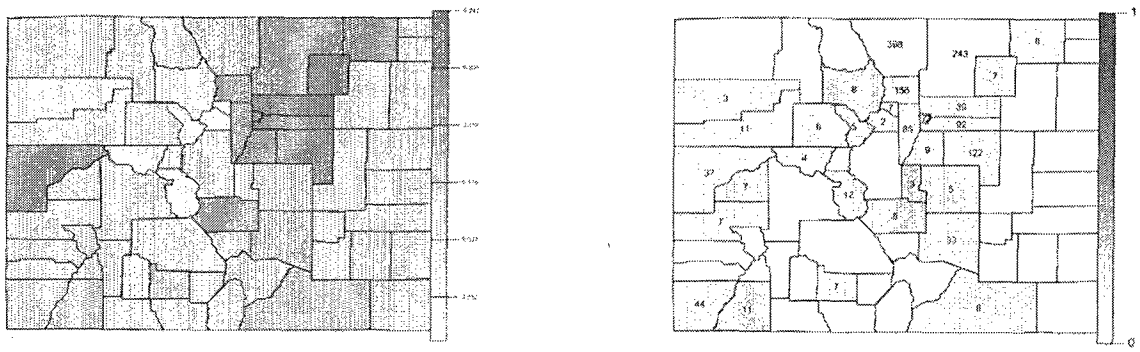
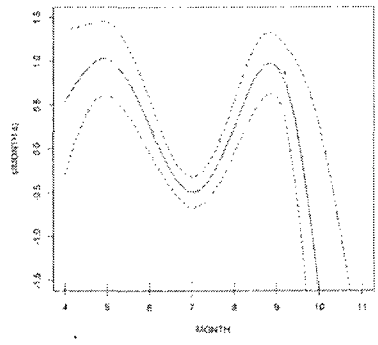


Figure 3.4. Model fit (circles and lines) versus observed (squares) prevalence by month for model 2. Model 7 produced similar results of bat rabies prevalence across months.

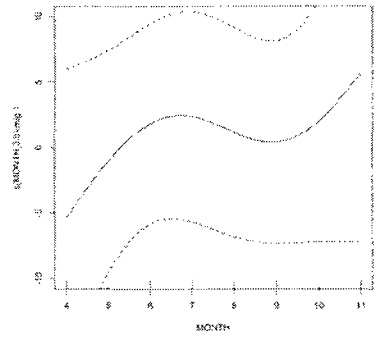


A. B.

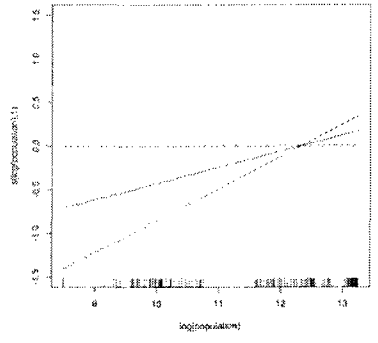
Figure 3.5. A. Map of the predicted prevalence for model 2 results showing each county in Colorado where red indicates prevalence level. Predicted prevalence was calculated as the average infection probability from bats in each county. Grey counties are those where no records were submitted. **B.** Map of model 2 results showing the absolute value of the residuals of model fit from each county, where residuals were calculated from observed and expected prevalence from each county, and bright blue indicates a lack of fit. The number of records is indicated within each county. Note the scales of the two maps differ. The maps for model 2 and model 7 are very similar.



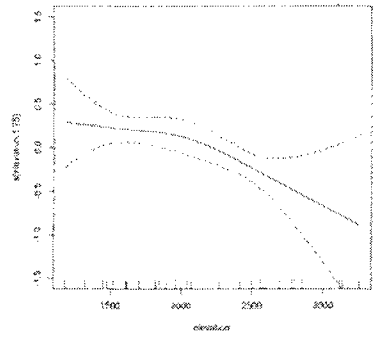
A.



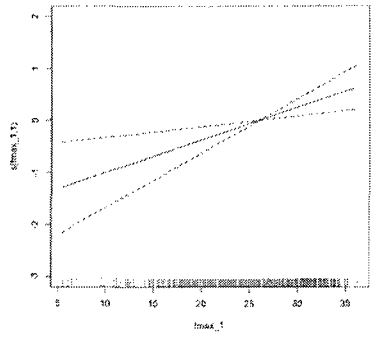
B.



C.



D.



E.

Figure 3.6. Model output for Colorado model 2 demonstrating predicted BRV prevalence across A. month B. migratory species by month C. human population D. elevation. Model output from model 7 showing E. maximum average temperature of the month.

Table 3.1: List of state from which passive surveillance samples were received

Quadrant	State	Number of samples		Number of species
		Positive	Total	
NW	WA	83	1059	14
	ID	55	490	14
	MT	58	665	8
	WY	0	1	1
	MN	4	59	4
	NE	7	299	9
	IA	17	560	8
	KS	18	309	8
SW	NV	20	74	9
	AZ	199	1373	24
	UT	48	348	13
	CO	225	1365	15
	NM	7	13	8
	OK	6	41	7
	TX	152	197	7
NE	WI	22	591	7
	IL	0	5	4
	MI	138	2150	6
	IN	24	467	9
	OH	3	107	6
	PA	57	234	7
	NY	543	15481	7
	VT	0	2	2
	NH	8	194	3
	ME	5	197	3
	MA	85	1847	7
	CT	41	1181	8
	RI	6	104	2
	NJ	33	747	5
SE	WV	20	179	4
	VA	50	1027	11
	MD	0	1	1
	KY	8	8	1
	DE	4	4	1

Table 3.2. Goodness of fit and model selection results.

Models		AUC	AIC	ΔAIC	w_{AIC}
Temporal					
1. CO:T1	migratory + *month* + *month*migratory* + *log(population)* + county	0.72	1325.06	20.27	0.00
2. CO:T2	**migratory + *month* + *month*migratory* + *log(population)* + elevation**	**0.70**	**1304.79**	**0.00**	**0.64**
3. CO:T4	migratory + *month* + *month*migratory* + *population* + *elevation* + county	0.72	1317.25	12.46	0.00
Environmental					
4. CO:E1	migratory + *tmax_3*migratory* + ppt_2 + *tmax_3*	0.61	1365.82	61.04	0.00
5. CO:E2	migratory + ppt_2 + *tmax_3* + *tmax_3*migratory* + county	0.68	1370.91	66.12	0.00
6. CO:E3	migratory + *tmax_3*migratory* + ppt_2 + *tmax_3* + *population* + *elevation*	0.64	1357.51	52.73	0.00
Combined					
7. CO:C1	**migratory + *month* + *month*migratory* + *log(population)* + *tmax_1***	**0.70**	**1306.24**	**1.45**	**0.31**
8. CO:C2	migratory + *month* + *month*migratory* + *tmax_3*+ ppt_2	0.69	1309.99	5.20	0.05
9. CO:C3	migratory + *month* + *month*migratory* + *population* + *tmax_3* + ppt_2 + county	0.72	1326.74	21.95	0.00
10. CO:C4	migratory + *month* + *month*migratory* + *tmax_3* + ppt_2 + county	0.72	1324.66	19.87	0.00

Note: Italicized covariates are smoothed covariates. Covariates for temperature (tmax) and precipitation (ppt) are followed by a number. This number indicates whether (1) the measurement was from the month the sample was submitted, (2) the previous month, or (3) two months previous. Thus, there are several temperature and precipitation covariates including: tmax_1, tmax_2, tmax_3, ppt_1, ppt_2, ppt_3. The models in bold face are those that were selected by AIC.

Table 3.3: Model output replicating general results of Mondul et al 2003 using general additive logistic regression

Formula:
POSITIVE ~ Migratory + s(MONTH, k = 5) + s(MONTH, by = mig.1,
k = 5) + REGION

Parametric coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	-3.18746	0.02412	-132.148	< 2e-16	***
Migratory	-0.66187	0.56421	-1.173	0.241	
REGIONNW	0.38738	0.05412	7.157	8.23e-13	***
REGIONSE	0.13597	0.09541	1.425	0.154	
REGIONSW	1.40132	0.04517	31.024	< 2e-16	***

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value	
s(MONTH)	4.000	4.500	194.2	<2e-16	***
s(MONTH):mig.1	3.922	4.422	101.4	<2e-16	***

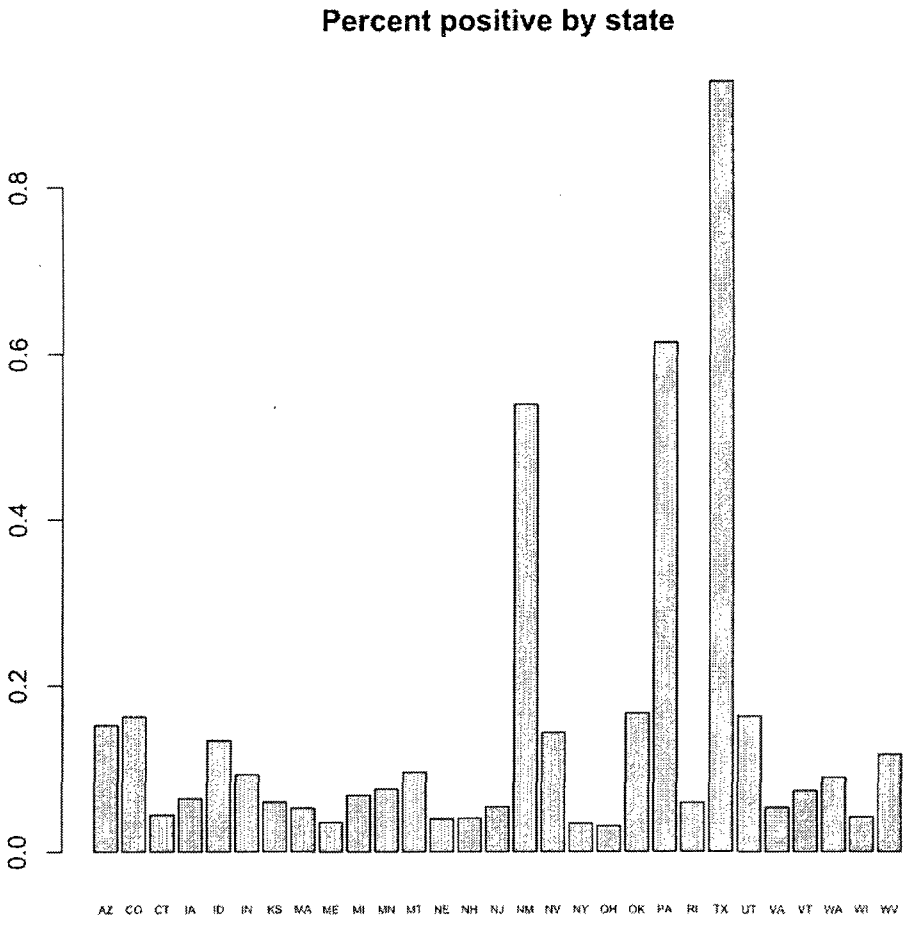
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) = 0.0891 Deviance explained = 11.2%
UBRE score = -0.56553 Scale est. = 1 n = 59841

Table 3.4. Parameter estimates of temporal model 2 for CO data (CO:T2) and combined model 1 (CO:C1)

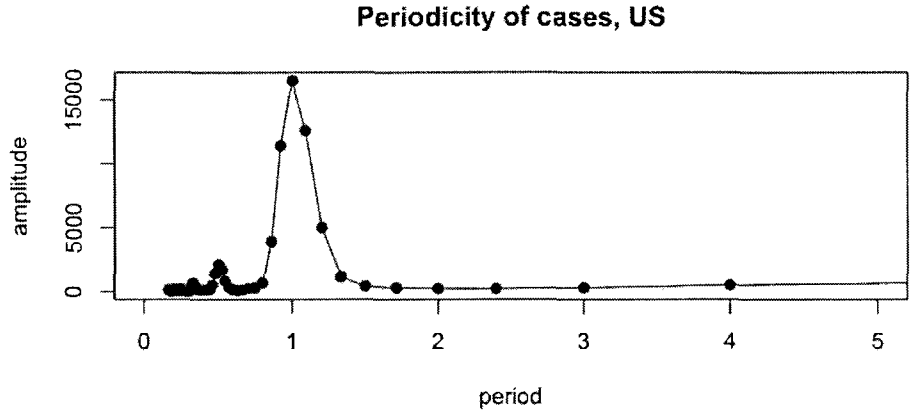
```
POSITIVE ~ Migratory + s(MONTH, k = 5) + s(MONTH, by = mig.1,
   k = 5) + s(log(population)) + s(elevation)

Parametric coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -1.9325     0.1062 -18.200  <2e-16 ***
Migratory   -0.4566     3.9176  -0.117   0.907
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
                edf Ref.df Chi.sq  p-value
s(MONTH)          4.000  4.500 48.363 1.57e-09 ***
s(MONTH):mig.1    3.500  4.000 25.246 4.49e-05 ***
s(log(population)) 1.002  1.502  4.187   0.0770 .
s(elevation)      1.751  2.251  9.837   0.0097 **


Formula:
POSITIVE ~ Migratory + s(MONTH, k = 5) + s(MONTH, by = mig.1,
   k = 5) + s(log(population)) + s(tmax_1)

Parametric coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -1.9419     0.1087 -17.866  <2e-16 ***
Migratory   -0.8294     4.0592  -0.204   0.838
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
                edf Ref.df Chi.sq  p-value
s(MONTH)          4.000  4.500 55.200 6.02e-11 ***
s(MONTH):mig.1    3.527  4.027 26.019 3.23e-05 ***
s(log(population)) 1.001  1.501  6.184  0.02627 *
s(tmax_1)         1.001  1.501  8.870  0.00637
```

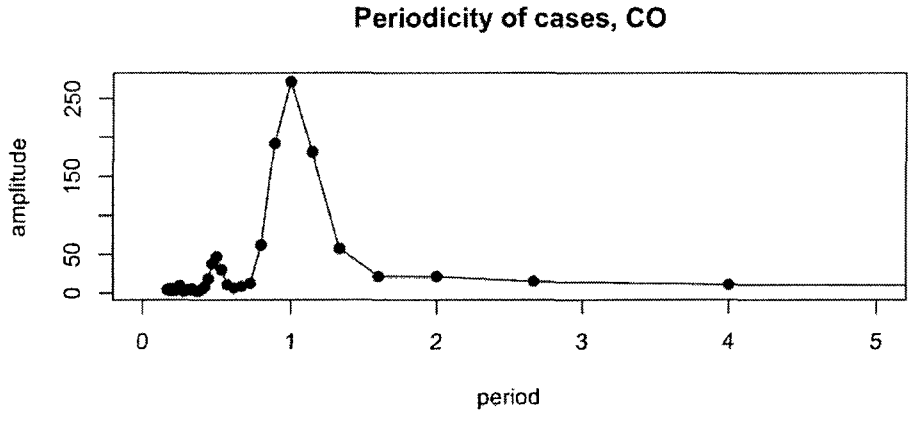
Appendix 3.1: Percent positive by state in passive surveillance data



Appendix 3.2: Periodicity of bat rabies cases

We analyzed the time series of bat rabies samples within the United States (1996-2003) to determine the extent we need to consider more complex non-linearities in describing seasonal bat rabies dynamics. We used a type of spectral analysis to generate periodograms that estimate significant periodicity in a time series. The periodogram exhibits how a times series can be broken into waves of different frequencies where frequency is the inverse of period. The importance of each frequency is measured by its amplitude where the larger the amplitude the greater the importance.



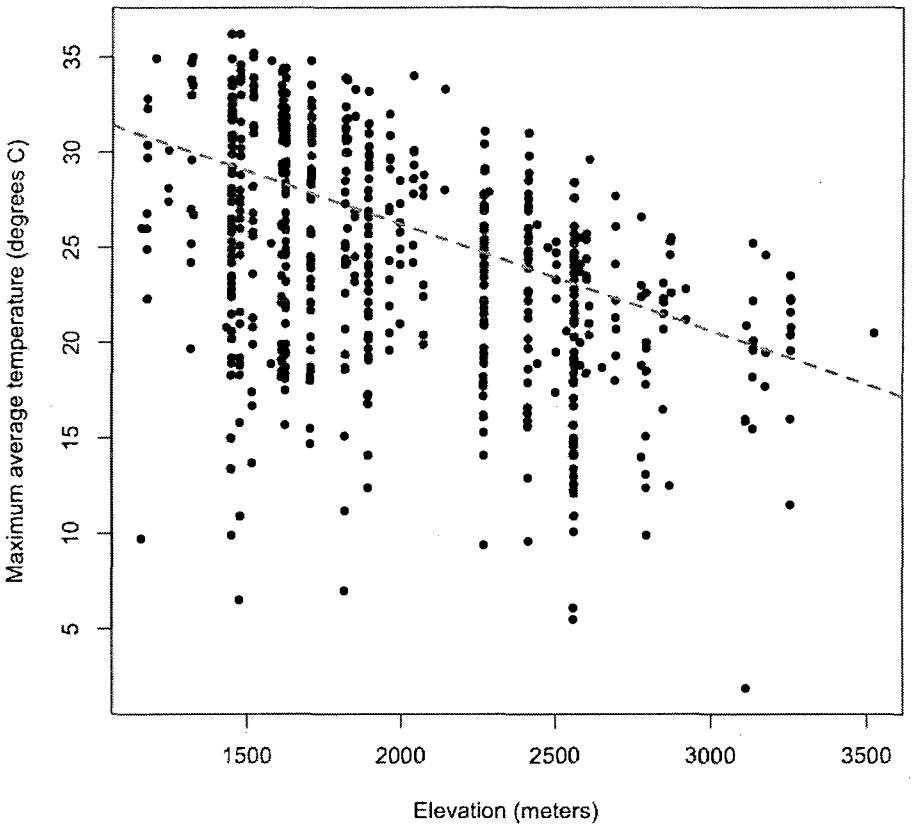


APPENDIX 3.3: Univariate tests of association among covariates

Wilcoxon tests
(univariate association of covariates to bat rabies prevalence)

	CO data
tmin_1	ns
tmin_2	p=0.040
tmin_3	p=0.079
tmax_1	ns
tmax_2	p=0.058
tmax_3	p=0.028
ppt_1	ns
ppt_2	p=0.047
ppt_3	ns
elevation	p=0.0010
population	p=0.035

Appendix 3.4: Correlation between elevation and average maximum daily temperature in Colorado where the dashed line indicates the significant linear relationship.



Call:
lm(formula = data$tmax_1 ~ data$elevation)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	37.3907630	0.4266011	87.65	<2e-16	***
data$elevation	-0.0055921	0.0002049	-27.29	<2e-16	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 4.51 on 2136 degrees of freedom
Multiple R-squared: 0.2585, Adjusted R-squared: 0.2581
F-statistic: 744.6 on 1 and 2136 DF, p-value: < 2.2e-16

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CHAPTER 4

SEASONALITY AND BAT RABIES: EFFECTS OF INCUBATION, ANNUAL REPRODUCTION AND SEASONAL MORTALITY ON RABIES PERSISTENCE

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INTRODUCTION

Many aspects of wildlife biology are strongly seasonal (Altizer et al. 2006). Mechanisms causing seasonal variation in the dynamics of human pathogens have been well explored (Anderson and May 1991; Keeling and Rohani 2007). Mechanisms generating seasonal patterns in pathogens circulating within wildlife populations, however, have not been as well investigated until recently (Swinton et al. 1998; Hosseini et al. 2004; Lass and Ebert 2006). Bat rabies dynamics exhibit a unique seasonal pattern in the number of cases relative to other wildlife reservoirs, indicating that rabies dynamics within bats behave differently than terrestrial carnivores (Blanton et al. 2007) (Fig. 4.1). Previous analyses using passive surveillance samples have demonstrated higher prevalence of bat rabies virus in the spring and especially fall throughout the United States (George et al. *in prep*; Mondul et al. 2003), yet no mechanistic explanation

for the seasonal pattern has been forwarded. Thus, why do bats have unique seasonal patterns of pathogen prevalence, and furthermore, what factors drive bat rabies virus seasonality?

Each year, rabies virus infection causes about 55,000 human deaths globally, mostly from dog bites in developing countries (Rupprecht et al. 1995). Successful vaccination programs have virtually eliminated dog rabies in the U.S. and Canada over the past 50 years and have the potential to control rabies virus in terrestrial carnivores (Rupprecht et al. 2002), but not in bats. Bat rabies virus (BRV) variants are currently the leading source of rabies in humans in the United States (Blanton et al. 2007). Therefore, bats provide a reservoir in which rabies virus will persist, and provide a source for new variants capable of re-infecting vaccinated populations (e.g., Leslie et al. 2006) and spreading to naïve populations.

Little is known about persistence mechanisms that allow BRV to be maintained enzootically in bat populations. There are few long-term data to address disease dynamics of these emerging agents in natural bat populations. Also, the majority of rabies modeling has focused on terrestrial carnivores (Lloyd-Smith et al. *in review*). Few BRV models that have been developed (Massad et al. 2001; Dimitrov et al. 2007; Dimitrov et al. 2008; Dimitrov and King 2008), and none explicitly consider the effects of seasonality. Modeling bat rabies dynamics has been underserved, leaving a paucity of knowledge about pathogen dynamics in this reservoir. In short, a detailed understanding of the pattern is missing, and, more importantly, the mechanisms that allow persistence and drive the seasonal pattern are poorly understood. In this paper, we address the mechanisms of BRV persistence and seasonality with a mechanistic model parameterized from empirical and experimental data. Specifically, we focus on how seasonal mortality, incubation periods, and reproduction could affect BRV persistence.

The paper is organized as follows. Initially, we describe the essential biology for big brown bats (*Eptesicus fuscus*) and bat rabies virus for a bat population studied for five years in Colorado. Subsequently, we incorporate this biology into a modified SLIR model designed to approximate BRV within big brown bats in Colorado. We compare the model predictions to empirical data and examine the impact of changes in the key factors (seasonal mortality, incubation periods, and bat reproduction) to bat population and viral persistence.

BAT AND BAT RABIES VIRUS BIOLOGY

Big brown bats, an insectivorous bat species, are wide-ranging and common in North America. This long-lived species can live up to approximately 20 years (Barbour and Davis 1969). In Colorado, their natural history can be described as a fusion-fission dynamic with intense periods of activity followed by an almost complete cessation of all activity, including physiological. Seasonal aggregation of individuals determines distinct periods within the year in which pathogen dynamics can be described by unique models representing disease dynamics within those periods. The three distinct ecological periods (transmission, hibernation, and pre-transmission seasons – Fig. 4.2) are associated with bat strategies to deal with seasonal climate. In Colorado, the transmission period begins as female bats form maternity roosts and lasts from approximately early June to mid-September. This period represents optimal climate conditions for big brown bats as well as optimal conditions for transmission of rabies virus among bats. During this period females create maternity colonies and give birth often in buildings near humans (Pape et al. 1999) (geometric mean: 47 adults in 53 colonies, max 219 in our area). In maternity roosts bats are in close contact allowing for maximum pathogen transmission. By mid-September bats

begin locating hibernacula and by mid-October all bats have gone until the following spring. In fall and winter, big brown bats (adult males, adult females and juveniles) enter the second period, hibernation (Barbour and Davis 1969), which can last up to six months (Beer and Richards 1956), and in Colorado they preferentially locate hibernacula in rock crevices at higher elevations (Neubaum et al. 2006). In early spring after bats arouse from hibernation, they enter the third period, pre-transmission, and they maintain use of torpor as females form maternity colonies (Grinevitch et al. 1995) close to humans. Ultimately, bat ecology, particularly seasonal life history patterns, can play a role driving seasonal bat rabies virus patterns (George et al. *in prep*).

(a) Seasonal mortality: Key factors affecting disease dynamics vary across the ecological periods (transmission, hibernation, pre-transmission). In particular, mortality rates vary across the different seasons, and previous research has allowed estimating seasonal variation of mortality rates in big brown bats (Ellison et al. 2007a). In brief, big brown bats effectively mitigate harsh climatic conditions in the winter months by hibernating. In addition, they escape the high mortality in the transmission season by entering hibernation with its associated lower mortality rates (Table 4.1, Fig. 4.3).

(b) Incubation period and facultative heterothermy: Rabies is a fatal disease caused by viruses in the genus *Lyssavirus* (Badrane and Tordo 2001), and is mainly transmitted through bites by infected saliva. Most lyssaviruses are Old World, but rabies virus also occurs in the New World, where it diversified into many host species, mostly bats (Hughes et al. 2005). Genetic evidence also suggests that host biology has a major influence on viral adaptation (Hughes et al. 2005; Franka et al. 2006). Only a proportion of bats exposed to rabies become infectious (Davis 2007). For those that do, incubation times in bats range from 2 – 25 weeks (Brass 1994) but can be greater than a year (Kaplan 1969). Upon

transmission, the virus needs to replicate, migrate to neural tissues, and ultimately the central nervous system for clinical signs to manifest. Sequestration in muscle tissues and the rate of viral replication will determine incubation times (Charlton et al. 1997).

Interestingly, big brown bats are facultative heterotherms. As such, their body temperatures maintain constant internal temperatures during optimal conditions and approach ambient temperatures during hibernation and torpor. During torpor, their metabolic rate also reduces to low levels correlating to ambient temperatures (Hock 1951; Speakman and Thomas 2003). Experimental research (Sadler and Enright 1959; Sulkin et al. 1960) suggests that cooler temperatures slow the rate BRV develops in bats. In short, cold temperatures enhance the effects of slow incubation times in bats and can act as a viral reservoir (Brass 1994).

(c) Annual birth pulse: Big brown bats give birth once annually (late June), and in our area the modal litter size is 1.0 (mean 1.1), and adult female survival is high (Ellison et al. 2007a). Lactation lasts approximately 32-40 days (Kunz 1974), and juveniles begin to fly in 18-35 days (Kurta et al. 1990). So by mid-to-late July, the juveniles born that year are volant and begin foraging for food. By early September, the juveniles are weaned and maternity colonies begin to disband. Also, there is no known vertical transmission of rabies within big brown bats (Brass 1994) but see (Steece and Calisher 1989), so the birth pulse supplies immunologically naïve individuals into the population each year.

MODEL

Bat ecology and rabies biology support the idea that local disease dynamics within this species can explain how BRV persists, and allows us to use a closed-system model to approximate population and disease dynamics. First, rabies virus variants are

species specific (Smith 1996; Hughes et al. 2005). Second, big brown bats have high roost fidelity across seasons (Ellison et al. 2007a). Thus, we can assume that we are capturing the dynamics of the same population through time. Third, big brown bats are non-migratory species (Shankar et al. 2004; Neubaum et al. 2006) such that they do not migrate on a continental or regional scale. Continental translocations such as those seen in ebolavirus or season influenza are not evident in bat rabies (Messenger et al. 2003). Thus, migration and long distance pathogen translocation can generally be dismissed as a major contributing factor to BRV disease dynamics in big brown bats (however, see (Cleaveland and Dye 1995) (Smith et al. 2005), and (Jeltsch et al. 1997). Fourth, there are no known multi-host dynamics for BRV in bats (Smith 1996; Shankar et al. 2005); which seems to be a requirement for rabies persistence in east African wildlife (Cleaveland and Dye 1995; Lembo et al. 2008). Alternative explanations of pathogen persistence such as multi-host transmission or consistent translocation of the pathogen from another location are not viable explanations based on bat ecology and molecular epidemiology. Therefore, modeling the population as a closed-system can provide insight regarding persistence mechanisms of BRV.

To accommodate the uniqueness of bat and BRV biology, we developed a model of BRV dynamics in big brown bats using a set of coupled sub-models to investigate seasonal mechanisms of persistence of BRV. The model consists of a set of sub-models that reflect discrete and different "seasons" of BRV within a year based on the ecology of big brown bats and biology of BRV (transmission season, hibernation, pre-transmission season, and birth pulse). Parameters are described in Table 4.1 and the parameterization section below. The seasonal BRV model (Fig. 4.4) we propose is characterized as follows:

Transmission season sub-model: (June 11 – October 1) The transmission season sub-model described in (1) follows females and juveniles in susceptible (S), latent (L_R and L_I), resistant (R), and infectious (I) disease classes (Anderson and May 1991) based on progression of rabies in bats. Bats that are infectious die quickly from the disease (Table 4.1), so natural mortality is ignored for infectious individuals. Density-dependent population regulation is a generally observed feature of wildlife populations (Keeling and Rohani 2007), so we included it in the transmission season submodel where it would be most readily apparent with a growing population that is aggregated for breeding.

$$\begin{aligned}
\frac{dS_j}{dt} &= -\beta S_j I - \mu_j S_j - \phi \mathrm{N} S_j \\
\frac{dL_{Rj}}{dt} &= (1-\rho)\beta S_j I - (\sigma_R + \mu_j + \phi \mathrm{N}) L_{Rj} \\
\frac{dL_{Ij}}{dt} &= \rho \beta S_j I - (\sigma_I + \mu_j + \phi \mathrm{N}) L_{Ij} \\
\frac{dR_j}{dt} &= \sigma_R L_{Rj} - \mu_j R_j - \phi \mathrm{N} R_j \\
\frac{dI_j}{dt} &= \sigma_I L_{Ij} - \upsilon I_j - \phi \mathrm{N} I_j
\end{aligned} \qquad (1)$$

where the *j* subscript indicates age classes: juvenile, year one adult females, year two and older adult females. Parameters are defined in Table 4.1. For simplicity in our model, we approximate the birth pulse in a single day (June 21) where births are added to the susceptible class. Because male bats roost separately and solitarily during the summer (Barbour and Davis 1969), we advance bats in each age class to the next highest age class and half of the juvenile age class (females) advances into the transmission season model to reflect the return of females to roost sites in spring. Following the birth pulse, the transmission model again is implemented.

Hibernation sub-model: (October 2-April 15) During hibernation disease dynamics are likely suspended because of the metabolic effects associated with torpor (Sadler and Enright 1959; Sulkin et al. 1960). Infectious bats die quickly from the disease (Table 4.1). Thus, the hibernation sub-model describes only overwinter mortality for each of the disease classes. Parameters are described in Table 4.1.

$$\begin{aligned}
\frac{dS_j}{dt} &= -\mu_{pj} S_j \\
\frac{dL_{Rj}}{dt} &= -\mu_{pj} L_{Rj} \\
\frac{dL_{Ij}}{dt} &= -\mu_{pj} L_{Ij} \\
\frac{dR_j}{dt} &= -\mu_{pj} R_j \\
\frac{dI_j}{dt} &= -\upsilon I_j
\end{aligned} \qquad (2)$$

Pre-transmission season sub-model: (April 16 – June 10) In early spring, BRV likely progresses within individuals, but transmission may be low because few individuals are interacting and the intermittent use torpor during this period will be more pronounces than during the transmission season. Thus, our model assumes BRV progresses within individuals, but no transmission occurs. Also, because pre-transmission occurs prior to the birth pulse (see below) it is assumed bat densities will be low which obviates the need for including density-dependence during this season. Parameters are described in Table 4.1.

$$
\begin{aligned}
\frac{dS_j}{dt} &= \mu_{pj} S_j \\
\frac{dL_{Rj}}{dt} &= -\sigma_{pR} L_{Rj} - \mu_{pj} L_{Rj} \\
\frac{dL_{Ij}}{dt} &= -\sigma_{pI} L_{Ij} - \mu_{pj} L_{Ij} \\
\frac{dR_j}{dt} &= \sigma_{pR} L_{Rj} - \mu_{pj} R_j \\
\frac{dI_j}{dt} &= \sigma_{pI} L_{Ij} - \upsilon I_j
\end{aligned}
\qquad (3)
$$

Parameterization: The model has been parameterized using literature and field data. Table 4.1 describes the main parameters in the model. Parameterization from literature is straightforward; however, we determined transmission rates from mark-recapture serology data from a five-year study of bat rabies within big brown bats in Fort Collins, Colorado (Appendix 4.A). Also, we were able to assess natural mortality rates at different times of the year from these same data (for details see (Ellison et al. 2007b)).

Sensitivity analysis and stochastic model: Sensitivity analysis was used to determine the quantitative impact of the uncertainty in these estimates on our results. Deterministic models can allow dynamics requiring few or partial individuals in a population. Stochastic simulations are required to consider models with integer individuals and thereby can be more realistically susceptible to extinction events when populations are small. Given there are few infectious individuals for a range of parameter space, we wanted to explore more fully how stochasticity would further restrict parameter space allowing pathogen persistence. The stochastic model allowed us to investigate enzootic fadeout dynamics, a well-known stochastic dynamic, and provide a more realistic consideration of parameter space that will drive host and viral extinction. We developed the stochastic implementation of the model using a Gillespie algorithm (Keeling and Rohani 2007).

Using the stochastic model, we explored parameter space generally, but with particular focus in the area of our estimated parameters, in order to determine what dynamical outcomes (e.g., viral and host persistence, disease free host, host population extinction) are possible and when they occur. Following this numerical stability analysis, sensitivity analysis was performed. Sensitivity is a measure of the relative importance of the input parameter with respect to the output variable. Following methods in Webb et al. (2006), sensitivity, S, is defined as the proportional change in an output variable, V, (e.g., persistence of BRV, persistence of bat population) for a given change in the value of a parameter, P:

$$\Sigma \approx \frac{\ln(V(P)) - \ln(V(P_0))}{\ln(P) - \ln(P_0)} \qquad (4)$$

This calculation is based on a pair of parameter values, the default value, P_0, and a second arbitrary value, P. The level of uncertainty in parameters can be used to inform the range of parameter values over which sensitivity is investigated. Uncertainty in parameters is more important for parameters to which the model output is highly sensitive. While we focused on the range of parameter values suggested from data, we also performed sensitivity analysis over a larger range of parameters in order to fully investigate the model's generality. Specifically, in our study the output variable was the percent of simulations (50 simulations per parameter combination) that maintain persistent (i) bat populations and (ii) viral dynamics.

RESULTS

Validation: The model quantifies the number of individuals in different disease classes (susceptible, latent, infectious, and recovered/resistant) through time. Both the deterministic and stochastic models generate results that are qualitatively similar.

Furthermore, we validated the model by comparing model output to empirical data from a five-year study on bat rabies in Colorado and estimates population sizes in the area. First, we considered how well the model predicted the bat population size. As a part of the five-year study on BRV in Colorado, researchers inspected 406 buildings of the approximately 65,000 addresses in the city limits of Fort Collins, CO. Of the buildings inspected 0.5-0.7% had a maternity colony at the time of the inspection. The observed roosts had a geometric mean size of 47 bats before the appearance of young in flight. Therefore, if we multiply the number of addresses by the proportion with maternity colonies and the mean number of bats (65,000 * 0.005 * 47 = 15,275) we generate a crude estimate of the number of bats in the system on the order of 15,000-20,000 bats in maternity roosts. Of course, following the birth pulse the population will increase substantially, perhaps doubling; however, this gives us a population estimate by which we can judge how well the model does in simulating a population similar to that seen in Fort Collins, CO. Figure 4.5 demonstrates that the model generates bat populations in the range of this estimate. Also, researchers sampled bat saliva with oral swabs at the maternity roosts and found approximately 0-1% of the bats presented rabies virus in their saliva (O'Shea et al. *in prep*). Similar studies on Brazilian free-tailed bats (*Tadarida brasiliensis mexicana*) found viral RNA in 0-2.5% of their salivary swabs (Dimitrov et al. 2007). Although infectious bats are highly seasonal, only within the transmission season, the model does generate approximately 1% of the total population (~200 bats) as infectious bats (Fig. 4.5). Our model predictions fell within the range of observed field dynamics such as the following: (1) estimate of total population of bats, ~20,000, and (2) proportion infectious, 0-2% (Fig. 4.5).

We also considered when the timing of the number of infectious individuals occurred each year. We compared model predictions to a time series of passive surveillance data from the Centers of Disease Control and Prevention exhibiting the number of positive cases of rabies in bats from Colorado (Fig. 4.6). The number of infectious bats generated by the model is larger than those in the empirical data but this is to be expected. Passive surveillance data is a sampling of the number of infectious cases while the model generates the predicted actual number of infectious in the population. Most importantly, the timing of the peak number of cases demonstrates that our model does well in generating a representative periodicity of infectious individuals (e.g., the predicted time of peak prevalences match well the observed time of peak prevalence in public health data). Lastly, we compared model predictions in the timing of prevalence in different age classes to rabies positive samples from the passive surveillance program of the Colorado Department of Public Health and Epidemiology (Fig. 4.7). The comparison demonstrated that adult female bats are infectious earlier in the year whereas infectious juveniles dominate samples in the late summer and autumn. Our model qualitatively reflects that female adults precede juveniles as the dominant group of infectious individuals, which gives us more confidence that the model does well representing the biology of this system. Thus, for a range of parameter values, including the default parameter set, a number of different model predictions quantitatively fit independent, empirical data on bat and bat rabies dynamics. Such model validation indicates that this model represents our current knowledge of the system and, hence, informs our understanding of bat rabies virus dynamics in big brown bats.

Model analysis and sensitivity analysis: Behavior of the seasonal deterministic model for parameter values surrounding the default parameter set demonstrates three general outcomes corresponding to three scenarios: (a) the bat population persists but BRV does not, (b) neither the bat population or BRV persists, and (c) the bat population and BRV persist (Fig. 4.8). These findings are consistent with analytical exploration of a simple SLIR model representing the transmission season (Dimitrov and King 2008, Appendix 4.B). Model behavior for the default parameter set provides model output where both bat population and BRV persist. Alternative model formulations were considered: pre-transmission sub-models with full disease dynamics but different parameter values (slower viral dynamics due to lower temperatures); and hibernation sub-models with transitions among disease classes but no new pathogen transmission (Appendix 4.C). However, overall dynamics did not differ qualitatively from those reported here. Model validation and sensitivity analysis results are also qualitatively invariant across these different model structures.

Using the stochastic implementation of the model, we considered how different parameters affected the model output. Sensitivity analysis on the stochastic implementation of the model demonstrated parameter thresholds for enzootic fadeout and bat population persistence. Sensitivity analysis demonstrated BRV extinction is affected strongly by three parameters (Fig. 4.9a): proportion of individuals infected that become infectious, or rather, the case-fatality rate ($\Sigma_r = 3.1$); the natural-mortality rate of juveniles during the transmission season ($\Sigma_{mj} = 3.8$); and the incubation period ($\Sigma_{sI} = 3.8$). Model output shows BRV persistence is determined by parameters that affect thresholds for population extinction (Fig. 4.10a), and thresholds for viral extinction (Fig. 4.10b). Also,

the case-fatality rate, ρ, affects both thresholds (Fig. 4.10c). Natural mortality rates, particularly for juveniles, and the disease-induced morality rates also were influential for both bat and rabies persistence (Fig. 4.9). Bat population extinction is largely insensitive to all parameters ($\Sigma < 0.5$; Fig. 6b) except the natural-mortality rate of juveniles during the transmission season ($\Sigma_{mj} = 3.3$) and proportion of individuals infected that become infectious ($\Sigma_r = 0.9$).

(a) Effect of seasonal mortality: Sensitivity analysis demonstrated that juvenile mortality rates, particularly in the transmission season, have a strong effect on model output. Big brown bat populations in Colorado experience substantial differences in mortality rates across a year (Fig. 4.3). To more fully consider the impact of variation in seasonal mortality, we explored model dynamics with and without pre-transmission and hibernation seasons. Thus, we constructed a non-seasonal model that used parameter values from the transmission season for 365 days with the birth pulse at the appropriate time. This non-seasonal model demonstrates long-term benefits of seasonally variable mortality rates. Bat populations in the model crash without lower mortality rates provided by the inclusion of hibernation and pre-transmission seasons (Fig. 4.11a). Sustained high mortality rates such as those in the transmission season move the bat population toward population extinction. To further demonstrate the influence of mortality rates on the population viability, we averaged mortality across the different seasons and projected the bat population for 100 years (Fig. 4.8c). The contrast of crashing (Fig. 4.8a) and a sustainable population (Fig. 4.8b,c) provide evidence for including hibernation and pre-transmission periods to mitigate transmission season mortality and allow bat population and, hence, pathogen persistence.

(b) Incubation period: Sensitivity analysis demonstrated that incubation period greatly impacts viral persistence. Figure 4.12 shows more explicitly how the incubation period affects viral dynamics. The length of the incubation period determines how many latent individuals are maintained in the population. Shorter incubation periods decrease the number of latent individuals, and longer periods increase number of latent individuals. Generally, the risk of pathogen extinction increases as the number of latent individuals decreases in the population.

(c) Effect of annual reproduction and age-structure: Annual reproduction serves as the main source of susceptible bats. Seasonal reproduction has been shown to be an important driver of pathogen dynamics (Hosseini et al. 2004; Altizer et al. 2006; Keeling and Rohani 2007). We considered how heterogeneity in immunity among age classes could allow rabies persistence. For the default parameter values, deterministic model output demonstrated that latent individuals enter and survive the hibernation period; however, infectious individuals do not (Fig 4.13a). Furthermore, latent adult bats emerge from hibernation, become infectious, and infect juvenile bats that amplify BRV infection quickly during the transmission period (Fig. 4.13b). As mentioned above, this interaction among age classes is consistent with independent empirical data from the Colorado Department of Public Health and Environment where age class data through time were available (Fig. 4.7).

DISCUSSION

This case study of rabies within a major reservoir in North America provides useful understanding of the general importance of seasonal factors in pathogen

persistence within wildlife systems. Using available empirical and experimental data we parameterized a theoretical model of disease dynamics to explore how these factors affect BRV persistence. We compared our model predictions to independent data from the field study and found our model does well in representing the biology of this system. Using this seasonal model, we explored the range of parameter space and characterized conditions that generated bat population viability and viral persistence in this system.

The mortality rate of the juveniles during the transmission period, incubation period in the transmission season, and case-fatality rate were the most important parameters driving bat population and pathogen dynamics. These parameters affect viral persistence in two different ways: (1) through effects on bat population viability, and (2) through effects on viral persistence within a viable bat population. Bat population extinction creates an envelope of opportunity for viral persistence. Generally, if the host population is not present then neither will be, ultimately, their directly transmitted pathogens. Juvenile mortality in the transmission season exemplifies how viral persistence is delimited by bat population viability (Fig. 4.10a). As the juvenile mortality rate increases past a threshold, bat population viability in the system, and therefore viral persistence, drastically declines (Fig. 4.10a). Also, variation in mortality rates across seasons plays a unique role in maintaining viable bat populations. Seasonal mortality allows bat populations to avoid extinction, or rather; mortality rates during the transmission season, particularly among juveniles, are too high for long-term population viability of big brown bats (Fig. 4.11). Hibernation provides a temporal refuge from seasonally harsh climate, and also a reprieve from high mortality during spring and

summer months with optimal climate. Bat population viability is a necessary but not a sufficient condition for viral persistence.

Rabies can be eliminated from the bat population without causing the bat population to crash. The incubation period does not affect population viability but does have a strong impact on viral persistence (Fig. 4.9,4.10b). Stochastic events such as epizootic fadeout can minimize viral persistence within a viable bat population. Epizootic fadeout is pathogen extinction because there are few susceptible individuals in the population immediately following an epizootic such that random fluctuations remove latent and infectious individuals and thereby the pathogen (Anderson and May 1991). The fewer latent or infectious bats in the population, the more prone it is to epizootic fadeout. The incubation period determines how quickly latent individuals, and ultimately infectious individuals, enter the population after transmission. Shorter incubation periods generate fewer latent individuals at any given time because latent bats progress to infectiousness quickly thereby leaving the latent class. And more importantly, in our model latent individuals follow an epizootic curve during the transmission season with the least number of latent individuals remaining in the population late in the summer (Fig. 4.13). Thus, the shorter the incubation period the more prone the population is to a rabies epizootic fadeout before the hibernation season occurs (Fig. 4.10b).

The interaction of incubation period with hibernation season generates an even more interesting dynamic. The longer the incubation period, the more likely infected bats will survive to hibernation and provide infectious contacts in the subsequent transmission season (Fig. 4.12). In essence, the longer the incubation period, the more infected individuals will enter hibernation, survive hibernation, and infect immunologically naïve

juveniles in the coming transmission season, thereby maintaining the pathogen in the system from year to year. The combination of long incubation period and the metabolic effect of cold temperatures during the hibernation season combine to make a temporal maintenance reservoir preserving rabies virus until the birth pulse provides a new supply of immunologically naïve bats.

The case-fatality rate generates more complex dynamics across its range (Fig. 4.10c) because it affects both bat population viability and viral persistence independently. At lower levels an insufficient number of infectious individuals are created, minimizing chains of transmission in the number of susceptible bats, and ultimately viral persistence. At the mid-range to higher values, bat population viability becomes increasingly uncertain as more and more bat individuals succumb to bat rabies and regulate the bat population. At high values of ρ, both the population and viral persistence are diminished simultaneously. For viral persistence, the value of ρ must be just right, not too high or too low.

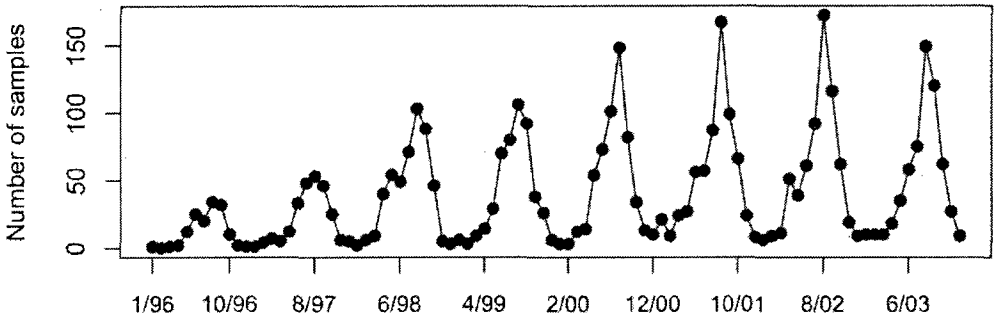
The annual birth pulse provides an immunologically naïve cohort in the spring which transmit BRV from infected adults surviving hibernation. The young of each year serve to amplify the pathogen in the system while the infected adults maintain bat rabies infections from the previous year (Fig. 4.13). This dynamic of amplifying and maintaining hosts is similar to multi-host persistence mechanisms in other host-pathogen systems (e.g., plague and prairie dogs (Gage and Kosoy 2005)). However, for big brown bats our model suggests age structure generates host that amplify and maintain the pathogen within the same species.

The interaction of hibernation with the incubation period and the birth pulse plays an important role in viral persistence and the seasonality of bat rabies cases. The use of hibernation, in part, dictates the seasonality of bat aggregation, reproduction, and cases of bat rabies. The combination of long incubation periods and the effects of cold during hibernation slowing viral activity allow BRV to avoid epizootic fadeout. Not only does hibernation allow big brown bats to escape sub-optimal climate conditions in the winter, it also allows bat populations to persist through high mortality during the optimal climate conditions in summer and until the next birth pulse in the spring. In this BRV system, if latent individuals survive through the transmission season (high mortality period) into the hibernation season (low mortality period) then many of those individuals, and the BRV they carry, can survive until a subsequent transmission season. Within infected bats, BRV survives hibernation, and infected bats become infectious thereby seeding bat rabies cases in the subsequent transmission season. Ultimately, a long-incubation period combined with the effects of slowing metabolisms during hibernation require few infectious individuals at any given point to maintain rabies within big brown bats indefinitely.

This research increases understanding of disease dynamics in wildlife populations and specifically within a significant disease reservoir. Given the potentially devastating effects of emerging diseases in bats on public health and wildlife conservation in the United States it is crucial that we improve understanding of how bat ecology relates to their propensity to serve as reservoirs for emerging pathogens (Messenger et al. 2002; Dobson 2005; Calisher et al. 2006; Wang et al. 2006). Because BRV shares many properties with other emerging pathogens associated with bats, this validated model can

be re-parameterized to predict dynamics of newly emerging diseases; this will be particularly important when less validation data are available and it is unclear which aspects of host ecology are important. Although we focus on a temperate zone bat species, our findings should also be informative in tropical systems where bats also exhibit seasonal migrations and changes in density and daily torpor driven by resource availability (Fleming and Eby 2003; Speakman and Thomas 2003).

(a)



(b)

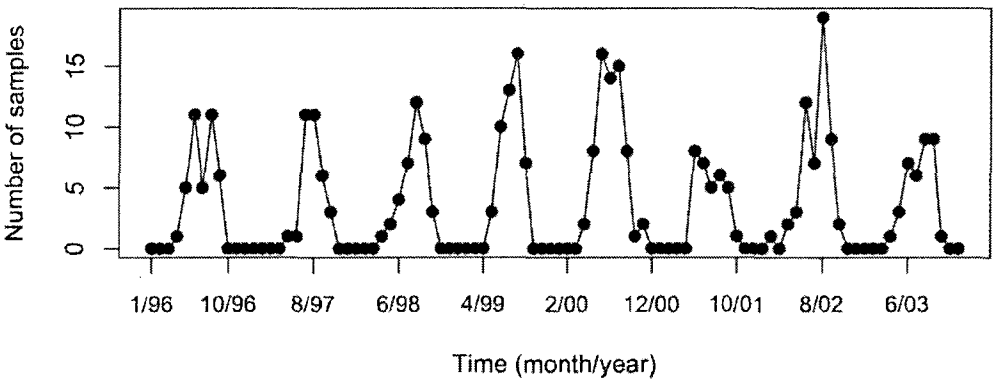


Figure 4.1. Time series of positive bat rabies samples by month from 1996-2003 in the (a) United States, and (b) Colorado.

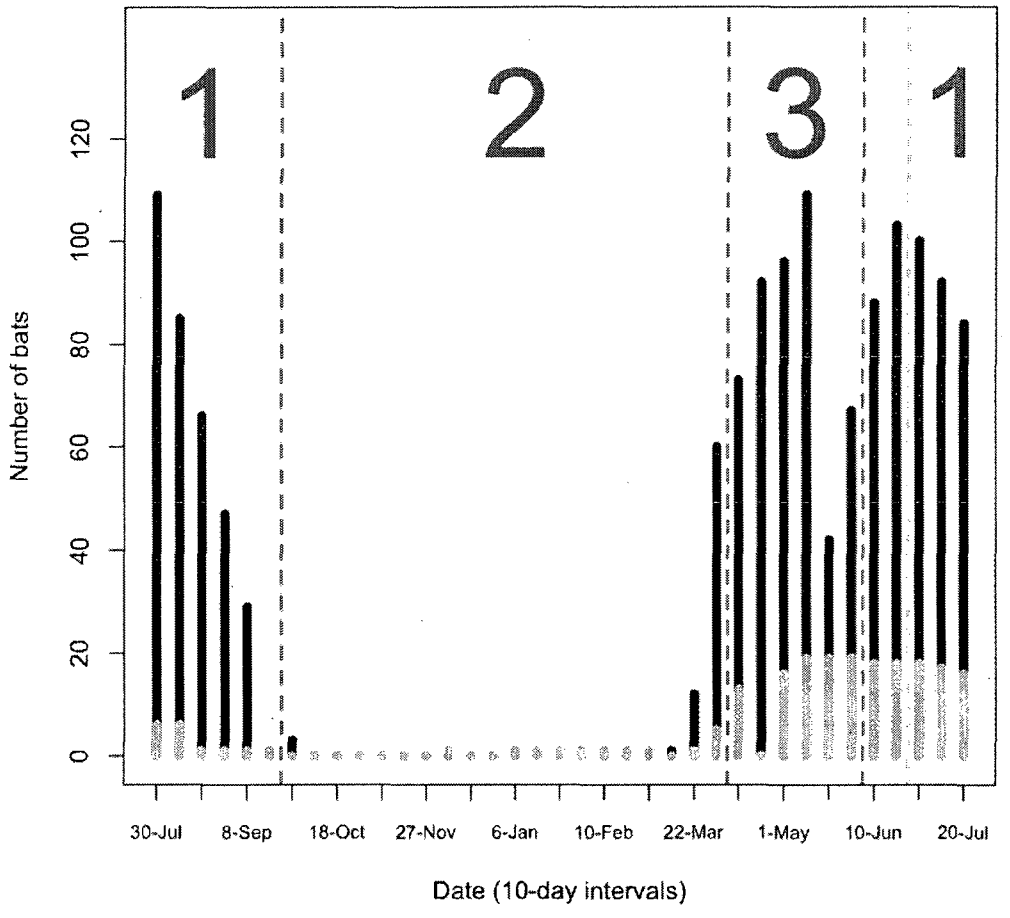


Figure 4.2. Annual cycle of attendance patterns of big brown bats *(Eptesicus fuscus)* in Fort Collins, Colorado (2004-2005) that were individually identifiable based on PIT-tags registered at two maternity colony sites: (grey bars) building GBH with 132 tagged known bats alive at the onset of monitoring, and (black bars) building PET with 22 tagged. Each bar represents the number of visits during ten-day intervals beginning on the date designated on the x-axis. The dotted line represents the approximate birth pulse (June 21) and the dashed lines delimit the different ecological periods: 1. transmission season, 2. hibernation season, 3. pre-transmission season. The blue numbers identify the ecological periods.

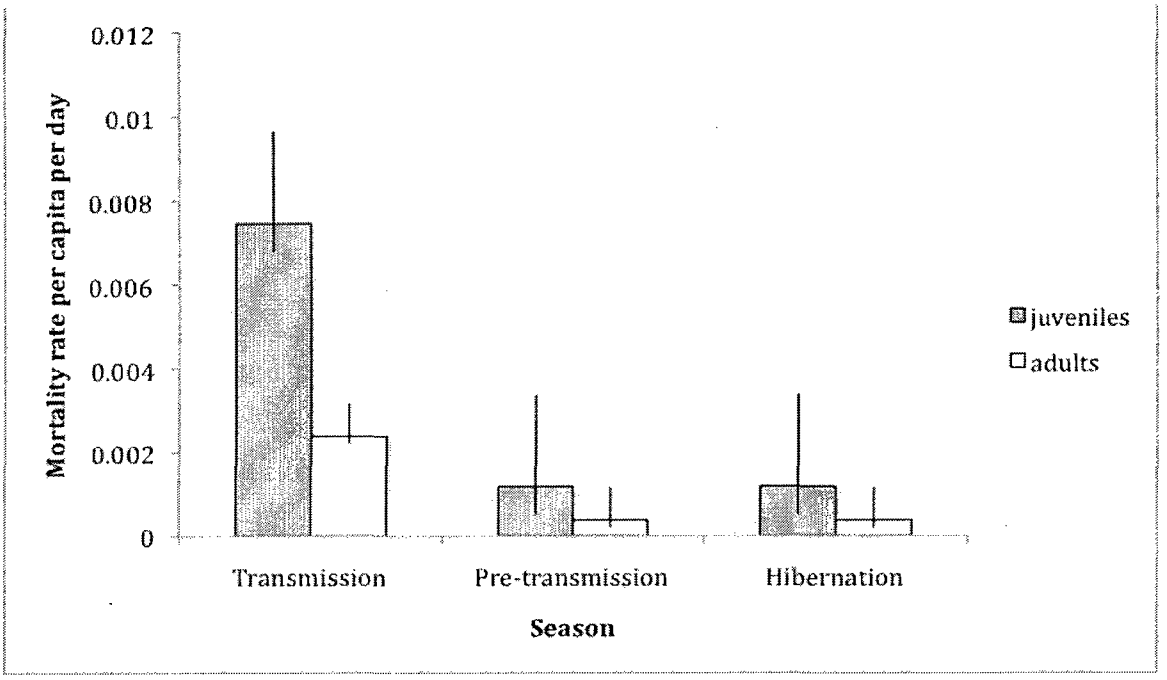


Figure 4.3. Seasonal variability of mortality rates for big brown bats *(Eptesicus fuscus)* by age class.

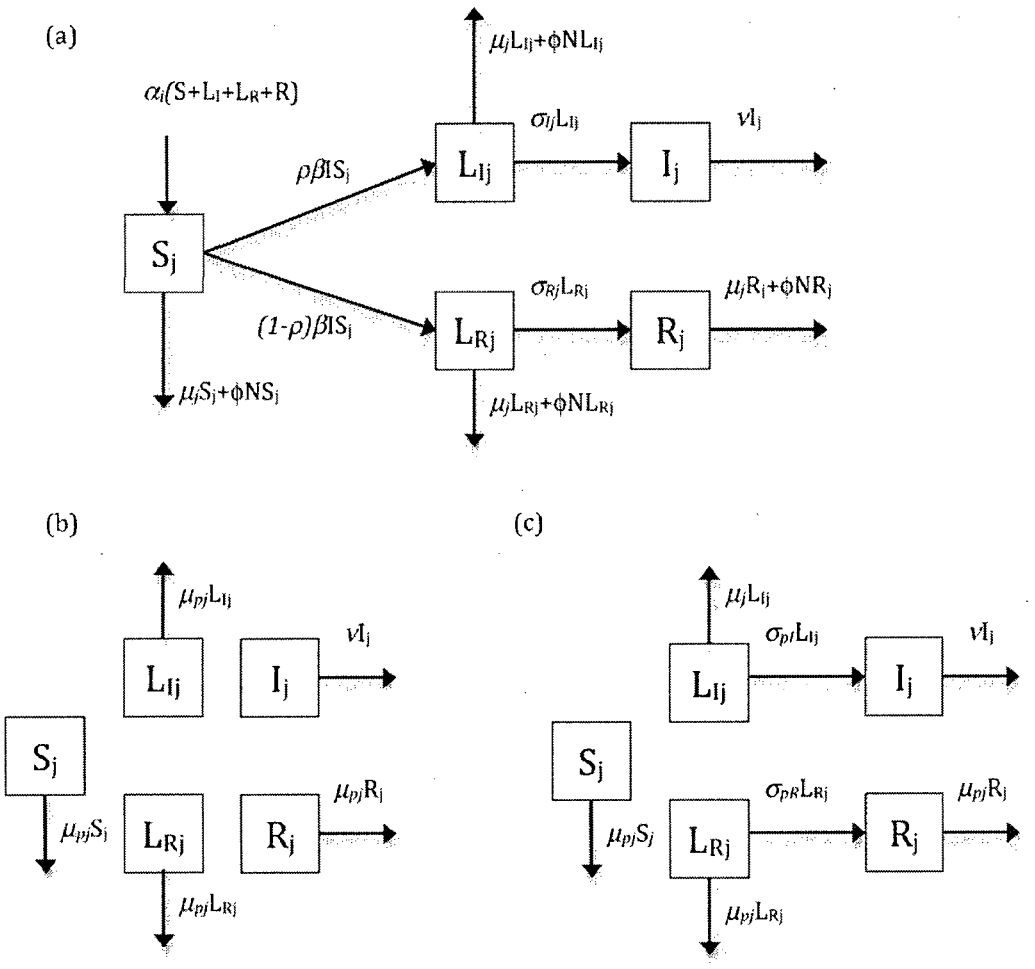
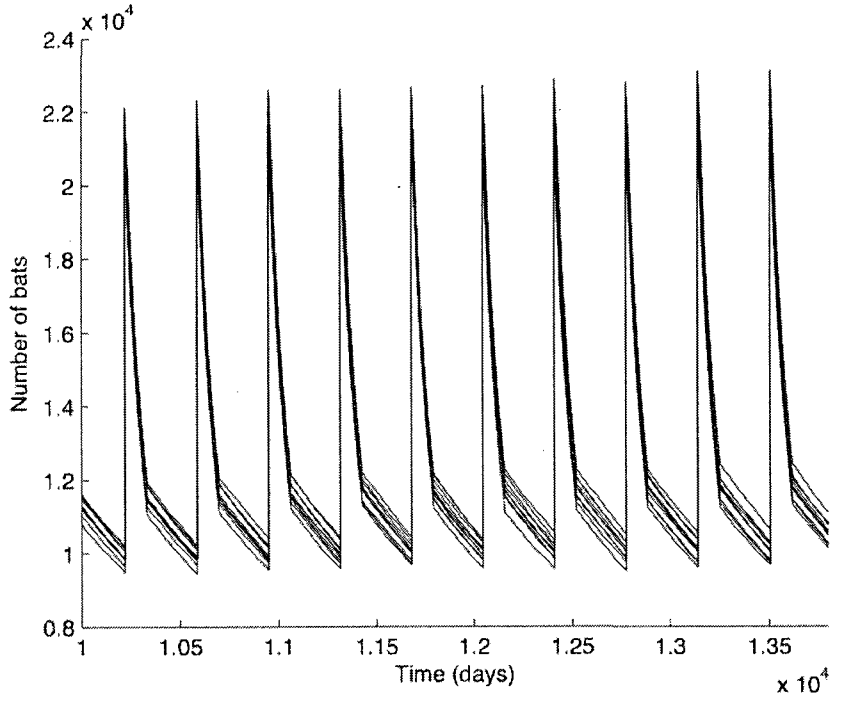
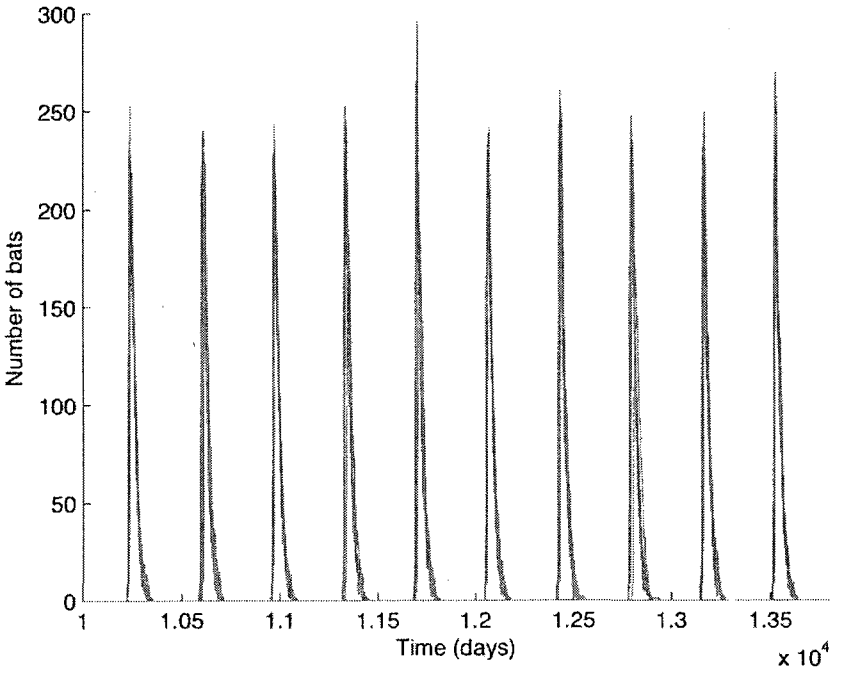


Figure 4.4. Diagram of a modified SLIR model representing generalized BRV dynamics in big brown bat (*Eptesicus fuscus*) populations in the (a) transmission season, (b) hibernation season, and (c) pre-transmission season.



(a)



(b)

Figure 4.5. Model dynamics, for the default parameters, over a ten-year period demonstrating (a) total bat population, and (b) number of infectious bats. The bat population is qualitatively similar to observed estimates of bat population size (~15,000-20,000 individuals) and proportion of infectious bats (0-1%).

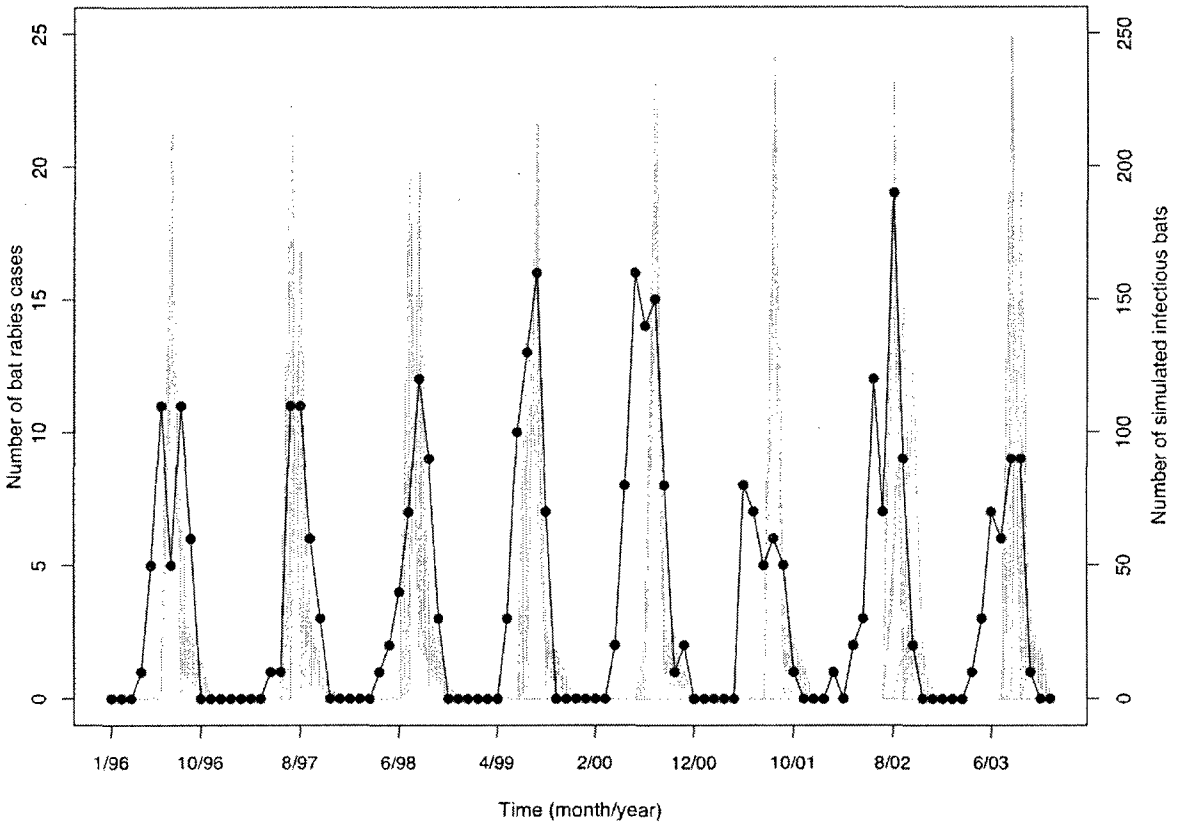
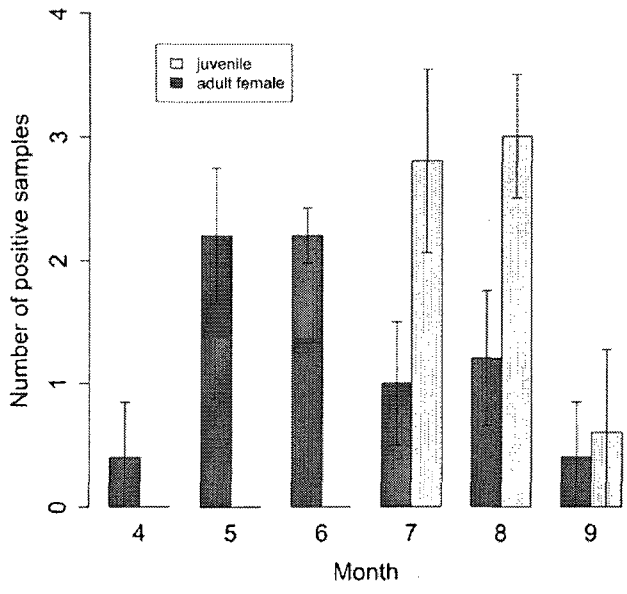


Figure 4.6. Comparison of empirical time series of monthly bat rabies cases in Colorado from 1996-2003 (black) and simulated time series of number of infectious bats (gray) from 100 runs of the model with default parameters. The simulation does well representing the timing of rabid bats in Colorado.

(a)



(b)

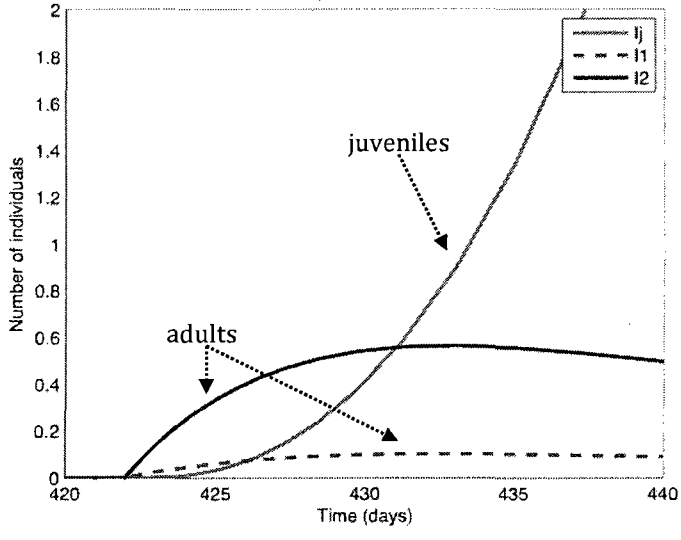
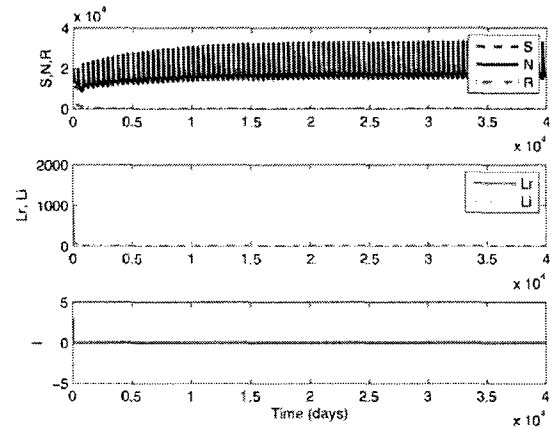


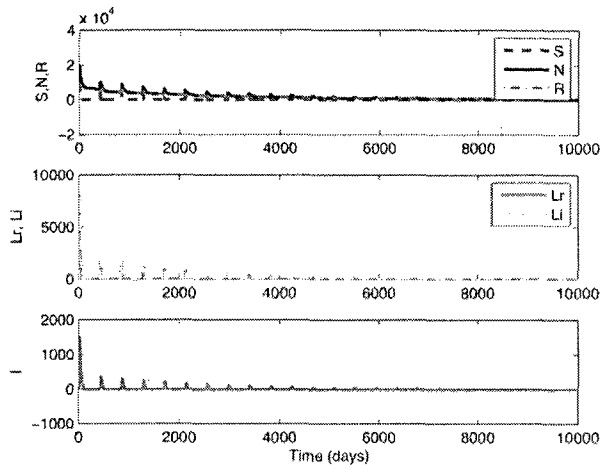
Figure 4.7. Comparison of empirical data to model output demonstrating the model qualitatively represents the system. (a) Average number of positive samples (bars are one standard deviation) submitted to the Colorado Department of Public Health and Epidemiology across months for the years 2001-2005. (b) Model output demonstrating adults (I1, I2) are infectious prior to juvenile bats. Empirical data provided by Tom O'Shea.

(a)



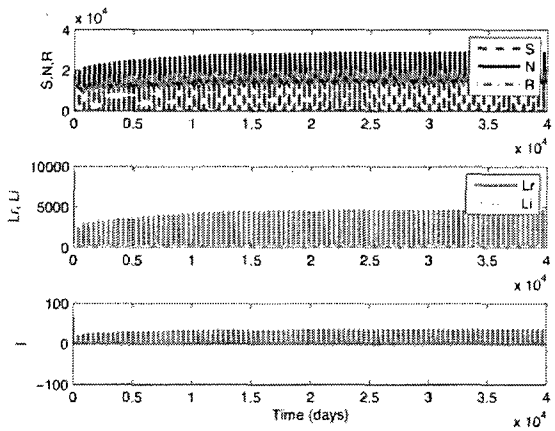
ρ = 0.001

(b)



ρ = 0.5

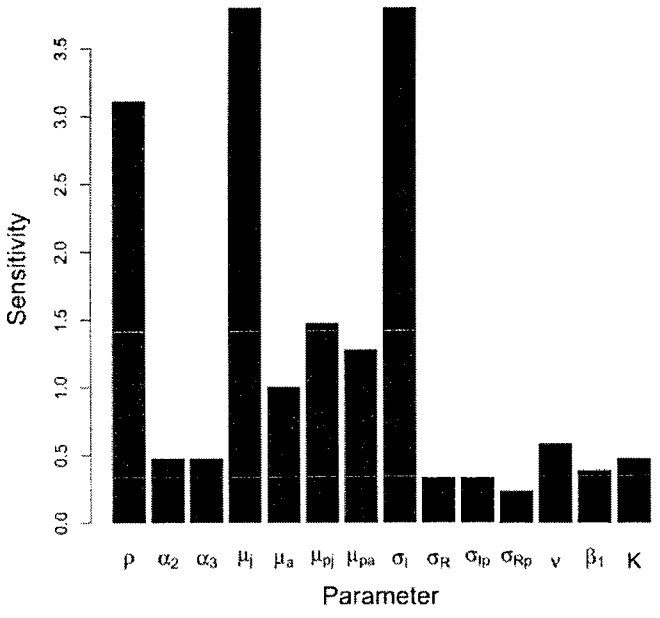
(c)



default parameters

Figure 4.8. General dynamical behavior for deterministic seasonal model demonstrating (a) bat population persists but BRV does not, (b) neither bat population or BRV persist, and (c) both persist.

(a)



(b)

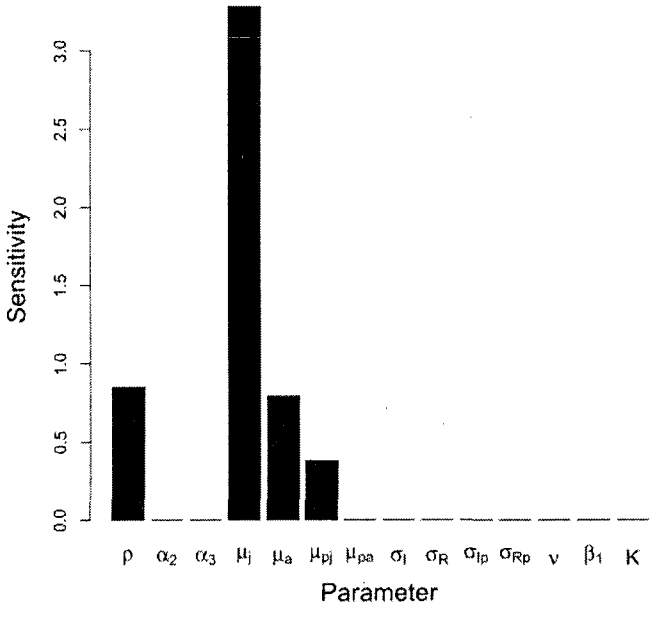


Figure 4.9. Sensitivity analysis for (a) pathogen extinction and (b) bat population extinction.

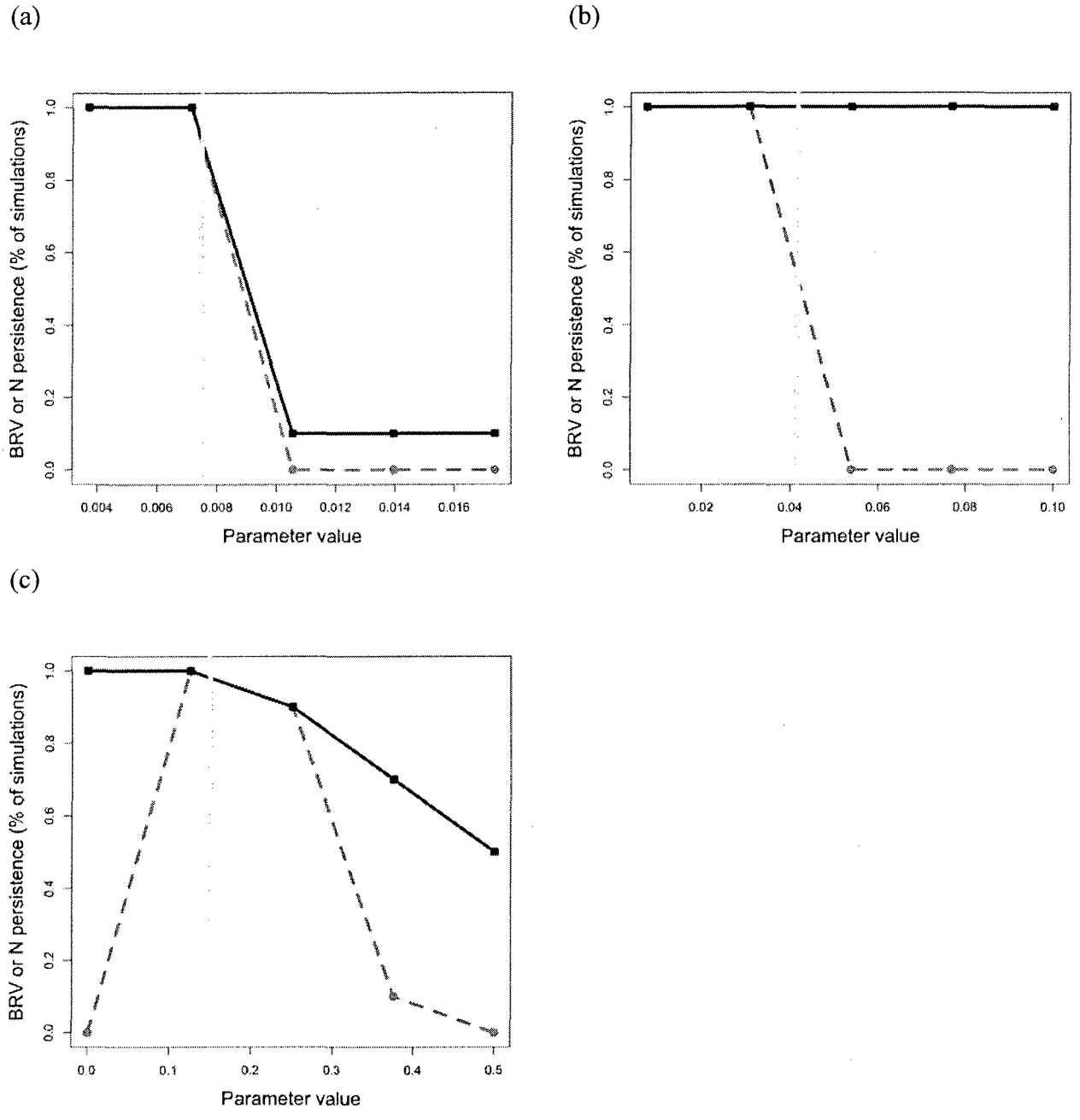


Figure 4.10. Model sensitivity (percent of simulations that have rabies or bat population persistence) for three key parameters: (a) juvenile mortality rate during the transmission season, μ_j; (b) incubation period, σ_I; and (c) the case-fatality rate, ρ. Black squares and solid lines represent the proportion of simulations with persistent bat populations. Gray circles and dashed line represent the proportion of simulations with persistent rabies. The vertical light gray bar identifies the default parameter value.

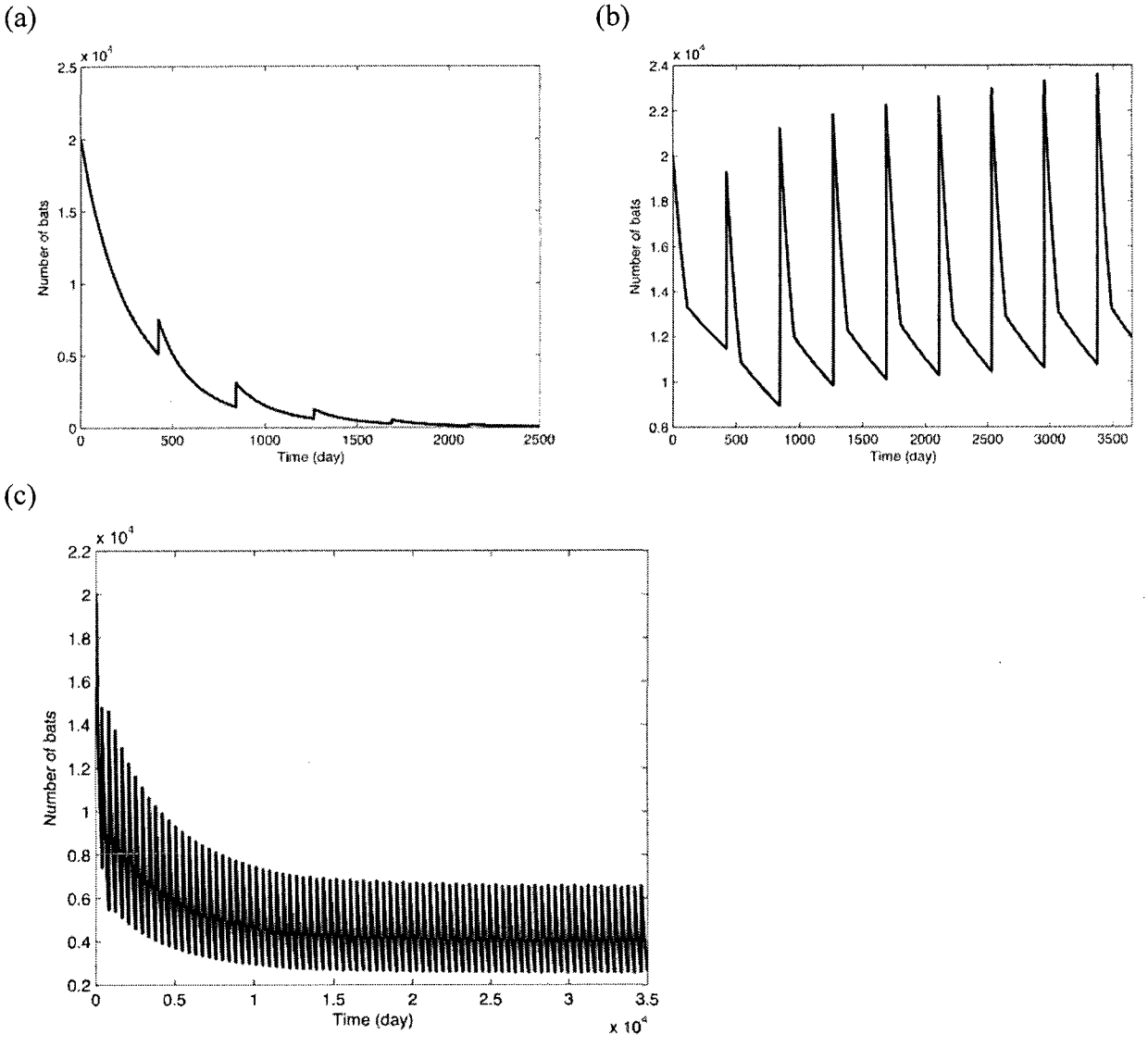


Figure 4.11. Bat population dynamics for (a) non-seasonal model that uses default parameters from the transmission season, (b) seasonal model with default parameters, and (c) non-seasonal model with averaged mortality rates. Without mitigating mortality rates in the transmission season, the model predicts bat populations will not be viable over the long-term.

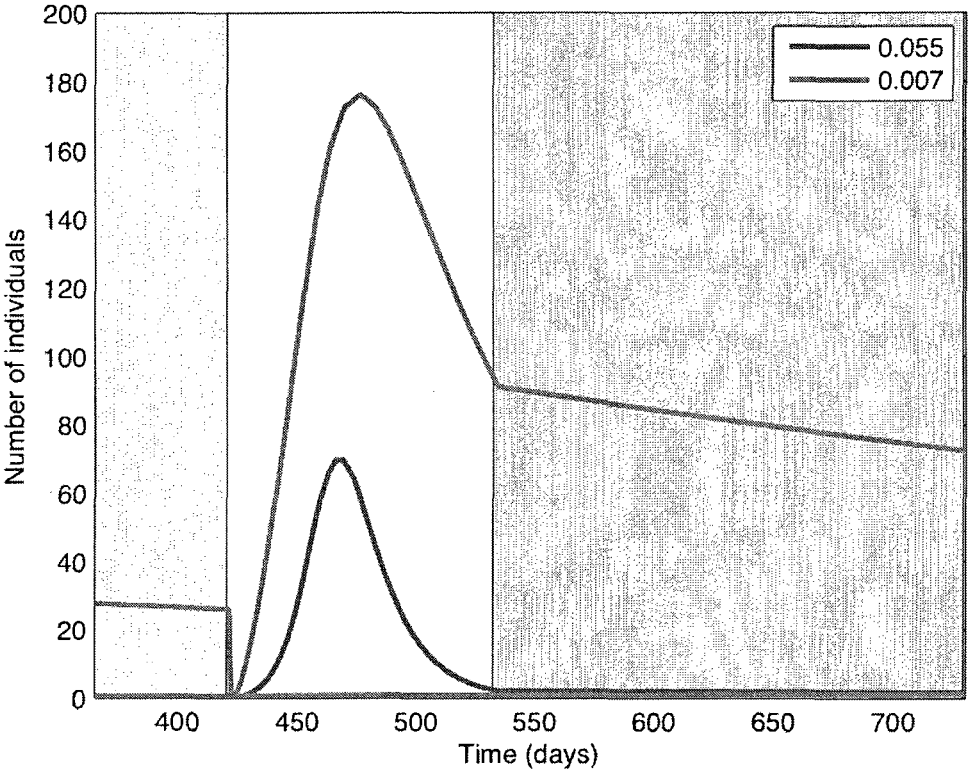
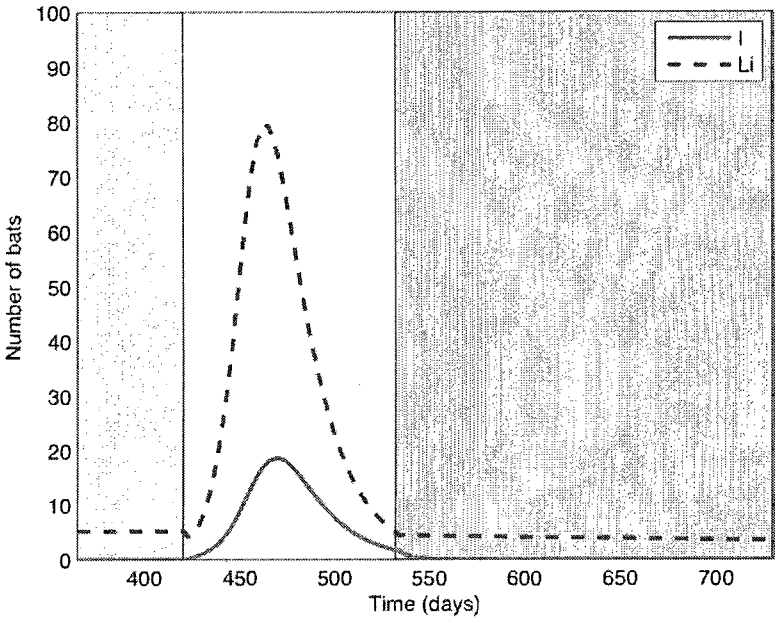


Figure 4.12. Deterministic dynamics of latent individuals that will become infectious evaluated at different incubation periods, s_I. The plot covers all three seasons of the model: pre-transmission (light gray), transmission (white), and hibernation (dark gray).

(a)



(b)

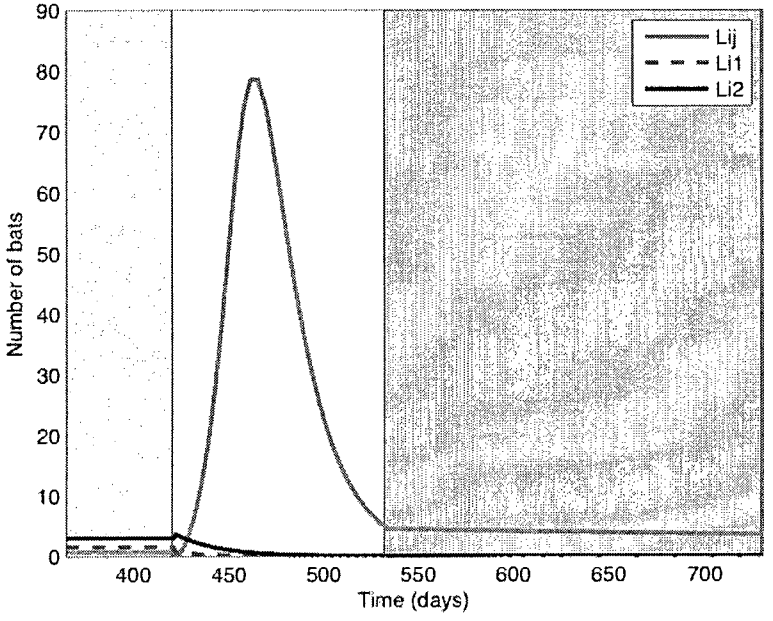


Figure 4.13. Deterministic model output for one annual cycle (using default parameter values) demonstrating (a) dynamics of infectious and latent bats, and (b) latent bats by age class. The shaded areas represent different ecological periods: pre-transmission (light gray), transmission (white), and hibernation (dark gray).

TABLE 4.1. BRV PARAMETER VALUES AND JUSTIFICATION.

Variable or Parameter	Description	Default value	Value variance	Current Reference
N	Total hosts	15,275	15,000-20,000	See text
$(1-\rho)\beta$	Transmission rate per day per capita for those that become infectious	1/190	1/265-1/187	Appendix 4.A
μ_j	Natural mortality rate per day per capita, juveniles in transmission season	0.0075	0.0037-0.017	Laura Ellison
μ_a	Natural mortality rate per day per capita, adults in transmission season	0.0024	0.0019-0.0030	Laura Ellison
μ_{pj}	Natural mortality rate per day per capita, juveniles in pre-transmission season AND hibernation season	0.0012	0.00067-0.0022	Laura Ellison
μ_{pa}	Natural mortality rate per day per capita, adults in pre-transmission season AND hibernation season	0.00037	0.00018-0.00078	Laura Ellison
ρ	Proportion of exposed hosts that eventually become infectious	0.15	0-0.5	Dick Bowen
σ_I^{-1}	Transmission season incubation rate per capita per day	24	10-130	Literature, Dick Bowen
σ_R^{-1}	Transmission season immunity rate per capita per day	14	2-365	Literature, Dick Bowen
σ_{Ip}^{-1}	Pre-transmission season immunity rate per capita per day. Also, for hibernation season.	48	10-130	Literature, Dick Bowen
σ_{Rp}^{-1}	Pre-transmission season incubation rate per capita per day. Also, for hibernation season.	28	2-365	Literature, Dick Bowen
ν^{-1}	Disease-induced morality rate per day per capita	6	2-10	Literature, Dick Bowen
α_2	Reproductive rate of first year adults per season per capita	0.79	0.75-0.82	Tom O'Shea
α_3	Reproductive rate of adults older than one year per season per capita	1.05	1.02-1.07	Tom O'Shea
K	Carrying capacity of the big brown bats	3e7	1e5-1e9	
ϕ	Density dependent mortality rate where ϕ = r/K, and r is the intrinsic growth rate which for our model is $r = (a_2+a_3)/2-(m_1+m_2)/2$	3.04e-8	9e-6 – 9e-8	
T_t	Length of transmission season in days, June 11- Oct 1	112	NA	Tom O'Shea
T_n	Length of pre-transmission season in days, April 16 - June 10	55	NA	Tom O'Shea
T_b	Length of birth pulse in days; June 21	1	June 10 – June 29	Tom O'Shea
T_h	Length of hibernation season, Oct 2 – April 15	197	NA	Tom O'Shea

Appendix 4.A: Estimation of transmission rates from serology data

We modified methodology for estimating nest survival (Stanley 2000) to estimate the rate of seroconversion in a bat rabies system. The utility of this method centers on dealing with data that comes from repeated sampling events. The time between sampling events is considered in the estimation of the parameter as follows:

$$P(Y = y \mid p) = (p^t)^y (1 - p^t)^{1-y}$$

where y is the fate of the nest (y=1 for survived or y=0 for failed), p is the daily survival probability, and t is the time interval between sampling events. So the likelihood function becomes:

$$\prod_{t \in T} (p^t)^y (1 - p^t)^{1-y}$$

Ultimately, using standard maximum-likelihood methods one can estimate p.

Additionally, p was further interpreted for the bat rabies virus (BRV) system within the context of a simple death process where $p = \exp(-\theta t)$. If we think of seroconversion as a simple death process that describes how individuals move from seronegative to seropositive, then we can provide more structure to p. Following Renshaw (1991) we can characterize a simple death process as follows:

$$q(t + h) = q(t)(1 - \theta t)$$

where

> *$q(t+h)$ = Pr(it is alive at time t and does not die in the subsequent small time interval h)*

On letting $h \rightarrow 0$:

$$\frac{dq(t)}{dt} = -\theta q(t)$$

which solves as:

$$q(t) = \exp(-\theta t)$$

and

$$\begin{aligned} p(t) &= Pr(\text{organism is dead by time } t) \\ &= 1 - q(t) \\ &= 1 - \exp(-\theta t) \end{aligned}$$

Thus, in our situation:

$$p = exp(-\theta t)$$

and this gives us the following likelihood function:

$$\prod_{t \in T} [\exp(-\theta t)]^{y} [1 - \exp(-\theta t)]^{1-y} \qquad (2)$$

We were able to estimate θ in a Bayesian framework in WinBUGS using the likelihood equation (2) giving us the following estimates:

Iterations = 5001:15000
Thinning interval = 1
Number of chains = 3
Sample size per chain = 10000

1. Empirical mean and standard deviation for each variable,
plus standard error of the mean:

	Mean	SD	Naive SE	Time-series SE
deviance	1.764e+02	1.3958771	8.059e-03	1.220e-02
theta	4.654e-03	0.0008416	4.859e-06	4.746e-06

2. Quantiles for each variable:

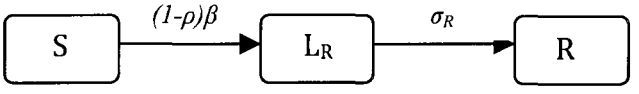
	2.5%	25%	50%	75%	97.5%
deviance	1.754e+02	1.755e+02	1.759e+02	1.767e+02	1.804e+02
mu	3.139e-03	4.058e-03	4.602e-03	5.196e-03	6.419e-03

Potential scale reduction factors:

	Point est.	97.5% quantile
mu	1.00	1.00
deviance	1.00	1.00

We were able to estimate $\theta = 0.00465$, which corresponds to a rate of something happening approximately every 214 days.

We gave further definition to the θ by considering which classes in the model can be considered seronegative and seropositive. The infectious and recovered individuals are considered seropositive and all other will be seronegative. We can reasonably exclude considering the pathway to infectious individuals when estimating the rate of seroconversion (Fig. 4a) because these individuals are short-lived and most likely will not be captured and represented in the data. Thus, for a seronegative bat to become a seropositive bat, it must follow the pathway from susceptible to recovered individual that is comprised of the following rates:



Thus, $\theta = (1-\rho)\beta + \sigma_R = 1/214$.

Because we have independent estimates of per capita per day immunity rate for BRV, $\sigma_R = 1/24$, we can further bound our θ estimate by subtracting the immunity rate which gives:

$(1-\rho)\beta = 1/190$

Appendix 4.B: Analytical evaluation of a modified SEIR model and R_0 calculations

Following Dimitrov and King (2008), we developed a simple SEIR model to explore general BRV dynamics during the transmission season in big brown bats (*Eptesicus fuscus*) in Colorado. An SLIR model structure is appropriate for bat rabies because bats exhibit an incubation period (Table 4.1) that has been documented by observational and experimental work (Shankar et al. 2004; Jackson et al. 2008). The SEIR model is as follows:

$$\dot{s} = \alpha - \mu s - \beta s i$$
$$\dot{I}_R = (1-\rho)\beta s i - \mu I_R - \sigma I_R$$
$$\dot{I}_I = \rho\beta s i - \mu I_I - \sigma I_I$$
$$\dot{i} = \sigma_L I_I - \upsilon i$$
$$\dot{r} = \sigma_R I_R - \mu r$$
$$\dot{n} = \dot{s} + \dot{I}_R + \dot{I}_I + \dot{i} + \dot{r} = (\alpha - \mu - \upsilon i)$$

where β is the transmission rate, μ is the natural mortality rate, ρ is the proportion of latent hosts that become infectious, σ_I is the incubation rate, σ_R is the immunity rate, α is the reproduction rate, and υ is the disease-induced mortality rate.

We can use this model to calculate R_0 (see below) in order to consider an enzootic threshold in terms of the case-fatality rate because BRV persistence requires $R_0 > 1$. Thus,

$$R_0 = \frac{\rho\beta\sigma_I}{\upsilon(\sigma_I + \mu)} = 1$$
$$\rho = \frac{\upsilon(\sigma_I + \mu)}{\beta\sigma_I}$$

determines an enzootic threshold, and, if

$$\rho < \frac{\upsilon(\sigma_I + \mu)}{\beta\sigma_I}$$

then $R_0 < 1$, and the bat population clears the BRV from the population. However, if the case-fatality rate, ρ, is high then the system is dictated by the infectious equilibrium, i^*, or rather, there is another threshold, the bat population survival threshold. If ρ, is high, then solving for the infectious equilibrium tells us about this threshold. Thus, if the population is to at least maintain itself then,

$$\dot{n} \geq 0$$

Using this requirement we can solve for the infectious equilibrium as follows:

$$\dot{n} = (\alpha - \mu - \upsilon i) = 0$$

$$i^* = \frac{\alpha - \mu}{\upsilon}$$

Furthermore, we can derive the requirement for bat population persistence; which is

$$i^* < \frac{\alpha - \mu}{\upsilon}$$

The combination of the two thresholds, survival and enzootic threshold, generate three possible outcomes including (a) a bat population goes locally extinct along with BRV creating an endangered population, (b) a growing bat population but BRV goes asymptotically extinct resulting in a recovering population, and (c) a persistent bat population and BRV dynamic that creates a reservoir population (Fig. 2).

R_0 calculations:

The basic modified SEIR model is as follows:

$$\dot{S} = \alpha N - \mu S - \beta S \, I/N$$

$$\dot{L}_R = (1-\rho)\beta S \, I/N - \mu L_R - \sigma L_R$$

$$\dot{L}_I = \rho\beta S \, I/N - \mu L_I - \sigma L_I$$

$$\dot{I} = \sigma_L L_I - \nu I$$

$$\dot{R} = \sigma_R L_R - \mu R$$

We re-arrange the model as follows:

$$\dot{L}_I = \rho\beta S \, I/N - \mu L_I - \sigma L_I$$

$$\dot{I} = \sigma_L L_I - \nu I$$

$$\dot{S} = \alpha N - \mu S - \beta S \, I/N$$

$$\dot{L}_R = (1-\rho)\beta S \, I/N - \mu L_R - \sigma L_R$$

$$\dot{R} = \sigma_R L_R - \mu R$$

Following (van den Driessche and Watmough 2002) we define the disease free equilibrium (DFE) and construct the vectors **F** and **V** as follows:

$$F = \begin{bmatrix} \rho\beta S \, I/N \\ 0 \\ 0 \\ 0 \end{bmatrix}, V = \begin{bmatrix} \mu L_I + \sigma_I L_I \\ \upsilon I - \sigma_I L_I \\ \mu S - \alpha N \\ \mu L_R + \sigma_R L_R - \beta S \, I/N \\ \mu R - \sigma_R L_R \end{bmatrix}, DFE = \begin{bmatrix} 0 \\ 0 \\ N \\ 0 \\ 0 \end{bmatrix}$$

With f, v evaluated at the DFE as follows:

$$f = \begin{bmatrix} \frac{dF_1}{dL_I} & \frac{dF_1}{dI} \\ \frac{dF_2}{dL_I} & \frac{dF_2}{dI} \end{bmatrix}_{DFE} = \begin{bmatrix} 0 & \rho\beta \\ 0 & 0 \end{bmatrix}$$

$$v = \begin{bmatrix} \frac{dV_1}{dL_I} & \frac{dV_1}{dI} \\ \frac{dV_2}{dL_I} & \frac{dV_2}{dI} \end{bmatrix}_{DFE} = \begin{bmatrix} \mu + \sigma_I & 0 \\ -\sigma_I & \upsilon \end{bmatrix}$$

$$fv^{-1} = \begin{bmatrix} \frac{\rho\beta\sigma_I}{\upsilon(\sigma_I + \mu)} & \frac{\rho\beta}{\upsilon} \\ 0 & 0 \end{bmatrix}$$

Giving R_0 as in the following form:

$$\rho(fv)^{-1} = R_0 = \frac{\rho\beta\sigma_I}{\upsilon(\sigma_I + \mu)}$$

Dynamics of the modified SEIR model demonstrate populations of the host population and pathogen that either (a) both do not persist, (b) the host population persists but the pathogen does not, or (c) both persist.

Appendix 4.C: Alternative model structures for the pre-transmission season

We considered different model structures for the pre-transmission season. We considered that the pre-transmission season was no different than hibernation in terms viral activity. Thus, none of the disease classes changed during the pre-transmission season.

$$\frac{dS_j}{dt} = -\mu_{pj} S_j$$
$$\frac{dL_{Rj}}{dt} = -\mu_{pj} L_{Rj}$$
$$\frac{dL_{Ij}}{dt} = -\mu_{pj} L_{Ij}$$
$$\frac{dR_j}{dt} = -\mu_{pj} R_j$$
$$\frac{dI_j}{dt} = -\upsilon I_j$$

An alternative form of pre-transmission season was also considered in order to test the importance of this season to overall pathogen dynamics. It assumes that rabies transmission occurs during the pre-transmission season albeit at lower levels because of lower temperatures, and is characterized as follows:

$$\frac{dS_j}{dt} = -\beta_p S_j I - \mu_{pj} S_j$$
$$\frac{dL_{Rj}}{dt} = \rho\beta_p S_j I - \sigma_{pR} L_{Rj} - \mu_{pj} L_{Rj}$$
$$\frac{dL_{Ij}}{dt} = (1-\rho)\beta_p S_j I - \sigma_{pI} L_{Ij} - \mu_{pj} L_{Ij}$$
$$\frac{dR_j}{dt} = \sigma_{pR} L_{Rj} - \mu_{pj} R_j$$
$$\frac{dI_j}{dt} = \sigma_{pI} L_{Ij} - \upsilon I_j$$

Lastly, we considered model without density-dependence in the transmission season. All of these models behaved qualitatively similar to those presented. The major difference was that in some regions of parameter space there was exponential population growth. However, this does not change the major findings of the paper.

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CHAPTER 5

GENERAL CONCLUSIONS

This dissertation focuses on determining mechanisms that allow two different pathogens (*Yersinia pestis* and rabies virus) to persist long-term in their respective wildlife systems. Within Chapter 1, the plague and prairie dog model, we considered the necessity of alternate host reservoirs for the persistence of plague. Classically, plague persistence is thought to occur because the bacterium is maintained at low levels in an enzootic cycle including partially resistant rodent hosts, with occasional spillover to highly susceptible hosts like humans and prairie dogs (Poland and Barnes 1979; Poland et al. 1994; Gage and Kosoy 2005). However, little direct evidence supports this classical view (Cully and Williams 2001; Gage and Kosoy 2005; Salkeld and Stapp 2006; Salkeld and Stapp 2008). We developed a stochastic patch occupancy model to determine if prairie dog populations could persist long-term given the effects of plague. Using this model, we demonstrated that prairie dogs can persist long-term and do not require an alternate host reservoir. The spatial structure of prairie dogs populations creates spatial refuges on the landscape that allow prairie dogs sub-populations to escape the effects of plague until depopulated towns are re-colonized. Hence, metapopulation structure allows long-term persistence of prairie dogs. Because plague causes the majority of local extinctions of prairie dog towns, we can also infer that plague persists long-term as well.

Alternative vector hosts create a refuge for the pathogen (like alternative disease hosts have already been shown to do) by decoupling local host extinction and landscape level pathogen transmission. Thus, spatial structure creates a host refuge and, more than likely, alternative vector hosts create a pathogen refuge, allowing prairie dog and plague persistence despite the high virulence of plague.

In Chapter 2, we considered whether life history or environmental factors primarily cause seasonal patterns of bat rabies cases. We developed statistical models to compete hypothetical drivers causing seasonality of bat rabies cases. Overall, the model containing temporal covariates best explains the variation in prevalence given the passive surveillance data. This suggests that intrinsic temporal factors, such as the seasonal dynamics of bat population biology and immunity, are most important in driving seasonal bat rabies prevalence.

In Chapter 3, we continued investigating the seasonality and persistence mechanisms of bat rabies by developing a mathematical model to represent big brown bat (*Eptesicus fuscus*) and rabies biology in northern Colorado. We demonstrated rabies persists in two distinct ways, (1) through effects on bat population viability, and (2) through effects on viral persistence within a viable bat population. Variation in mortality rates occurs across the year, and is primarily responsible for viable bat populations. Specifically, low mortality rates during hibernation allow bat populations to escape high summer mortality and ultimately facilitate long-term bat population viability. Within a viable bat population, lengthy incubation periods and the metabolic effects of host torpor maintain rabies virus n adult bats until the birth pulse of each year. After the birth pulse the young born that year amplify the pathogen within the population.

Traditional wisdom suggests that highly virulent pathogens frequently evolve lower levels of virulence due to the transmission-virulence tradeoff. Within these two systems, we see ecological mechanisms that allow virulent pathogens to persist (as opposed to the evolutionary mechanisms contained in the transmission-virulence tradeoff hypothesis). Within each of these two systems, we see a host refuge and a pathogen refuge. In the plague-prairie dog system, the host refuge is clearly spatial within the metapopulation context. In the bat-rabies system, a temporal refuge occurs for the host during the low mortality hibernation period. We also see a pathogen refuge within each of these systems. Within the plague-prairie dog system, alternative vector hosts could provide a refuge for plague. Within the bat-rabies system, a temporal refuge also occurs when viral replication is reduced during host hibernation. In both systems, large and new pools of susceptibles become available either spatially within the plague-prairie dog system or temporally due to the birth pulse in the bat-rabies system. Overall, we see that ecological mechanisms can facilitate persistence of highly virulent pathogens, and they require host refuges to maintain susceptible hosts in the system and pathogen refuges that decouple local host-pathogen extinction from landscape or long-temporal scale transmission chains.

Lastly, here is a word about data. Data limitations are a major limiting factor for many modeling efforts in disease ecology. In particular, quality data describing pathogen dynamics in wildlife systems over long periods of time are rare and valuable. This dissertation would not be possible without such data and the effort of many people in the field and laboratories throughout the United States. The Shortgrass Steppe Long Term Ecological Research, US Forest Service, and USDA Agricultural Research Service

Rangeland Resources Research Unit worked hard for many years to generate perhaps one of the best metapopulation data sets in the world. Their efforts through variation in funding cycles, priorities, and personnel have generated data that is furthering our understanding of prairie dog and disease ecology. These data were the backbone of the prairie dog metapopulation model. The data provided by the Centers for Disease Control and Prevention, United States Geological Survey, and Colorado Department of Public Health and Epidemiology were vital for Chapters 2 and 3. Many dedicated people working to improve public health, and ecological understanding have contributed from each of these agencies to generate vital data on rabies dynamics in bat populations. Recently bats have been implicated as reservoir hosts for many emerging pathogens that cause high human mortality and morbidity (Dobson 2005; Calisher et al. 2006; Wang et al. 2006). The data generated by the above agencies have extended our understanding of general pathogen dynamics in bats by exploring this case study of rabies virus in big brown bats in Colorado. Without it, we would not know as much as we do about a crucial reservoir of rabies virus in North America. The dedication of many people in the field and lab has provided a valuable resource.

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