Disease and Demography in the Woodchester Park Badger Population

SUBMITTED BY JENNIFER LESLIE MCDONALD TO THE UNIVERSITY OF EXETER

AS A THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BIOLOGICAL SCIENCE, MAY 2014

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

……

ABSTRACT

The topic of badgers in the UK is often a contentious one, dividing opinions and sparking political debate. On one hand, badgers represent an important part of the British ecosystem but on the other a wildlife reservoir of disease implicated in the transmission of bovine tuberculosis (TB) to livestock in the UK. This has prompted strong interest in their population dynamics and epidemiology. Using data from a long-term study of a naturally infected badger population in Woodchester Park, Gloucestershire, this thesis explores a range of capture-mark-recapture (CMR) models to further understand disease and demographic processes. The first section examines long term population dynamics, simultaneously estimating demographic rates alongside their drivers using integrated population models (IPMs). The findings provide new insight into badger demography, highlighting density-dependent mechanisms, vulnerabilities to changing climate and disease prevalence and subsequently how multi-factorial analyses are required to explain fluctuating badger populations. The following sections use multistate models to answer pertinent questions regarding individual disease dynamics, revealing rates of TB infection, progression and disease-induced mortality. A key finding was sex-differences in disease response, with males more susceptible to TB infection. After applying a survival trajectory analysis we suggest sex differences are due to male immune defence deficiencies. A comparative analysis demonstrated similarities between epidemiological processes at Woodchester Park to an unconnected population of badgers from a vaccine study, supporting its continued use as a model population. The final study in this thesis constructs an IPM to estimate disease and population dynamics and in doing so uncovers disease-state recruitment allocation rates, demographic and population estimates of badgers in varying health-states and predicts future dynamics. This model aims to encapsulate the more commonly held notion of populations as dynamic entities with numerous co-occurring processes, opening up avenues for future analyses within both the badger-TB system and possible extensions to other wildlife reservoir populations.

Acknowledgements

This thesis wouldn't have been the same without the help and support I have had along the way. First I would like to thank my supervisor Dave Hodgson, you have always provided great advice and there's no way I would have achieved what I have without your support and enthusiasm. Thanks for having faith in me and for all the opportunities you have given me. Second my supervisors, Graham Smith and Trevor Bailey, thank you for the time, feedback and guidance you have provided throughout, it has been invaluable. I also want to say a big thank you to Robbie McDonald and Dez Delahay, both of you have provided excellent insight and critique on manuscripts. My thesis is improved by the help and input of ideas from all of you.

Thanks to NERC for funding this project and FERA (now AHVLA) for the opportunity. Thank you to the Woodchester Park team, who collected the data on which my thesis is based and have always been a helpful point of contact. I am also grateful to Fernando Colchero for helpful advice.

My fellow PhDers and friends have provided support along the way, a special thank you to Katy Scott, Dom Henri, Chris Coles, Nicole Goodey, Clare Benton and Bea Downing. Also all the many other people who make CEC a friendly atmosphere to work, especially the 'Hodgson' research group, past and present, and the Farmhouse folk, you guys are fabulous!

Finally and by no means least my friends and family (and cats) outside the world of academia, who are always happy to listen to me waffle on and have given me so much moral support and encouragement. Mum, Dad, Katie, Apple, Nick thanks for venturing into Cornwall so regularly, your visits always energise me and fill me with happiness, not to mention the wine. I feel truly lucky to have you all in my life. Most importantly, I am thankful to my husband Neil thanks for turning your life upside down for me and moving with me to Cornwall. Without your love and support I would not be where I am today, thanks for always putting things into perspective and making me happy. This thesis is dedicated to you.

Contents

List of Tables

List of Figures

Authors Declaration

I had no involvement in the collection of field data. The source of data used throughout this thesis was collected by the Woodchester Park team, including all badger trapping, sampling, release and compilation of trapping records. Additional data used in Chapter 6, was also collected and compiled by a dedicated field team from Woodchester Park and AHVLA.

My supervisors commented on earlier drafts of this work. With the additional contribution from other co-authors who provided comments on manuscripts in Chapter 3 and Chapter 5. All of whom are credited in the acknowledgements.

With these exceptions, I declare that the work contained in this thesis is my own and has not been submitted for any other degree or award.

CHAPTER 1

General Introduction

Host ecology and epidemiology

Diseases capable of crossing species barriers are among the most threatening to wildlife populations and problematic to manage. Wildlife populations tolerant of infection can survive for prolonged periods, forming reservoirs of disease which can have disastrous consequences for sensitive hosts for whom infection has serious fitness costs. Wildlife reservoirs are responsible for the majority of emerging zoonotic diseases globally and of substantial concern when infection spills-over into domestic animals, wildlife of conservation concern and/or humans, presenting a challenge to managers, to policy-makers and to the public alike. In these scenarios management often focuses on the wildlife reservoir. But the outcome of control options is complicated by disease-specific parameters and/or by ecological influences, promoting in depth investigation into the epidemiology and ecology of these disease-host interactions.

To further our understanding, long-term data sets of disease and hosts in their natural setting are invaluable, allowing researchers to tease apart underlying regulatory factors. In this thesis I investigate what processes regulate a chronically infected Eurasian badger (*Meles meles*) population, reservoir hosts of bovine tuberculosis (TB caused by *Mycobacterium bovis*). Two broad objectives form the crux of this work.

(1) The estimation of fundamental rates influencing wildlife populations forms the foundation of reservoir-host ecology. I focus on the inference of robust disease-specific and demographic estimates and improving analytical methods where possible.

(2) An understanding of the processes that underlie the patterns observed can be applied to create a more complete and adaptive management approach as

well as contributing to fundamental ecology by highlighting life history strategies and evolutionary processes. This thesis focuses on identifying mechanisms driving individual-level disease impacts, badger demography, and long-term badger population dynamics.

Overall, I move between individual- and population-level impacts, from disease to demography, to explain underlying processes in a badger-TB system.

Badgers and tuberculosis

Bovine tuberculosis is a global concern. The zoonotic nature of *Mycobacterium bovis* had a significant impact on human health in the UK in the early 1900s with many deaths attributed to TB. However the advent of milk pasteurisation diminished the human health impact which is now considered only minor contributor to TB in humans (EFSA & ECDC, 2012), with *Mycobacterium tuberculosis* the main infective agent of humans. Presently the principal impacts of *M. bovis* are both economic, reducing domestic livestock profitability, and ecological, affecting species of conservation value. The generalist nature of TB and its wide host range promotes the creation of wildlife reservoirs (Coleman et al., 2006; Michel et al., 2006; Naranjo et al., 2008; Schmitt et al., 1997). These wildlife hosts sustain TB in the environment, increasing opportunities for interspecies transmission and generating spill over effects in, either ecologically vulnerable or economically important, species. Here I focus on TB infected badgers which form disease reservoirs of severe consequence to the health of livestock and profitability of pastoral farming in the UK and Ireland.

Tuberculosis is the most pressing animal health concern in the UK today (DEFRA, 2011). Attempts to reduce TB infection in cattle require substantial financial investment of tax payers' money, in addition to considerable emotional and financial losses to individual farmers. Costs contribute to routine herd surveillance including regular TB testing, slaughter of positive reactors and imposing movement restrictions in breakdown herds. Despite enforced testing regimes, TB in cattle is increasing in incidence and geographical distribution

(Abernethy et al., 2013). Reservoirs of TB in the environment may be responsible for dampening or even negating the positive impact of herd management by re-infecting livestock. Badgers are considered the main source of infection, with cattle farming providing favourable resources for badgers (Kruuk et al., 1979), and surrounding woodland providing the ideal habitat to construct their complex underground infrastructures. Living in close proximity and sharing foraging areas with cattle increases transmission opportunities with badgers directly involved in the transmission of TB to livestock (Donnelly et al., 2006). This has created a highly complex and emotive problem, with policies hotly debated due to the implications for livestock and the considered control strategies for badgers.

Control strategies

Bovine tuberculosis was discovered in badgers in England in 1971 (Murhead & Burns, 1974). Initially various culling methods formed the basis of control (Krebs et al., 1997). However the number of TB infected cattle continued to rise, bringing about an extensive review on the effectiveness of culling and the development of a large-scale field trial, the randomised badger culling trial (RBCT (Krebs et al., 1997)). The RBCT compared the effect of proactive, reactive and no culling strategies. Badger culling was found to have adverse effects; increasing herd breakdowns within reactive trial areas and in farms bordering proactively culled areas. Therefore despite an observable decrease in herd-breakdowns within proactive areas increased TB in farms surrounding the area reduced the overall benefit (Donnelly et al., 2006). Additionally, TB reductions within trial areas were not sustained in the years following the cull (Jenkins et al., 2010). These counterintuitive effects can be explained by an event termed ‘social perturbation’, whereby disruption to the typically discrete, stable social structure of badger populations leads to a behavioural change in those remaining (Carter et al., 2007; Pope et al., 2007). Specifically, disruption of territories results in increased ranging behaviour (Tuyttens et al., 2000; Woodroffe et al., 2006a) and mixing of social groups expanding the spatial spread of *M. bovis* (Jenkins et al., 2007) and ultimately increasing disease

prevalence in badgers (Woodroffe et al., 2009a) and livestock (Donnelly et al., 2007; Donnelly et al., 2006; Donnelly et al., 2003). Effective control is not as straightforward as simply reducing the number of hosts. Instead, current rationale suggests management strategies that minimise social perturbation of network structures would be the most effective.

Vaccination is less disruptive than culling and benefits badgers by slowing TB progression (Chambers et al., 2011) and providing an indirect protective effect in unvaccinated cubs (Carter et al., 2012). It is currently implemented at small scales whereby BCG is deployed via intramuscular injection. However trap and release strategies are too labour intensive and costly for large–scale applications and current development of vaccine delivery by oral baits for mass usage is complicated by technical and regulatory problems (Bourne, 2007). Biosecurity is another viable control option to reduce opportunities for transmission between wildlife reservoirs and cattle by restricting badger access to cattle barns, drinking troughs and cattle feed, and excluding cattle from areas of high badger activity. However, biosecurity measures require financial investment which farmers may not consider justifiable given that they may reduce the problem but are unlikely to resolve it alone. Currently, pilot culling has been implemented in England, sparking considerable debate and opposition. Concerns regarding the implementation and scientific merit of current culling procedures remain to be verified as evidence and further discussion emerge in the years to come. However, what is apparent is the intricate nature of this problem, due to both the complexities of the host and the polarized opinions regarding management. Further insights into the ecology and epidemiology of these hosts can only be of benefit to aid future management decisions of what is currently a highly convoluted problem with a very uncertain future.

Predictive models

Faced with significant contrasting views regarding management options, and ethical difficulties manipulating disease-host systems, predictive models provide

a useful tool to explore alternative options. In the absence of field data extensive mathematical models, ranging in complexity, endeavour to replicate the infection process and population dynamics of badgers (Bentil & Murray, 1993; Cox et al., 2005; Ruxton, 1996; Shirley et al., 2003; Smith et al., 1997; Smith et al., 2007; Smith & Cheeseman, 2002; Smith et al., 2001a; Smith et al., 2001b; White & Harris, 1995; White et al., 1997; Wilkinson et al., 2009; Wilkinson et al., 2004). These models have enabled major advances in our understanding of the disease, predicting the efficacy of control strategies, providing cost-benefit analyses, highlighting key processes that drive dynamics and contributing to the policy making process. However models have limitations: first, they often require the imputation of unknown parameters; second, being simplifications of reality, they risk ignoring key processes entirely, due to the lack of available information regarding epidemiological and demographic rates. This further highlights the need for improved analytical solutions to obtain updated parameter values directly from field data.

Ecology and epidemiology

Population dynamics

Individual-level infection processes including disease-transmission, disease-progression and disease-induced–mortality, operate simultaneously alongside other demographic mechanisms to regulate populations. Identifying key demographic traits and how they regulate and respond to environmental pressures, disease perturbation and anthropogenic change not only aids our understanding of wildlife populations but helps to predict future dynamics, identify evolutionary processes, and highlight the effectiveness of possible control strategies. Badgers have highly variable population densities both across their geographic range (reviewed in Roper (2010)) and within populations through time caused by fluctuations in demographic rates (Anderson & Trewhella, 1985; Cresswell et al., 1992). The question of what causes noisy dynamics requires consideration of the processes driving badger

demography. Focussing on temporal variation, this thesis attempts to answer this question (Chapter 2).

Population regulation is often framed in terms of density dependent and density independent processes. Density-dependent regulation is ubiquitous throughout wildlife systems (Brook & Bradshaw, 2006). High densities provide a negative feedback via demographic rates halting population growth (Lebreton et al., 1992). This mechanism of population limitation is classically thought to be due to resource limitation, with the feedback realised through competition for food via direct exploitation competition and interference competition caused by increased aggressive encounters at high densities. Also, disease and predation are more likely to operate at high densities, resulting in individuals competing for space to evade these threats, which are capable of amplifying any deleterious effects from poor nutrition that may also occur at high densities. Understanding mechanisms of density-dependence can answer ecological questions, revealing life-history strategies, but also has management applications, guiding predictive models. Density-dependence can influence the favourability of differing management options when modelled contrasting ways. For example, culling is deemed more favourable if density dependence acts on mortality (Barlow, 1996) and not when it acts on other processes such as fecundity or movement which may stabilize against the negative effect of culling due to reduced density-dependent pressure increasing birth rates and/or movement. Models of DFTD (devil facial tumour disease) in infected Tasmanian devils found that culling in the presence of density-dependent fecundity increased the proportion of susceptible individuals and in turn disease prevalence (Beeton & McCallum, 2011). Therefore, knowledge of compensatory mechanisms and at what intensity culling would need to be implemented to be of benefit, i.e. additive to natural mortality, is necessary to make informed predictions (Harrison et al., 2010). Badger TB models differ in their description of density-dependence (Bentil & Murray, 1993; Smith et al., 2001a; White & Harris, 1995) with further clarity required for effective modelling and to uncover other demographic drivers.

Alongside density dependence, extrinsic stochastic processes also drive populations such as weather and anthropogenic perturbations. Climatic conditions commonly act indirectly impacting resource availability, with changeable resources a determinant of reproductive success (Dasilva et al., 1993) and survival (Macdonald et al., 2010) in badger societies. Environmental heterogeneity arising from temporal weather variations are not included in any TB model predictions, and may be compensated or exacerbated by density-dependence, with parameter knowledge sparse regarding the interplay between these two processes. Additionally, disease itself may perturb populations. Currently disease is not thought to impact upon population dynamics (Wilkinson et al., 2000), however the impact of disease in the context of environmental drivers and density dependent processes is unknown. Understanding the force and relative impact of environmental, density-dependent and disease factors as drivers of badger abundance will reveal key regulating processes (Chapter 2).

Epidemiology

Animal populations respond to both density-dependent and -independent pressures, but alongside these large-scale processes individual-level disease effects can also change wildlife population dynamics. Epidemiological rates, including disease transmission, disease progression and disease-induced mortality, determine disease dynamics and the outcome of management strategies. Transmission routes of TB in badger populations can be airborne, through bite wounding, or ingestion of contaminated material. Opportunities for direct transmission within badger communities are high, differing considerably from between species transmission which is more likely to be indirect (Drewe et al., 2013). Tuberculosis affects most organs but emerges most commonly in badgers as a respiratory disease, infecting lung and thoracic lymph nodes (Gallagher & Clifton-Hadley, 2000; Gallagher et al., 1998). Excretion by respiratory routes likely exceeds that of other routes. Social grooming, group sleeping and a subterranean existence provide ideal conditions for disease spread and maintenance via airborne transmission, in addition to between

group territorial behaviour and within group competition which creates favourable circumstances for disease spread via bite wounding.

Once infected, the spectrum of individual responses varies widely, from latent, contained disease to a highly infectious, advanced stage with the formation and dissemination of lesions (Fig.1.1). Primary latent infection is generally asymptomatic, with many infected individuals never progressing to a severe disease state (Gallagher & Clifton-Hadley, 2000). As the disease advances internal lesions develop where bacteria have localized. These expel *M. bovis* which can spread to other parts of the body and be released into the environment such that the badger becomes infectious. The locations of the lesions determine the route of bacterial excretion. Respiratory lesions are the most common capable of transmitting TB directly via aerosol and bite wounding, as well as indirectly whereby swallowed tubercles are excreted in faeces. Other routes include; renal lesions prompting excretion via urine, and infected bite wounds generating wound exudates. Factors that trigger clinical disease are largely unknown, but stress due to nutritional depletion, territorial pressure, lactation and dominance have been suggested (Gallagher & Clifton-Hadley, 2000). The complex pathogenesis leaves many questions yet unanswered regarding the underlying cause of heterogeneity in infected states.

Defining heterogeneous disease states is valuable with variability in individual infectiousness an important determinant of disease persistence (Kramer-Schadt et al., 2009; Shirley et al., 2003), models accounting for heterogeneity in infectivity are vastly different from those that assume an average infection level (Lloyd-Smith et al., 2005). Complexity arises due to difficulties determining disease states of wild individuals. A wide spectrum of infected conditions and the absence of post-mortems leaves epidemiologists with diagnostic tests that have far from perfect detection (Drewe et al., 2010). Despite these difficulties, a higher infectious state has been included in numerous badger-TB models (Fig.1.1) (Shirley et al., 2003; Smith et al., 1997; Smith et al., 2001a; Smith et al., 2001b; Wilkinson et al., 2004). Current infectious states in badgers are defined by culture tests detecting active excretion of *M*. *bovis*. These infectious

badgers are divided into two categories, excretors and super-excretors, based on the number of culture positives detected. However with poor culture test sensitivity and intermittent bacterial excretion, infectious badgers are inclined to test positive sporadically with multiple positives not necessarily pointing to an increased severity of the disease. The work described in Chapters 3-4 expands upon current disease-state categorisation to estimate disease-specific parameters in badgers.

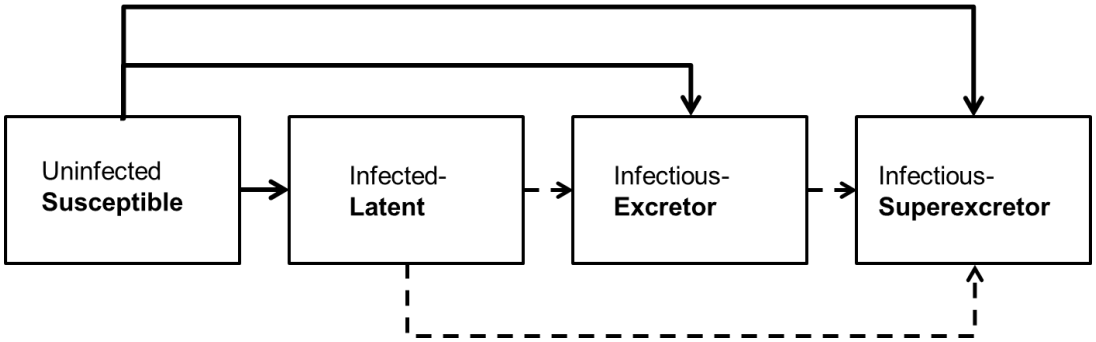

Figure 1.1 A diagram highlighting health-states occurring in an infected population of badgers. Infection processes are shown by a solid arrow and disease progression with a dashed arrow.

A defining characteristic of badgers is the formation of discrete social groups (Delahay et al., 2000; Rogers et al., 1997). Living within close proximity and sharing communal underground dens called setts provides the optimum environment for the spread and maintenance of infection within the group. Badgers spend up to 70% of their time in their setts (Roper, 2010) and maintain discrete territories mapped out by latrines on territory peripheries. This social spacing out mechanism (Kruuk, 1978) creates differential contact rates between individuals in separate groups ultimately leading to variation in opportunities for disease transmission, and uneven spread and clustering of TB within populations (Delahay et al., 2000). Although this thesis does not directly consider contact networks (other recent studies consider this aspect (Böhm et al., 2009; Weber et al., 2013)), we do consider the social grouping element to account for variability in transmission rates within and amongst groups. Social

organisation is a key determinant of TB distribution and spread, therefore its inclusion in analyses and/or modelling approaches may be influential. We incorporate indices of social structure to help absorb any social non-independence of badgers in our analytical models (Chapter 4).

Understanding factors that predispose individuals to become infected and advance from one TB state to another, particularly what makes a badger become infectious, could be important in predictive modelling, identifying areas at high risk of future herd breakdowns and aid the development of improved strategies for controlling TB. Current research shows that male badgers are more likely to become infected and die from infection (Wilkinson et al., 2000). Differential sex bias in both epidemiological and demographic traits are commonly observed in other species (Guerra-Silveira & Abad-Franch, 2013); however there are numerous possible causes. Explanations may be biological due to sex-hormones, with increased stress and testosterone in males linked to immunosuppression, increasing susceptibility to infection (Zuk & McKean, 1996) and reducing immune defences. But, social factors are also suggested to play a role. Male badgers generally exhibit more risk-taking behaviour, such as increased ranging and territorial behaviour and experience more aggressive encounters and bite wounds (Delahay et al., 2006; MacDonald et al., 2004). These behaviours may simultaneously increase exposure to infection whilst coincidentally predisposing males to unrelated mortality. Therefore both biological and cultural transmitted influences may exert different pressures on mortality rates between sexes. Understanding real mechanisms underlying disease patterns can be used to guide management, by promoting or discouraging targeted control. If immunosuppression is the underlying cause of sexual differentiation then, targeting males during management campaigns is likely to have the most impact on disease prevalence. Alternatively if males are more likely to experience a particular behaviour that coincidently predisposes them to infection then focussing on those that display this ‘risky’ behaviour may be a more appropriate course of action. Looking at the problem in immunological, behavioural and ecological contexts is an area of future discovery (Chapter 5).

Longitudinal studies

High quality individual-based data-sets provide invaluable information to help reveal population patterns and processes unobtainable by any other means. To answer ecological and epidemiological questions this thesis utilises data from a long-term capture-mark-recapture study of badgers in Woodchester Park, Gloucestershire, naturally infected with TB. Badgers of Woodchester Park have been studied intensively since 1975. The population is composed of over 20 social groups in an area 11km^2. It is the longest running badger survey in the UK consisting of naturally infected individuals, providing the most detailed information on any mammalian reservoir host. Unsurprisingly a large amount of research stems from this population which has allowed considerable advances in our understanding of badger ecology and epidemiology with details transferred to predictive models (Shirley et al., 2003; Smith & Cheeseman, 2002; Smith et al., 2001a). An ongoing concern is that Woodchester is an atypical population due to its undisturbed nature. No other long-term badger studies collect TB diagnostic data, therefore there is little opportunity to judge whether epidemiological parameters from Woodchester are representative of other populations. Such multiple population comparisons are rare due to difficulties gaining adequate data. Therefore, with arguments that single location data may not provide representative data for the species, any opportunity for comparison and validation should be applied (Chapter 6).

Although improvements in our understanding of badgers and TB have occurred in recent decades, there are still gaps and uncertainties in our knowledge. Consideration of the complete system, incorporating both ecological and epidemiological complexities of badgers, will provide detailed information aiding disease management decisions, predictive modelling and improve our comprehension of underlying mechanisms driving population dynamics in a disease-host. This thesis focuses on answering key epidemiological and ecological questions which require exploration of various analytical approaches to obtain further insight into badgers and TB.

Analysis of field data

Capture-Mark-Recapture procedures

Ecological systems vary over space, through time and among individuals, and are typically described incompletely by means of samples comprised of a fraction of the population. Development of techniques to extract demographic information from such data has allowed enormous advances in our understanding of wildlife ecology. In particular, long term monitoring of wildlife populations which track individuals over time provides data capable of mapping both individual- and population-level processes. However, only a portion of the population can be captured, motivating the development of capture-mark-recapture (CMR) models in the 1960s which accounted for detection bias, enabling robust estimates of survival, recruitment and population growth to be derived from longitudinal studies.

Initial consideration of survival and recruitment were as nuisance parameters in models developed to estimate population abundance from capture-recapture experiments (Jolly-Seber models; (Jolly, 1965; Seber, 1965)). Alongside these developments Cormack developed an approach to estimate survival of marked individuals (Cormack, 1964). The combined outcome was the Cormack-Jolly-Seber (CJS) model capable of estimating time-dependent survival and recapture probabilities. Over time, generalizations of this model were developed; incorporating age-dependency (Pollock, 1981) and inclusion of environmental and individual covariates. Additionally the model framework itself has evolved from CJS models (Lebreton et al., 1992), advancing to estimate recruitment and population growth (Pradel models (Pradel, 1996); Chapter 2), estimation of population abundance (POPAN models; (Schwarz & Arnason, 1996); Chapter 2), incorporation of multiple states and sites (multistate models; Chapter 3-4) and to estimate emigration (Robust design models (Pollock, 1982)).

Over the years CMR models have uncovered a host of regulatory processes and demographic trends occurring in multiple taxa. Their versatility allows discoveries regarding population regulation and limitation (Chapter 2) including but not exclusive to effects of climate change, impact of perturbation events and uncovering life-history strategies. Also the ability to not only map population processes but also individual variation through time allows transition models to follow individuals progressing through multiple states and/or sites. The application of these models to diseased individuals allows researchers to map the probability of infection, derive disease-specific survival estimates and even disease progression and recovery rates given the availability of data (Chapter 3-4).

This thesis explores a variety of different models using 3 different forms of statistical philosophy: (i) classical hypothetico-deductive tests of significance; (ii) frequentist approaches using information theoretic methods to compare likelihoods among models; (iii) Bayesian approaches to (a) determine the probability of truth of continua of parameter estimates, and (b) allow the construction of hierarchical state-space models with various mixed probability distribution functions.

Classical likelihood and program MARK vs. Bayesian and WinBUGS

Statistical analyses of survey data are used to estimate unknown population parameters (θ). Bayesians and frequentists take differing stances on how they view these parameters. Frequentist approaches regard them as discrete fixed values. Statistics associated with these estimates tell us how often we would observe these values if the experiment was repeated a large number of times. Proponents of Bayesian approaches argue it is more appropriate to express uncertainty in terms of the parameter values themselves opposed to replicate data sets. As such a Bayesian analysis focuses on the distribution of the calculated parameters and provides posterior distributions of the estimates thereby allowing probability calculations regarding the values position. This is the difference between the statements; “if this experiment were repeated the

parameter will lie within the confidence interval in 95% of the cases" using classical confidence intervals where the data is considered uncertain, and "there's a 95% probability that the parameter lies between these values" using Bayesian credible intervals where the parameter is the uncertain factor. A further fundamental difference is the incorporation of prior information with a Bayesian approach. Initial beliefs are specified before the data is observed, perhaps based on previous experiments, these combine with the likelihood of the data to provide the posterior distribution. Alternatively, vague prior distributions are often chosen to reflect limited parameter knowledge in an attempt to be non-informative when combined with the information from the data.

Comparing the analytical differences between these approaches, the likelihood function forms part of both Bayesian and frequentist inference. The likelihood is denoted $L(\theta \mid x)$ meaning the likelihood of the parameters (θ) given the data (x) and is considered proportional to the probability of the observed data (x) conditional on the parameter values $p(x \mid \theta)$. In a classical approach this likelihood plays a central role for inference by estimating parameters (θ) which maximize the likelihood of obtaining the observed data (x). This allows researchers to determine the best-fitting values (maximum likelihood estimates) that maximise the likelihood of the capture histories we observe. That is, it finds the parameter values such that the probability of the observed data is the highest. The likelihood function is not a density, therefore does not allow probability statements. Instead, Akaike weights are commonly used to represent the relative likelihood of obtaining the data given the hypothesis. This is generally done for a range of different models and models are compared in terms of their information criterion. Usually a single best model that describes the data is chosen. A problem with this approach is models with less support are often overlooked, and limited attention is given to the size of effects estimated. Additionally although a single best model that best reflects the data is chosen out of numerous candidate models it is unable to describe any degree of certitude (Ellison, 1996).

The aim of a Bayesian analysis is to estimate the joint posterior distribution of all model parameters $p(\theta \mid x)$ and calculate the probability of the parameters given the observed data. This is achieved by means of a simple relationship known as Bayes theorem.

$$p(\theta \mid x) = \frac{p(x \mid \theta)p(\theta)}{p(x)}$$

Again this theorem incorporates a form of the likelihood $p(x \mid \theta)$ representing the probability of the data given the parameters, however additionally prior information p(θ) is incorporated, and the denominator p(x) which acts as a scaling constant. This ensures the end result integrates to one and can be interpreted as a probability. As the denominator is only a function of the data (x) and not of the parameters Bayes theorem if often expressed as;

$$p(\theta \mid y) \propto p(x \mid \theta)p(\theta)$$

Utilising the likelihood and prior information the posterior distribution is calculated, which forms the basis for all Bayesian inference. However the posterior distribution is often complex and difficult to analyse. The development of Markov Chain Monte Carlo (MCMC) methods provided a solution to engage these high-dimensional integrations which were often analytically intractable. The aim of MCMC is to generate samples from the posterior distribution using Monte Carlo methods and construct Markov chains which upon reaching a stationary state and convergence (when multiple chains are run) can be considered independent from the starting distribution and an approximation of true posterior density. Researchers can sample from these posterior parameter distributions to answer various ecological questions. The width, peak and distribution of the posterior parameter distributions all provide useful information and allows for uncertainty in their interpretation. Therefore Bayesian posteriors may be more transferable to a management forum, whereby uncertainty can be considered in the decision making process e.g. “There’s a 99% probability that a decrease in density will result in increased fecundity”.

The development of specialised computer software for both methodological approaches enabled these complex calculations to be accessible to ecologists. Program MARK (White & Burnham, 1999) is a reliable software package with more than 20 classical likelihood models, all of which can be adapted to include fixed time effects, time dependency as a function of external variables, and individual covariates. It provides a robust analysis incorporating numerous goodness-of-fit testing procedures, and is constantly developing to incorporate new approaches. However, this framework has its limitations, with difficulties implementing mixed-effects models, and is currently unable to incorporate time-dependent individual effects, which may lead an ecologist to seek a more flexible approach.

The use of Bayesian models for ecologists has been greatly assisted by the development of MCMC algorithms incorporated into flexible software. Here we focus on the program WinBUGS (Lunn et al., 2000) which can implement complex algorithms and provides an adaptable framework which readily deals with models of increasing complexity. Individual and temporal characteristics can be incorporated using a structure similar to generalized linear models therefore moving from fixed effects models to mixed models is straightforward within WinBUGS (Royle, 2008). Additionally, missing data and small datasets can be handled easier than classical approaches. With its adaptability WinBUGS has made a whole host of complex models accessible to ecologists, one such analysis which builds upon earlier CMR models is an integrated population model (IPM).

Integrated Population Models

IPMs are a powerful statistical tool allowing different sets of data to be combined, to create a single unified analysis. This statistical development can estimate previously unidentifiable parameters (Besbeas et al., 2002; Besbeas et al., 2003). Its improved framework for analysing sparse data-sets has great potential to aid conservation biology when data acquisition is often limited

(Schaub et al., 2007), and also improves parameter precision (Brooks et al., 2004) which will benefit management decisions generally.

The ‘integrated’ part of IPMs refers to the linking of 2 models. Firstly, a state-space model is used to analyse count data via a state equation which describes population change as a consequence of demographic parameters. Specifically, using an observation process to account for observation error, population counts are linked to demographic rates via a population projection matrix model. A simple single-sex example of a lifecycle graph used to map population size is shown in figure 1.2. However, this can be developed further to constrain parameters across numerous classes such as incorporating two sexes, additional age-classes, life-stages and disease states.

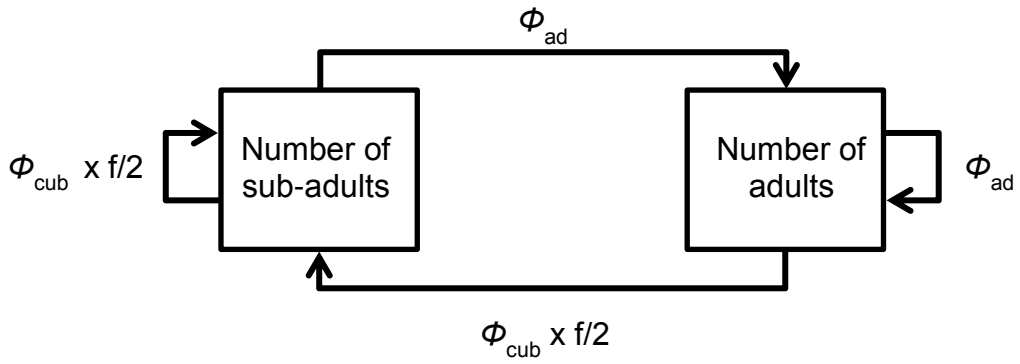

Figure 1.2. Example of a single-sex lifecycle graph used to map population counts as part of the state-space model. The nodes show age classes and the arrows show transitions including adult survival (Φ_{ad}), cub survival (Φ_{cub}) and productivity assuming an even sex ratio at birth (f/2).

Secondly, a CMR model is used, the most common being a CJS model, to analyse capture data to obtain estimates of other demographic parameters. These models are then combined to form a joint likelihood with each model fragment borrowing information from other model fragments (Fig. 1.3), resulting in higher precision parameter estimates (Schaub & Abadi, 2011).

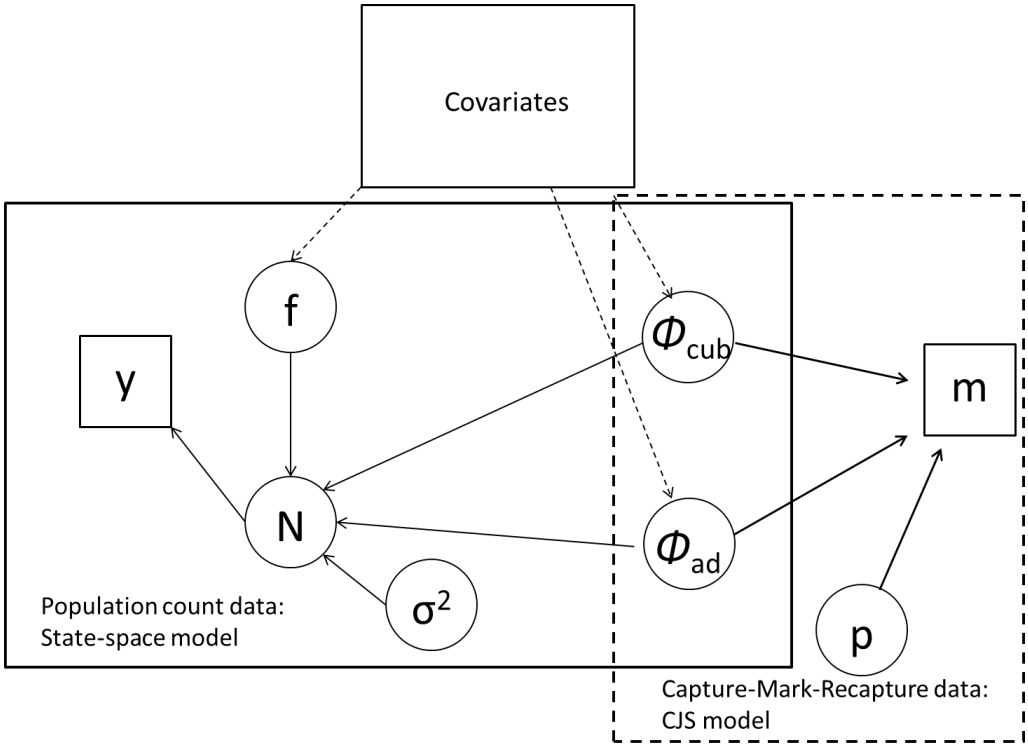

Figure 1.3. Example acyclic graph of an IPM using count data (y) modelled by a state-space model and CMR data (m) analysed using CJS model. Model fragments borrow from each other to identify parameters previously unidentifiable and improve precision of parameters, which are shown in circles, including adult survival (Φ_{ad}), cub survival (Φ_{cub}), productivity (f) and observation error (σ^2). Individual and/or temporal covariates can be naturally incorporated into this analyse to act on demographic components.

Integrated population models therefore force us to consider wildlife populations in their entirety, allowing any inconsistencies that might emerge from the disparate analyses to be resolved (Hoyle & Maunder, 2004). Additionally, these models allow for demographic and environmental stochasticity and take into account uncertainty in the data collection. This improves our ability to detect driving mechanisms, and map temporal synchrony between demographic parameters to density-dependent (Abadi et al., 2012; Peron et al., 2012), environmental or other driving covariates. The core output will also be beneficial as a management tool to forecast population dynamics and make management

predictions whilst accounting for uncertainty. Overall the benefits of IPMs should advance our understanding of population processes and allow researchers to consider more detailed ecological questions.

I explore IPMs and consider the importance of differing sources of variation and their individual and population level impacts to provide further insight into the ecology and epidemiology of badger populations. Utilising a combination of models I develop a CJS/state-space IPM (Chapter 2) and build up to a multi-state/state-space IPM to incorporate population dynamics along with individual level disease processes (Chapter 7).

Aims and structure of thesis

Using a combination of analytical techniques this thesis explores both disease and demographic traits of a badger population looking at both individual-level and population-level scales.

Chapter 2 investigates the key regulators of badger population dynamics. Using both a traditional and Bayesian IPM approach I assess how demographic rates respond to disease and density pressures, then build in weather variables to assess mechanisms by which badgers respond to changing climate.

Chapter 3 focuses on the individual consequence of disease using a multistate analysis. Disease transmission, progression and survival of TB infected badgers are estimated and disease states are reclassified. Building upon these results Chapter 4 moves from a likelihood framework to a Bayesian approach whereby social group is also considered as a random effect influencing these individual level disease processes.

In Chapter 5 I move from pattern to process. Previous chapters identified male-female differentiation in survival and transition rates, utilising a novel method to create survival trajectories of badgers in differing disease states, the possible

mechanisms resulting in male-female variation in infection response are considered.

Using data from a contrasting study site, chapter 6 provides the first comparative analysis of disease parameters between two naturally infected badger populations.

Chapter 7 integrates multi-state models explored in earlier chapters with demographic count data to create a state-space/multi-state IPM.

Finally, Chapter 8 presents a synthesis of results and implications for future work.

Throughout the following analytical chapters the term ”we” is used per publication standard and for consistency, it by no measure means that any part of this thesis is not my own work.

CHAPTER 2

The methods used in this thesis represent my progression through numerous analytical techniques. Although the first empirical chapter presented here, Chapter 2 adopts an innovative integrated population model approach, that wasn't implemented until the final year of my PhD. With this chapter I aim to introduce the Woodchester Park badger system by providing an overview of its population dynamics and demographic drivers.

Wildlife populations experience a heterogeneous world, with intrinsic and extrinsic pressures generating their population dynamics. Unfortunately the emerging literature on badger population dynamics lacks the coherence needed to determine the relative importance of driving parameters. This chapter is split into 2 sections. ***Chapter 2.1*** *explores the impact of disease and density on demographic rates and subsequent population dynamics, followed by* ***Chapter 2.2*** *which builds in weather variables to provide a comprehensive appraisal of abiotic and biotic drivers of badger population dynamics*.

Density, disease and weather influence demographic dynamics of a chronically diseased badger population

2.1: Density and disease

Summary

A quantitative understanding of multiple demographic drivers of wild reservoir hosts provides important information; identifying the relative importance of regulating processes, highlighting life history strategies, revealing mechanisms that drive changes in population abundance, and guiding control decisions. Applying a Bayesian integrated population model (IPM) to 23 years of capture-mark-recapture data and population counts taken from a population of naturally infected badgers, an important reservoir of bovine tuberculosis (TB), we simultaneously; (1) estimate survival and recruitment parameters; (2) determine whether density and/or disease drive changes in these demographic rates; and (3) calculate the amount of variation explained by these processes. Temporal variation was much greater in recruitment than in survival, with negative density-dependence explaining over 50% of inter-annual variation. Survival, whilst contributing more to population growth, was resistant to density pressures and demonstrated low levels of temporal variability, supporting the consensus that badgers are “slow” life history strategists. Disease prevalence negatively impacted survival with sex differences in the proportion of temporal variation explained, indicating increased vulnerability in female badgers during the overwinter period. Recruitment’s resilience to disease and its strong density-dependent mechanism promotes continued birth of cubs, both restricting the magnitude of disease prevalence and compensating for the loss of any infected individuals, favouring the persistence of chronically infected badger populations. This density-dependent mechanism also has relevance for future management decisions. We illustrate how IPMs can be used by researchers to assess a range of processes within a single modelling framework, which we suggest will generate more detailed ecological insights in future studies.

Introduction

Badgers in the UK rank high among the most important mammalian reservoirs of zoonotic disease (DEFRA, 2011), harbouring bovine tuberculosis (TB). For this reason badgers have been subject to intensive ecological and epidemiological research. Remarkably, however, to date there has been no comprehensive appraisal of the relative and combined influence of disease prevalence and density-dependence as drivers of badger population dynamics. This is unusual not just because an improved understanding of the drivers of badger demography could help to recommend evidence-based management strategies for the control of bovine tuberculosis, but also because long-term surveys of badgers and TB prevalence present a rare opportunity to understand the fundamental drivers of population dynamics in a diseased population of wild mammals.

Wildlife hosts are responsible for the majority of emerging zoonotic diseases, worldwide (Jones et al., 2008). The substantial economic costs they inflict, including impacts on human and livestock health, can alter the status of wild mammals from being of conservation concern to being serious pests. The transmission of TB between badgers and cattle (Donnelly et al., 2006; Jenkins et al., 2008b) in the UK and Ireland is of serious economic consequence for both farmers and other tax-payers. Despite enforced cattle restrictions the problem is not improving and initiatives targeting the wildlife reservoir are now central to current control strategies. Areas where badgers live at high densities pose the most threat to livestock (Krebs et al., 1997) emphasizing the applied value of furthering our understanding of the processes causing density changes within badger populations. Mammalian hosts of zoonotic disease can be long-lived with overlapping generations therefore our understanding of their population dynamics requires a consideration of age- and sex-dependent survival and reproduction, recognition of how density influences these demographic rates, and an appreciation of disease impacts. Empirical studies of badger demography to date have focused on individual drivers (Macdonald et al., 2010; Macdonald et al., 2002) and have ignored the interplay between dynamic components. We focus on temporal changes in survival and fecundity,

the principal demographic processes that yield badger population dynamics (Macdonald et al., 2009), in order to tease apart the underlying mechanisms generating fluctuations in population growth.

Mammals lie on a life-history axis known as the slow-fast continuum (Gaillard et al., 1989; Promislow & Harvey, 1990), which largely dictates the determinants of patterns of variation in demographic rates. Life-history buffering (or environmental canalization) is a corollary of this continuum (Gaillard & Yoccoz, 2003) which predicts lower temporal variance in vital rates that contribute most to population growth (Pfister, 1998). Broadly speaking, fast-living mammals buffer their reproductive effort against environmental fluctuations but suffer fluctuations in survival (Korpimaki et al., 2004; Luis et al., 2010) compared to slow-living species which buffer their survival and suffer fluctuations in recruitment (Gaillard et al., 1998; Gaillard et al., 2000). Consequently, there is a tendency to find density-dependent impacts on recruitment rates in populations of slow living species (Coulson et al., 2000; Hernandez-Pacheco et al., 2013) and often density-dependent survival in populations of fast living species (Kneip et al., 2011; Rodel et al., 2004). Most research on density-dependence in badgers has focused on the impact of intraspecific competition on body mass of adults and, despite links to fecundity (Anderson & Trewhella, 1985; Cresswell et al., 1992; Macdonald et al., 2002; Rogers et al., 1997), no empirical analysis combining the relative impact of density-dependence on demographic drivers has taken place. We expect that these medium sized mammals with small litters, high survival and relatively long-life spans will display lower variance in survival, with density pressures more likely to impact upon recruitment rates.

Several lines of evidence offer conflicting predictions regarding the importance of disease processes for inter-annual growth and decline of badger populations. Tuberculosis is a fatal disease of badgers in advanced stages (Graham et al., 2013), and might therefore be expected to drive fluctuations in their rates of survival. Direct population level responses to disease impacts are observed in other infectious disease systems including substantial population declines in Tasmanian devil populations due to devil facial tumour disease (DFTD) which brings about immediate population responses even at low prevalence (Lachish

et al., 2007), and cyclical dynamics in grouse (Redpath et al., 2006), partridge (Rosà et al., 2011) and Soay sheep (Gulland, 1992) caused by parasitic infections. Disease can also cause more subtle reductions in population growth, as observed in deer populations infected with chronic wasting disease (Dulberger et al., 2010). In contrast, the impact of TB infection on demographic rates in populations of brushtail possums, buffalo and bison do not propagate through to a population impact (Arthur et al., 2004; Cross et al., 2009; Jolles et al., 2005; Joly & Messier, 2005). This may be due to the typically low prevalence of endemic TB infection, whereby individual impacts of chronic TB infection are only observed at a population scale at high prevalence (Cross et al., 2009). Additionally, disease effects may be difficult to detect if compensated for by recruitment or improved survival of uninfected animals (Arthur et al., 2004; Muths et al., 2011).

Here we use a Bayesian approach to implement an integrated analysis of mark-recapture and census data from a long-term study of a chronically-infected badger population at Woodchester Park, Glos., UK. This method moves away from the commonly implemented approach whereby a range of capture-mark-recapture (CMR) models are used to estimate different demographic processes, such as CJS models for survival and reverse-time models for population growth (Lachish et al., 2007). Instead we advance from performing numerous discrete capture-mark-recapture (CMR) models (Lebreton et al., 1992; Pradel, 1996) to a Bayesian integrated population model (IPM (Besbeas et al., 2002)), creating a unified assessment of badger population dynamics. This integrated approach is of significant benefit to ecological analysis as it is able to simultaneously estimate population growth rate, survival and recruitment, whilst directly assessing the impact of individual and temporal covariates (Abadi et al., 2010a). Integrating data provides higher precision estimates (Johnson et al., 2010) and accounts for observation error deriving unbiased estimates, ensuring temporal fluctuations are truly reflective of population processes. Our goal is to use an IPM to tease apart the drivers of survival and recruitment, and therefore of net population dynamics, in this population. This represents a rare analysis of the impacts of chronic disease on wild mammal demography.

Using long-term CMR data from a naturally infected population of badgers this study addresses three major questions. (1) What is the impact and relative importance of disease and density on population dynamics of a wild reservoir of zoonotic disease? (2) Do these processes influence population dynamics via their influence on survival or recruitment rates? (3) How important is the contribution of these processes to overall temporal variation in demography? We use state-of-the-art Bayesian analyses to provide the most in-depth assessment of badger population dynamics to date, presenting additional insight into the main drivers, and discussion of the likely ecological processes, occurring in an important wildlife-disease system.

Methods

Study site and data characteristics

Data was collected from a long-term CMR study of a naturally infected badger population based at Woodchester Park, Gloucestershire (Cheeseman et al., 1987), from 1984 to 2005. Trapped badgers were anaesthetized and each given a unique identifying tattoo on their first capture occasion. At every capture event the location of capture, sex and age class (cub or adult) were recorded (for detailed methods see Delahay et al. (2000)). Infection status was determined by bacterial culture of *M. bovis* (Gallagher & Horwill, 1977) using samples of faeces, urine, sputum and pus from abscesses and/or bite wounds. The culture test was used as a proxy for infection due to its high specificity (Drewe et al., 2010), reducing the likelihood of incorporating uninfected individuals and, despite its low sensitivity, provides a useful index of infection (Delahay et al., 2013). Annual capture histories were created for each individual badger. Only 20 core social groups that were trapped consistently were included, therefore the terms density and population size are interchangeable.

Covariates

Prior to analysis annual population size was estimated, using the POPAN formulation (Schwarz & Arnason, 1996) of Jolly-Seber models in the program

MARK (White & Burnham, 1999). Models were fit using the log link function for population size and tested and adjusted for overdispersion using the program RELEASE.

The number of infectious badgers was calculated applying the same approach used to calculate total population size. These were then transformed into a proportion of infected individuals in the total population in a given year to provide a disease prevalence estimate.

We applied a comparable scale across covariates to emphasize the relative strength of the regression coefficients. Covariates were standardised to have a mean of 0 and a standard deviation of 1 using the following equation.

$$X_t = \frac{\Sigma(x_t - mean(x_t))}{s.d(x_t)}$$

X_t is the annual standardised variable and x_t is the true annual variable

Bayesian integrated population model

We built an IPM to evaluate the 'best' formulation of disease and density demographic impacts, thereby creating a single unified analysis able to estimate all demographic quantities simultaneously. More specifically, integrated models combine CMR data and population counts into a single model to calculate survival, recruitment rates and population change. We derive both count data and CMR data from the Woodchester study site. We used a pre-breeding census, of counts of unique badgers more than 1 year old caught annually. Capture histories were divided into males and females, and those first caught as adults and those first caught as cubs. Models were specified within R (R Development Core Team, 2013), using the package R2WinBUGS (Sturtz et al., 2005) to call WinBUGS (Lunn et al., 2000) within which the models were run and then results exported back to R.

Population model component

The core of the IPM is a population projection model which maps various demographic rates for different age classes. Most demographic models include only one sex, assuming that males and females have identical vital rates and population dynamics can be determined from one sex alone. However, badgers are amongst the many species that exhibit sex differences in mortality rates, with male mortality exceeding females (Graham et al., 2013). We use a 2 sex, 2 age structured population projection matrix with an annual time step (Fig. 2.1). The model assumes individuals start to reproduce at the age of 1 with an even sex ratio at the time of birth. For simplicity we assumed that the annual fecundity of both yearlings and adults were the same, thus our fecundity rate is per capita of the total population of males and females 1 year and older, creating a rate analogous to recruitment rates, i.e. a per capita rate of growth, which can be directly compared relative to survival in its contribution to population growth. The fecundity rate, also termed recruitment, used throughout this paper is an integrated measure of reproduction and early cub survival.

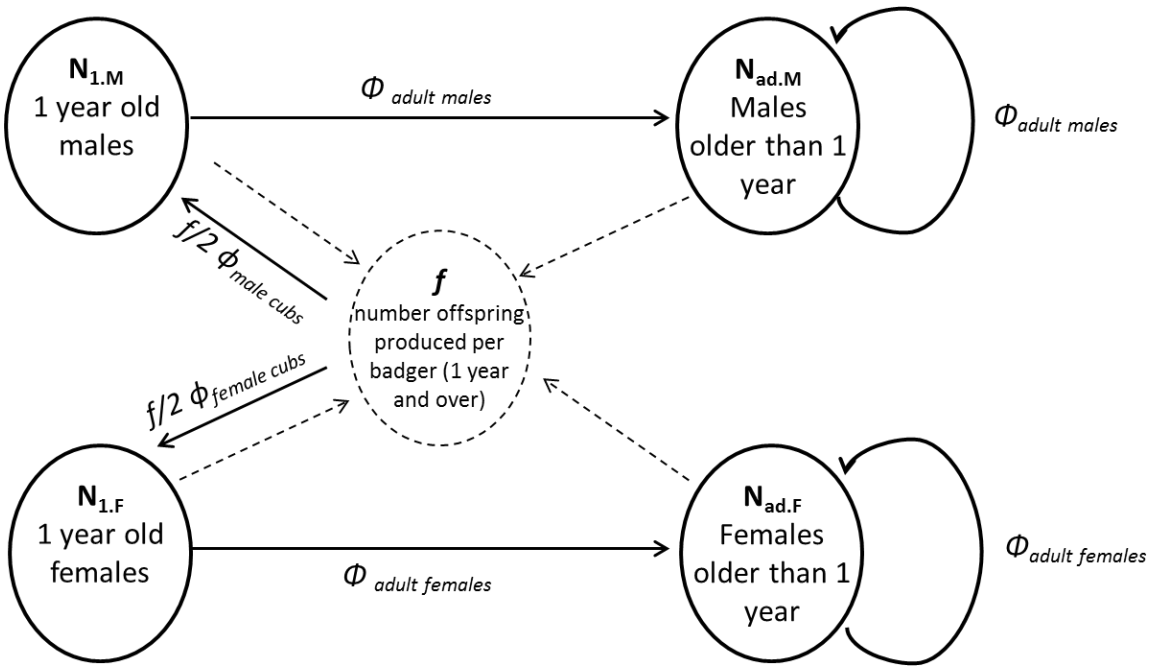

Figure 2.1. The life-cycle graph of a badger population, showing 2 age classes and sex-specific survival rates. N_1 represents the number of adults 1 year old, and N_{ad} the number of individuals older than 1 year. The solid arrows show the demographic processes that make up this population. Cub survival (ϕ_{cubs}) is the probability of a cub (a badger under 1 year) born in year t surviving to year t+1. Survival probability of adults (ϕ_{adults}) is the probability an adult badger (1+ year) surviving from year t to year t+1. The dashed lines represent the contribution of different sex- and age-specific stages to total population size, to calculate per capita fecundity (*f*). Fecundity (*f*) is the number of offspring (of both sexes) per individual (of both sexes) that are born and survive to emerge in the spring also termed recruitment.

Covariate structure

We tested assumptions regarding differential survival between ages (cub and adult) and between sexes, and whether covariates affect these cohorts equally (i.e. have additive effects) or differently (i.e. have interaction effect). We evaluated the best model structure using DIC values (Spiegelhalter et al., 2002).

We modelled the log of fecundity (*f*) parameters and the logit of survival (Φ) parameters as a linear function of covariates using the following linear relationships.

$$\text{logit}\,\phi_t = \alpha + \sum_{j=1}^{n} \beta_j x_{j,t} + \varepsilon_t$$

$$\log f_t = \alpha + \sum_{j=1}^{n} \beta_j x_{j,t} + \varepsilon_t$$

$$\varepsilon_t \sim N(0, \sigma^2)$$

Where $x_{j,t}$ are the values of the standardised j^{th} covariate over time t, βs are the regression coefficients for each covariate and ε is the residual temporal variation providing estimates of remaining variance (σ^2_{Model}). We also fit a model without covariate effects to gain an estimate of total temporal variance (σ^2_{Total}). The proportion of variance explained by the covariate effects is then identifiable using the following calculation $(\sigma^2_{Total} - \sigma^2_{Model}) / \sigma^2_{Total}$.

Vague N(0,10^4) priors truncated to lie in the interval (-5,5) were used for the unknown regression parameters (β). Uniform normal priors were used for mean survival U(0.4,0.95), vague N(0,10^4) prior for the logit of fecundity. Uniform U(0,10) priors were used for the variance parameters on the standard deviation scale.

The likelihoods and joint likelihood

An IPM constructs likelihoods for the two separate data sets (count data and CMR data) which are then combined to maximize the likelihood. Firstly, a state-space model and secondly a CMR model, the most common used being a Cormack-Jolly-Seber (CJS) model.

The state-space model analyses the count data (y), which is determined by the state process and the observation process. The state process describes the true but unknown population trajectory under the population model defined earlier (Fig. 2.1). Demographic stochasticity is included by modelling the

number of 1 year old badgers using a Poisson distribution and the number of adults using a binomial distribution. The observation process allows for observation error linking the observed census data (y) to the true population size: a Poisson distribution was used to account for observation error. The likelihood of the state space model is a product of the observation and process equations. This component of the IPM estimates total population size, survival of each age class (a), fecundity and observation error (N, Φ_a, f, σ^2_y).

The CMR data (m) was analysed via a CJS model which uses m-array formulations following a multinomial distribution including those badgers first caught as adults, and cubs of both sexes. It estimates survival parameters for both sexes (s) and age classes ($\Phi_{s,a}$) and recapture probability (p). Combining these likelihoods formulates the joint likelihood of the IPM (Fig. 2.2).

$$L_{IPM}(y,m \mid N,\phi_{a,s},f,p,\sigma^2_y) = L_{SS}(y \mid N,\phi_a,f,\sigma^2_y) \times L_{CJS}(m \mid \phi_{a,s},p)$$

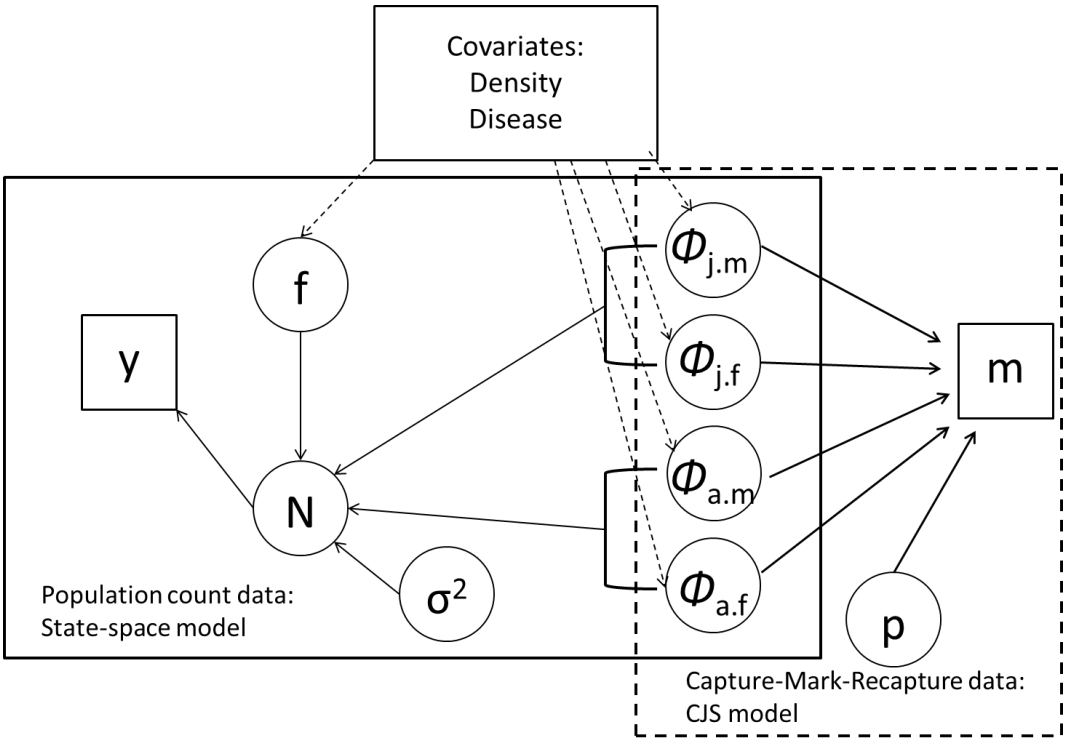

Figure 2.2. Graphical representation of the integrated population model. Squares represent the data and circles the parameters. Large squares represent the 2 different sub-models.

Model fitting and assumptions

Convergence of the chains was assessed by visually checking mixing of the chains and more formally using the Brooks-Gelman-Rubin criterion ($\hat{r}$ (Brooks & Gelman, 1998)) Initial trials with 3 independent chains found that convergence ($\hat{r}$ <1.02) was reached after 3000 iterations. Following the initial trial 3 chains of 10,000 with a burn-in of 3000 was run for each analysis and thinned such that every 10^{th} value was retained, thus giving a sample size of 2100 iterations. As there is no established goodness of fit test for integrated population model we tested the fit of the CMR model component in program MARK, which was not overdispersed ($\hat{c}$ < 1.9; appendix 2.1).

An assumption of IPMs is that demographic data and population counts are independent. Unfortunately due to the method of data collection we were unable to use independent data sets for census and capture history data. It has been suggested that non-independent datasets are unlikely to lead to biased estimates but might overestimate their precision (Schaub & Abadi, 2011), and a simulation study has shown that violating the independence assumption has almost no effect on parameter accuracy (Abadi et al., 2010a). However, we replicate the best model using a MARK analysis adjusted for GOF (Appendix 2.1) to provide an additional check for significance of effects.

Results

In the following results text, we refer to IPM results in which rates of survival (S) and recruitment (R) are subscripted by the covariates used in each model. Subscripts include the influence of the previous year's disease prevalence (D) and previous year's badger density (N).

Initial evaluation of IPMs found most support for sex-specific survival parameters but less support for incorporating differential cub and adult survival (ΔDIC > 23), in agreement with a prior survival analysis (Graham et al., 2013).

Overall population dynamics

Our best model structure when applied to an IPM provided estimates of population dynamics. Mean population growth over the duration of the study was, remarkably, exactly 1 (95% credible interval 0.988-1.013), However, yearly growth rates fluctuated between 0.84 and 1.3 (Fig. 2.3), coinciding with variable population estimate which can be considered an index of population size due to the tendency of CMR models to right censor and underestimate population size. The closeness of population estimates to population counts highlights a small observation error. This is perhaps unsurprising given the method of data collection, whereby counts are obtained from trapped badgers (Fig. 2.3).

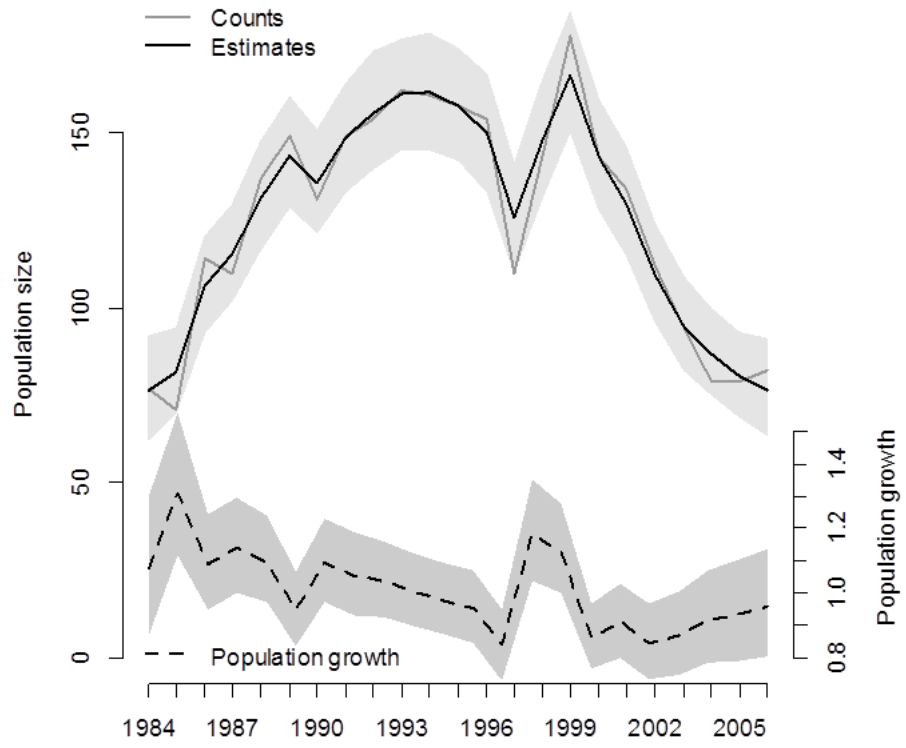

Figure 2.3. Observed counts and estimated population size, alongside between year population growth rates. Shaded regions represent 95% credible intervals (CRI) of estimates.

Creating the IPM: Density and disease

Density and disease effects on both survival and recruitment were explored using an IPM (S_{D+N},R_{D+N}). The posterior regression coefficients provide a measure of strength (i.e. how far the value is from 0) and an estimate of certainty (the posterior probability that the estimated effect is different from zero). The certainty of negative density-dependence was high for recruitment (0.98), and low for survival (0.47). Thus density dependent regulation appears to act mainly via recruitment, not survival. Conversely, the certainty of negative impacts of disease prevalence was low for recruitment (0.71) and high for survival (0.98). After standardising the covariates to have zero mean and unit variance, density and disease were found to have contrasting effects on survival and recruitment. Survival declined with increasing disease prevalence (posterior mean slope β_D = -0.149, Fig. 2.4) but was not affected by density (β_N = 0.007, Fig. 2.4). Recruitment declined with increasing density (β_N = -0.233, Fig.2.4) but not with disease prevalence (β_D = 0.057, Fig.2.4).

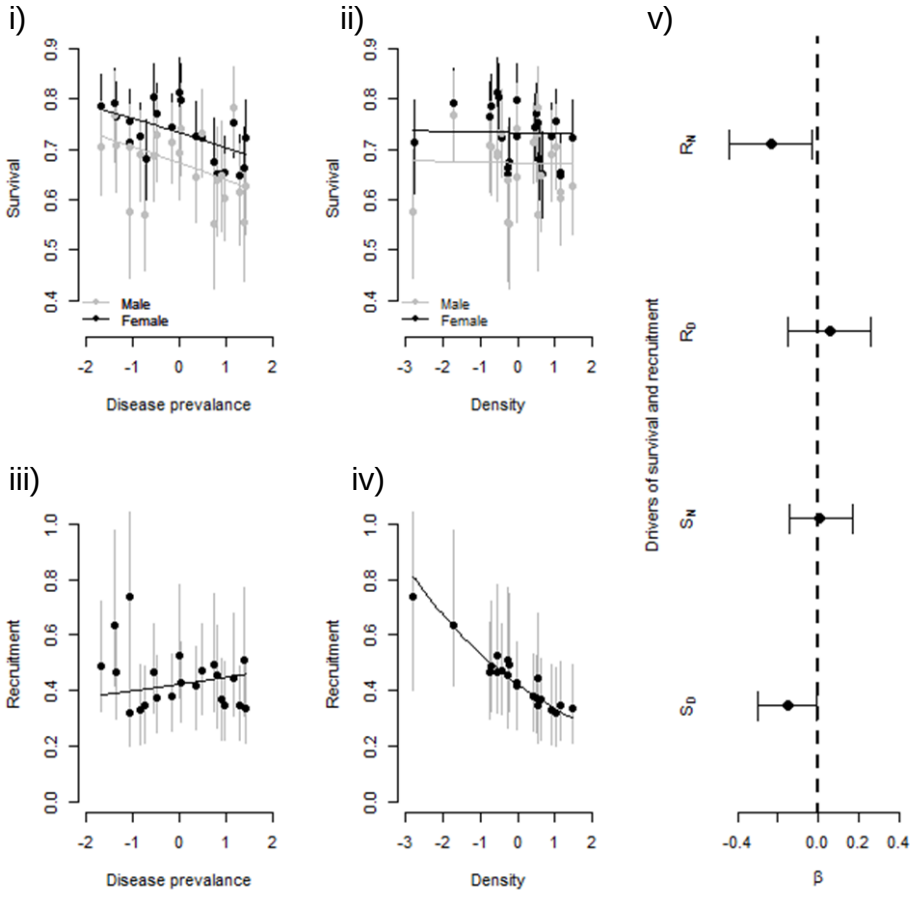

Figure 2.4. i-iv) The influence of density$_{t-1}$ and disease $_{t-1}$ on survival and recruitment rates in a badger population, showing the predicted relationship and the corresponding posterior means and 95% CRI from an IPM. v) Regression slopes describing the relationship between demographic rates; recruitment (R) and survival (S), and covariate effects; disease (D) and density (N). The posterior mean is displayed alongside the corresponding 95% credible intervals.

Dropping the uninformative density effects on survival, and disease prevalence on recruitment, was favoured by DIC selection (ΔDIC>8) and increased the strength of the informative covariates.

We used the IPM describing density-dependent recruitment and disease-dependent survival, to investigate the influence of demographic rates and covariates on population growth.

Demographic posteriors

Female badgers are more likely to survive the year than males (posterior mean female survival = 0.73, 95% CRI 0.70-0.77; posterior mean male survival = 0.67, 95% CRI 0.63-0.72). Mean annual per capita recruitment is 0.42 (95% CRI 0.37-0.48). Survival rates were higher therefore contributing more to population growth than recruitment.

Components of Variation in Recruitment and Survival

In order to calculate how much among-year variation in recruitment and survival is explained by density and disease, we fitted an IPM without any covariates, yielding residual temporal variance (σ^2_S and σ^2_R).

The IPM including density explained 59% between year variance in recruitment. Male and female badgers have similar levels of temporal variation in survival, but the sexes differed in the relative contribution of disease variables. 2% between year variance in male survival and 33% in female survival was explained by disease prevalence. We note that these discrepancies do not imply differential impacts of disease on males and females: models including interactions between disease prevalence and badger sex were not well supported by DIC values (ΔDIC>8) and did not result in any substantial change in the slope parameters (Appendix 2.2).

Correlations

Female survival and recruitment correlated most strongly with the population fluctuating growth rate (r=0.69 and r=0.63 respectively, Fig. 2.5). Male survival had the lowest correlation (r=0.58, Fig. 2.5). The coefficient of variation (CV) was calculated for all demographic rates revealing little year to year variation in male and female survival (CV < 10%) compared to recruitment (CV > 20%). Focussing on the main covariate effects; density and disease, density displayed the strongest negative correlation to population growth (r=-0.57, Fig.2.5), followed by disease prevalence (r=-0.50, Fig.2.5).

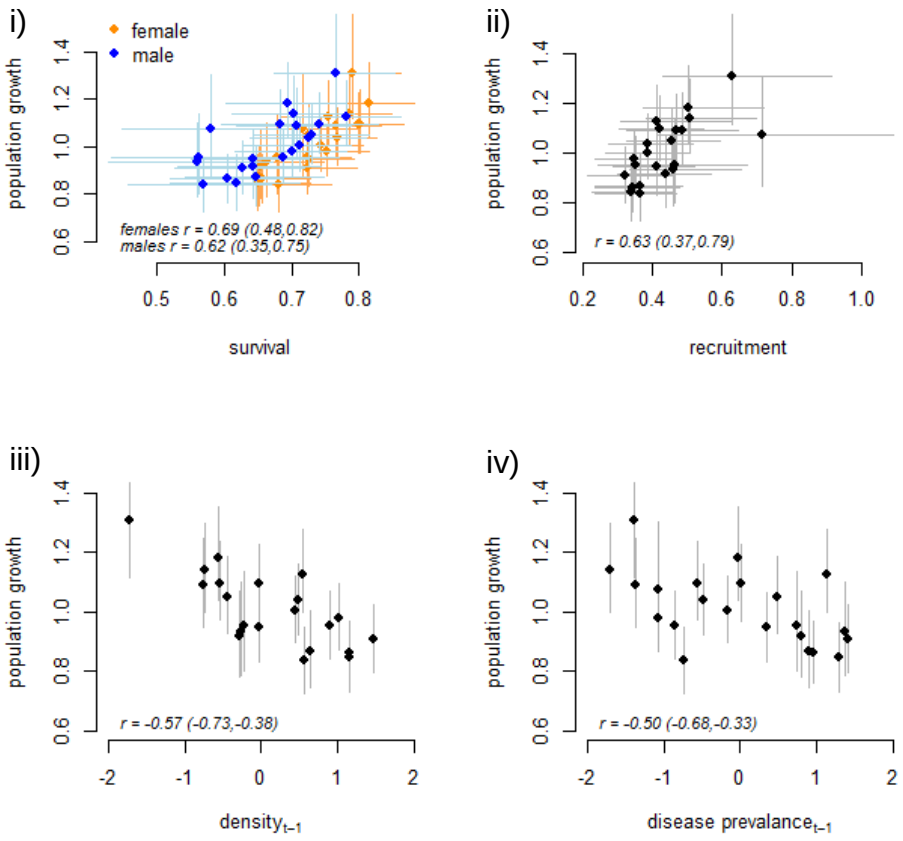

Figure 2.5. Estimates of annual demographic rates (i-ii) and main demographic drivers (iii-iv) plotted against population growth, including the posterior means and 95% CRI. The correlation coefficients (r) are shown along with their 95% CRI. Note from x-axis scaling that variation in recruitment is much greater than variation in survival.

Discussion

There are numerous statistical approaches available to obtain demographic rates from wild populations, but few capable of estimating individual and population processes simultaneously. In this study we demonstrate the capability of a Bayesian integrated population model to calculate key demographic quantities in a single framework, providing further insight into badger demography. Focussing on a high density population situated in a TB hotspot, we find overall population stability (mean λ= 1), but significant inter-annual changes. Key demographic characteristics including high survival rates

and low variable recruitment rates indicate badgers have evolved a relatively slow-life history strategy. Density-dependence had the greatest effect, acting via recruitment rates. Survival remained resilient to density pressures but was susceptible to TB prevalence. Better understanding of the complexities of reservoir host dynamics not only uncovers their underlying life history strategies but also reveals key population mechanisms which should contribute to evidence-based management strategies designed to reduce the prevalence of TB among badgers.

The demographic patterns reported here demonstrate how survival is the most influential component of badger population growth rate, as well as the most stable. In contrast to temporal variability in recruitment which is invariably higher, similar to life-history strategies of other slow-living mammals (Gaillard et al., 1998; Gaillard et al., 2000). Badgers adjust these recruitment rates under density pressures, which accounts for over half of all temporal variation. How could negative density-dependence act on badger recruitment rates? Numerous pressures are associated with life at high density including resource competition and increased aggressive encounters (Cresswell et al., 1992; Roper, 2010). These have associated fitness costs which can cause reductions in badger body condition (Anderson & Trewhella, 1985; Macdonald et al., 2002; Woodroffe & Macdonald, 1995). Resource limitation prevents organisms simultaneously maximising their own survival and their reproductive output, often resulting in inverse relationship between fitness components. In accordance with this we find badgers do not jeopardize their survival under density pressures, instead regulating their recruitment either limiting the ability of female badgers to successfully fulfil pregnancy (Anderson & Trewhella, 1985) or reducing pre-emergent cub survival Given survival's greater influence on population growth, its resistance to density pressures is advantageous, (Gaillard et al., 2000; Pfister, 1998), assisting the stability of badger populations (Saether et al., 2013). Survival's stability also provides more weight to the theory of environmental canalization as observed in other long-lived species (Gaillard & Yoccoz, 2003; Nevoux et al., 2010), whereby fitness components to which population growth is most sensitive are buffered against environmental variability (Gaillard & Yoccoz, 2003).

Survival, although unaffected by density, has a strong negative association with disease prevalence. We find sex bias in the amount of variation in survival explained by disease effects: 33% in females compared to just 2% in males. This result is surprising as males are known to be more vulnerable to infection (Graham et al., 2013; Wilkinson et al., 2000). However, investment in pregnancy may decrease tolerance of disease, with seasonal drops in female immunity over-winter known to occur in other mammals (Altizer et al., 2006). We speculate this result is an artefact of the between-year time-scale used which focuses on a period of vulnerability in female badgers, as opposed to males which are likely to be susceptible to disease effects year round. Further analysis into seasonal effects of TB prevalence may provide further illumination.

Although our analysis suggests that population growth rate may be reduced as a consequence of TB infection in badgers, we find population persistence and stability despite infection. Low disease prevalence likely prevents any large-scale population declines, and its lack of influence on recruitment rates promotes continued birth of uninfected cubs, restricting TB prevalence from escalating. Given that female reproductive success is not depressed by TB infection (Tomlinson et al., 2013), any loss of infected individuals is also likely compensated by density-dependent reproductive success, with density-dependence a stronger driver of population change than disease prevalence. Density-dependent compensation is likely to not only assist population persistence of infected hosts but also has implications for management. Density dependence may have a compensatory effect on culled populations with increased numbers of new-borns stabilizing perturbed populations. In certain scenarios success may still be achieved despite compensatory recruitment by reducing disease prevalence by dilution. However under density dependent transmission, culling may increase disease transmission opportunities if survival of cubs exceeds removal of adults (Potapov et al., 2012). Therefore neglecting density-dependent recruitment from the decision making process will result in exaggerated effects of culling on population size being predicted and in turn may underestimate levels of resulting disease prevalence.

The challenges involved in measuring population dynamics from longitudinal studies of animals in the wild are numerous, often requiring various single-process analyses to obtain all desired parameter estimates. In this paper we build upon traditional CMR models and develop a Bayesian IPM able to simultaneously estimate survival, recruitment and population growth along with individual-, temporal-, fixed- and random-effects. Comparison of these contrasting approaches illustrated that violating the assumption of independence in the IPM did not alter the core results. We suggest developing and utilising IPMs to answer ecological questions will be of immense benefit to understand other wildlife systems, due to the increased flexibility within the modelling framework, improved parameter precision and its capacity to account for both temporal stochasticity and observation error. Efforts to integrate analyses will be particularly relevant for populations of conservation or management concern.

Conclusion

Badgers lie on the slow side of the slow-fast life history spectrum: recruitment is highly variable, regulated primarily by density, while rates of survival contributed more to population growth whilst remaining relatively stable among years and was not affected by density. Disease prevalence influenced rates of survival and impacted on population dynamics, but was compensated for by density dependent recruitment. Our findings substantially improve our understanding of badger population dynamics, quantitatively describing the impact of disease and density as demographic drivers. Armed with a more precise understanding of the links between density and population growth we may be able to refine population models, allowing practitioners to improve strategies for the control of bovine tuberculosis. These results will be of interest both as an investigation into the regulators of badger population dynamics and to highlight the advantages of IPMs as an analytical tool to pose hypotheses in other study systems.

2.2: Incorporating weather in a density and disease IPM

Summary

The longevity of chronically infected populations permits numerous abiotic and biotic factors to act both additively and/or interactively to exert pressure on demographic rates. We build upon prior population analyses of the Eurasian badger to provide a comprehensive appraisal of the demographic consequences of weather in the presence of TB prevalence and density drivers. We apply weather variables considered important in a frequentist framework to an IPM to uncover their relative importance and reveal mechanisms to which badgers may respond to climate change. We find density-dependent recruitment remained a key regulating influence, with conditions over-winter and in spring further influencing cub-emergence rates. Autumn temperature negatively impacted annual survival rates and further interacted with disease. That is, disease prevalence had a strong negative effect at high temperatures. Our results highlight multiple mechanisms through which climate can impact badger demographic rates, including altering activity, limiting resource availability and increasing susceptibility to disease. In terms of climate change, the reduced capacity of badgers to adapt to varying autumn conditions, due to a physiological adaptations to gain weight during this period, may be a key mechanism through which climate change could influence a seasonally driven mammal. The addition of weather to our IPM provided insight into mechanisms by which a mammalian host population may respond to weather changes, and combined with density and disease pressures uncovered additional demographic intricacies.

Introduction

A review of the literature generally reveals two approaches to population analysis of reservoir hosts. Firstly, analyses which focus on the impact of disease itself on host population dynamics (Jolles et al., 2005; Joly & Messier, 2005; Lachish et al., 2007; Muths et al., 2011). Secondly, many demographic analyses of long-term hosts, including badger populations, disregard the impact

of disease and instead focus on density and/or weather processes as drivers of mammalian population dynamics (Frick et al., 2010; Luis et al., 2010; Macdonald et al., 2010). The first, disease focussed, approach may be relevant in studies of virulent pathogens which immediately propel populations to decline, or when parasites overtly drive cyclic dynamics, however is arguably inappropriate for long-term chronic conditions in long-lived hosts such as TB infected badgers. By the very nature of their longevity following the introduction of infection, badger populations will be subject to numerous co-occurring demographic processes. There are a number of reasons why we may be interested in multiple demographic drivers including, but not limited to, improving our understanding of complex interactions between environmental change and disease susceptibility, identifying compensatory processes that may buffer wild populations from demographic perturbations, revealing life-history strategies, and highlighting mechanisms by which populations will respond to climate change, all of which have relevance to predicting future population change for both management of pest species and to aid species of conservation concern.

Given that we know the importance of density-dependence and disease prevalence on badger demography (Chapter 2.1), our objective is to uncover what happens when we incorporate weather variables into these models. We build upon a prior Bayesian integrated population model (IPM) to provide a comprehensive appraisal of weather drivers. Studies from badgers at Wytham Woods have shown badger population dynamics to be sensitive to certain weather variables (autumn, winter and spring temperatures and rainfall (Macdonald et al., 2010; Nouvellet et al., 2013)), but there is little consensus regarding which environmental parameters have the greatest positive or negative impact on rates of survival and recruitment, or how they vary in the context of, and/or interact with, disease and density. Our objective is to assess the relative importance of weather changes in combination with known density and disease impacts on badger demographic dynamics.

Methods

Using the same dataset and integrated modelling approach from the preceding section (Chapter 2.1), we build upon the disease and density only IPM to create a model incorporating weather covariates present in the 'best' candidate model set indicated by a MARK analysis (see Appendix 2.3 for top models),

Climatic data was obtained from 5 x 5 km gridded observation data sets provided by the Met Office (http://www.metoffice.gov.uk/climatechange/science/monitoring/ukcp09/) for the time period 1984-2006 (mean annual temperature 9.6°C; mean annual precipitation 841.5mm). Priori hypotheses were made based on previous work to obtain meaningful candidate variables, avoiding the risk of over fitting (Knape & de Valpine, 2011). Autumn and spring are key seasons related to periods of high energy investment, with climatic conditions affecting badger survival (Macdonald et al., 2010). We also considered the winter period during which conditions have been suggested to alter the activity levels of badgers (Macdonald et al., 2010) perhaps having an adverse impact on fitness. We subsequently incorporated weather variables from spring (March-May), autumn (September – November) and winter (December-February). Mean temperature and summed precipitation are key weather features (Macdonald et al., 2010) and were tested for the time intervals of interest. Additionally the number of days of ground frost were included which we hypothesised may influence demographic rates of this ground-foraging and partly subterranean mammal. These covariates were standardised to have a mean 0 and standard deviation of 1.

As with the previous disease and density IPM, following checks for convergence, we ran 3 chains of 10,000 with a burn-in of 3000 and thinned such that every 10^{th} value was retained,

Results

In the following results text, we refer to IPM results in which rates of survival (S) and recruitment (R) are subscripted by the covariates used in each model. Subscripts include the influence of the previous year's disease prevalence (D), previous year's badger density (N), autumn temperature (AT), autumn rainfall (AR), frost-days in January (FJ) and spring temperatures (ST). Similar codes and subscripts are used to refer to MARK analyses (Appendix 2.3).

Weather drivers

We used the IPM describing density-dependent recruitment and disease-dependent survival, to investigate the influence of weather on these demographic parameters. Exploratory analysis using program MARK highlighted key climatic conditions effecting demographic rates. Autumn temperature and autumn rainfall were in the top candidate models influencing survival probability, along with an interactive effect between disease and autumn temperature. Key drivers of recruitment were January frost and spring temperatures but these only became important after accounting for density-dependence (Appendix 2.3). We analysed the full model using an IPM; $S_{D+AT+AR + (D: AT)}$; $R_{N+FJ+ST}$.

Overall dynamics

We find population dynamics similar to Chapter 2.1. The mean population growth rate over the duration of the study is 1 (95% CRI 0.989-1.013), with yearly growth rates fluctuating between 0.77, and up to 1.42.

Survival posteriors

Female badgers are more likely to survive the year than males (posterior mean female survival = 0.74, 95% CRI 0.71-0.77; posterior mean male survival = 0.68, 95% CRI 0.64-0.72). Autumn temperature had the strongest influence on survival (β_{AT} = -0.169, Fig.2. 6) with a high posterior probability that survival

declined with increasing temperature (99% of slopes were negative). Inclusion of this temperature effect reduced the impact of disease prevalence on survival (β_D changed from -0.149 to -0.038), and the posterior probability of negative disease-dependence reduced from 0.98 to 0.71 (Fig 2.6). This may be explained by the interactive effect of autumn temperature and disease prevalence (β_{D*AT} = -0.131, posterior probability of negative slope = 0.91), whereby under average conditions disease prevalence is limited in its effect on survival, but with increased autumn temperatures the negative effect of disease prevalence on survival rates also increases (Fig.2. 6). Autumn rainfall had a relatively weaker impact upon survival (β_{AR} = -0.067, posterior probability of negative slope = 0.86; Fig.2.6).

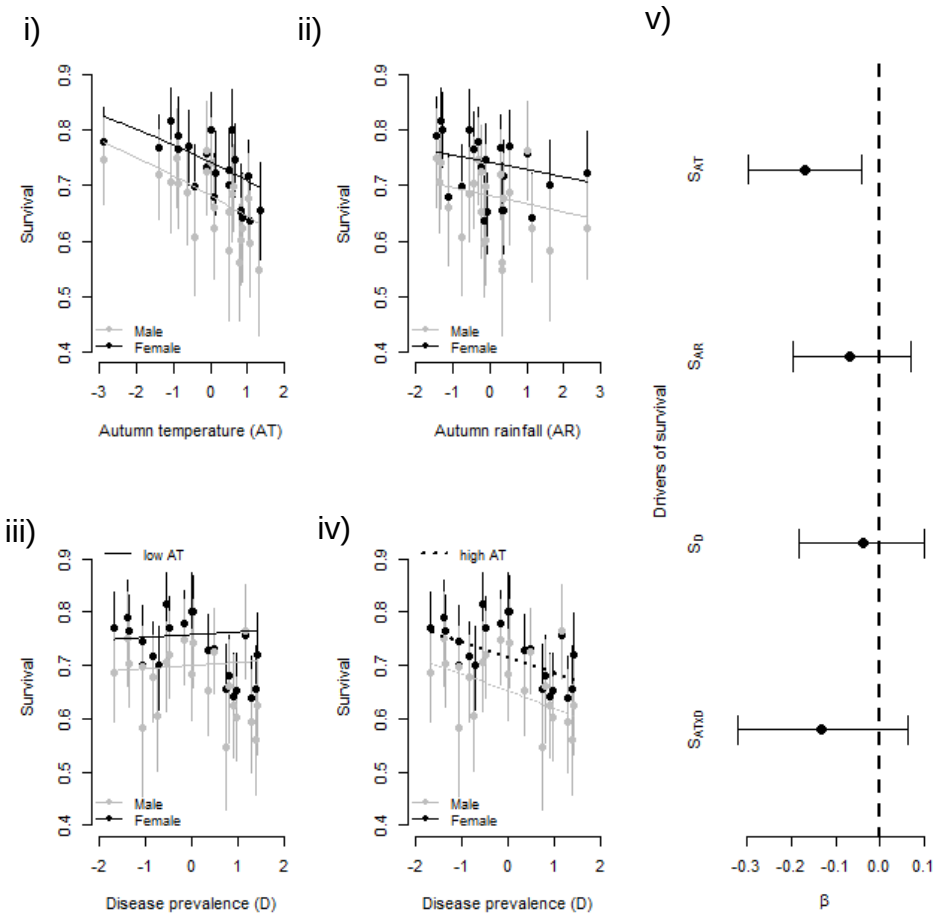

Figure 2.6. i-iv) The effect of covariates; autumn temperature (AT), autumn rainfall (AR) and disease (D), on survival rates as predicted by the IPM, iii-iv demonstrate how the effect of disease prevalence on survival is weak under low autumn temperatures but intensified at high autumn temperatures due to an interactive effect between disease and autumn temperature (AT x D). v) Followed by regression slopes describing the relationship between survival (S) and covariate effects. The posterior means are displayed alongside 95% credible intervals, on a logit scale.

Recruitment posteriors

Mean annual per capita recruitment is 0.41 (95% CRI 0.35-0.47). Rates of recruitment increased with increasing numbers of January frost days (β_{FJ} = 0.218, posterior probability 0.95; Fig.2.7). Recruitment increased with warmer

spring temperatures (β_{ST} = 0.199, Fig.2.7) but the posterior probability of this effect was 0.94. Even with the inclusion of these weather drivers, recruitment remained powerfully density-dependent (β_N = -0.239, posterior probability = 0.99; Fig.2.7). In models that ignored density-dependent recruitment, spring temperature effects were not identifiably different from zero.

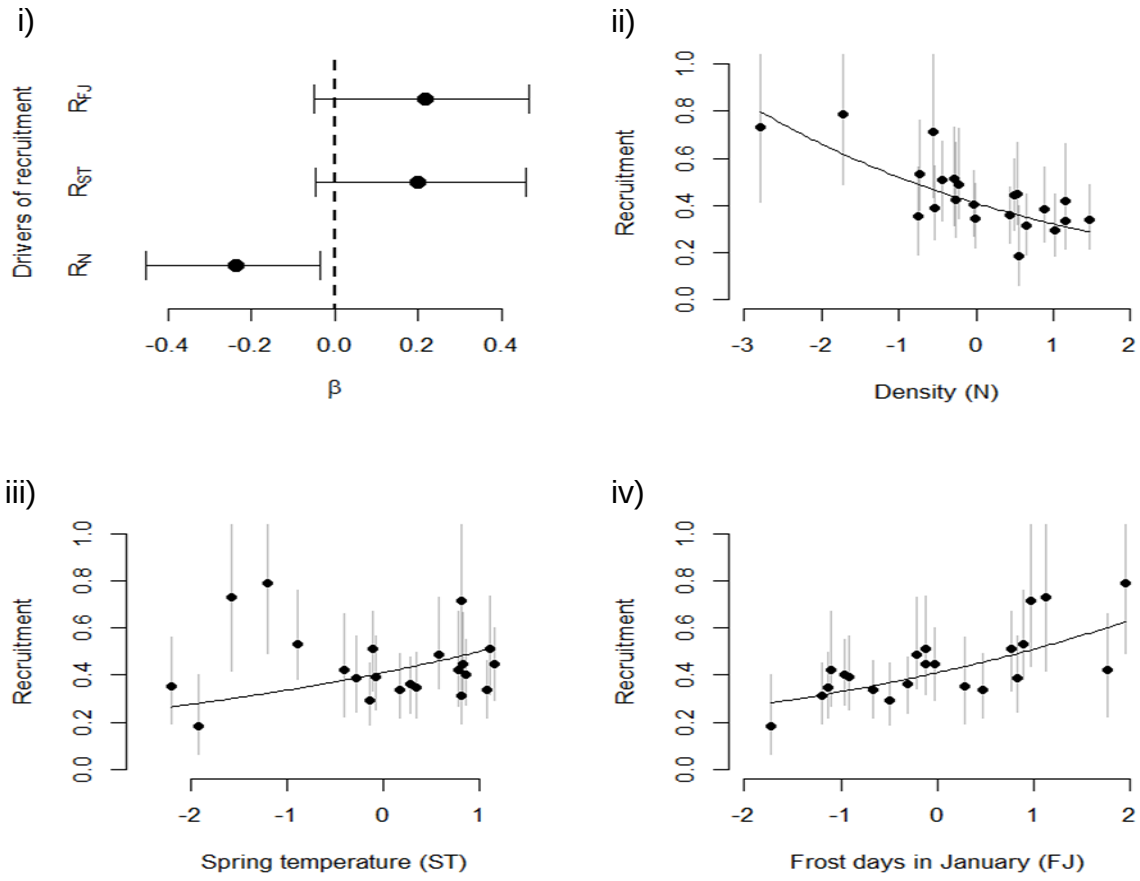

Figure 2.7. i) Regression slopes describing the relationship between recruitment (R) and covariate effects; density (N), frost days in January (FJ) and spring temperature (ST). The posterior mean is displayed alongside their 95% credible intervals on a log scale. ii-iv) Followed by their effects on recruitment rates predicted from an IPM.

Components of variation in recruitment and survival

The IPM including density, spring temperature and January frost days, explained 50% between year variance in recruitment. Models without density explained just 6% of variation in recruitment.

Male and female badgers have similar levels of temporal variation in survival, but the sexes differed in the relative contribution of disease and climatic variables. 38% between year variance in male survival and 47% in female survival was explained by autumn temperature, disease and autumn rainfall covariates. This contrast appears due to the effect of disease prevalence with disease only models accounting for more variation in female survival than variation in male survival (Chapter 2.1).

Correlations

Recruitment correlated most strongly with the population fluctuating growth rate (r=0.75, Fig. 2.8). Male survival had the lowest correlation (r=0.58, Fig. 2.9) marginally less than female survival (r=0.61, Fig. 2.9). The coefficient of variation (CV) was calculated for all demographic rates revealing little year to year variation in male and female survival (CV < 10%) compared to recruitment (CV>30%).

Focussing on the covariate effects; density displayed the strongest negative correlation to population growth (r=-0.51, Fig. 2.8) and frost days in January displayed the strongest positive correlation (r=0.55, Fig. 2.8), compared to spring temperature which was not identifiably correlated to population growth (r=-0.05, Fig. 2.8). Focussing on survival covariates disease prevalence had a negative correlation (r=-0.38, Fig. 2.9), slightly stronger than autumn temperature (r=-0.32, Fig. 2.9), followed by a weaker negative correlation with autumn rainfall (r=-0.28, Fig. 2.9).

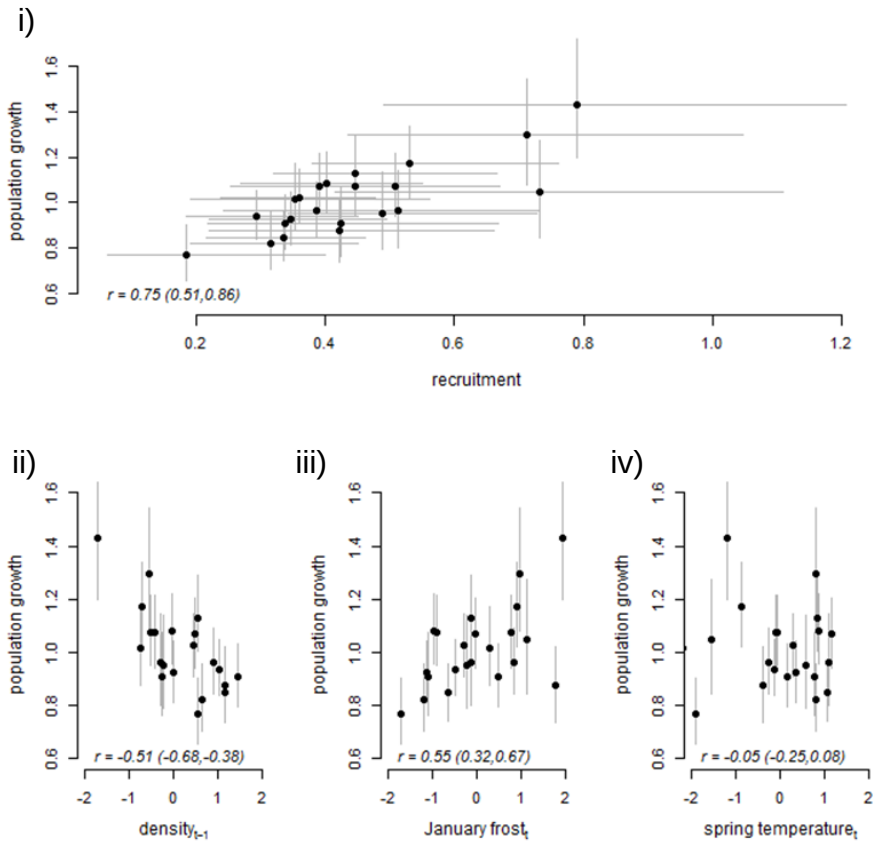

Figure 2.8. i) Estimates of annual recruitment rates ii-iv) and its drivers, plotted against population growth, including the posterior means and 95% CRI. The correlation coefficients (r) are shown along with their 95% CRI.

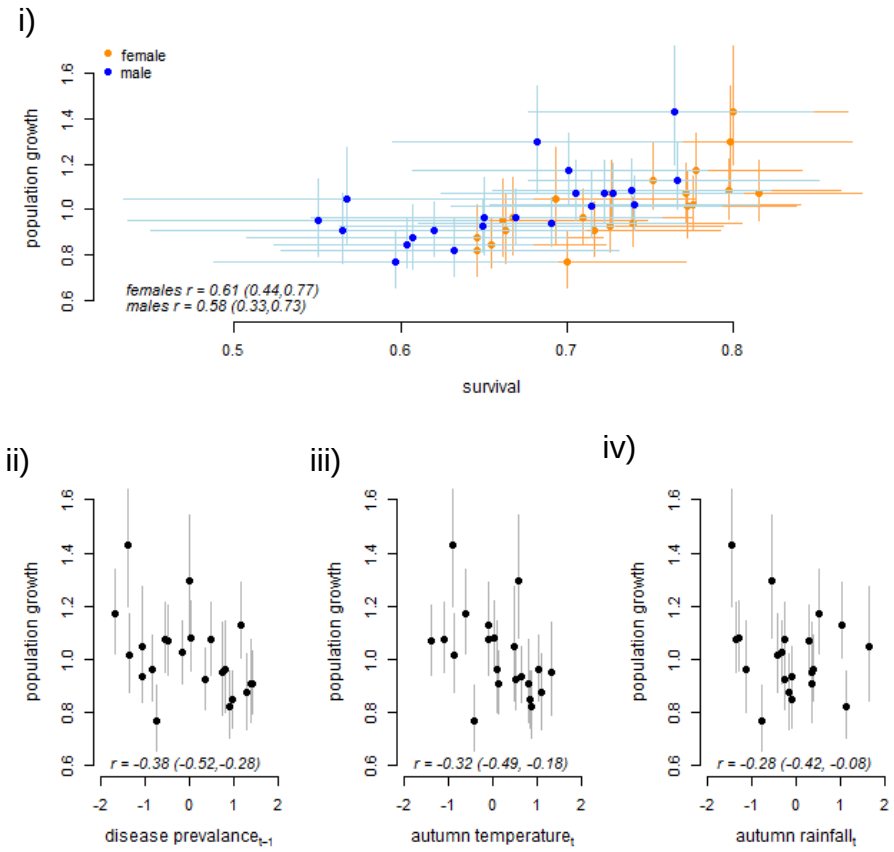

Figure 2.9. i) Estimates of annual survival rates ii-iv) and its drivers plotted against population growth, including the posterior means and 95% CRI. The correlation coefficients (r) are shown along with their 95% CRI.

Discussion

Studies able to disentangle the effects of weather changes on vital rates of wild populations provide important insights in to mechanisms that determine how populations will respond to climate change. In this paper we demonstrate how key demographic quantities and weather effects can be calculated using a Bayesian IPM. After accounting for disease and density, this study quantified how weather changes impact the vital rates of a long-lived reservoir host, supporting the hypothesis that badger populations are sensitive to weather conditions. We highlight that following density, overwinter frost and spring temperatures also drive recruitment rates. Fluctuations in survival were largely

driven by autumn temperature, followed by its interactive effect with disease prevalence. The effect of disease is intensified during years with high autumn temperatures, showing how weather interactions may facilitate disease-mediated mortality.

Effects on survival

Autumn temperature was the best predictor of badger survival rates. Autumn is a crucial time of fat deposition in badgers. The significant negative relationship between increased autumn temperature and badger survival is likely due to temperature shifts affecting foraging conditions, limiting resources required for badgers to obtain the weight they require to survive overwinter (Macdonald et al., 2010; Roper, 2010). Badgers have a wide and varied diet with weather effects capable of affecting multiple resources. Firstly, warmer temperatures may change the optimum microclimate for earthworm emergence. Alternatively increased temperatures may influence availability of plant material by delaying fruiting, mast seeding and windfalls, which badgers rely on more so when insect availability diminishes in the autumn (Roper, 2010). In contrast to previous analysis we find a weak negative association between autumn rainfall and survival, however a positive effect was demonstrated in other badger systems in an area where annual rainfall is lower (~200mm less per annum; (Macdonald et al., 2010; Nouvellet et al., 2013)) and more likely to be a limiting factor. From this result we can deduce that the effect of weather changes across different badger populations is likely to generate a range of population responses dependent upon local conditions, but periods of vulnerability remain similar across areas.

Inclusion of autumn temperature reduced the predicted negative impact of disease and revealed an interaction between temperature and disease prevalence. That is, the impact of disease prevalence on survival is negligible during average conditions, but the strength of the negative effect intensifies at high temperatures. Although the posterior probability of an interactive effect (91%) may remain unconvincing statistically, it may be biologically important if environmental processes that alter food sources increase badger susceptibility to disease, due to the nutritional costs of immune system functioning

(Lochmiller & Deerenberg, 2000; Plowright et al., 2008). This result adds to an increasing amount of evidence that climate change is an important driver of infectious disease (reviewed in Altizer et al. (2013)), due to harsh weather and poor nutrition weakening immune system functioning. Further investigation to uncover the direct causation of this relationship will require consideration of both within host disease effects along with large scale population processes.

Effects on recruitment

Density dependent recruitment is important in the regulation of badger populations. Adding environmental covariates to the model did not alter the pattern or strength of density-dependence. Relative to density, the strength of weather effects on recruitment rates are weaker but still deemed influential in our model estimates. Firstly, conditions in January were important for our measure of recruitment and correlated with population growth rates. That is, decreased ground frost during this overwinter period reduced reproductive success, with less cubs emerging in the spring. Milder overwinter conditions are linked to increased activity levels in badgers (Kowalczyk et al., 2003; Macdonald et al., 2010), contrary to their natural adaptation to remain dormant overwinter to conserve energy and sustain fat stores when resources are scarce (Fowler & Racey, 1988). We postulate that years with reduced frost may incite increased energy expenditure (Woodroffe & Macdonald, 1995) and have associated reproductive consequences (Bevanger & Broseth, 1998) either causing abortion or impairing parental care of new born cubs; we cannot establish whether this mechanism acts on pre- or post-natal losses due to the unknown time of parturition.

Secondly, cub emergence rates were susceptible to spring temperatures. Cubs favoured warmer conditions. The influence of spring conditions on cub emergence rates was only detectable after accounting for density dependence and not directly correlated to population growth rates, therefore its effect appears to dampen and elevate the consequences of changing densities, suggesting its main mechanism may be linked to resource availability and improvement of foraging conditions. Alternatively, warmer conditions may act directly decreasing the cost of thermoregulation, which has energetic

consequences in other mammals (Coulson et al., 2001; Rodel et al., 2004). These findings also show assumptions that weather effects can be detected independently are erroneous with true environmental stressors obscured by the lack of density in badger models. These results agree with the notion that fitting models with fewer parameters might restrict the ability to uncover ecological mechanisms occurring within populations, and a full model may be more equipped to capture population processes (Benton et al., 2006).

General implications

Although we are unable to predict long-term demographic patterns driven by climate change, by decomposing the effects of these small changes in weather on vital rates, we gain insight into the mechanisms determining population responses to climate. In general population dynamics of long-lived species, like badgers, are highly reactive to variations in factors regulating survival. In accordance with the environmental canalization hypothesis (Gaillard & Yoccoz, 2003), we would predict increased resilience in survival rates to environmental variation. Despite, survivals stability under density pressure and overwinter weather effects, it appears that badgers are compromised by their reduced capacity to adapt to changes in autumn conditions. A physiological adaptation to gain weight during autumn (Roper, 2010) increases their susceptibility to climatic perturbations during this essential time of fat deposition, which in turn is suggested to increase their vulnerability to infection. This highlights a mechanism through which climate change could influence a seasonally driven mammal, such as badgers, which have little adaptability to changing conditions during their annual periods of weight gain. Longer term consequences will depend on whether warmer autumns become more frequent which may delimit badgers seasonal physiological cycles, and be of negative consequence to their population growth.

Conclusion

Our findings substantially improve our understanding of badger population dynamics, quantitatively describing the impact of weather changes on demographic drivers. Weather fluctuations influence badger populations

through various mechanisms; impacting food availability, activity levels and even shown to intensify the negative impact of disease prevalence. Badgers have little adaptability to changes in autumn temperature and are likely to be most vulnerable to seasonal changes during this period. These results also confirm the importance of density-dependent recruitment in moderating badger abundance, which likely buffers against the negative influences on survival. The use of IPMs to uncover complex interactions between demographic and environmental components is becoming increasingly established especially with regard to avian populations (reviewed in Schaub and Abadi (2011)). We show how they provide insight into mechanisms by which a mammalian host population may respond to climate change, and uncover complex interactions between environmental change and infectious diseases. We encourage their use to uncover further complexities in both the badger-TB system and to pose hypotheses in similar study populations.

CHAPTER 3

The previous chapter took a scaled back look at the processes governing long-term badger population dynamics. In the following chapters I move away from population dynamics to focus on individual-level effects of disease. Exploration of various techniques to extract key information is a key theme throughout this thesis. This chapter introduces disease states of badgers and using a multistate model obtains rates of disease-induced mortality, disease transmission and progression, and forms the first published paper of this thesis.*

*Graham, J., Smith, G. C., Delahay, R. J., Bailey, T., McDonald, R. A., & Hodgson, D. (2013). Multi-state modelling reveals sex-dependent transmission, progression and severity of tuberculosis in wild badgers. *Epidemiology and infection*, *141*(07), 1429-1436.

Multistate modelling reveals sex-dependent transmission, progression and severity of tuberculosis in wild badgers

Summary

Statistical models of epidemiology in wildlife populations usually consider diseased individuals as a single class, despite knowledge that infections progress through states of severity. Bovine tuberculosis (bTB) is a serious zoonotic disease threatening the UK livestock industry, but we have limited understanding of key epidemiological processes in its wildlife reservoirs. We estimated differential survival, force of infection and progression among disease states in a population of Eurasian badgers *(Meles meles*), naturally infected with bTB. Our state-dependent models overturn prevailing categorisations of badger disease-states, and find novel evidence for early onset of disease-induced mortality among male but not female badgers. Males also have higher risk of infection and more rapid disease progression which, coupled with state-dependent increases in mortality, could promote sex-biases in the risk of transmission to cattle. Our results reveal hidden complexities in wildlife disease epidemiology, with implications for the management of TB and other zoonotic diseases.

Introduction

Many of the world's important diseases of humans and livestock are zoonotic, being harboured by and transmitted from wildlife reservoirs (Jones et al., 2008). Management of these diseases requires detailed understanding not just of their clinical epidemiology, but also the demographic processes of disease transmission, and of progression and disease-induced mortality, which may themselves vary among disease states, sexes or ages of hosts. Disease progression is commonly estimated and modelled in human-epidemiological studies (e.g. (Chen et al., 1996; Dasbach et al., 2006)). Few models of wildlife epidemiology consider disease states beyond the standard SIR/SEIR categories of classical models and we are not aware of any capture-mark-recapture (CMR) multi-state analysis that directly addresses parameterisation of

disease progression through intermediate disease states in wildlife populations. Predictions of effective disease management strategies, based on mathematical models, tend to be highly sensitive to transmission, progression and mortality parameters (Anderson & Trewhella, 1985; Kramer-Schadt et al., 2009; Shirley et al., 2003; Smith et al., 2001a). Therefore, better understanding of state-dependent epidemiology should improve management strategies, providing benefits to human wellbeing or the economic viability and health and welfare standards of livestock farming. Here we use state-dependent statistical models to reveal complexities in the ecological epidemiology of an important zoonotic disease: bovine tuberculosis in wild badgers.

Bovine tuberculosis (TB caused by *Mycobacterium bovis*) has severe consequences for the livestock industry in the UK. TB prevalence in cattle has increased in recent decades (Bourne, 2007; Gilbert et al., 2005), with substantial costs for farmers and other taxpayers. Badgers are a wildlife reservoir of TB in the UK and the Republic of Ireland and are strongly implicated in the transmission of *M. bovis* to cattle (Donnelly et al., 2006; Griffin et al., 2005). In addition to cattle control measures, badger culling has been used intermittently as a disease control option in the UK and Republic of Ireland (Gortazar et al., 2012). Additional strategies include enhanced bio-security measures and vaccination (Chambers et al., 2011; Judge et al., 2011).

Over the past 25 years, several models have been used to simulate the dynamics of TB in badger populations. In early susceptible-exposed-infectious models (Anderson & Trewhella, 1985; Bentil & Murray, 1993), badgers were considered to become infectious upon detection of *M. bovis* bacilli excreted from lesions. Estimates of disease-induced mortality in infectious badgers ranged from 0% (Bentil & Murray, 1993) to 100% (Anderson & Trewhella, 1985). Another long-standing categorisation of badgers divides the infectious category into “excretors” (badgers that are found to shed TB bacilli intermittently) and “super-excretors” which are assumed to be more consistently infectious (Shirley et al., 2003; Smith et al., 2001a). Super-excreting badgers have been modelled as experiencing enhanced disease-induced mortality ranging between 22.4-60% (Smith et al., 2001a; Smith et al., 1995). Parameter

estimates of transmission, disease progression and disease-induced mortality are pre-requisites for the prediction of TB prevalence in host populations (Anderson & Trewhella, 1985; Shirley et al., 2003). These parameters are drivers of disease incidence in the established badger-TB model (Smith et al., 2012) and rank among the key determinants of the rate of cattle herd incidence. Therefore, uncertainty in their magnitude and complexity needs to be reduced. A key question is whether the categorisation of TB infection in badgers, according to stages based on diagnostic test outcomes, reflects biologically relevant and discernible categories of host survival and disease progression.

Detecting population-level impacts of pathogens requires long-term studies of the host and infective agent in their natural environment. At Woodchester Park, Gloucestershire, UK, a population of naturally TB-infected badgers have been studied since 1976 (Cheeseman et al., 1987). Two main diagnostic approaches have allowed assessment of the TB status of each badger over most of this period. The Brock ELISA (Enzyme-linked immunosorbent assay (Goodger et al., 1994)) test detects *M. bovis* antibodies in blood serum. The second diagnostic test cultures *M. bovis* from sputum, faeces, urine, or swabs of wounds and abscesses (Clifton-Hadley et al., 1993). Although a relatively insensitive diagnostic approach (Drewe et al., 2010), positive culture gives an unequivocal indication of active excretion of *M. bovis* and hence an infectious state.

Only one previous study has attempted to parameterise badger mortality using demographic data from Woodchester Park (Wilkinson et al., 2000). The authors classified badgers as uninfected, Brock ELISA positive, single culture positive, and super-excreting. However, the definition of a super-excretor was a badger with more than one culture-positive result, from any sample. The inherent weakness in this approach is that it classified an animal which was excreting only intermittently from the same source, as a super-excretor, even if the disease had not progressed. As no alternative categorisations were considered the authors may have overlooked disease states of intermediate severity. In other host species, TB infection exhibits a wide spectrum of pathology (Blower et al., 1995; Thorns et al., 1982) and so exploration of disease-state-specific

mortality is likely to be productive in the badger-TB system. As TB infection in badgers progresses the number of sites of excretion increases (Corner et al., 2011; Gallagher et al., 1998), hence the existence of multiple excretion sources seems an obvious candidate proxy for disease severity.

Here we use state-dependent statistical modelling of the capture-mark-recapture histories of a marked population of wild badgers to assess sources of variation in class-specific epidemiological parameters, focusing on survival and disease progression (transition between disease states). We present a new classification of badgers based on disease severity, and provide estimates of mortality, force of infection and rate of disease progression. Our analyses improve upon previous estimates of TB-induced mortality in badgers, and more significantly will allow better evaluation of management strategies and improve our understanding of the outcomes of generalised or targeted management approaches to wildlife disease.

Methods

Recapture data

We used live capture data collected at Woodchester Park from 1984 to 2005 inclusive, as this period used consistent protocols consisting of quarterly trapping events at each social group's sett. Trapped badgers were anaesthetized and tattooed with an individual ID upon first capture. At every capture event the location, sex and age-class were recorded (for detailed methods see Delahay et al. (2000)). Blood samples were tested for antibodies to *M. bovis* using the Brock ELISA test (Goodger et al., 1994). Samples of faeces, urine, sputum and pus from abscesses and/or bite wounds were taken for culture of *M. bovis* (Clifton-Hadley et al., 1993).

Capture histories of 88 encounters (22 years x 4 trapping periods/year) were created for each badger. We considered a badger to be in one of four states on each encounter, classified according to the results of the diagnostic tests. A badger with no positive ELISA results and no positive culture results was classed as "test-negative" (N), while positive ELISA test result without positive

culture was classified as “ELISA positive” (P). Accurate diagnosis of TB in live badgers is difficult due to limitations in the performance of the diagnostic tests (Clifton-Hadley et al., 1995). To control for a specificity of 89-94% (Clifton-Hadley et al., 1995; Greenwald et al., 2003) of the ELISA test we considered badgers with only one ELISA positive result, followed by entirely negative results thereafter, to be false positives (Forrester et al., 2001), reducing the likelihood of misdiagnosis of infection. A positive culture result from a sample from one body site resulted in classification as a “one site excretor” (X) and if bacteria were isolated from more than one body site then the animal was classed as a “multiple site excretor” (XX). These categories (Fig. 3.1a) recognise that the number of excretory sites increases as TB infection progresses in badgers, indicating the spread of lesions or an increase in their severity (Corner et al., 2011; Gallagher et al., 1998). Models were also run using the standard definitions of “test negative”, “ELISA positive”, “excretor” and “super-excretor” (Wilkinson et al., 2000), to compare model fit with our proposed categorisation. The key difference is that the prevailing ‘super-excretor’ badger has multiple positive culture samples inclusive of culture positives from the same site, while our ‘multi-site excretor’ badger only includes multiple positives from different body sites. Additionally, to evaluate whether inclusion of multiple disease states provides important information, we compared standard susceptible-infected (SI) models with our proposed categorisation.

State-dependent statistical modelling framework

Data was analysed using multi-state models in the program MARK (White & Burnham, 1999) via the R interface (R Development Core Team, 2013) and the package RMark (Laake, 2011). Multi-state models (Lebreton et al., 2009) were used to analyse time-, age class (cub and adult)- , sex- and disease-state-specific variation in quarterly rates of survival, recapture and transition between disease states. We compared the performance of state-dependent models that included the established and the novel classifications of disease state. Models were assessed using Akaike Information Criteria (AIC) adjusted for overdispersion (QAIC) (Burnham & Anderson, 2002). ‘Better’ candidate models were indicated by their lower AIC values. Substantial support for the best model

alone is indicated when rival models all have QAIC > 2 units larger (Burnham & Anderson, 2002). We tested for overdispersion of models using the 'median c-hat' method as implemented in Program MARK (White & Burnham, 1999). We applied the highest estimate of overdispersion (1.28) to the results, which did not qualitatively change the findings but means that the significance of differences between parameter estimates is conservative. Significant differences among survival estimates of male and female badgers in different disease states were tested using z-scores with false discovery rate adjustment for multiple testing. Adjusted p-values less than 0.05 were considered significant.

Results

During the period 1984-2005 1640 badgers were trapped (674 males, 786 females). These individuals contributed 7699 capture events comprising 6739 uninfected occasions, 515 ELISA positive occasions, 285 one-site excretor occasions and 160 multi-site excretor occasions.

Best models

The best models indicated that survival (Φ) probabilities varied according to sex and disease status (Table 3.1). There was no evidence of age-specific mortality (Table 3.1). Recapture probabilities varied considerably over the 22 year period with apparent seasonality. Males had a consistently higher probability of recapture than females throughout all trapping sessions. Quarterly recaptures varied from 0.15±0.03 to 0.73±0.03 for females and 0.20±0.03 to 0.78 ±0.03 for males. Transition (Ψ) probabilities among states depended on sex and disease status (Table 3.1), but not age or time. The new categorisation of disease states improved model fit dramatically when compared to the previous categorisation of uninfected, ELISA positive, excretor and super-excretor (Wilkinson et al., 2000) (Table 3.1). There was also more support for the inclusion of multiple disease states (N, P, X and XX) than the standard, binary susceptible-infected (SI) epidemiological models (Table 3.1).

Table 3.1. Candidate multistate models of badgers categorised by disease state. Columns 1-3 describe the additive (+) or interactive (*) effects of sex, age and disease state on survival, transition and recapture probabilities. The 'best' two models classified badgers as negative (N), ELISA positive (P), one-site excretor (X) and multi-site excretor (XX). Competing models included: previous infectivity categorisation of uninfected, ELISA positive, excretor and super excretor; simplified categorisation of uninfected and infected; inclusion of age effects. Competing candidate models had zero model likelihood therefore only relevant examples are provided.

New Categorisation						
Survival	Transition	Recapture	QAIC	Number Parameters	QAIC Weight	Model Likelihood
disease * sex	**disease + sex**	**time + sex**	**20143.25**	**103**	**0.742**	**1**
disease + sex	**disease + sex**	**time + sex**	**20145.37**	**100**	**0.257**	**0.35**
disease*sex*age	disease + sex	time + sex	20957.96	111	0	0
age*sex	disease + sex	time + sex	20181.86	99	0	0
sex+age	disease + sex	time + sex	20179.91	98	0	0
***Prior Categorisation**:*						
disease * sex	disease + sex	time + sex	20624.15	109	0	0
Uninfected/Infected (SI) Categorisation						
disease*sex	disease + sex	time + sex	20166.88	99	0.00001	0

Survival

The severity of TB, as indicated by diagnostic test results, influenced quarterly survival probabilities in badgers. After adjustment for multiple comparisons, for both males and females the lowest survival probability occurred in multi-site

excretors (Figs. 3.1b, 3.1c, 3. 2). Quarterly survival probabilities of males in every infected state were significantly lower compared to uninfected male badgers (90.6% survival probability) and decreased from ELISA positive (86.7%, Z= -1.81, p = 0.035), to one site excretor (83%, Z= -2.59, p = 0.005) and finally to multi-site excretor (60.7%, Z= -6.06, p < 0.001). Female survival probability did not vary among uninfected and initial stages of disease progression (uninfected 92.6%, ELISA positive 92.4%, one-site excretor 92.8%), but a significant decrease in survival was observed between uninfected badgers (92.6%) and multi-site excretors (78.9%, Z= -5.36, p < 0.001).

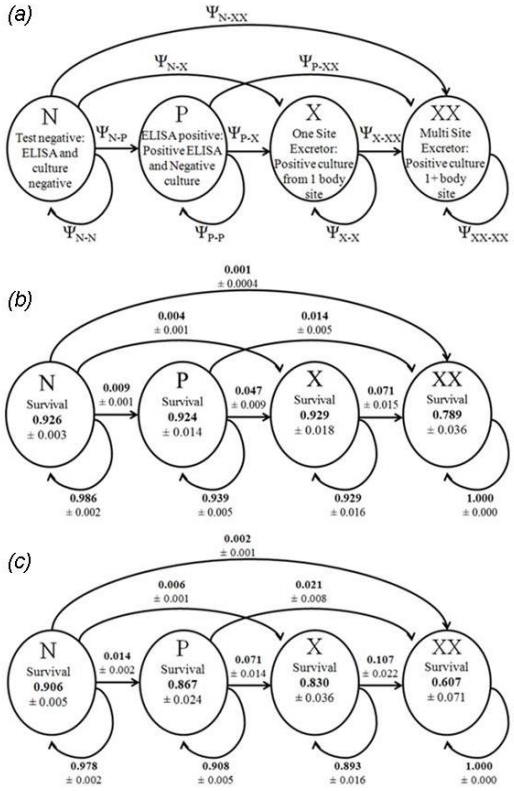

Figure 3.1. *(a)* Depiction of the multi-state model used for analyses. Transitions could only occur in the direction of the arrows. Quarterly estimates of state-transition rates and their standard errors for *(b)* female and *(c)* male badgers are provided, for surviving individuals.

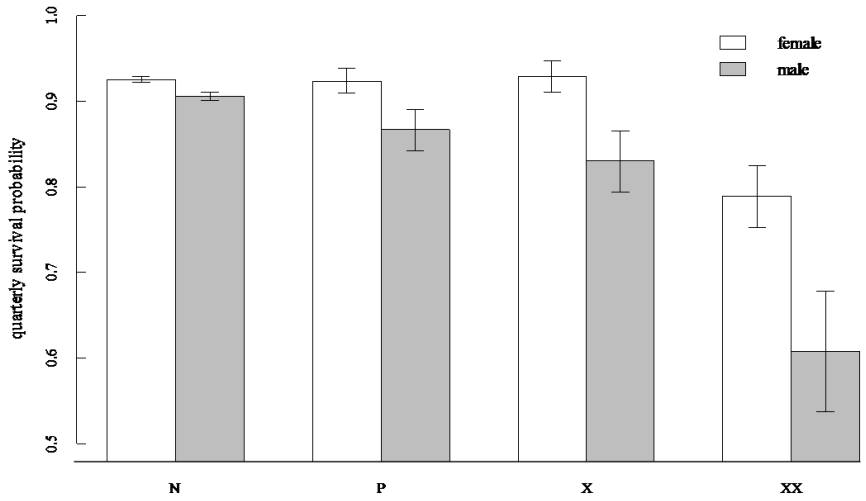

Figure 3.2. Quarterly survival estimates of female and male badgers when classed as: negative (N), ELISA positive (P), one-site excretor (X) and multi-site excretor (XX). In each case the parameter estimate is shown ± s.e.

Male badgers had significantly lower survival probability than females across all states (Fig. 3.2): uninfected state (Z=-2.54, p=0.005), ELISA positive state (Z= -2.14, p= 0.016), one-site excretor state (Z=-2.63, p=0.004) and multi- site excretor state (Z= -2.377, p = 0.034).

These results correspond to the following annual survival estimates, exclusive of cub-adult age classes, for males; uninfected 67.4%, ELISA positive 56.5%, one-site excretor 47.5% and multi-site excretor 13.4%, and females; uninfected 73.5%, ELISA positive 72.9%, one-site excretor 74.2% and multi-site excretor 38.7%.

Transition between disease states

Transition rates from multi-state models provide a measure of the probability of an individual becoming infected and also of the disease progressing. The best

supported models in the candidate set showed that transitions depended on the sex and disease state of the individual badger (Table 3.1).

The force of infection, i.e. the probability of moving from an uninfected to an infected state, was higher for males than females (Figs 3.1b and 3.1c). Hence, 2.2% of males became infected in any quarterly period compared to 1.4% of females. Males had a higher probability of disease progression than females: 7.1% of ELISA positive males progressed to be detected as a one site excretor in a quarterly period compared to 4.7% of females. Males were also more likely to become multi-site excretors with 10.7% of males in the one site excretor category progressing to this stage quarterly, compared to just 7.1% of females (Figs 3.1b and 3.1c).

Discussion

Studies of the epidemiology of zoonotic diseases have traditionally viewed the wildlife reservoir as a homogeneous population, with limited appreciation of variation in transmission, progression and mortality among demographic classes or disease states. In systems where stage-specific demographic information is available, state-dependent statistical modelling can reveal epidemiological complexities that could in turn be key drivers of disease persistence, and transmission between wildlife hosts and livestock or humans. Better understanding of these complexities should influence the assessment of disease management strategies. The badger-TB interaction exemplifies this argument: we have shown that key epidemiological parameters, to which current predictions of management options are highly sensitive (Smith et al., 2012), vary among disease states, and are sex-specific but not age-specific. These parameters will be incorporated into future TB models for improved evaluation of management strategies.

Male badgers suffer increased mortality during intermediate stages of disease progression, while females do not. Incorporating disease states of varying severity uncovered this additional variation and provides a better explanation of survival than a more traditional susceptible-infected approach. We have confirmed (Cheeseman et al., 1987; Wilkinson et al., 2000) that survival rates of

uninfected male badgers are lower than in females. We have also confirmed that survival rates of both sexes are significantly lower in multi-site excretors than in uninfected badgers (Wilkinson et al., 2000), and shown that multi-site excretor males suffered 29.9% additional mortality per quarter, double the additional mortality seen in females in the same state. Our results challenge the prevailing wisdom that cub survival rates are lower than those of adults (Anderson & Trewhella, 1985), although mark-recapture data cannot inform on mortality of offspring prior to emergence from natal setts.

This is the first study to provide empirical estimates of the force of infection, and rate of progression, of TB among badgers. Males were more likely to become test positive, suggesting that males are more liable to acquire infection. Further work is required to determine whether this force of infection is density- or frequency-dependent, *sensu* the transmission parameters of classic epidemiological models (McCallum et al., 2001). We also found that males progress through disease states more rapidly than females. Both behavioural and immunological mechanisms may cause the observation of higher infection risk and faster disease progression in male badgers. Males tend to range further than females (Delahay et al., 2006), perhaps increasing their risk of exposure to sources of TB. Males are more territorial (Delahay et al., 2006): associated incidence of bite wounds exposes them to a different route of infection compared to females, resulting in different patterns of disease progression (Cheeseman et al., 1988). Alternatively males may have weaker, or compromised, immune responses, which would increase all three epidemiological parameters. Teasing apart behavioural and immunological mechanisms will require detailed assay of infection and disease progression in individual badgers, and the answer could determine the efficacy of the various TB management strategies for badgers. It remains unclear whether males or females are most responsible for transmission of TB to other badgers or to cattle: males progress to infectious states more rapidly but are more likely to die; females spend more time in infectious states and might transmit infection to offspring; males might cause more transmission due to their wider-ranging movement. A complete demographic consideration of TB epidemiology will

require us to model state-dependent fecundity, recruitment and dispersal parameters.

Current tactical models that help inform UK policy related to bovine TB control have found that both disease prevalence and cattle herd breakdown rates are sensitive to badger TB transmission rates, mortality rates and disease progression (Smith et al., 2012). Our study contributes a significant revision of these key parameters, and yields novel demographic insight into the sex- and state-dependent epidemiology of TB in a wildlife reservoir. We recommend the use of this revised disease categorisation, and improved epidemiological parameters, to increase the predictive power of strategic models for control of bovine TB. Disease-transmission and disease-induced mortality are critical parameters in any infectious disease model, therefore we recommend multistate modelling for the study of the ecological epidemiology of wildlife reservoirs of any diseases that transmit to humans or livestock.

CHAPTER 4

This chapter follows an analytical progression towards a Bayesian philosophy. Performing the same multistate analysis in an alternative framework I explore the capabilities of Bayesian posteriors to provide additional conclusions. I also consider the possibilities of incorporating social structuring within these models due to the increased modelling flexibility.

Uncovering epidemiological heterogeneity using Bayesian multistate models

Summary

Obtaining epidemiological and demographic information from longitudinal field studies is integral to understanding disease spread and persistence within wild populations, but is often problematic with numerous spatial and temporal complexities. In Great Britain bovine tuberculosis (TB) persists in wildlife reservoirs, principally the Eurasian badger *Meles meles*. We apply capture-mark-recapture data to a Bayesian multistate model to estimate disease-state specific survival and transmission rates of badgers. Building in spatial complexities we attempt to account for heterogeneous associations between individuals which may have consequences for transmission dynamics. We first illustrate the similarities between frequentist and Bayesian models and confirm sex differences in epidemiological rates. We find high probabilities (>0.97) that males are more likely to become infected, infectious, and have reduced survival than females in all health states. Second, controlling for individual variation, we show disease-induced mortality impacts are intensified, reinforcing previous results that TB infection reduces badger survival in males during all infected states and in females in an advanced disease state. More than a fifth of variation in transmission rates was explained by social group. Sex differences remained, with males twice as likely to become infected compared to females. Overall, this study extends a Bayesian model to control for non-independence within badger populations, providing further corroboration that sex is an important determinant of disease susceptibility and social group associations affect infection risk.

Introduction

A major challenge in analysing time-series data is incorporating individual, temporal and spatial complexities, and deciphering the impact these have (Bjørnstad & Grenfell, 2001). This becomes critical when studying host-

pathogen interactions which can be highly heterogeneous, with infection risk and susceptibility often dependent upon, but not limited to, age, life-stage, sex, location, and social structuring. These factors can influence inherent immunology, incite specific behaviours from individuals, along with altering ecological conditions, all of which can bring about inequality in infection risk within populations. Using data from a longitudinal study of naturally infected badgers we explore this concept in a badger-TB system. Applying a Bayesian multistate analysis we consider epidemiological heterogeneity due to sex, as well as the impact of social grouping which may generate non-independence in infection risk among individuals.

Badgers are reservoir hosts of bovine tuberculosis and drivers of TB outbreaks in cattle in the UK and Ireland. Heterogeneities in disease susceptibility can significantly affect infection dynamics (Lloyd-Smith et al., 2005). Previous multistate analysis, using a classical maximum-likelihood approach (Chapter 3), uncovered sex-differences with male badgers experiencing elevated mortality rates and increased disease risk. Epidemiological differences between sexes are an important consideration that can be directly taken forward to guide management strategies. We add to this work and consider an additional possible source of bias in infection risk; social structure. A defining characteristic of badgers is their social living and use of shared underground dens called setts. Badgers form discrete social groups consisting of 2 -27 individuals (Rogers et al., 1997; Roper, 2010; Woodroffe et al., 2009b), with up to 70% of their time spent below ground sharing communal space (Roper, 2010). Social organisation can promote skewed contact rates with high levels of contact within groups and reduced direct contact between social groups. Across animal systems social behaviours have been linked to transmission (Rushmore et al., 2013; Wendland et al., 2010) driving spatial aggregation of disease across host populations (Blanchong et al., 2007; Joly et al., 2006). This represents a general movement away from the traditional view of randomly mixing homogeneous populations towards an acceptance that host association patterns may be heterogeneous. Variability in social structuring across a landscape may be responsible for the observed spatially patchy distribution of

TB (Delahay et al., 2000) and spatial clustering of strains of *M. bovis* (Kelly et al., 2010) across badger populations.

Tuberculosis infection can occur indirectly through contact with *M. bovis* in the environment, but direct transmission via contact with infectious individuals is suggested to be the dominant mechanism with the majority of lesion development consistent with aerosol transmission or bite wounding (Jenkins et al., 2008a). Therefore, accounting for social cohesion in disease analyses is likely to be particularly important if estimates of state-transitions, i.e. infection risk, are directly related to patterns of high within group social contact, with infection probabilities higher for individuals living in groups with TB already present (Vicente et al., 2007). White nosed syndrome in bats is found to be driven by similar social behaviour with clustering in hibernacula facilitating transmission rates (Langwig et al., 2012). However the impact of social structure on transmission will reduce the more individuals move between groups. Badgers although living in these discrete well-defined groups are suggested to move more than previously thought from trapping data alone with extra-group matings accounting for almost half of all paternity (Carpenter et al., 2005; Dugdale et al., 2007) and anecdotal evidence that more aggressive encounters occur between groups than within group (Roper, 2010). Additionally recent evidence suggests infected badgers may have a disproportionate contribution to infection between groups, due to their network position (Weber et al., 2013). If extra-group encounters are linked to transmission events then social partitioning may be trivial with regards to driving infection risk.

Despite a general consensus in the literature suggesting social structure is an important determinant of disease spread, there is a limited understanding on just how much social living contributes to disease dynamics. In the absence of detailed contact network analyses, accounting for non-independence amongst individuals within an analytical framework will be an essential step to uncover how much variation social structure accounts for in badger disease ecology. The incorporation of social group variation is an intractable parameter when using the prior traditional MARK approaches (Chapter 3). This is due to

analytical difficulties when incorporating it as a fixed effect due to data saturation at the social group level, and difficulties implementing individual covariates as random effects within this point-and-click program. We introduce a new approach using a Bayesian multistate model within program WinBUGS. Bayesian approaches are more flexible in their model set up, with mixed effect models coded similar to that of generalised linear mixed models. Creating a hierarchical model to account for multiple processes including social group as a random effect influencing TB transmission is unproblematic. Also, the resulting Bayesian inferences that can be made using the posterior values explicitly recognise parameter uncertainty, providing the probability of a given hypothesis, arguably improving interpretability of the results.

We apply a Bayesian multistate model to a population of naturally infected badgers. We first, compare results to prior classical approaches (Chapter 3) and second incorporate social group and individual variation as random effects to assess the amount of variation social non-independence explains in infection risk within badger populations.

Methods

Study population

The study population is the Woodchester badger society. We utilised the same subset of data (1984-2005), disease categorisation (N, P, X and XX) and quarterly capture histories used in the prior MARK analysis (for full details refer to Chapter 3). This consisted of 1640 badgers (674 males and 786 females). In addition to accounting for sex-related and disease-state differences we apply indices of social group to account for spatial heterogeneity between badgers in separate groups. The majority of badgers are only caught in one social group over their lifetime (82.5%), and the chance of permanent movement is generally considered small (Macdonald et al., 2008; Roper, 2010), therefore we assign group of first capture to each badger to provide an approximate index of social non-independence.

Multistate models

Multistate models build upon basic Cormack-Jolly-Seber (CJS) models which estimate survival and recapture probabilities, to also estimate transition probabilities allowing individuals to move among states or geographic locations. These models have numerous caveats assuming; i) individuals are subject to same capture probability; ii) marking doesn't affect survival, recapture or transitions; iii) marks are not lost; iv) the fate of each individual is independent. By applying this analysis to an infected badger population, this enables inclusion of time-varying discrete covariates specifying the disease state of the individual badger, estimating survival of badgers in progressive health states as well as infection and disease progression probabilities. We build upon the previous frequentist analysis implemented within the program MARK (Chapter 3) and utilise a Bayesian framework.

Bayesian approach

A Bayesian approach shifts the focus from estimating the best model whereby parameters are fixed and unknown quantities (Chapter 3), to providing probability distributions for the parameters themselves. Models were implemented using WinBUGS software (Lunn et al., 2000) via the R2Winbugs package (Sturtz et al., 2005) in program R (R Development Core Team, 2013). By providing a data-set, a statistical model and initial values in BUGS language WinBUGS provides the MCMC component of the analysis. The output is a long list of numbers for parameters of interest which, if convergence has occurred appropriately, represent the posterior distribution from which posterior summaries can be obtained. Specifically, with regards to this analysis we can use the posteriors to calculate the probability of survival rates differing between sexes and between progressive disease states. For the random effects model we can also use the posteriors to calculate the proportion of variation explained by social group.

Data

Male and female badgers were analysed separately due to known epidemiological differences between the sexes, additionally this helped reduce computation time. Input data for the model included;

- Individual capture histories with information regarding the health state of each badger on each capture occasion. This consisted of 4 states (N, P, X, XX) represented numerically as states 1 to 4.
- Vector with occasion of first capture
- Number of individuals
- Number of capture occasions
- Additional covariates: Vector of social group of first capture represented numerically 1:20 in conjunction with 20 different social groups.

Parameters

The model estimates 3 main parameters:

- $\Phi_{i,t}$= Probability that a badger in state i will survival time period t
- $P_{t,i}$= Probability that a badger in state i will be captured within time period t
- $\Psi_{t,i-j}$ = Probability that a badger in state i will move to state j within time period t

Model

Likelihood

The probability of capture history data (x) is equal to the joint probability of state process (z) and observation process (w).

Process model

We define 5 true states: alive test negative (N), alive ELISA positive (P), alive one-site excretor (X), alive multi-site excretor (XX) and dead.

The state equation describes the true development of states i.e. the state of an individual at time t+1, given its state at time t (Table 4.1). This is defined by a categorical distribution whereby state at $Z_{i,t}$ conditional upon state at $Z_{i,t-1}$.

Table 4.1. State transition matrix

		True state at Time t + 1				
		N	**P**	**X**	**XX**	**Dead**
True state at time t	**N**	$\phi_N\psi_{N-N}$	$\phi_N\psi_{N-P}$	$\phi_N\psi_{N-X}$	$\phi_N\psi_{N-XX}$	$1-\phi_N$
	P	0	$\Phi_P\psi_{P-P}$	$\Phi_P\psi_{P-X}$	$\Phi_P\psi_{P-XX}$	$1-\phi_P$
	X	0	0	$\Phi_X\psi_{X-X}$	$\Phi_X\psi_{X-XX}$	$1-\phi_X$
	XX	0	0	0	Φ_{XX}	$1-\phi_{XX}$
	Dead	0	0	0	0	1

The process matrix (Table 4.1) represents the probability of an individual in state S at time t depends on the combined probability of survival and transition at time t-1. The zeroes in the matrix denote that once a badger is infected and advancing through disease states it cannot recover and once dead it remains dead.

Observation model

The observation equation (Table 4.2) links the true state matrix (Table 4.1) with the observed state. We assumed homogeneous recapture across states in accordance with prior analyses (Chapter 3).

Table 4.2. Observation matrix

		Observation at time t				
		N	**P**	**X**	**XX**	**Dead**
True state at time t	**N**	*P*	0	0	0	1-*p*
	P	0	*P*	0	0	1-*p*
	X	0	0	*P*	0	1-*p*
	XX	0	0	0	*P*	1-*p*
	Dead	0	0	0	0	1

Priors

Bayesian inference requires the allocation of priors. Incorporating individual random effects meant survival was modelled on a logit scale to ensure realized individual specific survival was bound between 0 and 1. Priors for the back-transformed mean survival were modelled on a uniform distribution to lie between 0.5 and 1 (dunif~0.5, 1), this improved mixing of the chains, but still encompassed the broad range of survival parameters found previously (Chapter 3).

We use a multinomial logit link function for transition probabilities. For n-1 transitions we specified a normal dnorm(0,0.001) prior distribution corresponding to transition probabilities on a logit scale. Thus ensuring following back transformation their sum is less than 1 and allowing the calculation of the remaining transition parameter via 1 minus the sum of those already calculated.

Covariates

Transition probabilities between disease states incorporate both social group and individual variation as a random effect. Random effects were modelled with a standard deviation following a uniform distribution between 0 and 5, on a logit scale (σ~dunif(0,5)). Random effects were restricted to one type of transition, the probability of infection and assumed to be equivalent within these transitions (Fig. 4.1).

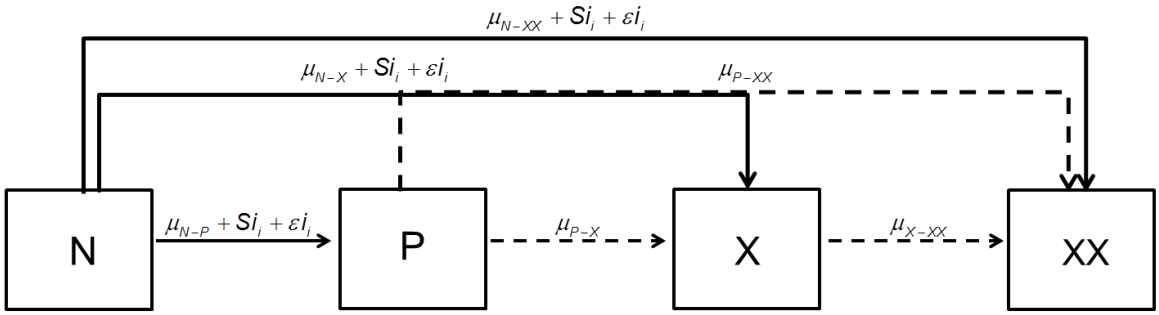

Figure 4.1. Schematic of possible transitions along with their composite equations used in the model, infection probabilities are shown via a solid line and progression probabilities via a dashed line. Transition probabilities (Ψ) are composed of the mean value for each transition (μ) plus variation caused by social group (S) acting on infection risk (Si) and individual variation influencing infection risk (εi). The probability of badgers remaining the same state is not shown here, but was estimated within the model by subtracting all other possible transitions from 1.

Survival was modelled on a logit scale with individual variation absorbed as a random effect using the following equations. The effects of individual variation on survival were assumed to be uniform across disease states.

$$\text{logit}\,\phi_i = \mu + \varepsilon_i$$
$$\varepsilon_i \sim N(0, \sigma^2)$$

Assessment of model's performance

Before obtaining model results there are a few pre-requisites that need to be satisfied. Firstly models need to have converged and an appropriate burn-in period needs to be selected to ensure the posterior distribution used for summary statistics is from the converged chain only. A failure to do this would result in the posteriors including the first part of the Markov chain and due to the autocorrelation in this process it will still have the impact of the arbitrary allocated starting values. We run more than 1 chain to check convergence both informally by looking at a time series plot of the sampled values to visually check chains converge and formally using the Brooks-Gelman-Rubin diagnostic which provides a value termed $\hat{r}$ which when equal to 1 indicates convergence has been reached, if $\hat{r}$ is high then chains need to be run for longer (Brooks & Gelman, 1998). Once convergence has been reached thinning can be used to reduce autocorrelation and save computer space whereby if data is thinned by k every kth sample is saved. After preliminary analysis we found convergence for the models minus random effects were reached after 10000 iterations and subsequently ran 3 chains of length=30000, burn-in=10000 and thinning = 100, all posterior values had converged ($\hat{r}$<1.01). The full model, with social group and individual variation incorporated as random effects, required longer convergence times, we ran 2 chains of length=60000 and burn-in=30000.

Once the model converged and an appropriate burn-in period utilised then summary statistics can be used. A problem with a Bayesian approach is the lack of test for overdispersion and model fit. We utilised the MARK analysis whereby the median c-hat overdispersion parameter <2 indicating the model was not substantially overdispersed (Chapter 3). Although, not accounting for overdispersion may result in variances too narrow, it has been suggested that incorporating individual variation in Bayesian models is analogous to using a variance inflation factor in frequentist approaches (Kéry & Schaub, 2012). We used deviance information criterion (DIC (Spiegelhalter et al., 2002)) to aid in model selection between models with and without random effects, however the value WinBUGS computes is suggested to be problematic in hierarchical

models and mixed effects models. Therefore although we utilise DIC values as a guide we additionally calculate the amount of variation in infection risk explained by social group.

Results

Constant model: comparison of methodological approaches

Our Bayesian model performed well in terms of precision and accuracy when compared to parameter estimation using a frequentist approach. Comparing the 'best' model highlighted from the prior MARK analysis (Chapter 3) with the equivalent Bayesian model we found almost identical results with less than 1% difference between mean quarterly survival values for all point estimates (mean difference= 0.3%).

Female badgers had similar probabilities of quarterly survival in uninfected and early disease stages (P & X) with probabilities of a decrease: rise in survival during these early stages nearer 50:50 than any notable direction of change. Once in an advanced disease state (XX) female survival had a 100% posterior probability of declining (mean survival; 0.93 (N), 0.92 (P) & 0.93 (X), 0.78 (XX); Fig. 4.2).

In comparison, we find survival rates in males had a 97.5% posterior probability of declining at early onset of infection (P & X). Similar to females, survival declined further during the final disease state with a 99.7% probability of male badgers experiencing reduced survival in the XX state (mean survival; 0.91 (N), 0.87 (P), 0.83 (X), 0.61 (XX); Fig. 4.2).

Comparing survival posteriors between sexes, males have lower survival than females in all disease states (posterior probability of reduced survival in males; N 100%; P 99.7%; X 100% & XX 99.5%).

i) ii)

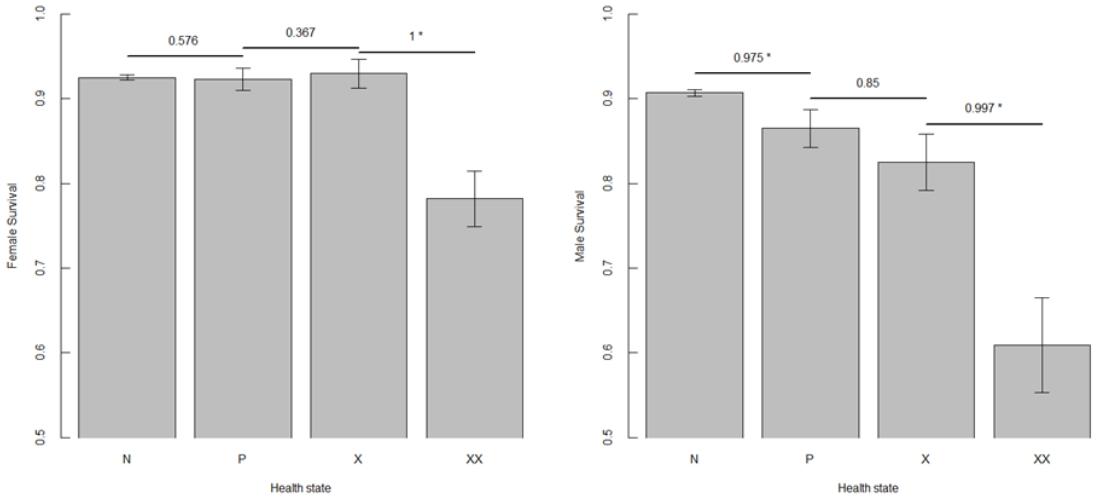

Figure 4.2. Mean quarterly survival estimates of i) female and ii) male badgers in disease states of increasing severity. Values shown are posterior probabilities that as disease states advance in severity that survival rates reduce. *indicate high probability (>0.95) of survival differences between states.

Comparing male and female quarterly infection rates, males are 98.5% more likely to have a higher rate of infection to an exposed class (N-P). They are 99.5% more likely to have a higher rate of infection to an early infectious class (N-X) and 96% more likely to go straight to a highly infectious class (N-XX). Combining all possibilities of infection males are 98% more likely to have a higher rate of infection than females. These infection probabilities are analogous to estimates found previously (Chapter 3), with 2.3% of males and 1.3% females becoming infected in any quarterly period.

Accounting for random effects

Incorporating social group and individual variation was favoured by DIC selection procedures (Δ DIC: males > 59; females > 1). 21.2% (95% CRI; 5, 46) variation in female infection risk and 24.4% (11, 42) variation in male infection risk was explained by social group (Fig. 4.3). After accounting for social group

and individual variation estimates of infection risk declined compared to constant models but still remained strongly sex dependent with 1.2% males (95% CRI 0.7, 1.8) and 0.5% females (0, 1) becoming infected in any given 3 month period.

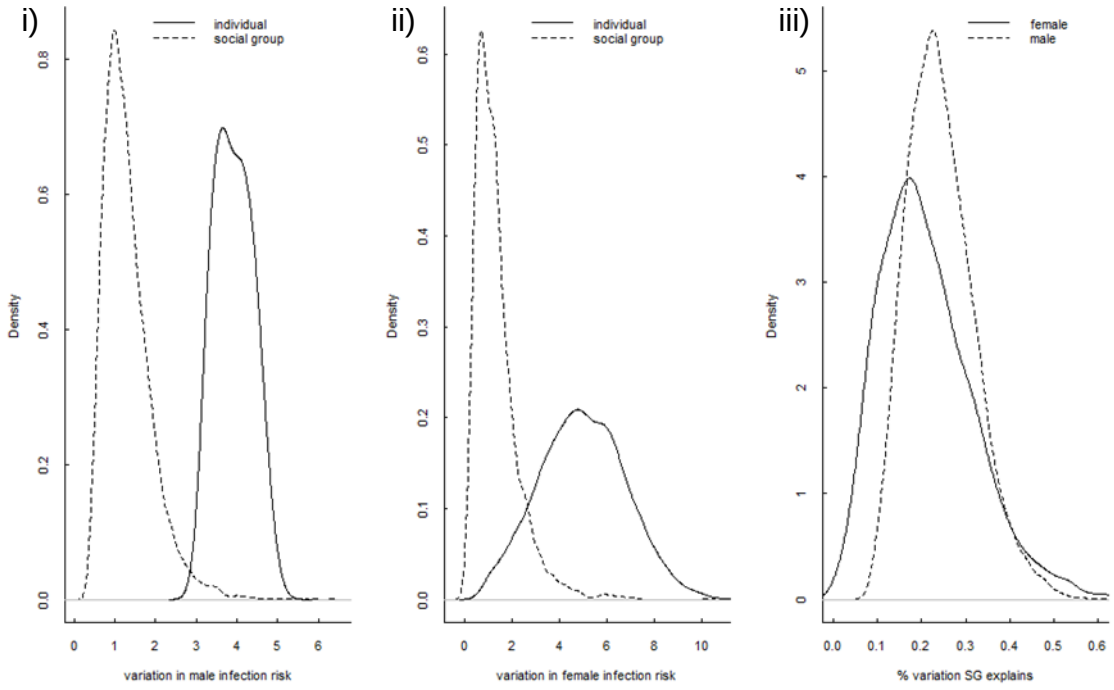

Figure 4.3. i-ii) Social group and individual variation in infection risk for (i) male and (ii) female badgers, iii) followed by proportion of variation explained by social group.

Incorporating individual variation in the model framework, along with social group as a random effect, did not alter mortality patterns with sex-differences in epidemiological traits remaining (Fig. 4.4). However it further exacerbated the impact of infection on badgers. Survival rates for uninfected badgers, remained unchanged, along with estimates of survival in female badgers during early infection states (P & X). However, where we previously found disease-induced mortality (XX females, P males, X males and XX males), the impact of disease

appears more severe (mean survival: female: 0.92 (N), 0.91 (P), 0.92 (X), 0.69 (XX); male: 0.91 (N), 0.83 (P), 0.77 (X), 0.58 (XX); Fig. 4.4).

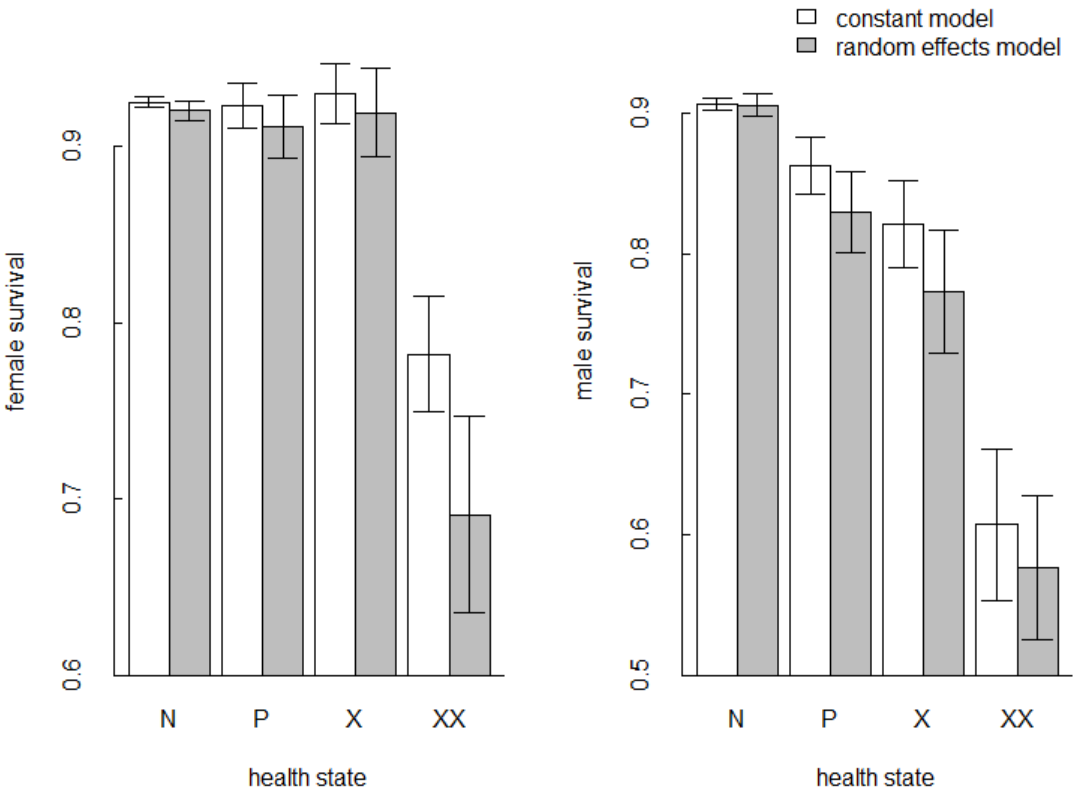

Figure 4.4. Quarterly survival estimates (± sd) of badgers from constant model and random effects model. Note the further reduction in survival estimates for female XX badgers and male P, X and XX badgers.

Discussion

We have explored one way in which non-independence between badger social groups can be incorporated in an epidemiological analysis, illustrating the importance of considering this alongside sex when estimating disease processes. Moving to a Bayesian analysis corroborated parameter estimates generated in previous studies (Chapter 3) and provided additional inference regarding sex-differences in epidemiological traits. By incorporating social structure in a relatively straightforward way we controlled for spatial heterogeneity in infection risk along with individual variation. Analyses showed sex remained an important determinant of disease processes, in addition to

social group which explained over a fifth of variation in infection risk in both sexes. Parameters generated from the mixed effects models varied slightly with reduced infection rates after controlling for non-independence, and intensification of disease-induced mortality estimates, increasing the estimated impact of TB on badger survival. This is an intriguing observation with the expected relationship between TB infection and survival perhaps more severe than previously thought.

Moving to a Bayesian framework provided similar survival and transition rates to a frequentist approach (Chapter 3). A notable difference is the ability to now assign measures of certainty, with high probabilities (>0.97) that males are more likely to become infected, infectious, and have reduced survival than females in all health states. In the absence of detailed contact data we explored one approach to control for non-independence in infection risk, incorporating social group as an index of spatial heterogeneity, alongside controlling for individual variation. Survival estimates in the mixed effects models intensified the impact of disease on badger survival. In previous analysis early disease stages reduced male survival by a few percent we now find a 7% reduction in male survival following infection increasing to a 33% reduction in advanced disease stages. Equally we find female mortality patterns similar to previous studies, that is survival rates remain unaffected and equivalent to estimates from constant models during early infection stages, however controlling for individual variation we find they too have intensified disease-induced mortality rates, albeit not until the advanced disease state, reducing their survival by 23% (equating to an additional 9% reduction when compared to the constant model).

Social organisation directly influences disease persistence and spread across wild animal populations (Altizer et al., 2003). Badgers conform to this ecological principle with social grouping explaining 21-24% of variation of TB infection risk. Social structuring likely impacts host association patterns and host proximity by driving high within group contacts and low between group contacts. A mechanism suggested to have evolved to reduce the spread of disease throughout socially partitioned populations (Loehle, 1995). In the light of this,

within group dynamics may be important in driving epidemiology and more appropriate than mass action models which are likely a crude approximation of infection processes. Yet, before making definitive predictions about how social structuring affects epidemiological processes we must understand how contact mechanisms translate to transmission events, requiring more behavioural studies.

Along with social group structure, movement between groups has also been associated with a rise in TB incident cases (Rogers et al., 1998; Vicente et al., 2007). Inclusion of individual time-varying random effects can be incorporated within this Bayesian framework and was explored prior to this study, but allowing social group to change over time for each individual resulted in lengthy model runs impractical to implement as part of this project. Given adequate computational power these models may be worth considering in future analyses. An additional factor to take into account in a multi-host system, where pathogens infect more than one host, is interspecies transmission. Proximity logger data has suggested that badgers may experience more interspecies contact than between social group contact (Böhm et al., 2009). Therefore social structuring may not only impact intra-species contact patterns but also badger-cattle contact events. Our approach of incorporating an index of social group may absorb some of this variation if badgers living in close proximity to cattle are more likely to become infected.

Moving to a Bayesian philosophy corroborated with previous studies highlighting sex-differences as an important driver of heterogeneity in disease susceptibility. In the absence of contact data capture-mark-recapture can provide information regarding location of captures which can be used to account for spatial heterogeneity providing robust epidemiological estimates. It appears that badger social group accounts for more than a fifth of variation in transmission rates. However, a greater understanding of interactions between these variation patterns and empirical contact data will provide further insight into observed epidemiological patterns and the social implications of disease dynamics.

CHAPTER 5

The previous chapters have obtained key epidemiological parameters. These rates can be a vital tool used to guide future management decisions. However, management arguably also requires knowledge of the underpinning mechanisms. Having identified sex as an important determinant of disease induced mortality, disease transmission and disease progression, I use a novel technique to turn these discrete rates into trajectories and investigate the underlying cause of sex-related differences in disease response, a general move from identifying patterns to uncovering processes.

This chapter is split into 2 sections. ***Chapter 5.1*** *focuses on uninfected and infected categories of male and female badgers, and forms the second manuscript of this thesis which is currently in submission*.* ***Chapter 5.2*** *provides further detail focusing on specific disease states, and despite being second in this chapter line-up was actually the first survival trajectory analysis implemented.*

*A version of this chapter is now in print:

McDonald, J. L., Smith, G. C., McDonald, R. A., Delahay, R. J., & Hodgson, D. (2014). Mortality trajectory analysis reveals the drivers of sex-specific epidemiology in natural wildlife–disease interactions. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1790), 20140526.

5.1: Mortality trajectory analysis reveals the drivers of sex-specific epidemiology in natural wildlife-disease interactions

Summary

In animal populations, males are commonly more susceptible to disease-induced mortality than females. However, three competing mechanisms can cause this sex-bias: weak males may simultaneously be more prone to exposure to infection, and mortality; being “male” may be an imperfect proxy for the underlying driver of disease-induced mortality; or, males may experience more progressive disease than females. Here we infer the drivers of sex-specific epidemiology by decomposing fixed mortality rates into mortality trajectories and comparing their parameters. We applied Bayesian survival trajectory analysis to a 22-year longitudinal study of a population of badgers (*Meles meles*) naturally infected with bovine tuberculosis. At the estimated point of infection, infected male and female badgers have equal mortality risk, refuting the hypothesis that acquisition of infection occurs in males with coincidentally high mortality risk. Males and females harboured similar levels of heterogeneity in mortality risk, refuting the hypothesis that being male is only a proxy for disease susceptibility. Instead, sex-differences were caused by a more rapid increase in male mortality rates following infection. Males are indeed more susceptible to bovine tuberculosis, probably due to immunological differences between the sexes. We recommend this mortality trajectory approach for the study of zoonoses in wild animal populations.

Introduction

There is increasing epidemiological evidence of sex-related differences in host-pathogen interactions in animal populations. Males are usually more likely than females to acquire infection, and die from disease once infected (Guerra-Silveira & Abad-Franch, 2013). However, the mechanisms that drive these sex biases remain poorly understood. By changing our view of mortality parameters, from fixed rates in discrete stage classes to mortality trajectories, we aim to

deconstruct the mortality process in infected males and females, uncovering when in the infection process sex-differences arise, and helping to identify the mechanisms that generate such variation.

The most obvious driver of sex-differences in infectious disease-induced mortality is that disease affects males more than it does females, due to weaker, or simply different, physiologies (Zuk & McKean, 1996). Genetic differences between sexes may directly impact disease susceptibility, with X-linked genes a determinant of immune functioning (Markle & Fish, 2013). Sex-hormones have also been linked to male-biased mortality due to their role in determining immunocompetence (Schuurs & Verheul, 1990). Androgens, in particular testosterone, are known to regulate male reproductive trade-offs (Bouman et al., 2005; Klein, 2000) suppressing disease defences (Folstad & Karter, 1992; Zuk & Stoehr, 2002). Indirect mechanisms of sex-differences include the possibility that infection itself causes sex-biased changes in behaviour, for example increased fighting or ranging, exposing males and females to differential risk of mortality as infections progress.

Alternatively, behavioural and ecological differences between the sexes might indirectly make males simultaneously more likely to acquire infection and die from other causes. In many species, including humans (Byrnes et al., 1999), males are more likely to engage in risk-taking behaviours increasing their disease exposure. Risky behaviours such as higher levels of aggression (Delahay et al., 2006) and wider ranging movements (Delahay et al., 2006; Macdonald et al., 2008) may simultaneously raise male mortality and increase infection risk, giving rise to a correlation between infection risk and increased mortality but no direct causality.

A third potential driver of sex differences is that infection may have disproportionate effects on substandard males that are already in poor body condition, resulting in observable differences in heterogeneity in response to infection, between sexes. In this case, maleness is simply a proxy for

susceptibility to disease: the true driver is poor body condition, but more males than females tend to be in this state.

Classical statistical approaches, to demographic or epidemiological analysis of surveys of wild animal populations, tend to estimate fixed mortality parameters for pre-defined classes of population members (e.g. male vs female; age classes). Fixed mortality parameters assume that infected individuals experience an exponential decay in survival over the infection period, and thus fail to consider infection as a dynamic process, and fail to reveal immunological or behavioural causes. In reality, mortality trajectories will be more complicated than the exponential process, and differences in the parameters of survival curves, among classes of individuals, can reveal important epidemiological processes. If males are coincidentally more likely to develop disease and die of other causes, we predict elevated male mortality at the point of infection. If males are more susceptible to disease because the male class harbours the greater share of substandard individuals, we predict less heterogeneity in disease-induced mortality among males than among females. Finally, if males are genuinely more susceptible to disease progression, we predict that the rate of increase in mortality, post-infection, will be greater in males than in females.

One reason for the paucity of time-varying mortality trajectories of infected hosts, in wild populations, is that individuals cannot be monitored continuously from time of infection to death. However, age-specific mortality functions are commonly used in human and wildlife demographic analyses, and a recently developed method can estimate age-specific mortality trajectories whilst accommodating uncertainty in dates of birth and death (Colchero & Clark, 2012). For the first time, we employ this Bayesian trajectory framework (BaSTA; (Colchero et al., 2012)) to describe disease-induced mortality trajectories, accounting for uncertainty in date of infection, and apply this method to obtain mortality patterns for different health states in a population of wild badgers (*Meles meles*) naturally infected with *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB).

Sex-differences in epidemiological traits have been observed in bTB-infected badgers, with males suffering increased mortality during early disease stages and faster progression into advanced diseased states (Graham et al., 2013) where they experience double the rate of disease-induced mortality , when compared to females (Graham et al., 2013; Wilkinson et al., 2000). We test contrasting hypotheses and describe sex-related differences in the mortality trajectories of badgers. Given the economic importance and high public profile of badgers as a reservoir of bTB (Donnelly et al., 2006), it is critical that we better understand the epidemiology of this disease. Teasing apart the behavioural, ecological and physiological drivers that divide the wildlife population into categories of susceptibility to disease may promote improved, targeted strategies to reduce rates of transmission to livestock (Woolhouse et al., 1997).

In summary, we have applied a new methodology for analysing longitudinal demographic data which provides mortality trajectories rather than discrete rates of mortality during different stages of disease progression. We suggest this methodology can be used to obtain mortality trajectories that depend on infection duration, rather than age *per se*. We show that bTB infection alters mortality trajectories of badgers. We describe differences in mortality trajectories between uninfected and infected states and by focusing attention on variation over time, the role of sex in shaping heterogeneity in disease response. The ability of BaSTA to account for unknown date of infection provides opportunities to explore disease-specific mortality trajectories in this and other wild mammal populations, paving the way for a better understanding of the role of sex in epidemiological processes.

Methods

Ecological data

We used capture-recapture data collected from an intensively studied natural population of badgers in Woodchester Park, Gloucestershire for the period 1984

to 2005. Twenty social groups that were trapped consistently throughout the study period were incorporated in this analysis. Badgers were trapped approximately quarterly. They were anaesthetized and each was given a unique identifying tattoo on its first capture (for detailed methods see Delahay et al. (2000)). Blood samples were taken and tested for antibodies to *M. bovis* using an enzyme-linked immunosorbent assay (the Brock ELISA (Goodger et al., 1994)). Samples of faeces, urine, sputum and pus from abscesses and/or bite wounds (where relevant) were taken for bacterial culture of *M. bovis* (Clifton-Hadley et al., 1993; Gallagher & Horwill, 1977). Badgers were categorised according to these diagnostic test results as either uninfected (U) defined as a test-negative badger and infected (I) including badgers that test positive to the ELISA test and/or culture. In our categorisation we made two assumptions. First, as TB is a progressive disease in badger populations, once classified as infected we assumed that a badger did not recover (in accordance with previous studies (Delahay et al., 2000; Vicente et al., 2007)). Second, we assumed accuracy of diagnostic tests. Accurate diagnosis in live badgers is difficult due to limitations in the performance of the ELISA test which has a specificity of 89–94% (Clifton-Hadley et al., 1995; Greenwald et al., 2003), and culture which despite high specificity has low sensitivity (Drewe et al., 2010). Violation of these assumptions due to error ascribing infection status would only act to weaken the signal of mortality effects in infected badgers, thus making our results conservative. Individual quarterly capture histories were created for uninfected and infected badgers with sex incorporated as a covariate, totalling 7957 capture occasions across 1460 individual capture histories for 786 females (125 of which were ‘infected’) and 674 males (124 of which were ‘infected’). Survival analysis was then applied to the separate data sets.

Modelling Framework

To account for uncertainty in infection date, we fitted a Bayesian survival trajectory analysis (BaSTA) (Colchero et al., 2012) to capture data for infected badgers, using the software R (R Development Core Team, 2013). BaSTA utilises a CMR approach incorporating recapture probabilities less than one,

thereby providing a powerful analysis that can account for variable recapture rates. Recapture probability was kept fully time-dependent throughout the analysis accounting for any temporal recapture bias.

BaSTA models “birth” years (in this case year of infection) and death years as latent variables, drawing inference on age- or time-since-infection-specific mortality despite missing data. For the uninfected badgers analysed, prior information on the year of birth was obtainable when badgers were first caught as cubs or yearlings, therefore under such circumstances birth dates were incorporated, consisting of 1011 known birth dates. With regard to the infected badgers we cannot be certain when an individual entered a disease state, therefore no date was included. Dates of death were recorded when badgers were found dead: time of death was known for 214 uninfected badgers and 48 infected badgers.

Four mortality functions, each able to describe different trends in mortality, were compared (Colchero et al., 2012) :

Exponential. The simplest trajectory models consist of a single constant mortality parameter that assumes mortality is independent of the duration of infection, equivalent to the fixed discrete rates we commonly see in wildlife disease analyses.

Gompertz. These models consist of two parameters; an initial mortality and an exponential increase in mortality parameter (Colchero et al., 2012).

Weibull. This model has two parameters, a shape and a scale (Colchero et al., 2012; McDonald et al., 1996). The versatility of the model means it can show accelerating increase, decelerating increase, decreasing or constant mortality.

Logistic. This model has three parameters (Colchero et al., 2012). It is similar to a Gompertz model with an additional deceleration parameter whereby mortality levels off over time. In terms of mortality trajectories of an infected population

this levelling off could represent a reduction in mortality at advanced duration of infection i.e. an improvement in survival, or (more likely) heterogeneity in disease response (Vaupel et al., 1979).

To ensure model convergence, initial trials of four Markov Chain Monte Carlo (MCMC) iterated samplings (chains) were run for each model, followed by 100,000 iterations on four chains, with a burn-in of 20,000 for each analysis. Convergence was assessed both visually ensuring mixing of the chains and formally within the model calculating the potential scale reduction ($\hat{R}$ (Colchero et al., 2012)). When $\hat{R}$ is close to 1 we can be confident that convergence has been reached, the burn-in period was determined when $\hat{R}$ <1.01. We also tested mortality parameters for prior sensitivity, running the model for both uninfected and infected badgers under four different prior structures. The choice of prior did not influence the identification of mortality parameters or differences among them. The deviance information criterion (DIC) (Spiegelhalter et al., 2002) was used to assess model fit. Additionally BaSTA provides a diagnostic tool based on Kullback–Leibler discrepancies (Kullback & Leibler, 1951) calibrated to reduce asymmetry (KLDC), which provides an assessment of the extent of overlap of posterior distributions of parameter estimates for categorical variables. This is a value between 0.5 and 1: a value of 0.5 indicates identical distributions, and 1 that there is no overlap between them (Colchero et al., 2012). This allows us to determine the magnitude of the effect of sex on the parameters of mortality trajectories.

Predictions

Using a logistic model to represent mortality trajectories following infection (Fig. 5.1), we formulated hypotheses regarding the cause of the established sexual dimorphism in infection response among badgers. The logistic model relates mortality rates (μ) to time-since-infection(x),

$$\mu(x) = \frac{e^{b_0 + b_1 x}}{1 + b_2 \frac{e^{b_0}}{b_1}(e^{b_1 x} - 1)}$$

in which b_0 represents mortality at the point of infection, b_1 describes the rate of mortality increase post-infection and b_2 highlights deceleration in mortality rates.

Hypothesis 1: If sex-differences in mortality are caused by a coincidental predisposition to die and to also become infected, we would expect to find differences in mortality at the point of infection (b_0; Fig.5.1).

Hypothesis 2: If sex-differences are caused directly by disease, we would expect similar intercept values (b_0) but sex-related differences in the subsequent rate of increase in mortality post infection (b_1; Fig.5.1).

Hypothesis 3: If maleness is a proxy for infection susceptibility to disease with the male sex harbouring a greater proportion of substandard individuals, we would expect reduced heterogeneity in response, indicated by a reduction in the deceleration parameter (b_2; Fig.5.1). It should also be noted that if the male sex tends to harbour weaker individuals, we might also observe higher male mortality at the point of infection (hypothesis not graphed).

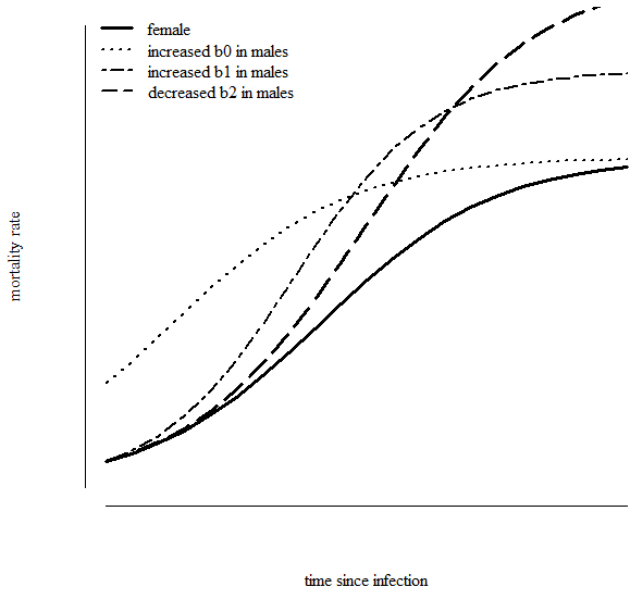

Figure 5.1. Hypothetical, sex-specific, logistic mortality trajectories driven by different mechanisms. Hypothesis 1: The sexes are differentiated by rates of mortality at the point of infection (b0). Hypothesis 2: Sexes are differentiated by the rate of increase in mortality post-infection (b2). Hypothesis 3: Sexes are differentiated by their degree of deceleration post infection, an artefact of heterogeneity in disease response.

Results

The Gompertz model was most supported for uninfected badgers (Table 5.1, Fig. 5.2), consisting of just two parameters: initial mortality at the point of birth (b_0) and the rate of mortality increase (b_1). Once infected, the logistic mortality function was most supported, consisting of an additional deceleration parameter (b_2; Table 5.1, Fig. 5.3). As the use of DIC values has been considered controversial (Celeux et al., 2006; Colchero & Clark, 2012) further support for a logistic trajectory in infected badgers is provided in Table 5.2, wherein b2 is identifiably different from zero, upholding the rejection of the simple Gompertz model. These results were robust under four different prior structures (Gompertz priors b_0, b_1: (3, 0.01), (-3, 1), (-2, 1), (-2, 0.01); Logistic priors b_0, b_1, b_2: (-3, 0.01, 0), (-3, 1, 0.01), (-3, 1, 1), (-2, 1, 0.01)).

Table 5.1. Candidate mortality functions for survival trajectories of male and female badgers in two health states (infected and uninfected), and their corresponding differences in deviance information criterion (∆DIC). Substantial support for the 'best' model alone is indicated when rival models all have ∆DIC > 3 (Spiegelhalter et al., 2002). **the most supported model

mortality function	Uninfected	infected
Exponential	9.1	26.7
Gompertz	0**	49.4
Logistic	21.5	0**
Weibull	29.4	6.7

Table 5.2. Posterior means and 95% credible intervals of mortality trajectory parameters for uninfected and infected badgers, including intercept (b0), increase mortality rate (b1) and for infected badgers a deceleration parameter (b2).

		uninfected			infected		
		mean	**lower**	**upper**	**mean**	**lower**	**upper**
b0	**male**	-2.426	-2.56	-2.297	-3.538	-4.464	-2.721
	female	-2.635	-2.762	-2.507	-3.231	-4.064	-2.477
b1	**male**	0.006	-0.003	0.015	0.847	0.513	1.238
	female	0.002	-0.005	0.01	0.481	0.202	0.768
b2	**male**	-	-	-	2.833	1.682	4.147
	female	-	-	-	2.626	1.122	4.104

Inferred life expectancies decreased once badgers became infected. Life expectancies were consistently shorter in males than in females. To the nearest month, male average life expectancies were estimated to be 32 months for uninfected badgers and 22 months for infected badgers. Female average life

expectancies were found to be 40 months for uninfected badgers and 35 months for infected badgers. When they are uninfected, this equates to males having on average a 20% lower life expectancy compared to females, with the acquisition of infection increasing this difference to 37%.

Sex-related differences amongst uninfected badgers were due to higher initial mortality parameter values in males, suggesting that they are predisposed at birth to have a higher initial mortality than females (KLDC; 1, Fig. 5.2, Table 5.2). Their subsequent life-time rate of mortality increase was similar to that of females, with a high degree of overlap between posteriors (KLDC; 0.68, Fig. 5.2, Table 5.2).

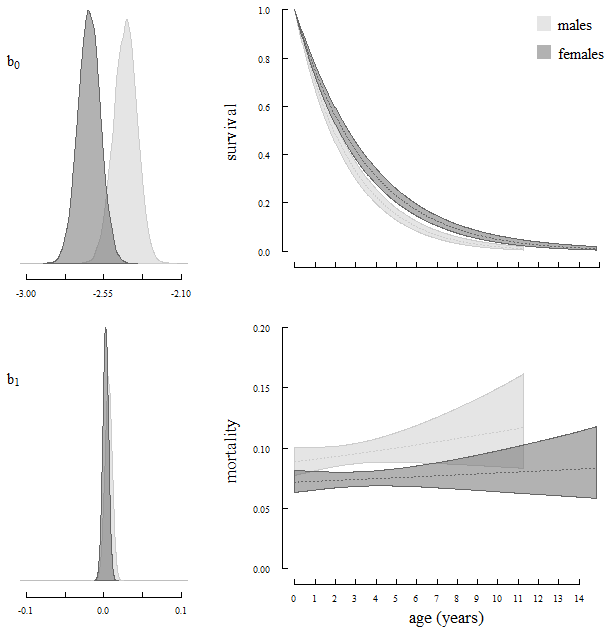

Figure 5.2. Age-dependent survival and mortality trajectories of uninfected male and female badgers. Initial mortality values (b_0) at point of birth were higher for males than females, but the rate of mortality increase (b_1) was similar between the sexes. Uninfected mortality trajectories were best described by Gompertz functions.

Infection alters mortality patterns in badgers, with trajectories of infected animals supported by a logistic framework, consisting of an additional deceleration parameter (b_2). At the point of infection, there is no identifiable difference in mortality between the sexes, with significant posterior overlap (KLDC; 0.62, Fig. 5.3, Table 5.2), suggesting that infected males do not represent a biased subset of more susceptible individuals in the population. Following infection, the mortality rate in males increases substantially faster than in females (KLDC; 0.96, Fig. 5.3, Table 5.2). The degree of deceleration or heterogeneity was similar in males and females once infected (KLDC; 0.54, Fig. 5.3, Table 5.2) indicating a similar spectrum of responses to disease in both sexes. The absence of the deceleration parameter (b_2) in the trajectories of uninfected individuals suggests that infection promotes an increase in heterogeneity in mortality amongst badgers, i.e. a wider spectrum of mortality responses. These results support our hypothesis 2, that differences between male and female infected badgers are due to a substantial difference in the post-infection rate of increase of mortality (b_1).

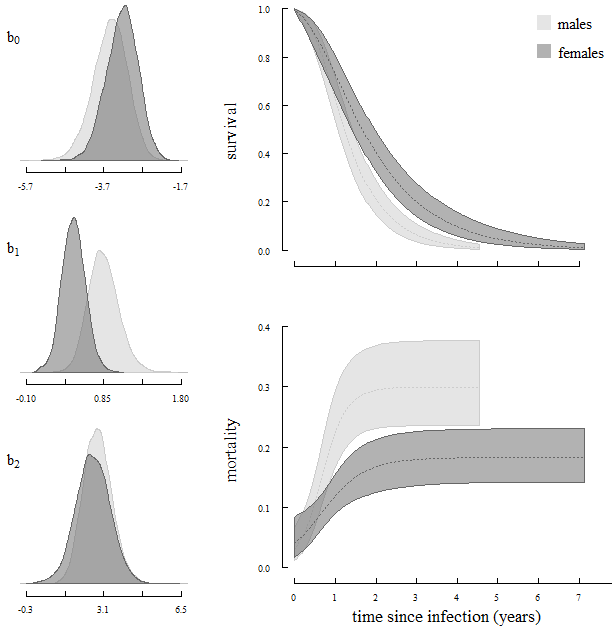

Figure 5.3. Logistic survival and mortality trajectories of badgers following infection. At the point of infection there is no discernible difference between sexes (b_0), however males have elevated rates of increase in mortality following infection (b_1), and males and females display similar levels of heterogeneity (b_2) in disease responses.

Discussion

While increased mortality due to bTB infection is already known to occur in male badgers (Graham et al., 2013; Wilkinson et al., 2000) we have now located where in the infection process these sex-related differences arise, and can begin to infer the mechanisms that might generate this variation. Mortality rates at the point of infection are very similar between the sexes, suggesting that elevated mortality in infected males is not due to the coincidental risks of natural mortality and infection. We also found no evidence that infected male and female badgers differ in the degree of heterogeneity among individuals in their responses to infection. Instead, the distinction we see between the sexes is that males experience a faster rate of increase in mortality with increasing time-since-infection. This suggests a difference in immunological or other physiological response to bTB infection between males and females, and/or that

infection itself might cause male badgers to behave in ways that increase their risk of death.

Uninfected badgers do demonstrate sex-based differences in mortality and senescence-related mortality. However, the increase in mortality with age in the best supported Gompertz framework is not substantial compared to other mammals (Colchero & Clark, 2012), i.e. evidence of senescence is weak amongst uninfected wild badgers. Uninfected males are predisposed to higher mortality from the time of birth, resulting in a 20% shorter life expectancy, possibly due to increased competitive encounters and bite wounding among males, a phenomenon also found in other mammals (Clutton-Brock & Isvaran, 2007). Uninfected badgers have very different mortality trajectories to infected badgers, characterised by substantially lower mortality rates. Infection with bTB clearly alters prognoses of life expectancy and exaggerates sex differences in survival rates.

Mortality trajectories of infected badgers were best described by logistic curves, whereby after an increase in mortality the trajectory decelerates and reaches a plateau. Although this pattern implies that susceptibility to disease progression declines with duration of infection in all infected badgers, a rival and more biologically plausible explanation is heterogeneity in individual response to infection (Vaupel et al., 1979), whereby the most susceptible individuals die early on in the infection process, leaving the more resilient to die later. Male and female badgers show similar levels of heterogeneity in mortality rates. This indicates a comparable spectrum of immune responses, and suggests that a broad array of individuals of both sexes become infected rather than just a biased sample of males that are already predisposed to high mortality rates.

We suggest that sex-related variation in immunocompetence is likely to be the main mechanism for observed differences between the epidemiology of bTB in males and females. Mortality patterns highlight raised mortality in males following infection but otherwise comparable trajectories, suggesting weaker immunological defences. This is consistent with results of prior studies that

showed males suffering rapid disease progression (Graham et al., 2013) and substantial weight loss (Tomlinson et al., 2013) following infection. Immunological defences are costly and can trade off with other physiological processes (Graham et al., 2013; Lochmiller & Deerenberg, 2000; Sheldon & Verhulst, 1996) perhaps resulting in differential investment between the sexes. Although, chromosomal differences, and other physiological processes cannot be discounted sex-hormones are suggested to be strong determinants of immune response to mycobacterial infection across study species (Markle & Fish, 2013). Male immune suppression is commonly found in other mammals (Mills et al., 2010; Moore & Wilson, 2002), whereby the cost of allocating resources to reproductive activity (e.g. male ornamentation (Verhulst et al., 1999), singing (Saino et al., 1997), territorial behaviour (Svensson et al., 2001) and aggressive encounters (Cavigelli & Pereira, 2000)) suppresses immune defences. Such a trade-off, also known as the immunocompetence handicap (Folstad & Karter, 1992), may explain why male badgers are more likely to become infected and die from bTB (Graham et al., 2013; Wilkinson et al., 2000). The investment in reproductive effort in male badgers is not expressed as extravagant ornamentation as in some species (Folstad & Karter, 1992) but more likely by competitive and/or aggressive behaviour (Delahay et al., 2006), maintaining territories, ranging behaviour (Vicente et al., 2007) and the associated investment in a larger body size compared to females (Rogers et al., 1997). Speculatively, investment in growth and reproduction in male badgers may contribute more to fitness than investment in immunological defence against diseases such as bTB.

An intriguing additional, and not exclusive, explanation for higher rates of increase in mortality risk in male badgers is the possibility that infection itself causes changes in behaviour that increase the likelihood of death. Pathogens can manipulate host behaviour (Klein, 2003; Vyas et al., 2007) increasing risk-taking behaviours such as aggression in order to increase physical contact and transmission opportunities between individuals. The possibility that infection drives behavioural changes in male badgers cannot be discounted, with increased aggression one suggested mechanism explaining why infectious

male badgers are more likely to be bitten (Jenkins et al., 2012) reducing their survival. However, determining the causality is problematic as higher prevalence of bite wounds may also be due to disease-driven reductions in body condition impacting the social status (Delahay et al., 2006) and competitive ability (Jenkins et al., 2012) of infected males.

We uncover the counter-intuitive result that males have similar mortality to females upon becoming infected, despite having higher natural mortality. This may be due to earlier onset of infection in males. That is, increased probabilities of infection in males (Graham et al., 2013) may incite infection at a younger age when their background age-dependent survival will be naturally lower. An alternative explanation may be that females with high mortality, due to other mortality pressures, have increased infection risk. Generally females have reduced susceptibility to disease therefore those under additional pressures or comorbidities, for example due to nutritional stress and/or reproduction-mediated drops in immunity (Altizer et al., 2006), may be at higher risk of infection. Given that multiple mechanisms may drive similar patterns, an individual-level disease analysis may be useful to observe drivers of bTB in female badgers. Unfortunately, there is no current means to incorporate time-varying individual covariates within the BaSTA framework, but such a development would allow variation in body condition and reproductive status to change over time addressing these questions.

Understanding how the risk of mortality changes, as infection progresses, provides a key to explaining and predicting the population dynamics of infected hosts, and ultimately informs the development of better intervention strategies for disease control. We demonstrate the utility of a Bayesian modelling framework, developed specifically for age-related survival analysis, but translated here for the analysis of disease-induced mortality trajectories in wildlife populations. Trajectories, as opposed to discrete rates of mortality, can highlight heterogeneity in disease response, stages of maximum vulnerability, and allow comparison of mortality trends between cohorts and classes of infected hosts. Trajectory analysis has revealed key sex-related differences in

bTB epidemiology in badgers, and we recommend its application to surveys of disease-induced mortality in other populations and species.

5.2: Extending BaSTA into discrete infected states reveals further deviance in sex-specific mortality trajectories

Methods

Adopting an identical BaSTA modelling framework (Colchero et al., 2012) described in the preceding section (Chapter 5.1) we delve further into the complexity of disease state specific analysis and advance from an uninfected-infected comparison to compare sex-specific survival trajectories in three contrasting infected states. Infected badgers were categorised according to diagnostic test results as ELISA positive (P), one-site excretor (X) and multi-site excretor (XX) (see Table 5.3 for full definitions (Graham et al., 2013)). Once a badger tested positive (P, X or XX) they were included in the analysis for the corresponding health-state.

Table 5.3. Definitions of infected states based on diagnostic test results in order 1:3 based on levels of disease severity

disease state	diagnostic meaning
1. ELISA positive (P)	Positive ELISA and negative culture
2. One-site excretor (X)	Positive culture from one body site
3. Multi-site excretor (XX)	Positive culture from more than one body site

Once categorised in to the 3 progressive diseased states quarterly capture histories were created for each state with sex incorporated as a covariate. Only badgers that did not progress into a more severe state were included totalling; 118 ELISA positive; 68 one-site excretors; 63 multi-site excretors. We cannot be certain when an individual enters a disease state therefore no date of infection was included. When death dates were known they were included, consisting of 20 ELISA positive; 17 one-site and 11 multi-site individuals with known deaths.

Results

The logistic mortality function (Colchero et al., 2012) was the most supported mortality function for badgers in all infected states (Table 5.4, Figs 5.4, 5.5 &

5.6), in agreement with the ‘best fit’ mortality pattern for the grouped infected category (Chapter 5.1).

Table 5.4. Candidate mortality functions for badgers in various health states and their corresponding ΔDIC values

mortality function	P	X	XX
Logistic	0**	0**	0**
Weibull	4.9	3.0	11.4
Exponential	9.0	3.4	23.6
Gompertz	22.8	11.5	8.06

Inferred life expectancies decreased during disease progression however there was no recognizable difference between life expectancies during early and intermediate disease states (P and X). Male life expectancies were consistently shorter than females. Male average life expectancies through progressive disease states were estimated to be 18 months (P), 18 months (X) and 11 months (XX). Female average life expectancies were found to be 27 months (P), 27 months (X) and 16 months (XX). Therefore despite no detectable difference between intermediary disease states, once in an advanced disease state both sexes encountered a ~40% decrease in life expectancy.

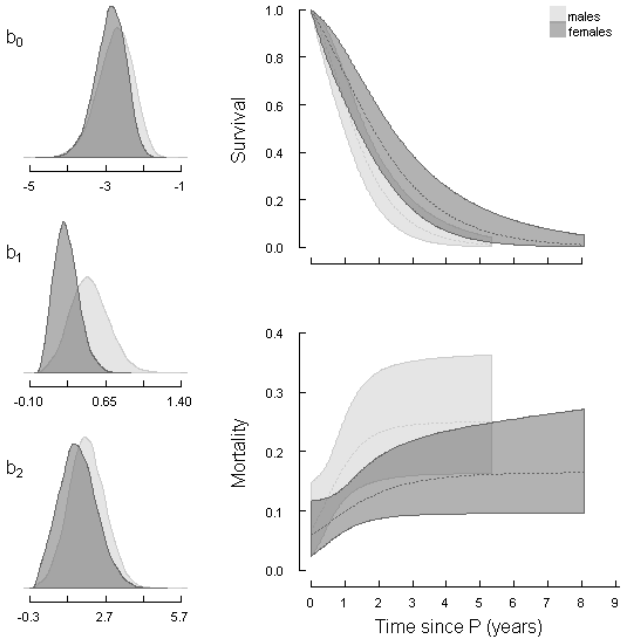

Figure 5.4. Survival and mortality trajectories of badgers during early infection (P) stages. Initial mortality (b0) and rate of deceleration (b2) parameters did not differ however rate of mortality increase (b1) was higher in males.

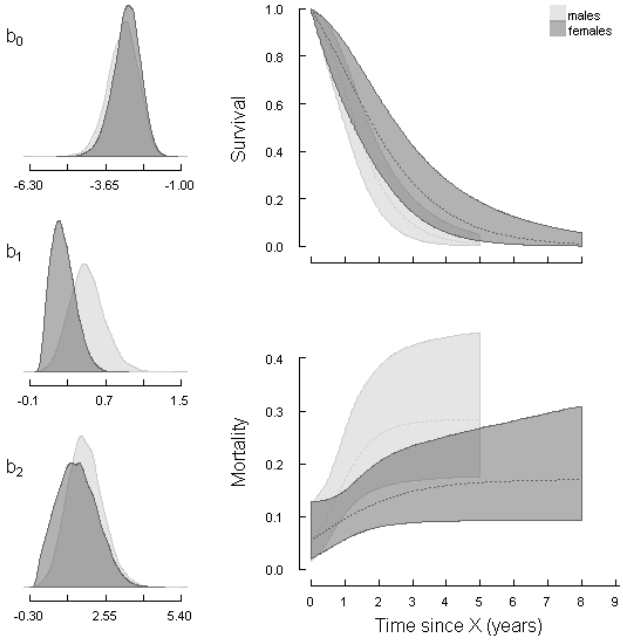

Figure 5.5. Survival and mortality trajectories of badgers during intermediary infection (X) stages. Initial mortality (b0) and rate of deceleration (b2) parameters did not differ however rate of mortality increase (b1) was higher in males.

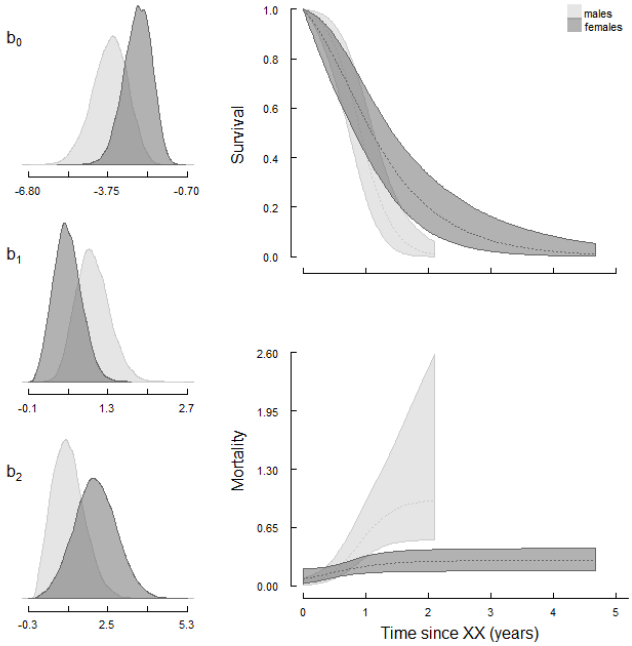

Figure 5.6. Survival and mortality trajectories of badgers in health state XX. Initial mortality (b0) and rate of deceleration (b2) was lower in males and rate of mortality increase (b1) was higher in males.

The logistic model consists of 3 parameters which represent initial mortality (b0), an initial exponential increase in mortality (b1) and a deceleration (heterogeneity) parameter (b2). In concurrence with the previous section, the initial mortality values (b0) at point of infection i.e. when badgers enter early disease states did not differ substantially between sexes (Fig. 5.4, KLDC; P=0.53). Males had consistently higher mortality rates (b1) than females throughout the 3 infected states (Fig. 5.7, KLDC values; P=0.86, X=0.88, XX=0.86), coinciding with rapid increase in disease-induced mortality rates in males.

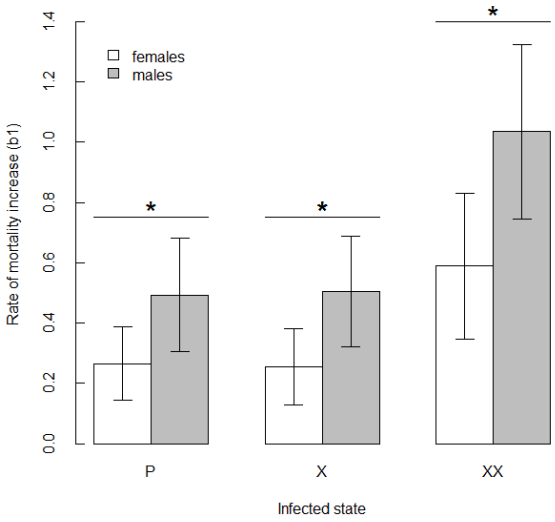

Figure 5.7. Rate of mortality increase (b1) parameter ± standard errors in male and female badgers during progressive infected states. *indicates substantial differences in mortality rates between sexes inferred from KLDC value >0.8.

The degree of deceleration or heterogeneity (b2) males and females experienced were similar during early and intermediary disease stages (KLDC; P=0.55, X=0.54), ruling out hypothesis 3, however subdividing badgers into various disease states has uncovered additional complexity with males in a more severe disease state demonstrating a substantial reduction in their deceleration parameter, in other words reduced heterogeneity (Fig. 5.6 & 5.8, KLDC; XX=0.84).

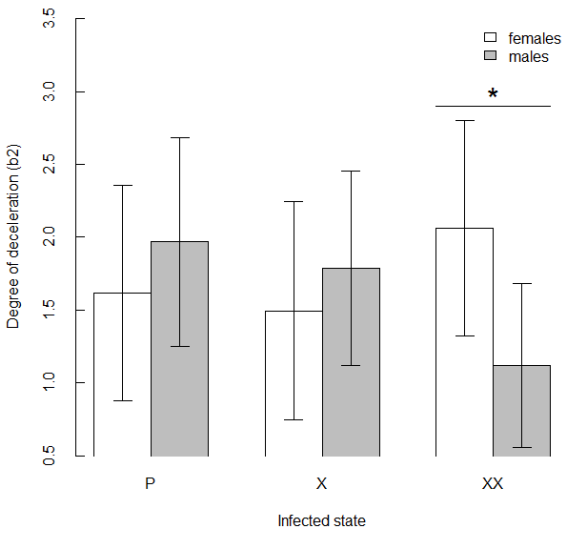

Figure 5.8. Degree of deceleration or heterogeneity (b2) parameter ± standard errors in male and female badgers during progressive stages of disease severity. *indicates substantial differences between sexes inferred from KLDC value >0.8, which were only observable in advanced (XX) state.

Discussion

In this sub-chapter we demonstrate how quantities describing disease-state specific mortality trajectories can be calculated using a novel BaSTA framework. Previous work suggested that sex biases in survival are exacerbated by the infection process most likely due to immunological differences (Chapter 5.1). Dividing the infected class into multiple disease states provided an opportunity to compare mortality processes in early, intermediary and advanced disease states.

During early and intermediary disease states initial mortality values were not substantially different. This result illustrates that male and females are not under any differential mortality pressure at the point of infection, therefore male susceptibility to infection does not appear to be coincidentally linked to a mortality-inducing behaviour e.g. risk taking behaviours. Instead the distinction we see between males and females is due to a higher rate of mortality increase in males following infection. Raised male mortality rates were found across disease states, indicative of immune deficiencies. Mortality trajectories of

badgers in all infected states were most supported by a logistic pattern whereby after an initial increase in mortality the trajectory decelerates and plateaus. The degree of deceleration provides an indication of heterogeneity to infection (Vaupel et al., 1979). Males and females show similar levels of heterogeneity during early infection stages suggesting a comparable spectrum of immune responses between sexes with a broad array of both males and females becoming infected and not just a bias sample of 'weak' males already predisposed to high mortality values.

The results so far further corroborate with the preceding section (5.1). However by separating badgers into numerous disease states we uncovered an additional mortality difference; male mortality rates are more homogeneous during advanced disease stages. In other words, males with advanced disease all have high frailty and poor disease defences to the extent that their average life expectancy is less than a year. Comparatively, females in the same advanced state still display a variety of responses to advanced infection. We suggest this sex-difference provides further evidence for inferior male immunocompetence as the main mechanism for sex differences in TB epidemiology whereby androgen-mediated investment into reproductive behaviours or other physiological processes reduce immune system functioning (Klein, 2000; Schuurs & Verheul, 1990). This hypothesis coincides with raised mortality in males following infection but otherwise comparable mortality patterns between sexes during intermediary stages and high frailty in males during advanced states.

There is a caveat worthy of discussion. For badgers in intermediary disease states (P and X) only those that did not progress into a more severe state were incorporated thus ensuring disease progression and survival were not confounded. As a result only those that died in the state were included, consequently this may create artificially higher mortality rates. Despite this possible skew our mortality trajectories do represent the majority of badgers which due to the chronic nature of infection never advance into an infectious state. Of those that become clinically infectious less than 15% of males and 9%

of females progress to the more advanced multi-state stage in any given year (Graham et al., 2013), thus minimising the likely bias.

We provide empirical evidence suggesting sex differences in immune defences yields the observed sex differences in mortality rates in TB infected badgers. Our results agree with infected-uninfected analysis (Chapter 5.1) by demonstrating male and females differ greatly in their rate of mortality increase but otherwise follow similar mortality patterns during early and intermediary disease states. However by accounting for disease states of varying severity we find that males become more homogeneous in their disease response during severe disease states. That is, all infected individuals are at high risk compared to females that still display a variety of responses. These results point to deficiencies in male immune defence as the most probable mechanism responsible for sex biases in survival in TB infected badgers.

CHAPTER 6

Exploring patterns of disease dynamics naturally led to questions regarding the applicability of Woodchester-specific parameters to populations occurring elsewhere. Replicated experimentation of longitudinal survey data is not feasible and can question the atypical nature of single location studies such as the Woodchester population. The provision of an additional dataset of badgers caught as part of a badger vaccination study provided an opportunity for me to compare epidemiological processes between populations.

A comparison of demography and epidemiology between two badger populations

Summary

Long-term surveys of reservoir hosts in their natural setting play a critical role in disease ecology, revealing epidemiological processes that would otherwise be unknowable. The relevance of extrapolating from such single studies to make broad inferences regarding populations can be a concern but is often unavoidable when few opportunities exist for comparison. We address the question; what is the applicability of epidemiological rates, estimated for the Woodchester badger population, to other study areas? Using a Bayesian multistate model we estimate and compare epidemiological parameters from the intensively studied Woodchester badger society, with epidemiological traits of an unconnected study population taken from a badger vaccine study (BVS). Survival, infection rates and disease progression were estimated for each site and sex, and post-hoc calculations provided probabilities of differences between sites in addition to calculating Kullback-Leibler divergence (KLD) to formally measure the difference between distributions. We show that posterior infection rates and disease progression probabilities were similar across studies, with probabilities of posterior differences between all analogous parameters less than 85% and KLD values ranging between 0.02-0.49. These similarities in key parameters highlight the applicability of Woodchester Park as a model system. Survival parameters also overlapped between survey sites but were less similar (KLD 0.2-0.9), with the exception of infectious badgers which displayed similarities in their distribution (KLD; males; 0.02, females; 0.08). Ecological, social and landscape factors will also influence survival rates, and weren't accounted for in this study. Future use of parameters originating from Woodchester Park badgers, for example to inform landscape models of badger-TB-livestock management strategies, is supported by results from this study.

Introduction

Gaining reliable estimates of epidemiological parameters requires long term monitoring of infected populations in their natural setting. These individual-based surveys provide unique insight into population processes, but are logistically challenging to implement and require considerable funding to set up and maintain long-term (Clutton-Brock & Sheldon, 2010). Therefore, it is unsurprising that when such studies do occur and epidemiological traits are inferred, replication and validation of parameters are rarely possible. A lack of experimental replication can propagate scepticism of single location data, especially within controversial disease-host systems, questioning the applicability of scaling up from a single study to make generalizations across populations. Although this doubt is invariably unavoidable and great value can be taken from longitudinal individual-based studies, opportunities for corroboration should also be taken whenever possible.

When infected wildlife populations threaten human health and/or livestock health, management commonly focuses on the wildlife reservoir, hence demanding research into the ecology of these infected hosts. The Eurasian badger (*Meles meles*) is one example which has been the target of intense scientific activity and political debate due to the close relationship between badgers, cattle and the pathogen *Mycobacterium bovis*, the causative agent of bovine tuberculosis (TB). Infection of cattle by badgers (Donnelly et al., 2006) contributes to the persistence of TB in cattle, reducing the success of current cattle test and slaughter programs which, to date, have failed to control the problem. Much attention has been paid to uncovering epidemiological information in badgers with the goal to reduce between-species transmission. A long-term badger study at Woodchester Park, Gloucestershire (Rogers et al., 1997; Rogers et al., 1998) was initiated in 1975 with the objective to provide a detailed resource to understand epidemiological processes. These in turn can feed into models to explore management strategies and guide TB policy. It is the longest running badger survey in the UK, consisting of naturally infected individuals and providing arguably the most detailed information on any mammalian reservoir of disease. A considerable amount of our current

knowledge regarding TB in badgers originates from this population. However opportunities for comparison between other sites are minimal, with few individual-based longitudinal studies contributing to badger ecology (but see Macdonald and Newman (2002)) and no current studies obtaining long-term epidemiological data. We aim to appraise the representativeness of Woodchester Park as a study population.

The Woodchester Park study collects both ecological and epidemiological data. This involves monitoring individual badgers across their lifetime by capture-mark-recapture (CMR) methods and collecting information including demographic traits, location data and their TB status using clinical sampling (Delahay et al., 2000). This information has generated much knowledge of both individual- and population-level processes, leading research in badger epidemiology. It has been the subject of more than 100 publications, with top papers in this field cited extensively throughout the literature (e.g. (Rogers et al., 1997)). Epidemiological parameters occurring within this population are used to predict population and disease dynamics under a variety of management scenarios (Smith et al., 2001a; Smith et al., 2001b; Wilkinson et al., 2004) and in turn have the potential to inform policy changes. However, badgers at Woodchester could be considered an atypical population due to its unmanaged, high-density nature, casting doubt on the whether epidemiological processes underlying disease dynamics represent what's occurring within other badger societies. We pose a key question: what is the applicability of epidemiological rates from Woodchester to other study areas? We attempt to answer this question by extending this single study system to a paired population study.

With such longitudinal data, replicated experimentation is unfeasible and robust comparisons of epidemiological rates with other badger populations have so far been impossible with no other long-term badger study collecting TB diagnostic data. Our alternate population therefore consists of a subset of badgers from a badger vaccine study (BVS) set up for the different purpose of investigating the effect of vaccination on TB transmission and progression (Chambers et al., 2011). The BVS is a 4 year monitoring programme, comparing vaccinated and unvaccinated (control) badgers. We use data from the control badgers to form a

comparable (un-manipulated) population to the Woodchester Park badgers. Despite the smaller scale of this study it provides a unique opportunity for comparative analysis, with similar trapping methods and diagnostic regimes used across both study sites.

Using a state-dependent Bayesian model we analyse data taken from two unconnected populations to extract comparable epidemiological parameters. A Bayesian approach is favourable to frequentist methods due to improved analytical ability to cope with the sparse data of the BVS, whilst also allowing probabilistic statements. Specifically we apply post-hoc calculations using the posterior samples of state-dependent survival, disease transmission and progression to compare epidemiological processes across sites.

Methods

Study sites

Both studies collected data from marked individuals, whereby badgers were trapped and given a unique identifying tattoo on their first capture occasion. Individual information including, but not limited to, sex and disease status was collected on every occasion. Both sites are situated in independent locations in Gloucestershire, England.

The BVS study site is located in a 55km^2 area of a mixture of agricultural and mixed woodland. Trapping commenced over a period of 4 years (2006-2009) totalling 8 capture occasions (full details see (Chambers et al., 2011)). Only unvaccinated badgers from the study's control group were included in this analysis, consisting of 150 males and 170 females.

The Woodchester study site is an area 7km^2 consisting of pasture and mixed woodland. Data was taken from 2006 to 2011 to include comparable diagnostic test data, consisting of 6 years of trapping information (for detailed methods see (Delahay et al., 2000)). This incorporated 207 male and 243 female badgers.

TB is endemic at both study sites, allowing clinical samples to be taken and individual TB statuses to be recorded at every capture event. Badgers are classified according to the results from 2 diagnostic tests: Brock Stat-Pak, and microbial culture. Stat-Pak detects TB antibodies (Carter et al., 2012; Chambers et al., 2009). Badgers testing positive for TB antibodies were classified as Stat-Pak positive (P). Culture tests detect active excretion of the bacterium, indicating an advanced infectious disease state. Badgers can be classified as either one-site (X) or multi-site (XX) excretors according to whether their culture positive results reside from single (X) or multiple (XX) body sites (Chapter 3). Due to the short duration of the study, sample sizes for progressed disease states were insufficient to analyse separately therefore culture positive states we grouped to form one infectious class (X/XX). Badgers neither Stat-Pak positive nor culture positive were classified as test negative (N) (Fig 6.1).

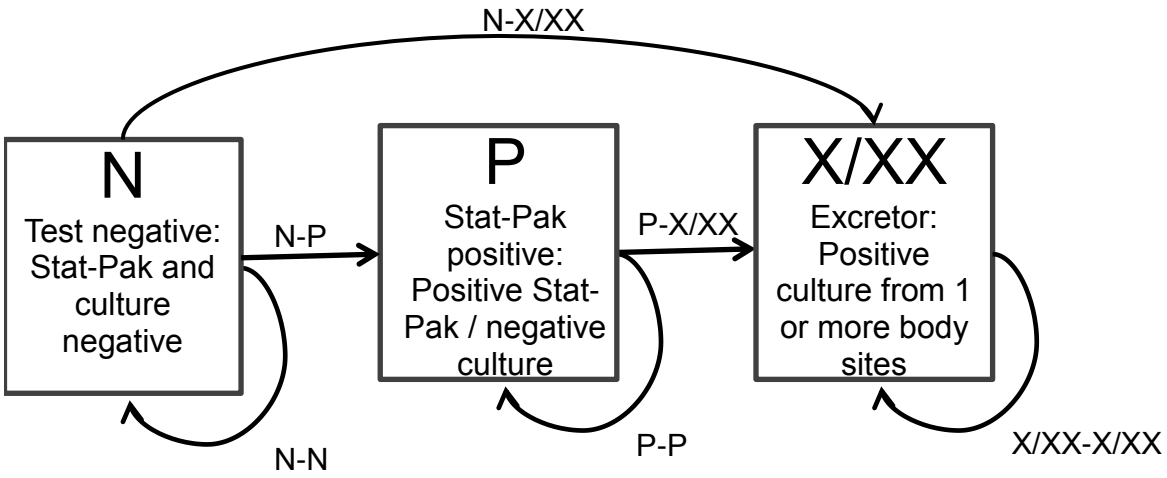

Figure 6.1. Disease states and transitions incorporated in the state-dependent model.

Multistate mark-recapture model

Multistate models estimate state-dependent survival and transition probabilities of survivors between multiple disease states. Bayesian inference using Markov chain Monte Carlo procedures within the program WinBUGS (Lunn et al., 2000) was used to analyse multistate CMR data (for full details on the Bayesian multistate framework see Chapter 4). WinBUGS was called via an R interface

(R Development Core Team, 2013) allowing post hoc comparison of epidemiological rates across the two unconnected geographic locations. Males and females were analysed separately due to known epidemiological differences between the sexes (Graham et al., 2013) which was also beneficial for reducing computation time.

Badgers were caught at regular intervals at Woodchester Park. Annual capture histories were used to obtain yearly parameter estimates. Capture occasions in the BVS study were unevenly distributed (Fig.6.2). Consequently, we adjusted for unequal time-steps to estimate the probability of surviving 12 months (ϕ_{12}), using the following equation

$$\phi_{12} = \phi_u^{\frac{12}{u}}$$

In words; the survival estimate for the time interval *U* (ϕ_U) is raised to the exponent of 12 divided by the length of the unequal time interval (which varied between 3-12 months, Fig.6.2).

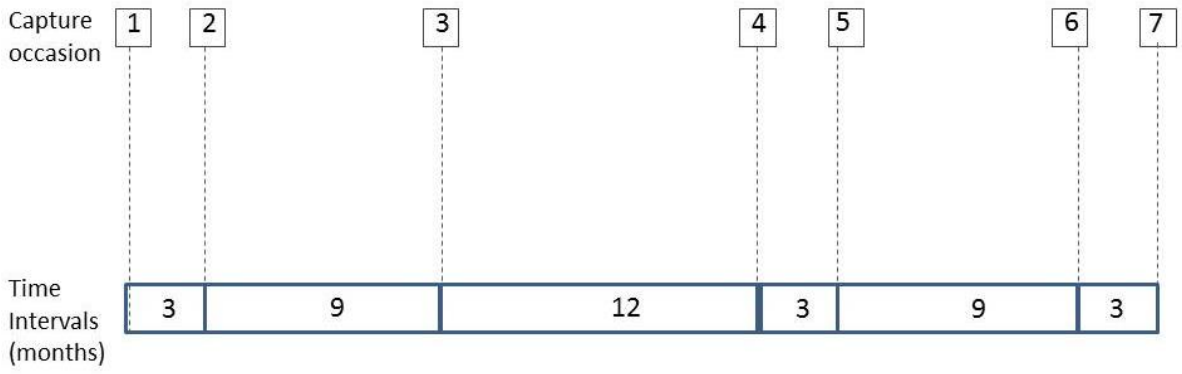

Figure 6.2. Schematic of the time-intervals occurring between capture occasions implemented in the BVS study.

Applying a correction is inappropriate for estimating transition parameters as it is unable to account for all possible transitions (see Fig 6.1 for transitions). To estimate movement between states we used grouped yearly capture histories, resulting in sparser data but not in violation of any assumptions.

Model specification

Due to difficulties testing for goodness-of-fit with Bayesian models, we recreated the models within the program MARK (White & Burnham, 1999) and found the data was not overdispersed for either the BVS or the Woodchester Park data (variation inflation factor $\hat{c} < 1.3$).

Initial model runs suggest convergence was reached after 15000 iterations. We therefore ran 60000 iterations with a burn-in of 15000 for each model. To work out the probability of dissimilar epidemiological rates occurring between the two populations, we calculated post-hoc differences between posterior samples of corresponding parameters taken from each study site. In addition to this probability we technically measure the discrepancy using Kullback-Leibler divergence (KLD (Kullback & Leibler, 1951)). A high KLD would indicate substantial divergence between 2 distributions, whereby 0 indicates identical distributions and the KLD value increases with the discrepancy between the distributions.

Results

Posterior differences between parameters (θ) at contrasting study sites were calculated $(\theta_{WP} - \theta_{BVS})$. That is, the proportion of values above zero provide the probability of parameters being higher for Woodchester badgers compared to BVS badgers and vice versa for proportion of values that lie below zero. Broadly we find the calculated posterior differences between BVS and WP badgers all overlapped zero (Figs 6.3-6.6).

Survival

Survival posterior across study sites overlapped (Fig. 6.3, 6.4). Focussing on the posterior differences between parameters, for badgers in infectious states (X/XX) the certainty that survival probabilities differed between sites was below 0.73, i.e. fewer than 73% of values lay either side of zero and below the classical 0.95 one-sided significance threshold, corresponding with relatively low KLD values (KLD X/XX; males; 0.02; females; 0.08, Figs 6.3 & 6.4).

Uninfected females and Stat-Pak positive males and females were the most divergent groups when comparing between sites with more than 95% values lying above zero, suggesting Woodchester Park badgers have higher survival than their BVS counterparts. Uninfected female badgers in the BVS study have on average a 17% decrease in annual survival compared to Woodchester badgers, with posterior distributions considerably different (KLD; 0.9). Mean posterior differences between stat-Pak positive females found a similar 18% decrease in survival of badgers situated in the BVS study site compared to females at Woodchester Park, (KLD; 0.5, Fig 6.3).

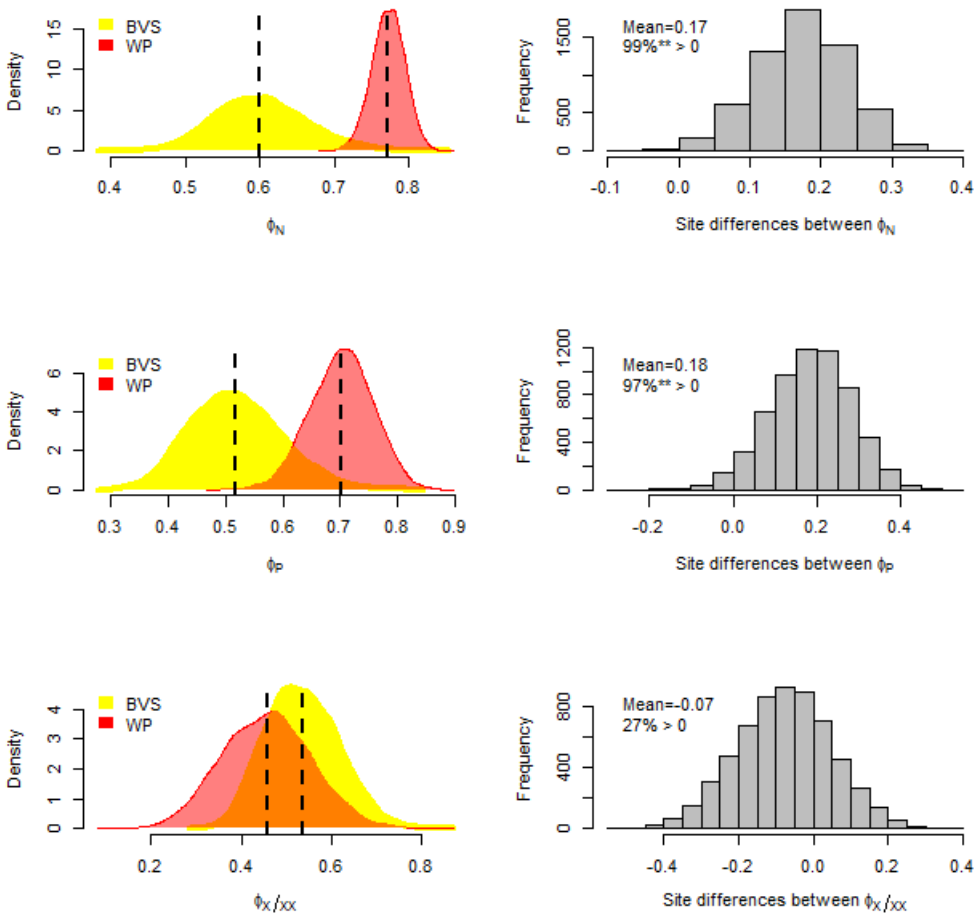

Figure 6.3. Comparison of posterior female annual survival estimates in progressive disease states between 2 sites with mean survival estimates shown by dashed lines. Showing overlaps of posterior densities, and histograms of differences between survival rates along with mean and proportion of values greater than zero. ** highlights probabilities more than 95% which coincides with KLD values greater than 0.5

Male posterior annual survival rates between sites all broadly overlapped when comparing between sites, with near identical estimates for uninfected badgers (mean values; 0.68 (WP), 0.64 (BVS)) and infectious badgers (mean values; 0.47 (WP), 0.44 (BVS)). However, Stat-Pak positive male survival was on average 20% lower at the BVS study site compared to the Woodchester Park males, similar to the difference found in Stat-PAK positive females, with similar discrepancies between distributions (KLD; 0.67, Fig 6.4).

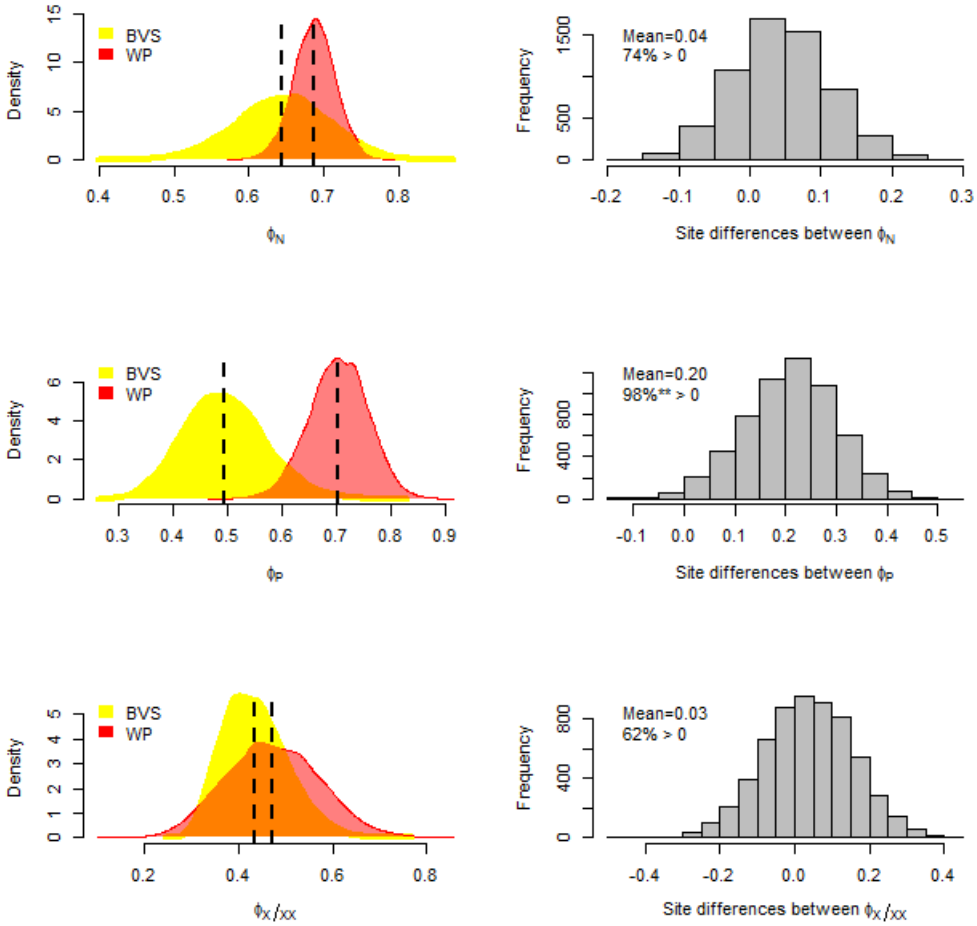

Figure 6.4. Comparison of annual survival posteriors in male badgers in differing health states between study sites. Showing overlaps of posterior densities, and histograms of differences between survival rates along with mean and proportion of values greater than zero. ** highlights probabilities more than 95% which coincides with KLD values greater than 0.5

Transition

The probability of becoming infected (N-P & N-X/XX) and disease progression (P-X/XX) is comparable between study sites, with no inferable differences between posteriors (Fig 6.5, 6.6). Focussing on the posterior differences between both male and female transition parameters, the certainty that transition probabilities differed between sites was below 0.85 for all transitions. Similarly KLD values were relatively low (Females; N-P 0.05, N-X/XX 0.16, P-

X/XX 0.06; Males; N-P 0.07, N-X/XX 0.02, P-X/XX 0.49), with the obvious exception of male P-X/XX transitions, wherein the posterior for BVS badgers was only weakly identifiable.

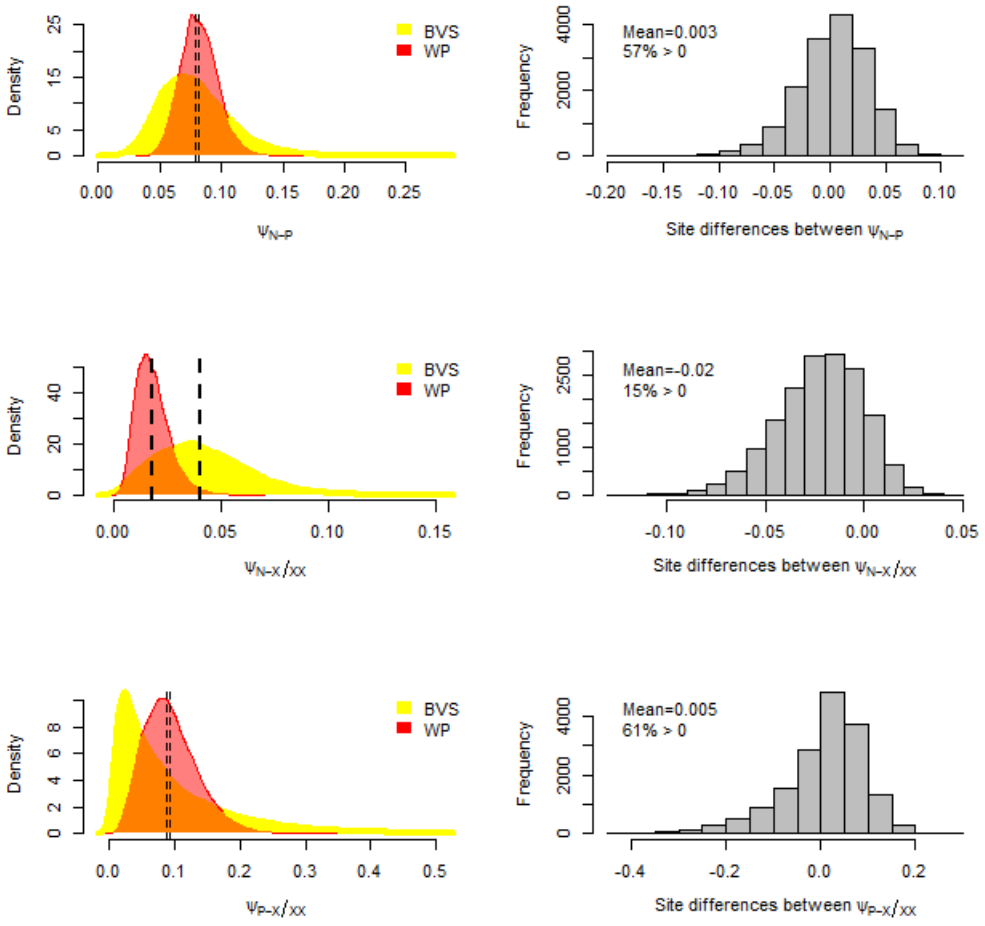

Figure 6.5. Comparison of infection and disease progression probabilities of female badgers between 2 sites. Showing overlaps of posterior densities, and histograms of differences between transition rates along with mean difference and proportion of values greater than zero.

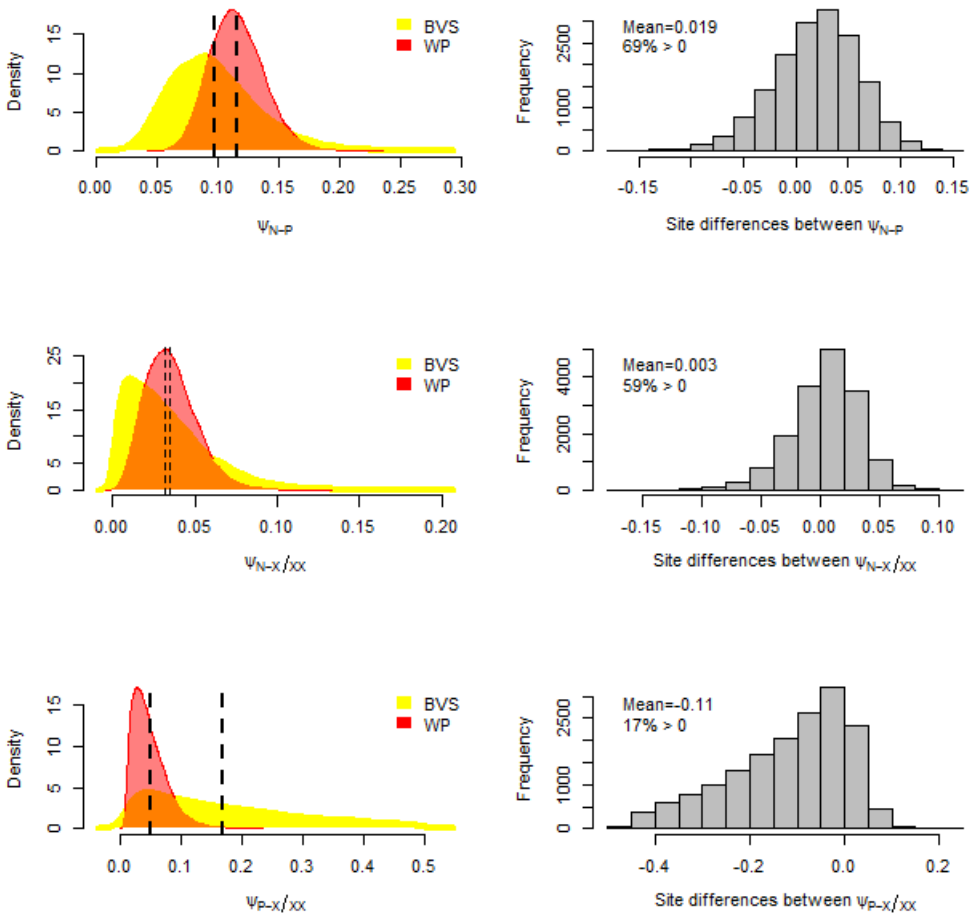

Figure 6.6. Comparison of infection and disease progression probabilities of male badgers between 2 sites. Showing overlaps of posterior densities, and histograms of differences between transition rates along with mean difference and proportion of values greater than zero.

Discussion

The Woodchester Park badger survey is a unique data-set, containing the TB statuses of individual badgers across numerous generations, and uncovering key epidemiological processes that might otherwise be inestimable. This detailed information has come at substantial financial cost, fraught with logistical challenges; consequently replication of the survey on a similar scale is infeasible. The BVS provided a unique, albeit limited, opportunity to compare epidemiological parameters between two independent badger populations.

Applying a Bayesian multistate analysis we estimated and compared epidemiological parameters from CMR data taken from two sites over a comparable time period with identical diagnostic test procedures. We uncover similarities between epidemiological processes across populations.

Infection rates and progression probabilities to infectious stages are of high importance in terms of predicting and modelling viable control strategies (Shirley et al., 2003; Smith et al., 2001a). Disease transmission and progression were not unique to a particular location, with similar estimates found in both study areas. This transferability of key epidemiological parameters between populations confirms the applicability of using disease rates obtained from Woodchester Park to make generalizations regarding TB epidemiology across populations.

Survival estimates showed the most variation between sites, most notably occurring between Stat-Pak positive badgers. During this early infection state BVS badgers had lower mean survival estimates than their WP counterparts. One factor that may explain the observable heterogeneity between early infection survival estimates is the impact of differing trapping effort and study duration on disease detection. Woodchester Park is a longer term study with increased annual trapping events, thus greater opportunities for detection of infected badgers. This is most relevant for detection of advanced infectious badgers (X/XX), as culture tests have notably poor probability of detection due to its low sensitivity (8% (Drewe et al., 2010)). Consequently, there is a real possibility that badgers that advance into infectious stages are not detected as effectively in the BVS study and are included in our early infection (P) survival estimates, bringing about an observable reduction in survival of Stat-Pak positive badgers in the BVS study. However, if this were the case we would arguably expect the effect be more pronounced in males than females with males more vulnerable to infection with higher mortality occurring earlier on in the infection process (Chapter 3). Additionally a multitude of factors, other than disease, will also impact survival rates with ecological, social, and habitat features not accounted for in this present study. Therefore with numerous additional extrinsic influences acting on survival, it is unsurprising that we find

differences in survival rates among populations. Contrastingly we find infectious badgers have similar survival rates and distributions across study systems. Speculatively this may be due to the overriding effect of infection in advanced disease states. That is, badgers in this advanced state remain unaffected by alternative mortality pressures that uninfected badgers and early disease stage badgers may experience. We suggest this may be an additional explanation for the observed similarities during advanced but not early disease stages.

Here, we have compared two high density badger populations which occur at densities much higher than populations occurring elsewhere in the species' geographic range. This undoubtedly fuels concerns regarding whether these populations provide exemplars of a typical badger population. However, their densities are not atypically high for regions in which TB is now a major problem, i.e. much of western Britain (Roper, 2010). Therefore, both populations can be considered credible model populations in terms of identifying those present in areas where TB is endemic and badgers present a legitimate risk to cattle.

Our results further confirmed the accuracy of epidemiological rates of badger populations that pose the greatest threat to cattle and those that should be considered when planning future control strategies. Despite largely concluding a lack of detectable difference between study sites, we are unable to give clear parameter values for either site due to the sparse data and short time period used. Therefore further comparisons would be beneficial. Overall, our approach supports conclusions that Woodchester badgers have similar epidemiological traits to badgers occurring outside the study region.

CHAPTER 7

Having utilised methods to investigate both population dynamics using IPMs and disease processes using multistate models I wished to integrate these analyses to answer further ecological queries. To do this I nudge IPMs into the novel realms of disease dynamics, replacing the usual demographic CJS model, with a multistate model. Although advances in computation power support these more complex analyses running this analysis on the full badger dataset was beyond the time constraints of this thesis. Instead I use simulated data which provides not only a smaller sample size and more manageable analysis but also allows assessment of the reliability of the technique to estimate disease and population processes, identify productivity parameters and finally its ability to forecast dynamics into future years.

A novel framework for estimating and predicting disease and population dynamics

Summary

The dynamics of natural pathogen-host systems are complex and problematic to disentangle due to multiple demographic and epidemiological processes occurring simultaneously. This study links disease and population ecology into a single integrated statistical framework. We advance integrated population models (IPMs) into an epidemiological context, by combining multistate capture-recapture data with multistate population counts. Using a simulated data set, motivated by the study of Eurasian badgers (*Meles meles*), we draw inferences about demographic and epidemiological parameters, along with population abundance. We show how even under various prior structures, previously unidentifiable disease-specific productivity parameters can be inferred. We further highlight the extension of these models as a predictive tool to estimate disease and population dynamics in future years. We suggest the IPM presented here should be used to pose further hypotheses in the badger-TB system. This approach also has the potential to shed light on disease and population dynamics in other study systems with structural flexibility enabling researchers to tailor IPMs to their specific disease-host scenario.

Introduction

Quantifying rates of disease spread and associated population dynamics of hosts is integral to conservation and management but remains a great challenge in field ecology. Demographic models have moved away from exclusively estimating population size to pay attention to underlying causes of population dynamics and the deeper mechanisms, reflecting a move in ecological studies from pattern to process. This coincides with the methodological advancement of capture-mark-recapture (CMR) models which now incorporate a wide and varied range of frameworks providing the tools to address a range of ecological questions directly from samples of wild

populations. Multistate models are an example of how methods have developed to answer increasingly complex ecological questions by exploring site-specific variability (Arnason, 1973; Lebreton & Cefe, 2002; Schwarz et al., 1993). State-dependent models simultaneously estimate state-specific survival and recapture rates, along with transition probabilities of survivors between states. The state variable in these models is largely interchangeable and easily adapted to study geographic site- , breeding state-, and disease state-specific systems. Their application in an epidemiological context reveals rates of disease-induced mortality, infection and progression in a wildlife-host system (Chapters 3-5).

The logical tendency to focus on a single-process model inevitably leads researchers to overlook the reality that individual processes, such as epidemiological rates, are just one component of a much larger interacting system. CMR models can quantify key processes and reveal patterns of causation but they are unable to account for processes occurring in parallel within the system which is problematic when population and disease parameters co-vary. Environmentally driven fluctuations in population growth may be mediated via disease dynamics: for example declines in amphibian populations are linked to climate change which acts by generating increased disease outbreaks (Pounds et al., 2006), and seasonal weather changes can drive population abundance indirectly due to its direct effects on host-pathogen interactions (Altizer et al., 2006). Equally, studies focusing on the individual impact of infection on survival and fecundity rates may not directly translate to population level effects, due to compensatory population responses (Muths et al., 2011) or the intensification of disease impacts by environmental stressors. Improvements in computational power and development of new methodologies now provide solutions to quantify a combination of demographic traits, and despite analytical models only ever providing a caricature of wildlife population dynamics, a combined approach will minimize discrepancies that may arise from analyses focussing on single processes. Here we move away from quantifying disease effects as an exclusive process. Instead we consolidate previous discrete analyses into a common hierarchical framework to identify

both demographic and epidemiological rates, capturing the key dynamics of wildlife-reservoir populations.

Integrated population models (IPM) analyse demographic and census data in a single model, providing precision estimates of demographic parameters and population abundance, free from observation error. The most common approach applies Cormack-Jolly-Seber (CJS) models to analyse demographic data and a state-space model to gain population estimates from census data. However, a shortfall of this approach is the inability of the CJS model component to account for individual infection processes (Chapter 2) preventing consideration of the full demographic mechanisms of reservoir host populations. We expand upon existing IPM research replacing the CJS model component, which only considers a single state, with a multistate model. This adaptation allows individuals to move through different disease states, whilst simultaneously accounting for population level dynamics provided via the census data. Previous studies have merged these models (Péron et al., 2010) however we extend these into a Bayesian framework and apply it for the first time to multiple disease states opposed to geographic sites or breeding states (Borysiewicz et al., 2009; McCrea et al., 2010). Being able to investigate individual disease processes alongside population dynamics is a useful step, bridging the gap between population and disease ecology.

Integrating data provides meaningful estimates of previously unidentifiable parameters such as, but not limited to, immigration (Abadi et al., 2010b) and productivity (Besbeas et al., 2002). We apply this to a naturally infected badger population to explore productivity, specifically the disease-state into which offspring born are recruited, a quantity termed ‘reproductive allocation’ in other study systems (Coulson et al., 2010). Badgers give birth below ground so, in the absence of genetic studies, pedigree is unidentifiable and shared communal setts provide opportunities for disease transmission throughout the social group preventing clear knowledge of the disease state of parents. In the absence of allocated parentage we focus on a per- capita reproductive rate, analogous to recruitment rates, i.e. a per capita rate of growth, that is subsequently

partitioned to represent the proportion of cubs entering each discrete disease state.

At the core of an IPM is a population model which, combined with the multistate model, provides the natural framework for estimating the state-based parameters of population projection models. This built-in projection model can utilise the estimated state-dependent demographic rates and any uncertainty attached to them, to project population dynamics into future years. Thus avoids the necessity for any post-hoc population modelling and allows noise in the system to be propagated naturally into forecasts. In these scenarios IPMs can be used as a decisive tool to aid practitioners and conservationists by providing probabilistic statements regarding future changes in population abundance and disease prevalence. We will draw on the IPM results in our example to demonstrate how they can be interpreted to make predictions regarding reservoir host population dynamics.

The construction of models to estimate both disease and population aspects of reservoir-host systems will provide further understanding of wild host-pathogen dynamics. A simulated data set approximating a naturally infected badger population is used to demonstrate how an IPM, composed of multiple state CMR data and census data, can be analysed and adapted to a specific study system to obtain robust epidemiological and demographic parameter estimates and predict future dynamics. To summarise, our aims are three-fold. First, we seek to build a model which considers multiple interacting processes that occur within badger populations by simultaneously estimating individual disease and population level parameters. Second, we are interested in whether per-capita reproductive allocation rates are identifiable and consistent under different prior structures. Finally we introduce the predictive capability of IPMs.

Methods

Integrated Population model

Integrated population models analyse two or more different data types, obtaining parameter estimates that maximize the joint likelihood of the models. We use state-dependent census data (yearly post-breeding counts of the number of individual badgers caught in different disease states) and multi-state CMR data, tracking an individual's TB status over time, analysed using a state-space model and multistate model respectively. Multistate models are structured models which categorise individuals into discrete classes. Four health states are considered based on categories established in prior studies (Graham et al., 2013; Wilkinson et al., 2000). These include; negative (N), ELISA positive (P), one-site excretor (X) and multi-site excretor (XX). Badgers are able to progress into more severe disease states, but we assume once infected a badger does not recover (for all possible transitions see Fig. 7.1).

By integrating a state-space model and a multistate model the IPM calculates total population size (N_{tot}), abundance of badgers occupying each i^{th} health state (N_i), productivity rates (f_i), disease-specific survival (Φ_i), and transition between health states ($\Psi_{i\text{-}i}$). Sex was incorporated as a covariate in the model structure for survival, transition and recapture estimates. Due to the anonymity of parentage and evidence suggesting infection does not affect fecundity (Chapter 2) (Tomlinson et al., 2013), we calculate a per capita productivity rate whereby individuals in all health-states (N_{tot}) are equally capable of producing young. This was then subdivided to reflect the allocation of cubs to each disease state upon entering the population generating estimates of per capita reproductive allocation (f_i) to each disease state (Fig. 7.1), as our measure of productivity.

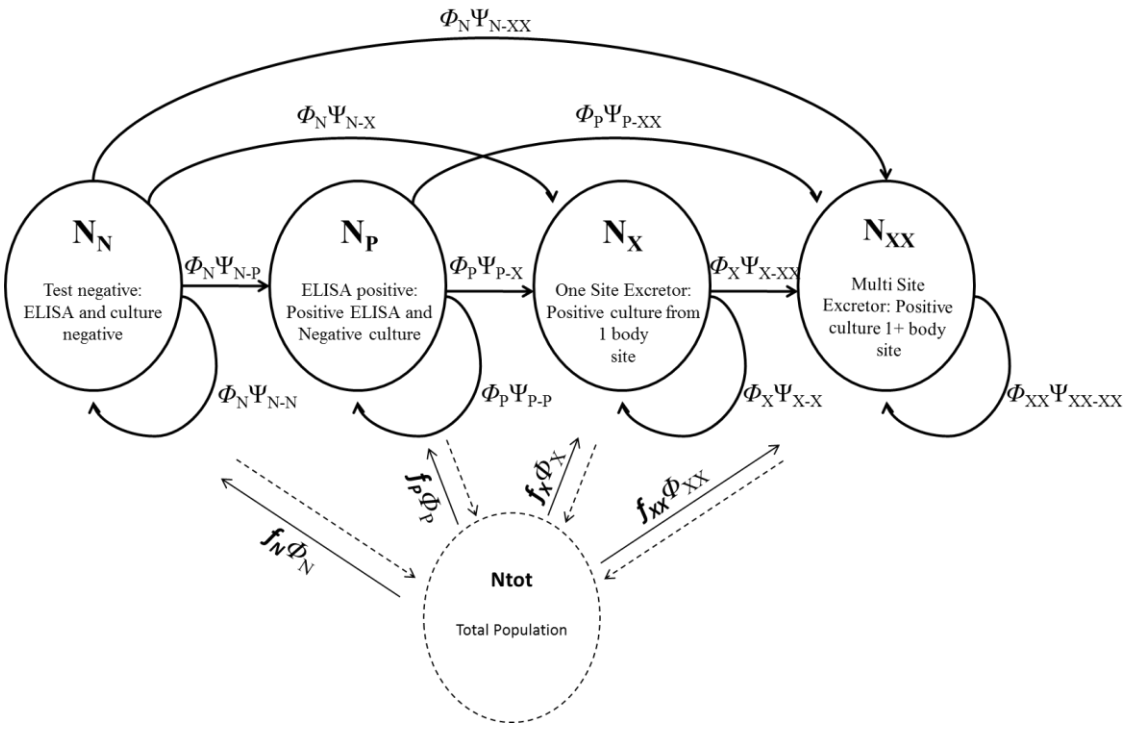

Figure 7.1. Graphical representation of the core population model used to map the number of badgers in each disease state. The nodes show 4 disease classes and the arrows transition probabilities between infected states along with an index of productivity (*f*) to estimate the number of cubs entering each disease class wherein all badgers (N_{tot}) contribute to productivity.

Multistate model

We used the state-space formulation of a multistate model, consisting of a state process and observation process. The state equation uses state-dependent survival (Φ) and transition (Ψ) probabilities to describe the true progression through states. We constrain the state matrix to only allow biological reasonable transitions between disease states (Fig. 7.1 shows possible transitions). However the system description is inaccurate as badgers have variable recapture probabilities less than 1. The observation process therefore links the true state process with the states observed in the environment, by taking into account recapture probabilities (*p*). The likelihood uses a categorical distribution

to estimate the likelihood of capture history data (x) which is equal to the joint probability of state process (z) and observation process (w).

$L_{MS}(x|\ \Phi, \psi, p) = L(z|\ \Phi, \psi) \times L(w|\ p)$

State-space formulation

State-space models are also hierarchical models consisting of an observed time series of counts which is broken down into process variation (what is actually happening in the environment) and observation error. The change in population size over time is a Markovian process with each year dependent on population size the previous year. This approach provides unbiased population estimates or population indices if detection probability is less than 1. The process equations are analogous to a population projection model (Caswell, 2001), describing the true but unknown population trajectory of badgers in various health states by linking changes in population size with demographic rates. Demographic and environmental stochasticity are taken into account within the process equations by relating the population sizes of infected individuals between years using a Poisson distribution.

Not only do these models account for demographic stochasticity, they also allow for uncertainty in the data collection. We use an observation process to link the observed counts of individuals to the true population sizes which is achieved by allowing for observation error. The choice of the observation model often has no strong effect on the parameter estimates (Kéry & Schaub, 2012). We assume the counts (y) of badgers in disease state i over time t follows a Poisson distribution, $y_{i,t} \sim Po\ (N_{i,t})$. The likelihood of the state-space model is the product of the observation and state process, calculating population estimates (N), indices of productivity (f) and survival probabilities (Φ).

$L_{SS}(\mathbf{y} \mid \mathbf{N}, \mathbf{\Phi}, \mathbf{f}) = L_O(\mathbf{y} \mid \mathbf{N}) \ \ X \ \ L_S(\mathbf{N} \mid \mathbf{\Phi}, \mathbf{f})$

Combined likelihood

The likelihood of these two separate analyses share common parameters. Thus the joint likelihood of the IPM is obtained as the product from the 2 data sources (Fig. 7.2).

L_{IPM} (x, y | **Ψ, p, N, Φ, f**) = L_{MS}(**x | Φ, Ψ, p**) X L_{SS} (**y | N, Φ, f**)

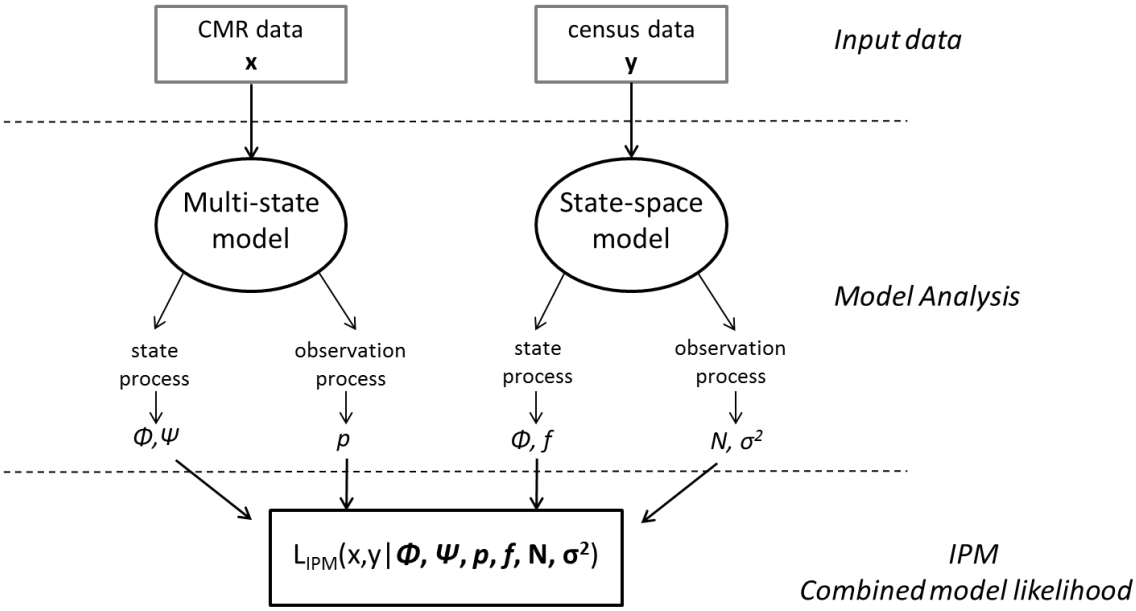

Figure 7.2. Structure of the integrated population model combining a multistate model and a state space model.

Simulation study

A simulated data set was used to test this model. Data were simulated, using functions adapted from Kéry and Schaub (2012), to replicate realistic values of a naturally occurring infected badger population (for full R code see appendix 7.1). 200 simulated badgers consisting of 100 females and 100 males were marked and data for this population was simulated for a six year period. Badgers were able to be in 1 of any 4 discrete disease states (N, P, X, XX) on

each capture occasion. The probability of badgers surviving and moving between disease states were incorporated using parameter values based on prior analyses in order to reflect biological reasonable estimates. In accordance with earlier studies (Chapters 2, 3) survival probabilities were assumed to vary according to sex and disease state, but were not time- or age-dependent. Male survival was simulated to be lower than female survival in all disease states, with male survival gradually decreasing during disease progression and female survival remaining constant until the final disease state (Table 7.1). Recapture was sex-dependent but not state- or time-dependent.

Census data was simulated for each disease state over a 6 year period, with initial population sizes simulated to reflect the proportion of badgers caught in each health state. 78% of badgers were counted in state N ($N_{N,1}$=96) and the number of badgers in the infected states decreased with advancing disease similar to that observed in the Woodchester park study. 13% of total population counts were made up of badges in state P ($N_{P,1}$=17), 5% in state X ($N_{X,1}$=6) and 4 % in state N XX ($N_{XX,1}$=5). Population growth was set to 1 with temporal variation incorporated as 0.02 resembling rates obtained in a prior population analysis (Chapter 2).

Parameter estimation and sensitivity analysis

In Bayesian analyses a parameter is considered identifiable if its distribution differs from the prior specified for it. We asked the question; are reproductive allocation rates identifiable under different prior structures? If per capita reproduction is deducible we would expect priors to have a limited impact upon its posterior distribution. We specified three different prior distributions (two with a uniform distribution; between 0 and 3 (U(0,3)), and 0 and 1 (U(0,1)); and one with a normal distribution with a mean 0 and variance 0.25, truncated to lie between 0 and 3 (N(0,0.25) I(0,3)). The uniform prior assumes that fecundity is equally likely throughout the range of the prior, with U(0,3) the most uninformative prior. In contrast the normal prior is our most informative, assuming that low fecundity is more likely than high fecundity. We adopted non-

informative priors for the remaining parameter estimates, with the exception of starting densities.

Bayesian analyses can efficiently cope with unknown parameters therefore by placing unknown quantities following the raw data we extend the model to forecast population size over future years. We specify 2 additional years in the data following the observations to predict future dynamics. For more information on data simulation, IPM model structure and prior choice refer to the R script in appendix 7.1.

Model Implementation

Models were implemented using Bayesian MCMC methods within program WinBUGS called via an R interface. Convergence was reached following 1000 iterations as indicated by the visible mixing of posterior chains and r-hat < 1.1. For the main model we ran two MCMC chains of 10000 and discarded the first 1000 thinning every 5 samples, such that we retained 1800 samples from each chain (a total of 3600 samples). These chains took 4 days to run.

Results

Parameter estimation

By combining both counts and multistate CMR data, key epidemiological and demographic parameters were estimated simultaneously. The 95% CRI parameter estimates encompassed the true simulated estimates in all cases (estimates shown in Table 7.1).

Table 7.1. Simulated CMR survival estimates and IPM estimates with corresponding 95% CRI

	Female		Male	
Parameter	**Parameter used in simulated CMR data**	**IPM estimate (95% CRI)**	**Parameter used in simulated CMR data**	**IPM estimate (95% CRI)**
Φ_N	0.73	0.769 (0.695, 0.837)	0.67	0.689 (0.611, 0.762)
Φ_P	0.73	0.673 (0.457, 0.86)	0.57	0.662 (0.48, 0.831)
$\Phi_{X,}$	0.73	0.81 (0.555, 0.945)	0.47	0.414 (0.153, 0.723)
Φ_{XX}	0.37	0.452 (0.168, 0.783)	0.14	0.311 (0.105, 0.75)
$\Psi_{N\text{-}N}$	0.945	0.951 (0.908, 0.981)	0.914	0.915 (0.856, 0.96)
$\Psi_{N\text{-}P}$	0.035	0.033 (0.01, 0.07)	0.055	0.069 (0.029, 0.125)
$\Psi_{N\text{-}X,}$	0.016	0.012 (0.002, 0.032)	0.023	0.015 (0.001, 0.044)
$\Psi_{N\text{-}XX}$	0.004	0.004 (0, 0.015)	0.008	0.001 (0, 0.008)
$\Psi_{P\text{-}P}$	0.77	0.861 (0.641, 0.981)	0.67	0.773 (0.569, 0.928)
$\Psi_{P\text{-}X,}$	0.175	0.104 (0.014, 0.277)	0.25	0.185 (0.053, 0.372)
$\Psi_{P\text{-}XX}$	0.055	0.035 (0.001, 0.121)	0.08	0.042 (0.001, 0.134)
$\Psi_{X\text{-}X}$	0.689	0.579 (0.503, 0.732)	0.636	0.718 (0.513, 0.945)
$\Psi_{X\text{-}XX}$	0.311	0.421 (0.268, 0.497)	0.364	0.282 (0.055, 0.487)

Disease-specific reproductive allocation rates

The multi-state model alone was able to estimate all survival and transition probabilities, but when combined with census data was also able to identify per capita reproductive values, a parameter not directly simulated, but identifiable through an integrated analysis. The estimated per capita reproductive rate allocated per disease state was; 0.368 (95% CRI; 0.244, 0.51) for negative badgers, 0.061 (95% CRI; 0.009, 0.135) for P badgers, 0.026 (95% CRI; 0.001, 0.072) for X badgers and 0.044 (95% CRI 0.002, 0.154). This means for every member of the population in year t, 0.368 of the population were recruited as cubs into the uninfected state at t+1. The choice of prior had minimal impact on our reproductive allocation rates with the posterior means and CRI were the same under different priors (Fig. 7.3). Therefore reproductive allocation is an identifiable parameter despite the lack of explicit productivity data.

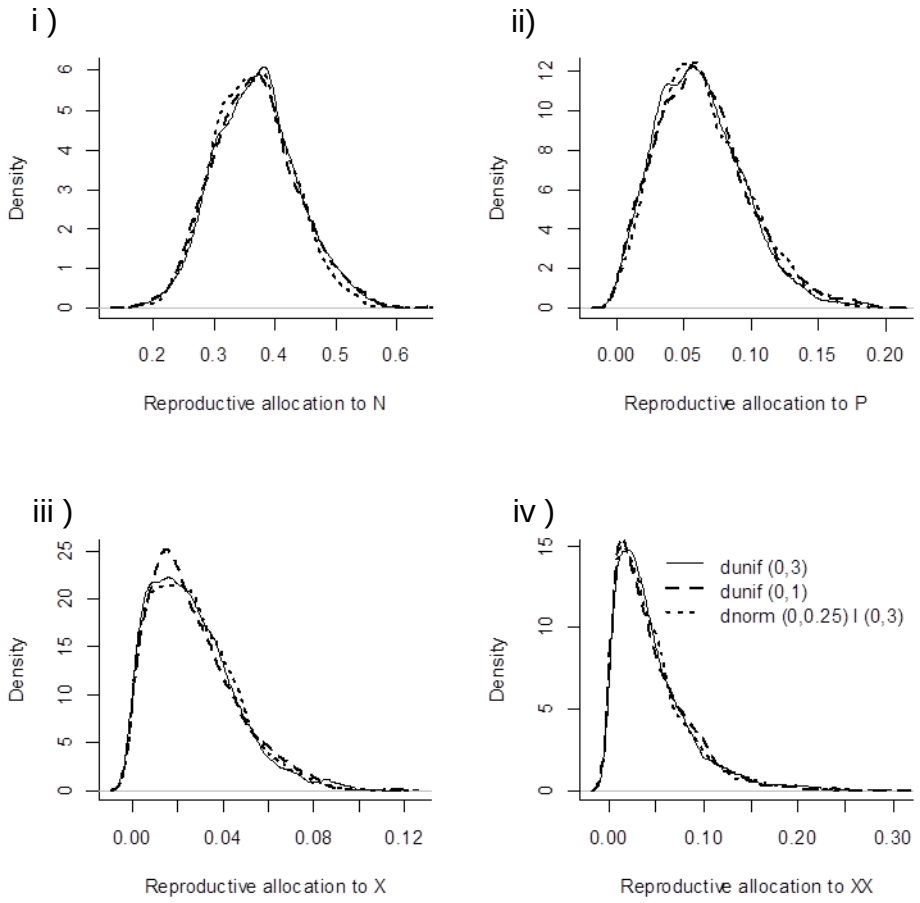

Figure 7.3. Posteriors of per capita reproductive rates under different prior structures, subdivided according to allocation of cubs to specific health states; i) N; ii) P; iii) X & iv) XX.

Predictive capabilities

The mean population growth for this population was estimated to be 1.02. The model performed well at predicting future dynamics (Fig. 7.4). The mean predicted growth for future years was 0.95, however this methodological approach not only provides a mean, but also a measure of certainty. Using data from the simulated data set we find a 69.5% probability of population decline between years 6 and 7, and a 63.6% chance of decline between years 7 and 8. This is calculated by the proportion of posteriors that lie below 1 (the probability

of negative growth) and above 1 (the probability of positive growth), and can be visualised in figure 7.4.

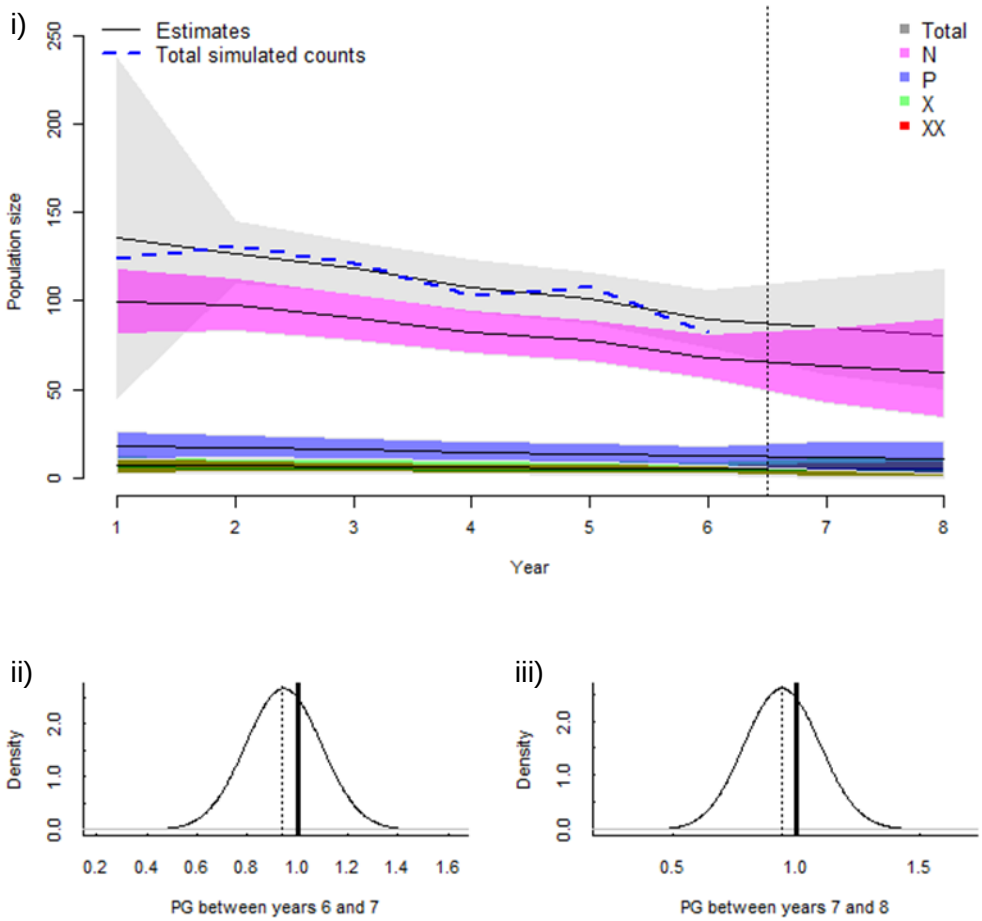

Figure 7.4.i) Estimated and simulated (years 1-6) and predicted (years 7-8) total population size and abundance of badgers occupying each health state. ii-iii) Posteriors of predicted between year population growth between years ii) 6 and 7 and iii) 7 and 8, Mean population growth is indicated by dashed line, the stable population growth (PG=1) is shown by a solid line and can be used as a reference point to predict probabilities of populations undergoing an increase or decrease in population size.

In addition to predicting population size, the number of infected badgers can be predicted into forth coming years (Fig.7.4), along with disease prevalence and

corresponding probabilities of growth/decline in the number of infected badgers (Fig. 7.5).

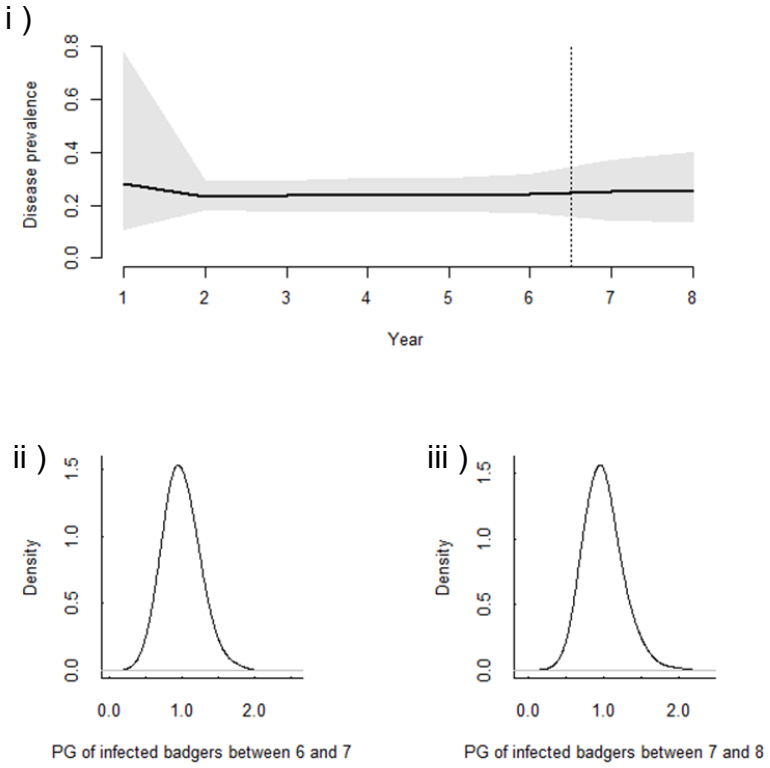

Figure 7.5. i) Estimated disease prevalence in years 1-6 and predicted prevalence for years 7 to 8. Posteriors of predicted population growth of infected badgers between years ii) 6 and 7 and iii) 7 and 8

Ignoring for now that this is a simulation study, we can now make statements regarding the population's previous and future, disease and population dynamics:

'This badger population has remained relatively stable with an overall population growth of 1.02 and a corresponding growth of infected badgers of 1.03 (Fig. 7.4). Consequently, disease prevalence has remained stable (μ= 0.24, 95% CRI 0.15, 0.38).

The predicted population growth rate over the next two years is 0.95, with a 69% chance of population decline next year and 63% probability of a decline the following year. Disease prevalence is predicted to remain stable (μ=0.25, 95% CRI 0.14, 0.39), with a (57:43 chance of a rise: fall in disease prevalence). There is no evidence to suggest the number of infected individuals will increase (overall predicted population growth of infected badgers = 1.006, Fig. 7.5).'

Discussion

Integrated population models are developing as a promising tool in ecological research. Their flexibility and accessibility open up a host of complex analyses to ecologists. This work extends current IPM research and describes one way a demographic and disease model can be derived and parameterised to allow coherent estimation of population dynamics alongside epidemiological processes. This study has only touched the surface with regard to the possibilities and adaptabilities of this framework, but has hopefully highlighted the potential of IPMs to capture dynamics in other study systems. We discuss results from this study and how IPMs can be developed to incorporate further ecological complexities.

Parameter estimation

A key advantage of IPMs is their ability to estimate parameters for which data has not been specifically collected. Estimating productivity is often problematic, however due to the integrated nature of the analysis estimates can be calculated without explicit fecundity data. Measures of productivity are user-driven within the model and could be comfortably altered to provide alternate indices. In this study we estimate per capita reproductive rates partitioned relative to the proportion of cubs entering each disease state, which are identifiable under numerous prior structures. Future analyses can build upon these to model reproductive allocation as a function of environmental and/or individual covariates, revealing whether any correlations exist between variations in the number of infected cubs and environmental or intrinsic

processes. From a management perspective this approach could identify possible cues indicative of infection in cubs.

What is particularly illuminating is the ease in which these models can be tailored to the study organisms' specific population processes as well as the ecologists' particular research question. Here we've focused on adaptations in a badger-context however this methodological approach can be tweaked to be of immense benefit to other ecological systems. The state-space component can be adapted to model alternative forms of productivity and incorporate additional disease states and age or life-stages allowing researchers to address questions specific to their animal system. For example, we may be able to make assumptions regarding parentage such that only infectious adults produce infected juveniles and incorporate not just reproductive allocation but also state-specific fertility. This can be implemented by revising the state-process equation of the state-space model. The number of disease states and feasible transitions between them can be altered in the state-process of the multi-state model for example; including a recovered class, allowing infected individuals to revert back to an uninfected class or reducing the number of health states to a standard susceptible-infected categorisation (Faustino et al., 2004; Lachish et al., 2007), these adaptations may be relevant in other wildlife-pathogen systems. The capabilities of these models are extensive and can be built upon through stages to answer ecological questions of increasing complexity unidentifiable from previous analysis.

Hypothesis testing

These models are the ideal framework to pose ecological hypotheses. Although not covered here IPMs have improved precision compared to individual CMR models, with improved power to detect covariate effects (Schaub & Abadi, 2011). We have developed a model to estimate disease and population dynamics of an infected wildlife reservoir, with only sex incorporated as a covariate due to known epidemiological differences between male and female badgers. However, a range of statistical models can be fit to these demographic

characters within the prior structure to include fixed and random effects. As with survival and productivity in standard IPMs (Chapter 2), probability of infection and reproductive allocation can now also be studied as a function of density, time, individual effects and environmental covariates. A range of hypotheses can now be addressed to disentangle multiple drivers of population dynamics. For example, determining drivers of infection risk and susceptibility to disease in cubs and adults and how they translate to population dynamics will unravel likely co-varying mechanisms, such as interactions between weather and disease processes (Chapter 2.2).

Along with improving our understanding of pest species, these models can be applied to species of conservation concern when pathogens pose an immediate threat to biodiversity, such as the well documented decline of Tasmanian devils due to infectious facial tumours (McCallum et al., 2007), and global declines in amphibian populations due to chytridiomycosis (Schloegel et al., 2006). Pathogens can interact with other driving factors, facilitating disease-mediated extinction risk in species of conservation concern (Kriger et al., 2007). Exploratory questions can be posed within our model framework, explicitly modelling transmission and survival rates of those infected as a function of plausible drivers of infection risk, alongside population dynamics will provide a quantitative understanding of how environmental change influences disease emergence and susceptibility and is worth consideration for the study of species of conservation concern (Smith et al., 2009).

How can IPMS help practitioners?

Infection can spark a multitude of population changes in their host, from immediate population declines to apparent population stability. Understanding the impact of disease on population dynamics is vital for populations of conservation or management concern. One appealing trait of an IPM is its ingrained ability to predict population and disease dynamics into future unknown years, equipping researchers with the means to answer concerns with probabilistic statements such as; ‘there is a 68% probability that this population

is going to decline next year'. This study showcases the ability of IPMs to not only predict total population size, but also the number of infected individuals and subsequently disease prevalence. We find linking disease with population level dynamics as part of the estimating procedure provides the ideal structure to forecast into future years. Population projection models usually fulfil this predictive role, but their fixed transition rates prevent any degree of uncertainty in their predictions. However IPMs provide a measure of uncertainty within the posteriors of state-based parameters, which can be propagated through into predictions of future dynamics.

When more than just predictions of population abundance and disease prevalence are required, IPM posterior estimates and their inherent uncertainty can be transferred into external models. Uncertainty in population responses to disease prompts indecision in management strategies, with predictive models highly sensitive to changes in disease-induced demographic characters and heterogeneity in parameter estimates greatly altering the predicted success of modelled control options (Smith et al., 2012). Consequently, uncertainty in commonly used fixed estimates reduces our ability to accurately and confidently model infected populations. However, incorporating uncertainty in demographic characters within the model will account for any doubt surrounding parameter estimates and assign quantities of certitude to predictions. This information would be more intuitive to assist management decisions, after all although we can never be certain in our model predictions this approach can at least assign a measure of how confident we are.

Conclusion

IPMs benefit from being intuitive and tractable, and equipped with an understanding of a basic IPM available from concise literature (Kéry & Schaub, 2012; Schaub & Abadi, 2011) researchers can tailor models to their specific study system. We advance IPMs into an epidemiological framework. These early results suggest that these models can accurately assess disease dynamics along with population processes including productivity rates, and

there is no reason why these models cannot be extended further to include immigration, and other modelling frameworks. Additionally and perhaps the most appealing aspect of IPMs are their predictive capabilities, and the transference of doubt in parameter estimates to provide posteriors of future dynamics. Future use of these models can be extended into other animal systems and to pose a range of hypotheses regarding disease and population dynamics of badger populations.

CHAPTER 8

Discussion

Overview

Capture-mark recapture (CMR) models are a central tool in ecology, used widely to uncover and understand key ecological processes of wild populations. This thesis has presented a variety of CMR methods to quantify and understand processes underpinning disease and demography of Eurasian badgers (*Meles meles*). First, using an integrated population model (IPM), I provided an overview of badger population dynamics, revealing intrinsic and extrinsic drivers of demographic change (Chapter 2). Adopting a state-structured framework, I moved to individual effects of TB infection and quantified disease-specific mortality rates, infection risk and disease progression probabilities in males and females, highlighting sex-differences in TB epidemiology (Chapter 3 & 4). Progressing into a Bayesian framework can help researchers build more complex mixed-effects models. I used this improved flexibility to account for social structuring of badger populations in the multistate analysis (Chapter 4). A key finding throughout these chapters was sex-differences in infection response. However, discrete survival parameters, despite highlighting sex-differences in TB epidemiology, were unable to explain the underlying causality. I have shown how generating survival trajectories of infected badgers can infer the mechanism underpinning sex differences in disease susceptibility (Chapter 5). A separate badger population provided an opportunity to assess the transferability of epidemiological rates obtained from the Woodchester population, supporting its continued use as a model population (Chapter 6). Finally, I provide a method that links disease and population ecology, estimating disease dynamics and demographic processes, uncovering previously unidentifiable productivity rates and predicting future population dynamics (Chapter 7).

Epidemiology

Epidemiological studies traditionally view wildlife reservoirs as homogeneous populations, with infection imposing a fixed effect across individuals, as illustrated by the standard susceptible-infected paradigm. Due to detailed longitudinal data and multiple diagnostic test procedures, badgers form one of the few wild population studies in which multiple disease states have been described (Wilkinson et al., 2000). I presented a new classification of badgers consisting of four health states using diagnostic results from ELISA and culture tests; negative (N), ELISA positive (P), one-site excretor (X) and multi-site excretor (XX). State-dependent statistical models revealed further epidemiological complexities. Epidemiological parameters vary among disease-states and are highly sex-specific. By obtaining empirical estimates of the force of infection and TB progression I not only found males to have a high likelihood (0.99) of suffering from raised mortality rates than females throughout the infection process, whereby male survival rates reduced by 2%, 6%, 10%, 18% compared to female survival for N, P, X & XX health states respectively, but they also have a high likelihood (>0.96) of increased infection rates and progression rates (Chapters 3 & 4).

Sex-related differences are an important contributor to TB disease dynamics (Chapter 3, Chapter 4 & Chapter 5). Dissimilarities in infection response between sexes is a generally accepted phenomenon across mammal species (Guerra-Silveira & Abad-Franch, 2013; Hazel et al., 2000), however the underlying cause is often poorly understood. Understanding the mechanism driving epidemiological heterogeneity may prove imperative for management considerations for example, targeting specific cohorts that contribute proportionally more to disease transmission, or individuals that exhibit certain behaviours that coincidentally predispose them to infection risk.

Determining a causative link between sex-differences and possible immunological, behavioural and ecological traits is a perplexing problem with limited solutions. Manipulating this scenario experimentally is logistically challenging and achieving it in a laboratory setting is infeasible given the nature

of our hypotheses which require individuals to behave naturally. Obtaining information directly from natural populations would appear a logical approach but requires exploration of analytical tools. I have shown Bayesian survival trajectory analysis (BaSTA (Colchero et al., 2012)) to be useful in comparative analyses of males and females using data direct from a wild population. By decomposing fixed mortality rates into trajectories and comparing parameters of male and female infected badgers I find mortality patterns suggestive of male immunological defects as opposed to any behavioural or ecological predisposition to die (Chapter 5). Diminished immune system functioning in males is likely mediated by androgens, particularly testosterone, although behavioural changes driven by infection cannot be discounted. BaSTA could be used to elucidate the causes of sex-related heterogeneity in other field systems, with male-biased responses to disease occurring across many wildlife populations (Guerra-Silveira & Abad-Franch, 2013). Additional exploration of trajectory parameters can highlight heterogeneity in disease response, stages of maximum vulnerability, and be developed to compare mortality trends between alternative cohorts of infected hosts.

Despite the universal acceptance that long-term studies are vital to uncover ecological and epidemiological processes, longitudinal field projects are limited in their funding opportunities (Clutton-Brock & Sheldon, 2010). An inability to replicate field surveys commonly breeds scepticism of results from single population studies. Much of our current knowledge regarding TB infection in badgers stems from the Woodchester badger society. A comparative analysis (Chapter 6) of Woodchester Park badgers and control badgers captured as part of a vaccination trial in an unconnected region in Gloucestershire highlighted consistencies among epidemiological processes, especially disease transmission and progression rates, instilling confidence in Woodchester Park as a model population. Unfortunately as both populations were in regions of high density I am unable to infer whether these rates are relevant to populations living at lower density. However, low density badger populations are not typically associated with areas posing the highest risk to livestock (Roper, 2010) therefore in terms of using model parameters to assess disease prevention

measures the evidence points to Woodchester Park as a typical population. Of course this result is just a snap shot of badger society with comparisons limited to a short time period and to specific diagnostic tests, therefore further opportunities for comparison will be valuable.

Population dynamics

Despite decades of research, a comprehensive appraisal of demographic drivers of badger population dynamics had not been implemented. I built on previous studies (Macdonald et al., 2010) to provide a more complete understanding of causes underpinning fluctuating badger abundance, capturing a significant amount of variation in key demographic rates (Chapter 2). Considering disease, weather and density as long-term drivers of badger population dynamics I demonstrated how explanations of population dynamics must be based on a multi-factorial analysis, with low-powered statistical approaches unable to encompass the interactions between demographic drivers. Badgers conform as slow life-history strategists, regulating their recruitment, but not survival, under environmental pressures. Density-dependent constraints acted upon recruitment, explaining a large proportion of demographic variability, and its inclusion uncovered influential climatic conditions; over-wintering and spring conditions influenced the number of cubs emerging from setts in the spring. Survival appeared resilient to density changes, showing reduced variability compared to recruitment. Survival contributes proportionally more to population growth, making its reduced temporal variance advantageous due its greater potential to influence fitness (Gaillard et al., 2000; Pfister, 1998) and likely contributes to their observed population stability as a long-lived species (Saether et al., 2013). However, I did uncover a chink in the badgers' evolutionary armour: autumnal conditions. Survival, although resilient to the onslaught of the majority of environmental and density changes, was affected by changes in autumn conditions, indicating an adaptation to gain weight during this period increases their vulnerability to environmental changes. Although these results highlight the sensitivities of badger populations to weather changes, the mechanisms that these act on

remain unclear, some such as autumn conditions seem intuitive due to the known importance of autumn conditions to weight gain (Roper, 2010). But more sophisticated mechanisms are also likely to be at play including changes in activity levels, and covariance between disease and weather effects.

In accordance with studies of TB in other long-lived species (Arthur et al., 2004; Cross et al., 2009; Jolles et al., 2005; Joly & Messier, 2005), disease prevalence had only a limited effect on population dynamics. Recruitment rates were completely unaffected by changes in disease prevalence therefore promoting continued birth of cubs which may in turn restrict levels of disease prevalence from escalating. Although survival appeared strongly disease-dependent, the inclusion of autumnal conditions in the model dampened the effect such that its influence was only felt during periods of unfavourable autumn temperatures. In vertebrates, immune system functioning not only varies between individuals, but also due to a number of factors including, but not limited to, nutritional stress, severe weather, and seasonally with changes in reproductive activity and human disturbance. This study highlights an interaction between disease and weather conditions, suggesting that disease effects are intensified during periods of nutritional stress. However, the direct causality of this influence was undetectable within this analysis. Consideration of individual-level disease processes and weather interactions as well as these large-scale dynamics are required to unravel this interaction and shed further light on the cause of disease-weather complexities.

One solution would be to analyse individual disease processes alongside population dynamics. I demonstrated such an analysis within an IPM, merging individual multistate data and population counts (Chapter 7). Using simulated data I demonstrated how a demographic and disease model can be derived and parameterised to allow simultaneous estimation of disease processes alongside population dynamics. This model should be taken forward to analyse badger population dynamics and pose further hypotheses, perhaps disentangling any co-variation between environmental and disease processes.

Management

The cost of TB to the farmer and other taxpayers is escalating, yet the complexity of the problem has to date prevented control initiatives succeeding, with TB prevalence in cattle increasing and TB hotspots spreading geographically. To combat diseases that affect multiple hosts, management will likely require both cattle and badger focussed interventions. Ideally, any management initiatives would not take place until the host community is fully understood, however the practicalities of obtaining such detailed knowledge make this goal unrealistic and managers need to act despite limited and often polarized information. Although studies in this thesis are unable to devise a clear route for management, all scientific evidence, particularly parameter estimates, are easily transferable into predictive models for future design of management strategies. Having quantified both disease and population processes, to which current predictions of management are highly sensitive (Smith et al., 2012) these can now be applied in a predictive context.

I have identified numerous mechanisms that may be used to guide management including seasonality in disease effects, sex-differences in TB epidemiology and density-dependent compensation. Here I suggest how these could be viewed from a management perspective.

This thesis highlighted that autumn conditions may exacerbate the impact of TB. While exact connections between disease and seasonal patterns are largely unknown, if seasonal drivers of TB do exist this could guide intervention strategies with control targeting infection peaks (Altizer et al., 2006; Joseph et al., 2013). BCG vaccination has a largely unknown duration of effect, and it is not certain how much protection occurs over time following vaccination (Brown et al., 2013). However, if there is a peak level of protection this may be used to time vaccination. For example, vaccinations could be timed to occur prior to the onset of regular outbreaks (Altizer et al., 2006). TB vaccination is suggested to slow the progression of disease (Chambers et al., 2011) therefore,

speculatively, vaccinating before autumn may prevent disease progression to infectious stages even when individuals are subjected to nutritional stress.

Additionally, I identified increased susceptibility to TB in male badgers. That is, males are more likely to become infectious once they have been infected. Individuals that account for a large proportion of transmission are often termed super-spreaders (Lloyd-Smith et al., 2005), with targeted vaccination of these individuals likely to reduce disease spread more effectively than population wide strategies. However this requires an understanding of factors that drive infectiousness. I uncovered maleness as a predictive correlate of higher infectiousness, but also found results suggesting this is due to immunological mechanisms, and not an observable behavioural or ecological difference. Therefore, pinpointing identifiable traits in these individuals is problematic. Recent analysis has highlighted further complexities with TB positive badgers likely to be more socially isolated from their social group (Weber et al., 2013) consequently they may contribute to between-group transmission proportionally more than within-group. Therefore, badgers don't appear to conform to the archetype of a super-spreader. Difficulties pinpointing high-risk individuals, along with this social complexity, indicate population-wide vaccination may be the more effective strategy, as well as preventing perturbation of the intricate badger social systems.

Density-dependence is an important consideration both for supporting populations of conservation concern (Carrete et al., 2006), when density-dependent constraints may restrict population growth despite conservation initiatives, and for pest populations with compensatory density-dependence alleviating the negative consequences of management strategies (Beeton & McCallum, 2011). Previous studies of badger populations have highlighted how perturbation can induce dispersal patterns (Woodroffe et al., 2006b), which can be considered a density-dependent change in social behaviour. Our results further highlight density-dependent compensation, such that culling may release populations from density-dependent pressure increasing birth and survival rates of cubs. This can have implications for population size; stabilizing perturbed

populations and possibly even increasing disease transmission (Potapov et al., 2012). Overcoming these perturbation effects would require marked eradication of badger populations to reduce the viable breeding population along with dispersal opportunities. Arguably this would be both unethical and infeasible logistically given recent failures: a recent culling operation in Gloucestershire was unsuccessful at reaching its target of a 70% reduction in population size (Independent Expert Panel, 2014). Although formulating precise control outcomes is beyond the scope of this thesis, I propose that density-dependent mechanisms will play a pivotal role in determining the success of modelled control strategies and due to its compensatory impact its exclusion may result in exaggerated effects of culling being estimated. Again in this scenario the limited impact of vaccination on population dynamics may be more favourable, avoiding any compensatory effects.

Predictive models were only touched upon in this thesis, but I highly advocate applying the uncertainty expressed in Bayesian posteriors to future predictive models. Although by their very nature predictions are uncertain, more reasonable assertions can be made by assigning a measure of doubt to them. I glimpsed at one way this can be achieved in Chapter 7 where an IPM was used to forecast over future years, providing both mean estimates and a posterior summary. These models can express a ‘best’ and ‘worse’ scenario i.e. the probability of population rise or fall, or probability of an increase or decrease in disease prevalence. In this context the IPM took into account uncertainty in the parameters of epidemiological processes, differences between sexes and disease-specific reproductive allocation rates which were then used to predict not only the population size but also the number of badgers in each disease state. This can be used as a tool not only for reservoir host management, but also conservation initiatives where data may be sparse, or to assess whether a management intervention is required. This is a novel opportunity to make predictions of future population size with full propagation of parameter uncertainty and an important next step should focus on ways to utilise these parameters to consider uncertainty when assessing the benefits of proposed management strategies.

Method development

Using analytical approaches that are able to reveal underlying mechanisms direct from wildlife populations is a worthwhile venture providing more 'realistic' answers to ecological questions than can be achieved by any other means. The work presented here applies new CMR modelling approaches to generate updated robust estimates of processes underlying an infected badger population. The natural progression of analyses led to a move from a frequentist to Bayesian philosophy and through single state to multiple states to integrated models.

A general benefit of Bayesian analyses is the ease to which individual and time-varying covariates, both fixed and random, can naturally be incorporated in a style analogous to coding generalised linear mixed models. This modelling flexibility increased the inferential capability of both the multistate and IPM models. Although novel in the context of badger studies, multistate models have been used across wildlife systems (Faustino et al., 2004), however I expand them to estimate force of infection and disease progression across multiple health states. An area for future development within the multistate framework would be to account for imperfect diagnostic tests by incorporating sensitivity and specificity of the diagnostic tests that guide state assignment. Current research on state uncertainty in multistate models is based on incomplete records (Conn & Cooch, 2009) or uncertainty in detection probability (Jennelle et al., 2007), with diagnostic uncertainty a relatively recent consideration (McClintock et al., 2010).

Moving on to using IPMs for population analyses had numerous advantages, including the acceptance and incorporation of observation error and stochastic variation in population dynamics into the model framework. In this thesis I applied two forms of IPMs to the badger data, first using a standard CJS and state space model to investigate broad population dynamics and detect temporal drivers of fluctuations in badger abundance. This IPM explained a considerable amount of variation in population fluctuations but was unable to

firmly describe, and therefore disentangle, a causative link with disease dynamics. Second, I developed a multistate and state space model to account for epidemiological processes alongside population dynamics. The state matrix in the multistate framework was altered to reasonably represent the infection process in badgers. The state process in the state-space component was altered to estimate abundance of badgers in each health state and provide indices of productivity. Creating a state-structured IPM provided estimates of disease dynamics, alongside population processes, directly linking population ecology (Chapter 2) with disease ecology (Chapters 3 & 4). This model can now be used to pose hypotheses in a badger-TB system to uncover individual and temporal complexities and provide a direct link between disease-dynamics and population change.

New developments in the field of IPMs, including those in this thesis, should help broaden the use of these models in understanding other disease-host systems. With a basic understanding IPMs can easily be adapted, for example by providing alternate indices of fecundity, different disease states and incorporating immigration rates (Abadi et al., 2010b). There would be significant merit in applying IPMs to other disease systems: their ability to predict alone is a novel component. I have also shown how a multistate model can replace a CJS model in an IPM, therefore combining additional models such as ring recovery models, and robust design models which are able to separate survival and emigration estimates may be more appropriate and/or advantageous in other animal systems.

There are challenges that lie ahead with methodological development. Although evidence points to IPMs as a very promising tool for ecological studies there are currently no appropriate goodness-of-fit testing procedures which may restrict its mass usage. In this thesis the addition of a likelihood framework using program MARK provided validation of modelling results, however this feels rather counterintuitive and statistical advancement in this area is likely required before these models are commonplace. Additionally, I found that convergence was slow in individual multistate studies taking from weeks up to months to run,

especially when random effects were incorporated which required a large number of samples before converging. This may dictate whether a model is practical to run or not. Therefore further tuning of the parameters may optimise the convergence rate. Alternatively, modelling outside a WinBUGS framework may improve computation time with MCMC methods running quicker in different programing languages. This would reduce the problem of long convergence times but requires direct coding of the 'black box' MCMC algorithm that WinBUGS provides.

Conclusion

The work presented here has improved our understanding of disease and demography in a badger society. I have emphasized the importance of sex-specific epidemiology and provided inference regarding the causation of these patterns. I also have a more complete understanding of the drivers of large-scale population dynamics and developed a more intricate modelling framework which will hopefully shed light on some mechanisms that still remain uncertain. Capture-mark-recapture analysis is a constantly developing field providing a broad range of tools to answer our ever growing list of ecological questions. The methods in this thesis to some extent display the statistical evolution occurring within CMR analyses, progressing from long-established CJS models to highly computational IPMs. I suggest that pursuing IPMs will yield major advances in ecology as they increasingly become part of the population ecologists' toolbox. Results presented in this thesis have unravelled demographic and disease mechanisms and hopefully highlighted the potential for these to be carried forward to aid management decisions along with answering key ecological and epidemiological questions.

APPENDICES

Appendix 2.1 MARK: measuring survival and recruitment

Survival was modelled using the Cormack-Jolly-Seber model (Lebreton et al., 1992) in the program MARK (White & Burnham, 1999) using the logit link function. Recruitment was analysed in a Pradel model (Pradel, 1996) using the log link function. These models were used to recreate the IPM model to test for goodness of fit, and the significance of disease and density. Disease and density were incorporated by altering the design matrix

The disease model for survival was recreated in a CJS model. After adjusting for overdispersion (c-hat=1.9), disease coefficient parameters are similar to those obtained within the IPM (β_D=0.145 (95% CI -0.24 to -0.04))) with disease explaining a significant amount of variation in the survival model (χ^2_1= 8.58, p=0.003).

Pradel models were used to recreate the effect of density on recruitment. After adjusting for overdispersion (1.9) density was still found to be an important regulatory factor influencing recruitment (β_N = -0.18 (95% CI -0.26 to -0.09), explaining a significant amount of variation (χ^2_1= 12.89, p<0.001).

Appendix 2.2 Sex differentiated covariates

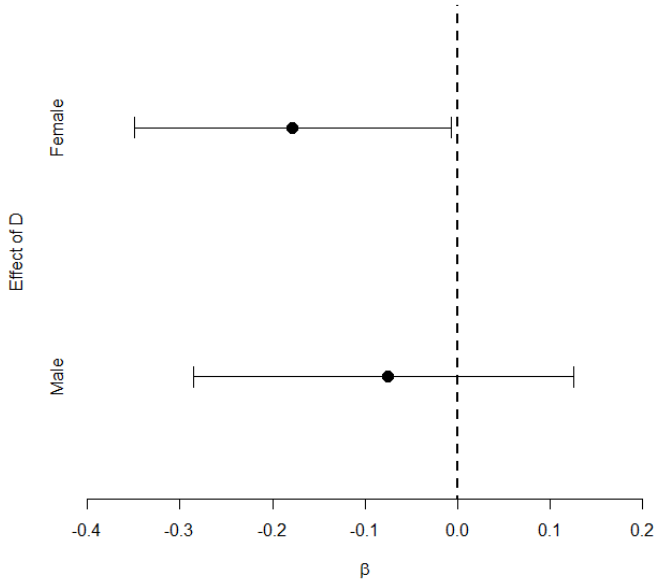

Figure A2.1. Sex-specific effect of disease (D) on survival rates modelled separately for male and female badgers, displayed using posterior means and 95% CRI.

Appendix 2.3 MARK: measuring effect of weather on survival and recruitment

Survival was modelled using the Cormack-Jolly-Seber model (Lebreton et al., 1992) in the program MARK (White & Burnham, 1999) using the logit link function. Recruitment was analysed in a Pradel model (Pradel, 1996) using the log link function. These models were used to analyse time-, sex-, weather-disease prevalence- and density-specific variation in survival and recruitment probabilities. Covariates were incorporated by altering the design matrix. Models were assessed using Akaike Information Criteria (AIC) adjusted for overdispersion (QAIC). 'Better' candidate models were indicated by their lower QAIC values. QAIC weights were used to select the best model (Burnham & Anderson, 2002). Where the difference between the QAIC values for best

approximating model and the next most competitive model was greater than 2, this indicates substantial support for the first model alone (Burnham & Anderson, 2002). In this situation, parameter estimates were derived solely from this most supported model and covariates were taken forward into the IPM. When the difference in QAIC was less than 2 model covariates from the top candidate model set were considered and taken forward into the IPM.

RESULTS

Survival was tested for against different climatic factors, disease prevalence, sex and density in a CJS model. The minimum adequate model found that survival is influenced by autumn temperature (β_{AT}=-0.19 (95% CI -0.33 to -0.07)), disease (β_{D}=- -0.04 (95% CI -0.16 to 0.07)) and sex (Table A2.1), with an interaction between disease and autumn temperature ($\beta_{AT \times D}$=-0.14 (95% CI -0.31 to 0.02)). Autumn temperature was in all the top models. Autumn rainfall appeared in the candidate model set (ΔQAIC<1) but were not as well supported with beta parameters spanning zero (β_{AR}=-0.066 (95% CI -0.19 to 0.05)).

Table A2.1. Summary of the top survival models. Models are ranked by ascending QAICc.

Model (Φ)	Delta QAICc	AICc Weights	Num. Par	QDeviance
S+D+AT+(D:AT)	0	0.31969	7	1096.8883
S+AR+D+AT+(D:AT)	0.948	0.19901	8	1095.8283
S+D+AT	0.9589	0.19793	6	1099.8541
S+D+AR+AT	2.3606	0.0982	7	1099.2488
S+D	6.7753	0.0108	5	1107.6766
S+D+AR	7.6202	0.00708	6	1106.5154
S+D+(S:D)	8.0362	0.00575	6	1106.9315

Pradel models were used to determine the effect of weather variables upon recruitment in a model with density dependence. After adjusting for overdispersion (1.9) the most parsimonious model indicated that density is an important regulatory factor influencing recruitment (β_{N} = -0.18 ±0.04), present in all of the top models with a combined weighting of 99.8% (Table A2.2). By

accounting for density dependence spring temperature and January frost days were found to have a positive effect on recruitment (β_{ST} = 0.145 ± 0.06; β_{FJ} = 0.139 ± 0.06). Spring temperature and January frost days were in the top candidate model with density, however they did not explain a significant amount of variation in models without density dependence (χ^2_2= 3.46, p= 0.18).

Table A2.2. Best models of recruitment (*f*). Survival and recapture remained the same for all the models (Survival $_{Sex}$, Recapture $_{Sex}$)

Model (*f*)	Delta QAICc	AICc Weights	Num. Par	QDeviance
N + ST + FJ	0	0.4928	8	1191.9236
N + FJ	1.4917	0.23375	7	1195.4234
N	2.3376	0.15313	6	1198.2759
N + ST	2.8654	0.11761	7	1196.7969
FJ	11.8765	0.0013	6	1207.8146
.	13.2254	0.00066	5	1211.1695
ST + FJ	13.7785	0.0005	7	1207.7099
ST	15.1986	0.00025	6	1211.1367

Appendix 7.1. RCODE for simulating data and analysing within IPM

Detailed information for simulating multi-state CMR and census data and their subsequent analysis using an IPM combining a multi-state model and census data.

The following presents the code to simulate multistate capture recapture data

Simul.ms is the function to simulate multistate capture-recapture data and was taken directly Kéry and Schaub (2012)

Define mean survival (phi), transitions (psi) and recapture (p) per disease state (a, b, c, d, analogous to N, P, X, XX health states)

```
# Estimates specified for males
        phiA<-0.67
        phiB<-0.57
        phiC<-0.47
        phiD<-0.14
        psiAA<-0.914
        psiAB<-0.055
        psiAC<-0.023
        psiAD<-0.008
        psiBB<-0.67
        psiBC<-0.25
        psiBD<-0.08
        psiCC<-0.636
        psiCD<-0.364
        p<-0.93

# Estimates specified for females
        phiA<-0.73
        phiB<-0.73
        phiC<-0.73
        phiD<-0.37
        psiAA<-0.945
        psiAB<-0.035
        psiAC<-0.016
        psiAD<-0.004
        psiBB<-0.77
        psiBC<-0.175
        psiBD<-0.055
        psiCC<-0.689
        psiCD<-0.311
        p<-0.88

#Define number of occasions (years =6), number of states (a, b, c, d, dead), number of
possible observations and number of individuals released into each state on each occasion
        n.occasions<-6
        n.states<-5
        n.obs<-5
        marked<-matrix(NA,ncol=n.states,nrow=n.occasions)
        marked[,1]<-rep(17,n.occasions)#number released each state
        marked[,2]<-rep(2,n.occasions)
        marked[,3]<-rep(1,n.occasions)
        marked[,4]<-rep(0,n.occasions)
        marked[,5]<-rep(0,n.occasions)
##Define matrices with survival, transition and recapture probabilities
#These are 4-dimensional matrices, with
#Dimension 1: state of departure
#Dimension 2: state of arrival
#Dimension 3: individual
#Dimension 4: time
#1. Define state process matrix- adapted to allow transitions observed in badger
#populations
        totrel<-sum(marked)*(n.occasions-1)
        PSI.STATE<-array(NA,dim=c(n.states,n.states,totrel,n.occasions-1))
        for(i in 1:totrel) {
        for (t in 1:(n.occasions-1)) {
        PSI.STATE[,,i,t]<-matrix(c(
        phiA*psiAA,     phiA*psiAB,     phiA*psiAC,     phiA*psiAD,     1-phiA,
        0,              phiB*psiBB,     phiB*psiBC, phiB*psiBD, 1-phiB,
        0,              0,              phiC*psiCC,     phiC*psiCD,     1-phiC,
        0,              0,              0,              phiD,           1-phiD,
        0,              0,              0,              0,              1),
        nrow=n.states,byrow=TRUE)
        } #t
```

```
        }#i
#2. Observation process matrix
        PSI.OBS<-array(NA,dim=c(n.states,n.obs,totrel,n.occasions-1))
        for(i in 1:totrel) {
        for (t in 1:(n.occasions-1)) {
        PSI.OBS[,,i,t]<-matrix(c(
        p,0,0,0,1-p,
        0,p,0,0,1-p,
        0,0,p,0,1-p,
        0,0,0,p,1-p,
        0,0,0,0,1),nrow=n.states,byrow=TRUE)
        } #t
        }#i
##Execute simulation function for males and females
        sim<-simul.ms(PSI.STATE,PSI.OBS,marked)
        FCH<-sim$CH
        MCH<-sim$CH
        badg<-rbind(FCH,MCH)
#Replace zeroes with 5
        badg[badg==0]<-5
```

#The following presents the code to simulate census data

```
# specify number of years
        n.years<-6
# specify mean annual population growth rate
        mean.lambda<-1
# specify process (temporal) variation of the growth rate
        sigma2.lambda<-0.02
# specify variance of the observation error
        sigma2.y<-0
#Specify initial population size of uninfected badgers, simulate population size in future
#years, generate observed data conditional on true population size in this scenario there
#observation error=0 so no difference
        NA1<-96
        yA<-Na<-numeric(n.years)
        Na[1]<-NA1
        lambda<-rnorm(n.years-1,mean.lambda,sqrt(sigma2.lambda))
        for (t in 1: (n.years-1)){
        Na[t+1]<-Na[t]*lambda[t]
        }
        for(t in 1:n.years) {
        yA[t]<-rnorm(1,Na[t],sqrt(sigma2.y))
        }
# Repeat previous steps for badgers in each disease state
        NB1<-17
        yB<-NB<-numeric(n.years)
        NB[1]<-NB1
```

```
for (t in 1: (n.years-1)){
NB[t+1]<-NB[t]*lambda[t]
}# Simulate population sizes
for(t in 1:n.years) {
yB[t]<-rnorm(1,NB[t],sqrt(sigma2.y))
}
NC1<-6
yC<-NC<-numeric(n.years)
NC[1]<-NC1
for (t in 1: (n.years-1)){
NC[t+1]<-NC[t]*lambda[t]
}
for(t in 1:n.years) {
yC[t]<-rnorm(1,NC[t],sqrt(sigma2.y))
}
ND1<-5
yD<-ND<-numeric(n.years)
ND[1]<-ND1
for (t in 1: (n.years-1)){
ND[t+1]<-ND[t]*lambda[t]
}# Simulate population sizes
for(t in 1:n.years) {
yD[t]<-rnorm(1,ND[t],sqrt(sigma2.y))
}
```

The following presents the code to run the model in R

Step 1. Prepare data and covariates

```
# Compute vector with occasions of first capture
    get.first <- function(x) min(which(x!=5))
    f <- apply(badg, 1, get.first)
#load library
    library(R2WinBUGS)
#specify directory containing winbugs program
    bugs.dir<-"//isad.isadroot.ex.ac.uk/UOE/User/Desktop/WinBUGS14/"
#Create indices of sex (1=female, 2=male) called 'group' to incorporate as covariate
    fe<-c(rep(1,100))
    m<-c(rep(2,100))
    group<-(c(fe,m))
#For predicting create a vector of length 2 corresponding to the number of years to
#predict containing NA (NA, NA) and add to end of census data
    pyears<-2
    yA2<-c(yA,rep(NA,pyears))
    yB2<-c(yB,rep(NA,pyears))
    yC2<-c(yC,rep(NA,pyears))
    yD2<-c(yD,rep(NA,pyears))

#Create a 2 x 200 matrix of NAs and bind to individual badger capture histories
    B = matrix(rep(NA,400), nrow=200, ncol=2)
```

```
        badg2<-cbind(badg,B)
# Function to create known latent states z taken
        known.state.ms <- function(ms, notseen){
          # notseen: label for 'not seen'
          state <- ms
          state[state==notseen] <- NA
          for (i in 1:dim(ms)[1]){
          m <- min(which(!is.na(state[i,])))
          state[i,m] <- NA
           }
          return(state)
          }

# Function to create initial values for unknown z
        ms.init.z <- function(ch, f){
         for (i in 1:dim(ch)[1]){ch[i,1:f[i]] <- NA}
         states <- max(ch, na.rm = TRUE)
         known.states <- 1:(states-1)
         v <- which(ch==states)
         ch[-v] <- NA
         ch[v] <- sample(known.states, length(v), replace = TRUE)
          return(ch)
          }
# Step 2. Specify model- Including priors, state-space model and multistate model
        sink("IPM-MS4.bug")
        cat("
        model {

#PRIORS AND CONSTRAINTS
# Initial population sizes
        Na[1] ~ dnorm(30, 0.0001)I(0,)    #Negative
        NB[1] ~ dnorm(10, 0.0001)I(0,)    # P
        NC[1] ~ dnorm(7, 0.0001)I(0,)    # X
        ND[1] ~ dnorm(5, 0.0001)I(0,) # XX
        Ntot[1] ~ dnorm(50, 0.0001)I(0,)
#Transition probabilities and survival probabilites
        for (i in 1: nind){
        for (t in 1:(n.occasions-1)){

        psiAA[i,t] <- exp(lpsiAA[t,group[i]]) / (1 + exp(lpsiAA[t,group[i]]) +
        exp(lpsiAB[t,group[i]])+ exp(lpsiAC[t,group[i]]))
        psiAB[i,t] <- exp(lpsiAB[t,group[i]]) / (1 + exp(lpsiAA[t,group[i]]) +
        exp(lpsiAB[t,group[i]])+ exp(lpsiAC[t,group[i]]))
        psiAC[i,t] <- exp(lpsiAC[t,group[i]]) / (1 + exp(lpsiAA[t,group[i]]) +
        exp(lpsiAB[t,group[i]])+ exp(lpsiAC[t,group[i]]))
        psiBB[i,t] <- exp(lpsiBB[t,group[i]]) / (1 + exp(lpsiBB[t,group[i]])+
        exp(lpsiBC[t,group[i]]))
```

```
        psiBC[i,t] <- exp(lpsiBC[t,group[i]]) / (1 + exp(lpsiBB[t,group[i]])+
        exp(lpsiBC[t,group[i]]))
        psiAD[i,t]<-1-(psiAA[i,t]+psiAB[i,t]+psiAC[i,t])
        psiBD[i,t]<- 1-(psiBB[i,t]+psiBC[i,t])

        logit(phiA[i,t]) <-eta.phiA[t,group[i]]
        logit(phiB[i,t]) <-eta.phiB[t,group[i]]
        logit(phiC[i,t]) <-eta.phiC[t,group[i]]
        logit(phiD[i,t]) <-eta.phiD[t,group[i]]
        logit(psiCC[i,t])<-eta.psiCC[t,group[i]]
        }}
#Recapture probabilities
        for (i in 1: nind){
        logit(p[i]) <- mu[group[i]]
        }

        for (u in 1:g) {
        for (t in 1:(n.occasions-1)){
        eta.phiA[t,u]<-mu.phiA[u]
        eta.phiB[t,u]<-mu.phiB[u]
        eta.phiC[t,u]<-mu.phiC[u]
        eta.phiD[t,u]<-mu.phiD[u]
        eta.psiCC[t,u]<-mu.psiCC[u]
        lpsiAA[t,u] <-mu.psiAA[u]
        lpsiAB[t,u] <-mu.psiAB[u]
        lpsiAC[t,u] <-mu.psiAC[u]
        lpsiBB[t,u] <-mu.psiBB[u]
        lpsiBC[t,u] <-mu.psiBC[u]
        }
        mean.psiCC[u]~ dunif(0.5,0.99)
        mu.psiCC[u] <- log(mean.psiCC[u]/(1-mean.psiCC[u]))
        mean.psiCD[u]<-1-mean.psiCC[u]
        mean.phiA[u] ~ dunif(0.1,0.95) # Priors for mean state-spec. survival (at A)
        mean.phiB[u]  ~ dunif(0.1,0.95)# Priors for mean state-spec. survival (at B)
        mean.phiC[u]  ~ dunif(0.1,0.95)# Priors for mean state-spec. survival (at C)
        mean.phiD[u]  ~ dunif(0.1,0.95)# Priors for mean state-spec. survival (at D)
        mu.phiA[u]  <- log(mean.phiA[u] /(1-mean.phiA[u] ))
        mu.phiB[u] <- log(mean.phiB[u] /(1-mean.phiB[u] ))
        mu.phiC[u] <- log(mean.phiC[u] /(1-mean.phiC[u] ))
        mu.phiD[u] <- log(mean.phiD[u] /(1-mean.phiD[u] ))
         mu.psiAA[u] ~ dunif(0,10)
         mu.psiAB[u] ~ dunif(0,10)
         mu.psiAC[u] ~ dunif(0,10)
        mu.psiBB[u] ~ dunif(0,10)
        mu.psiBC[u] ~ dunif(0,10)
        mean.psiAA[u] <- exp(mu.psiAA[u]) / (1 + exp(mu.psiAA[u]) +
        exp(mu.psiAB[u])+ exp(mu.psiAC[u]))
```

```
mean.psiAB[u] <- exp(mu.psiAB[u]) / (1 + exp(mu.psiAA[u]) +
exp(mu.psiAB[u])+ exp(mu.psiAC[u]))
mean.psiAC[u] <- exp(mu.psiAC[u]) / (1 + exp(mu.psiAA[u]) +
exp(mu.psiAB[u])+ exp(mu.psiAC[u]))
mean.psiBB[u] <- exp(mu.psiBB[u]) / (1 + exp(mu.psiBB[u])+ exp(mu.psiBC[u]))
mean.psiBC[u] <- exp(mu.psiBC[u]) / (1 + exp(mu.psiBB[u])+ exp(mu.psiBC[u]))
mean.psiAD[u]<-1-(mean.psiAA[u]+mean.psiAB[u]+mean.psiAC[u])
mean.psiBD[u]<- 1-(mean.psiBB[u]+mean.psiBC[u])
mu[u]<-log(mean.p[u]/(1-mean.p[u]))
mean.p[u] ~ dunif(0.4, 0.96)
}#g
for (t in 2:(nyears)){
fa[t]<-mean.fa
fb[t]<-mean.fb
fc[t]<-mean.fc
fd[t]<-mean.fd
}
#use different states to look at recruitment, fecundity per disease state
mean.fa~dunif(0,3)
mean.fb~dunif(0,3)
mean.fc~dunif(0,3)
mean.fd~dunif(0,3)

#Specify likelihood for population count data: the state-space model
# System process specifying the true state can be adapted to include alternate indices
  for (t in 2:nyears){
  for (i in 1:nind){
  mean1A[i,t] <- (fa[t]*Ntot[t-1]*phiA[i,t-1]) + (phiA[i,t-1]* Na[t-1]*psiAA[i,t-1])
 mean1B[i,t] <- (fb[t]*Ntot[t-1]*phiB[i,t-1])+(phiB[i,t-1]*NB[t-1]*psiBB[i,t-
1])+(Na[t-1]*phiA[i,t-1]*psiAB[i,t-1])
    mean1C[i,t] <- (fc[t]*Ntot[t-1]*phiC[i,t-1])+(phiC[i,t-1]*NC[t-1]*psiCC[i,t-
1])+(phiA[i,t-1]*Na[t-1]*psiAC[i,t-1])+(phiB[i,t-1]*NB[t-1]*psiBC[i,t-1])
    mean1D[i,t] <- (fd[t]*Ntot[t-1]*phiD[i,t-1])+(phiD[i,t-1]*ND[t-1])+(phiC[i,t-
1]*NC[t-1]*(1-psiCC[i,t-1]))+(phiA[i,t-1]*Na[t-1]*psiAD[i,t-1])+(phiB[i,t-1]*NB[t-
1]*psiBD[i,t-1])
        }
}
#Include stochasticity
for (t in 2:nyears){
N1A[t]<-mean(mean1A[,t])
N1B[t]<-mean(mean1B[,t])
N1C[t]<-mean(mean1C[,t])
N1D[t]<-mean(mean1D[,t])
Na[t] ~ dpois(N1A[t])
NB[t] ~ dpois(N1B[t])
NC[t] ~ dpois(N1C[t])
ND[t] ~ dpois(N1D[t])
```

```
        Ntot[t] <- Na[t]+NB[t]+NC[t]+ND[t]
        }
# Observation process
        for (t in 1:nyears){
        yA[t] ~ dpois(Na[t])
        yB[t] ~ dpois(NB[t])
        yC[t] ~ dpois(NC[t])
        yD[t] ~ dpois(ND[t])
            }

#Specify likelihood for multistate capture data
# Define state-transition matrices
        for (i in 1:nind){
        for (t in f[i]:(n.occasions-1)){

# Define probabilities of state S(t+1) given S(t)
            ps[1,i,t,1] <- phiA[i,t] * psiAA[i,t]
            ps[1,i,t,2] <- phiA[i,t] * psiAB[i,t]
            ps[1,i,t,3] <- phiA[i,t] * psiAC[i,t]
           ps[1,i,t,4] <- phiA[i,t] * (1-psiAA[i,t]-psiAB[i,t]-psiAC[i,t])
            ps[1,i,t,5] <- 1-phiA[i,t]
            ps[2,i,t,1] <- 0
            ps[2,i,t,2] <- phiB[i,t] * psiBB[i,t]
            ps[2,i,t,3] <- phiB[i,t] * psiBC[i,t]
            ps[2,i,t,4] <- phiB[i,t] * (1- psiBB[i,t]-psiBC[i,t])
            ps[2,i,t,5] <- 1-phiB[i,t]
            ps[3,i,t,1] <- 0
            ps[3,i,t,2] <- 0
            ps[3,i,t,3] <- phiC[i,t] * psiCC[i,t]
            ps[3,i,t,4] <- phiC[i,t] * (1-psiCC[i,t])
            ps[3,i,t,5] <- 1-phiC[i,t]
            ps[4,i,t,1] <- 0
            ps[4,i,t,2] <- 0
            ps[4,i,t,3] <- 0
            ps[4,i,t,4] <- phiD[i,t]
            ps[4,i,t,5] <- 1-phiD[i,t]
            ps[5,i,t,1] <- 0
            ps[5,i,t,2] <- 0
            ps[5,i,t,3] <- 0
            ps[5,i,t,4] <- 0
            ps[5,i,t,5] <- 1

# Define observation matrix, the probabilities of O(t) given S(t)
            po[1,i,t,1] <- p[i]
            po[1,i,t,2] <- 0
            po[1,i,t,3] <- 0
            po[1,i,t,4] <- 0
```

```
            po[1,i,t,5] <- 1-p[i]
            po[2,i,t,1] <- 0
            po[2,i,t,2] <- p[i]
            po[2,i,t,3] <- 0
            po[2,i,t,4] <- 0
            po[2,i,t,5] <- 1-p[i]
            po[3,i,t,1] <- 0
            po[3,i,t,2] <- 0
            po[3,i,t,3] <- p[i]
            po[3,i,t,4] <- 0
            po[3,i,t,5] <- 1-p[i]
            po[4,i,t,1] <- 0
            po[4,i,t,2] <- 0
            po[4,i,t,3] <- 0
            po[4,i,t,4] <- p[i]
            po[4,i,t,5] <- 1-p[i]
            po[5,i,t,1] <- 0
            po[5,i,t,2] <- 0
            po[5,i,t,3] <- 0
            po[5,i,t,4] <- 0
            po[5,i,t,5] <- 1
            } #t
          } #i
# Likelihood
        for (i in 1:nind){
# Define latent state at first capture
        z[i,f[i]] <- q[i,f[i]]
         for (t in (f[i]+1):n.occasions){
# State process: draw S(t) given S(t-1)
        z[i,t] ~ dcat(ps[z[i,t-1], i, t-1,])
# Observation process: draw O(t) given S(t)
         q[i,t] ~ dcat(po[z[i,t], i, t-1,])
         } #t
         } #i
        }
        ",fill = TRUE)
        sink()
#END OF MODEL
# Step 3. Bundle data, specify parameters to be monitored, initial values and MCMC settings
# Bundle data
        bugs.data <- list(nyears=8, q = badg2, group=group, g=length(unique(group)),
        yA=yA2, yB=yB2, yC=yC2, yD=yD2,  f = f, n.occasions = dim(badg2)[2], nind =
        dim(badg2)[1], z = known.state.ms(badg2, 5))
#specifiy initial values
        inits <- function ( ) { list(z = ms.init.z(badg2, f),  Na = rpois(dim(badg2)[2],15),
        NB = rpois(dim(badg2)[2],8), NC = rpois(dim(badg2)[2],5), ND =
        rpois(dim(badg2)[2],5))
```

```
# Parameters monitored
        parameters <- c("mean.phiA", ”mean.phiB", ”mean.phiC", "mean.phiD", "mean.fa",
        "mean.fb", "mean.fc", "mean.fd", "mean.psiAA", "mean.psiAB", "mean.psiAC",
        "mean.psiAD", "mean.psiBB", "mean.psiBC", "mean.psiBD", "mean.psiCC",
        "mean.psiCD", "Na", "NB", "NC", "ND", "Ntot", "mean.p")
# MCMC settings
#specify number of iterations
        ni <- 10000
#specify thinning rate
        nt <- 5
#specify burn-in
        nb <- 1000
#specify number of chains
        nc <- 2
# Step 4. Call WinBUGS from R
        ms42 <- bugs(bugs.data, inits, parameters, "IPM-MS4.bug", n.chains = nc, n.thin =
        nt, n.iter = ni, n.burnin = nb, debug = TRUE, bugs.directory = bugs.dir,
        working.directory = getwd())
#Following model run, visually check for convergence of chains and then print summary
to get your posteriors
        print(ms42, digits = 3)
```

REFERENCES

Abadi, F., Gimenez, O., Arlettaz, R., & Schaub, M. (2010a). An assessment of integrated population models: bias, accuracy, and violation of the assumption of independence. *Ecology, 91*(1), 7-14.

Abadi, F., Gimenez, O., Jakober, H., Stauber, W., Arlettaz, R., & Schaub, M. (2012). Estimating the strength of density dependence in the presence of observation errors using integrated population models. *Ecological Modelling, 242*, 1-9.

Abadi, F., Gimenez, O., Ullrich, B., Arlettaz, R., & Schaub, M. (2010b). Estimation of immigration rate using integrated population models. *Journal of Applied Ecology, 47*(2), 393-400.

Abernethy, D. A., Upton, P., Higgins, I. M., McGrath, G., Goodchild, A. V., Rolfe, S. J., Broughan, J. M., Downs, S. H., Clifton-Hadley, R., Menzies, F. D., de la Rua-Domenech, R., Blissitt, M. J., Duignan, A., & More, S. J. (2013). Bovine tuberculosis trends in the UK and the Republic of Ireland, 1995-2010. *Veterinary Record, 172*(12).

Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M., & Rohani, P. (2006). Seasonality and the dynamics of infectious diseases. *Ecology letters, 9*(4), 467-484.

Altizer, S., Nunn, C. L., Thrall, P. H., Gittleman, J. L., Antonovics, J., Cunningham, A. A., Cunnningham, A. A., Dobson, A. P., Ezenwa, V., & Jones, K. E. (2003). Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annual Review of Ecology, Evolution, and Systematics*, 517-547.

Altizer, S., Ostfeld, R. S., Johnson, P. T., Kutz, S., & Harvell, C. D. (2013). Climate change and infectious diseases: From evidence to a predictive framework. *Science, 341*(6145), 514-519.

Anderson, R. M., & Trewhella, W. (1985). Population-dynamics of the badger (*Meles meles*) and the epidemiology of bovine tuberculosis (*Mycobacterium bovis*). *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, 310*(1145), 327-381.

Arnason, A. N. (1973). The estimation of population size, migration rates and survival in a stratified population. *Researches on population ecology, 15*(2), 1-8.

Arthur, A., Ramsey, D., & Efford, M. (2004). Impact of bovine tuberculosis on a population of brushtail possums (*Trichosurus vulpecula* Kerr) in the Orongorongo Valley, New Zealand. *Wildlife Research, 31*(4), 389-395.

Barlow, N. D. (1996). The ecology of wildlife disease control: Simple models revisited. *Journal of Applied Ecology, 33*(2), 303-314.

Beeton, N., & McCallum, H. (2011). Models predict that culling is not a feasible strategy to prevent extinction of Tasmanian devils from facial tumour disease. *Journal of Applied Ecology, 48*(6), 1315-1323.

Bentil, D. E., & Murray, J. D. (1993). Modelling bovine tuberculosis in badgers. *Journal of Animal Ecology, 62*(2), 239-250.

Benton, T. G., Plaistow, S. J., & Coulson, T. N. (2006). Complex population dynamics and complex causation: devils, details and demography. *Proceedings of the Royal Society B: Biological Sciences, 273*(1591), 1173-1181.

Besbeas, P., Freeman, S. N., Morgan, B. J. T., & Catchpole, E. A. (2002). Integrating mark-recapture-recovery and census data to estimate animal abundance and demographic parameters. *Biometrics, 58*(3), 540-547.

Besbeas, P., Lebreton, J. D., & Morgan, B. J. T. (2003). The efficient integration of abundance and demographic data. *Journal of the Royal Statistical Society Series C-Applied Statistics, 52*, 95-102.

Bevanger, K., & Broseth, H. (1998). Body temperature changes in wild-living badgers *Meles meles* through the winter. *Wildlife Biology, 4*(2), 97-101.

Bjørnstad, O. N., & Grenfell, B. T. (2001). Noisy clockwork: time series analysis of population fluctuations in animals. *Science, 293*(5530), 638-643.

Blanchong, J. A., Scribner, K. T., Kravchenko, A. N., & Winterstein, S. R. (2007). TB-infected deer are more closely related than non-infected deer. *Biology Letters, 3*(1), 104-106.

Blower, S. M., McLean, A. R., Porco, T. C., Small, P. M., Hopewell, P. C., Sanchez, M. A., & Moss, A. R. (1995). The intrinsic transmission dynamics of tuberculosis epidemics. *Nature Medicine, 1*(8), 815-821.

Böhm, M., Hutchings, M. R., & White, P. C. (2009). Contact networks in a wildlife-livestock host community: identifying high-risk individuals in the transmission of bovine TB among badgers and cattle. *Plos One, 4*(4), e5016.

Borysiewicz, R., Morgan, B., Hénaux, V., Bregnballe, T., Lebreton, J.-D., & Gimenez, O. (2009). An integrated analysis of multisite recruitment, mark-recapture-recovery and multisite census data *Modeling Demographic Processes in Marked Populations* (pp. 579-591): Springer.

Bouman, A., Heineman, M. J., & Faas, M. M. (2005). Sex hormones and the immune response in humans. *Human Reproduction Update, 11*(4), 411-423.

Bourne, F. J. (2007). *Bovine TB: the Scientific Evidence.*: Final Report of the Independent Scientific Group on Cattle TB.

Brook, B. W., & Bradshaw, C. J. A. (2006). Strength of evidence for density dependence in abundance time series of 1198 species. *Ecology, 87*(6), 1445-1451.

Brooks, S. P., & Gelman, A. (1998). General methods for monitoring convergence of iterative simulations. *Journal of computational and graphical statistics, 7*(4), 434-455.

Brooks, S. P., King, R., & Morgan, B. J. T. (2004). A Bayesian approach to combining animal abundance and demographic data. *Animal Biodiversity and Conservation, 27*(1), 515-529.

Brown, E., Cooney, R., & Rogers, F. (2013). Veterinary guidance on the practical use of the BadgerBCG tuberculosis vaccine. *In Practice, 35*(3), 143-146.

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: a practical information-theoretic approach*: Springer-Verlag, New York.

Byrnes, J. P., Miller, D. C., & Schafer, W. D. (1999). Gender differences in risk taking: A meta-analysis. *Psychological Bulletin, 125*(3), 367-383.

Carpenter, P. J., Pope, L. C., Greig, C., Dawson, D. A., Rogers, L. M., Erven, K., Wilson, G. J., Delahay, R. J., Cheeseman, C. L., & Burke, T. (2005). Mating system of the Eurasian badger, *Meles meles*, in a high density population. *Molecular Ecology, 14*(1), 273-284.

Carrete, M., Donázar, J. A., & Margalida, A. (2006). Density-dependent productivity depression in Pyrenean bearded vultures: implications for conservation. *Ecological Applications, 16*(5), 1674-1682.

Carter, S. P., Chambers, M. A., Rushton, S. P., Shirley, M. D. F., Schuchert, P., Pietravalle, S., Murray, A., Rogers, F., Gettinby, G., Smith, G. C., Delahay, R. J., Hewinson, R. G., & McDonald, R. A. (2012). BCG Vaccination Reduces Risk of Tuberculosis Infection in Vaccinated Badgers and Unvaccinated Badger Cubs. *Plos One, 7*(12).

Carter, S. P., Delahay, R. J., Smith, G. C., Macdonald, D. W., Riordan, P., Etherington, T. R., Pimley, E. R., Walker, N. J., & Cheeseman, C. L. (2007). Culling-induced social perturbation in Eurasian badgers *Meles meles* and the management of TB in cattle: an analysis of a critical problem in applied ecology. *Proceedings of the Royal Society B-Biological Sciences, 274*(1626), 2769-2777.

Caswell, H. (2001). Matrix population models. Second edition. *Sunderland, MA: Sinauer Associates*.

Cavigelli, S. A., & Pereira, M. E. (2000). Mating season aggression and fecal testosterone levels in male ring-tailed lemurs (*Lemur catta*). *Hormones and Behavior, 37*(3), 246-255.

Celeux, G., Forbes, F., Robert, C. P., & Titterington, D. M. (2006). Deviance information criteria for missing data models. *Bayesian Analysis, 1*(4), 651-673.

Chambers, M. A., Rogers, F., Delahay, R. J., Lesellier, S., Ashford, R., Dalley, D., Gowtage, S., Dave, D., Palmer, S., Brewer, J., Crawshaw, T., Clifton-Hadley, R., Carter, S., Cheeseman, C., Hanks, C., Murray, A., Palphramand, K., Pietravalle, S., Smith, G. C., Tomlinson, A., Walker, N. J., Wilson, G. J., Corner, L. A. L., Rushton, S. P., Shirley, M. D. F.,

Gettinby, G., McDonald, R. A., & Hewinson, R. G. (2011). Bacillus Calmette-Guerin vaccination reduces the severity and progression of tuberculosis in badgers. *Proceedings of the Royal Society B-Biological Sciences, 278*(1713), 1913-1920.

Chambers, M. A., Waterhouse, S., Lyashchenko, K., Delahay, R., Sayers, R., & Hewinson, R. G. (2009). Performance of TB immunodiagnostic tests in Eurasian badgers (*Meles meles*) of different ages and the influence of duration of infection on serological sensitivity. *BMC veterinary research, 5*(1), 42.

Cheeseman, C. L., Wilesmith, J. W., Ryan, J., & Mallinson, P. J. (1987). Badger population dynamics in a high-density area *Symposia of the Zoological Society of London* (Vol. 58, pp. 279-294): Clarendon Press.

Cheeseman, C. L., Wilesmith, J. W., Stuart, F. A., & Mallinson, P. J. (1988). Dynamics of tuberculosis in a naturally infected badger population. *Mammal Review, 18*(1), 61-72.

Chen, H. H., Duffy, S. W., & Tabar, L. (1996). A Markov chain method to estimate the tumour progression rate from preclinical to clinical phase, sensitivity and positive predictive value for mammography in breast cancer screening. *Statistician, 45*(3), 307-317.

Clifton-Hadley, R. S., Sayers, A. R., & Stock, M. P. (1995). Evaluation of an ELISA for *Mycobacterium bovis* infection in badgers (*Meles meles*). *Veterinary Record, 137*(22), 555-558.

Clifton-Hadley, R. S., Wilesmith, J. W., & Stuart, F. A. (1993). *Mycobacterium bovis* in the European badger (*Meles meles*) - epidemiologic findings in tuberculosis badgers from a naturally infected-population. *Epidemiology and infection, 111*(1), 9-19.

Clutton-Brock, T., & Sheldon, B. C. (2010). The seven ages of Pan. *Science, 327*(5970), 1207-1208.

Clutton-Brock, T. H., & Isvaran, K. (2007). Sex differences in ageing in natural populations of vertebrates. *Proceedings of the Royal Society B-Biological Sciences, 274*(1629), 3097-3104.

Colchero, F., & Clark, J. S. (2012). Bayesian inference on age-specific survival for censored and truncated data. *Journal of Animal Ecology, 81*(1), 139-149.

Colchero, F., Jones, O. R., & Rebke, M. (2012). BaSTA: an R package for Bayesian estimation of age-specific mortality from incomplete mark-recapture/recovery data with covariates. *Method in Ecology and Evolution, 3*(3), 466-470.

Coleman, J. D., Coleman, M. C., & Warburton, B. (2006). Trends in the incidence of tuberculosis in possums and livestock, associated with differing control intensities applied to possum populations. *New Zealand Veterinary Journal, 54*(2), 52-60.

Conn, P. B., & Cooch, E. G. (2009). Multistate capture–recapture analysis under imperfect state observation: an application to disease models. *Journal of Applied Ecology, 46*(2), 486-492.

Cormack, R. M. (1964). Estimates of survival from sighting of marked animals. *Biometrika, 51*(3-4), 429-438.

Corner, L. A. L., Murphy, D., & Gormley, E. (2011). *Mycobacterium bovis* Infection in the Eurasian Badger (*Meles meles*): the Disease, Pathogenesis, Epidemiology and Control. *Journal of Comparative Pathology, 144*(1), 1-24.

Coulson, T., Catchpole, E. A., Albon, S. D., Morgan, B. J. T., Pemberton, J. M., Clutton-Brock, T. H., Crawley, M. J., & Grenfell, B. T. (2001). Age, sex, density, winter weather, and population crashes in Soay sheep. *Science, 292*(5521), 1528-1531.

Coulson, T., Milner-Gulland, E. J., & Clutton-Brock, T. (2000). The relative roles of density and climatic variation on population dynamics and fecundity rates in three contrasting ungulate species. *Proceedings of the Royal Society B-Biological Sciences, 267*(1454), 1771-1779.

Coulson, T., Tuljapurkar, S., & Childs, D. Z. (2010). Using evolutionary demography to link life history theory, quantitative genetics and population ecology. *Journal of Animal Ecology, 79*(6), 1226-1240.

Cox, D. R., Donnelly, C. A., Bourne, F. J., Gettinby, G., McInerney, J. P., Morrison, W. I., & Woodroffe, R. (2005). Simple model for tuberculosis in cattle and badgers. *Proceedings of the National Academy of Sciences of the United States of America, 102*(49), 17588-17593.

Cresswell, W. J., Harris, S., Cheeseman, C. L., & Mallinson, P. J. (1992). To breed or not to breed - an analysis of the social and density-dependent constraints on the fecundity of female badgers (*Meles meles*). *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, 338*(1286), 393-407.

Cross, P. C., Heisey, D. M., Bowers, J. A., Hay, C. T., Wolhuter, J., Buss, P., Hofmeyr, M., Michel, A. L., Bengis, R. G., Bird, T. L. F., Du Toit, J. T., & Getz, W. M. (2009). Disease, predation and demography: assessing the impacts of bovine tuberculosis on African buffalo by monitoring at individual and population levels. *Journal of Applied Ecology, 46*(2), 467-475.

Dasbach, E. J., Elbasha, E. H., & Insinga, R. P. (2006). Mathematical models for predicting the epidemiologic and economic impact of vaccination against human papillomavirus infection and disease. *Epidemiologic Reviews, 28*, 88-100.

Dasilva, J., Woodroffe, R., & Macdonald, D. W. (1993). Habitat, food availability and group territoriality in the European badger, *Meles meles*. *Oecologia, 95*(4), 558-564.

DEFRA. (2011). *The Government's policy on Bovine TB and badger control in England*.

Delahay, R. J., Langton, S., Smith, G. C., Clifton-Hadley, R. S., & Cheeseman, C. L. (2000). The spatio-temporal distribution of *Mycobacterium bovis* (bovine tuberculosis) infection in a high-density badger population. *Journal of Animal Ecology, 69*(3), 428-441.

Delahay, R. J., Walker, N., Smith, G. S., Wilkinson, D., Clifton-Hadley, R. S., Cheeseman, C. L., Tomlinson, A. J., & Chambers, M. A. (2013). Long-term temporal trends and estimated transmission rates for *Mycobacterium bovis* infection in an undisturbed high-density badger (*Meles meles*) population. *Epidemiology and infection, 141*(7), 1445-1456.

Delahay, R. J., Walker, N. J., Forrester, G. J., Harmsen, B., Riordan, P., MacDonald, D. W., Newman, C., & Cheeseman, C. L. (2006). Demographic correlates of bite wounding in Eurasian badgers, *Meles meles* L., in stable and perturbed populations. *Animal Behaviour, 71*, 1047-1055.

Donnelly, C. A., Wei, G., Johnston, W. T., Cox, D. R., Woodroffe, R., Bourne, F. J., Cheeseman, C. L., Clifton-Hadley, R. S., Gettinby, G., Gilks, P., Jenkins, H. E., Le Fevre, A. M., McInerney, J. P., & Morrison, W. I. (2007). Impacts of widespread badger culling on cattle tuberculosis: concluding analyses from a large-scale field trial. *International Journal of Infectious Diseases, 11*(4), 300-308.

Donnelly, C. A., Woodroffe, R., Cox, D. R., Bourne, F. J., Cheeseman, C. L., Clifton-Hadley, R. S., Wei, G., Gettinby, G., Gilks, P., Jenkins, H., Johnston, W. T., Le Fevre, A. M., McInerney, J. P., & Morrison, W. I. (2006). Positive and negative effects of widespread badger culling on tuberculosis in cattle. *Nature, 439*(7078), 843-846.

Donnelly, C. A., Woodroffe, R., Cox, D. R., Bourne, J., Gettinby, G., Le Fevre, A. M., McInerney, J. P., & Morrison, W. I. (2003). Impact of localized badger culling on tuberculosis incidence in British cattle. *Nature, 426*(6968), 834-837.

Drewe, J. A., O'Connor, H. M., Weber, N., McDonald, R. A., & Delahay, R. J. (2013). Patterns of direct and indirect contact between cattle and badgers naturally infected with tuberculosis. *Epidemiology and infection, 141*(7), 1467-1475.

Drewe, J. A., Tomlinson, A. J., Walker, N. J., & Delahay, R. J. (2010). Diagnostic accuracy and optimal use of three tests for tuberculosis in live badgers. *Plos One, 5*(6).

Dugdale, H. L., Macdonald, D. W., Pope, L. C., & Burke, T. (2007). Polygynandry, extra-group paternity and multiple-paternity litters in European badger (*Meles meles*) social groups. *Molecular Ecology, 16*(24), 5294-5306.

Dulberger, J., Hobbs, N. T., Swanson, H. M., Bishop, C. J., & Miller, M. W. (2010). Estimating chronic wasting disease effects on mule deer

recruitment and population growth. *Journal of Wildlife Diseases, 46*(4), 1086-1095.

EFSA, & ECDC. (2012). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. *EFSA Journal, 10*(3).

Ellison, A. M. (1996). An introduction to Bayesian inference for ecological research and environmental decision-making. *Ecological Applications, 6*(4), 1036-1046.

Faustino, C. R., Jennelle, C. S., Connolly, V., Davis, A. K., Swarthout, E. C., Dhondt, A. A., & Cooch, E. G. (2004). *Mycoplasma gallisepticum* infection dynamics in a house finch population: seasonal variation in survival, encounter and transmission rate. *Journal of Animal Ecology, 73*(4), 651-669.

Folstad, I., & Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. *American Naturalist, 139*(3), 603-622.

Forrester, G. J., Delahay, R. J., & Clifton-Hadley, R. S. (2001). Screening badgers (*Meles meles*) for *Mycobacterium bovis* infection by using multiple applications of an ELISA. *Veterinary Record, 149*(6), 169-172.

Fowler, P. A., & Racey, P. A. (1988). Overwintering strategies of the badger, *Meles meles*, at 57-degrees-N. *Journal of Zoology, 214*, 635-651.

Frick, W. F., Reynolds, D. S., & Kunz, T. H. (2010). Influence of climate and reproductive timing on demography of little brown myotis *Myotis lucifugus*. *Journal of Animal Ecology, 79*(1), 128-136.

Gaillard, J.-M., Pontier, D., Allaine, D., Lebreton, J., Trouvilliez, J., & Clobert, J. (1989). An analysis of demographic tactics in birds and mammals. *Oikos*, 59-76.

Gaillard, J.-M., & Yoccoz, N. G. (2003). Temporal variation in survival of mammals: a case of environmental canalization? *Ecology, 84*(12), 3294-3306.

Gaillard, J. M., Festa-Bianchet, M., & Yoccoz, N. G. (1998). Population dynamics of large herbivores: variable recruitment with constant adult survival. *Trends in Ecology & Evolution, 13*(2), 58-63.

Gaillard, J. M., Festa-Bianchet, M., Yoccoz, N. G., Loison, A., & Toigo, C. (2000). Temporal variation in fitness components and population dynamics of large herbivores. *Annual Review of Ecology and Systematics, 31*, 367-393.

Gallagher, J., & Clifton-Hadley, R. S. (2000). Tuberculosis in badgers; a review of the disease and its significance for other animals. *Research in Veterinary Science, 69*(3), 203-217.

Gallagher, J., & Horwill, D. M. (1977). Selective oleic-acid albumin agar medium for cultivation of *Mycobacterium bovis*. *Journal of Hygiene, 79*(1), 155-160.

Gallagher, J., Monies, R., Gavier-Widen, M., & Rule, B. (1998). Role of infected, non-diseased badgers in the pathogenesis of tuberculosis in the badger. *Veterinary Record, 142*(26), 710-714.

Gilbert, M., Mitchell, A., Bourn, D., Mawdsley, J., Cliton-Hadley, R., & Wint, W. (2005). Cattle movements and bovine tuberculosis in Great Britain. *Nature, 435*(7041), 491-496.

Goodger, J., Nolan, A., Russell, W. P., Dalley, D. J., Thorns, C. J., Stuart, F. A., Croston, P., & Newell, D. G. (1994). Serodiagnosis of *Mycobacterium bovis* infection in badgers - development of an indirect ELISA using a 25-kda antigen. *Veterinary Record, 135*(4), 82-85.

Gortazar, C., Delahay, R. J., McDonald, R. A., Boadella, M., Wilson, G. J., Gavier-Widen, D., & Acevedo, P. (2012). The status of tuberculosis in European wild mammals. *Mammal Review, 42*(3), 193-206.

Graham, J., Smith, G. C., Delahay, R. J., Bailey, T., McDonald, R. A., & Hodgson, D. (2013). Multi-state modelling reveals sex-dependent transmission, progression and severity of tuberculosis in wild badgers. *Epidemiology and infection, 141*(7), 1429-1436.

Greenwald, R., Esfandiari, J., Lesellier, S., Houghton, R., Pollock, J., Aagaard, C., Andersen, P., Hewinson, R. G., Chambers, M., & Lyashchenko, K. (2003). Improved serodetection of *Mycobacterium bovis* infection in badgers (*Meles meles*) using multiantigen test formats. *Diagnostic Microbiology and Infectious Disease, 46*(3), 197-203.

Griffin, J. M., Williams, D. H., Kelly, G. E., Clegg, T. A., O'Boyle, I., Collins, J. D., & More, S. J. (2005). The impact of badger removal on the control of tuberculosis in cattle herds in Ireland. *Preventive Veterinary Medicine, 67*(4), 237-266.

Guerra-Silveira, F., & Abad-Franch, F. (2013). Sex bias in infectious disease epidemiology: patterns and processes. *Plos One, 8*(4).

Gulland, F. (1992). The role of nematode parasites in Soay sheep (*Ovis aries* L.) mortality during a population crash. *Parasitology, 105*(03), 493-503.

Harrison, A., Newey, S., Gilbert, L., Haydon, D. T., & Thirgood, S. (2010). Culling wildlife hosts to control disease: mountain hares, red grouse and louping ill virus. *Journal of Applied Ecology, 47*(4), 926-930.

Hazel, S. M., Bennett, M., Chantrey, J., Bown, K., Cavanagh, R., Jones, T. R., Baxby, D., & Begon, M. (2000). A longitudinal study of an endemic disease in its wildlife reservoir: cowpox and wild rodents. *Epidemiology and infection, 124*(3), 551-562.

Hernandez-Pacheco, R., Rawlins, R. G., Kessler, M. J., Williams, L. E., Ruiz-Maldonado, T. M., Gonzalez-Martinez, J., Ruiz-Lambides, A. V., & Sabat, A. M. (2013). Demographic variability and density-dependent dynamics of a free-ranging rhesus macaque population. *American Journal of Primatology, 75*(12), 1152-1164.

Hoyle, S. D., & Maunder, M. N. (2004). A Bayesian integrated population dynamics model to analyze data for protected species. *Animal Biodiversity and Conservation, 27*(1), 247-266.

Independent Expert Panel. (2014). Pilot badger culls in Somerset and Gloucestershire: Department for Environment Food and Rural Affairs.

Jenkins, H., Morrison, W., Cox, D., Donnelly, C., Johnston, W., Bourne, F., Clifton-Hadley, R., Gettinby, G., McInerney, J., & Watkins, G. (2008a). The prevalence, distribution and severity of detectable pathological lesions in badgers naturally infected with *Mycobacterium bovis*. *Epidemiology and infection, 136*(10), 1350-1361.

Jenkins, H. E., Cox, D. R., & Delahay, R. J. (2012). Direction of association between bite wounds and *Mycobacterium bovis* infection in badgers: Implications for transmission. *Plos One, 7*(9).

Jenkins, H. E., Woodroffe, R., & Donnelly, C. A. (2008b). The effects of annual widespread badger culls on cattle tuberculosis following the cessation of culling. *International Journal of Infectious Diseases, 12*(5), 457-465.

Jenkins, H. E., Woodroffe, R., & Donnelly, C. A. (2010). The duration of the effects of repeated widespread badger culling on cattle tuberculosis following the cessation of culling. *Plos One, 5*(2).

Jenkins, H. E., Woodroffe, R., Donnelly, C. A., Cox, D. R., Johnston, W. T., Bourne, F. J., Cheeseman, C. L., Clifton-Hadley, R. S., Gettinby, G., Gilks, P., Hewinson, R. G., McInerney, J. P., & Morrison, W. I. (2007). Effects of culling on spatial associations of *Mycobacterium bovis* infections in badgers and cattle. *Journal of Applied Ecology, 44*(5), 897-908.

Jennelle, C. S., Cooch, E. G., Conroy, M. J., & Senar, J. C. (2007). State-specific detection probabilities and disease prevalence. *Ecological Applications, 17*(1), 154-167.

Johnson, H. E., Mills, L. S., Wehausen, J. D., & Stephenson, T. R. (2010). Combining ground count, telemetry, and mark-resight data to infer population dynamics in an endangered species. *Journal of Applied Ecology, 47*(5), 1083-1093.

Jolles, A. E., Cooper, D. V., & Levin, S. A. (2005). Hidden effects of chronic tuberculosis in African buffalo. *Ecology, 86*(9), 2358-2364.

Jolly, G. M. (1965). Explicit estimates from capture-recapture data with both death and immigration-stochastic model. *Biometrika, 52*, 225-&.

Joly, D. O., & Messier, F. (2005). The effect of bovine tuberculosis and brucellosis on reproduction and survival of wood bison in Wood Buffalo National Park. *Journal of Animal Ecology, 74*(3), 543-551.

Joly, D. O., Samuel, M. D., Langenberg, J. A., Blanchong, J. A., Batha, C. A., Rolley, R. E., Keane, D. P., & Ribic, C. A. (2006). Spatial epidemiology of

chronic wasting disease in Wisconsin white-tailed deer. *Journal of Wildlife Diseases, 42*(3), 578-588.

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature, 451*(7181), 990-U994.

Joseph, M. B., Mihaljevic, J. R., Arellano, A. L., Kueneman, J. G., Preston, D. L., Cross, P. C., & Johnson, P. T. (2013). Taming wildlife disease: bridging the gap between science and management. *Journal of Applied Ecology, 50*(3), 702-712.

Judge, J., McDonald, R. A., Walker, N., & Delahay, R. J. (2011). Effectiveness of Biosecurity Measures in Preventing Badger Visits to Farm Buildings. *Plos One, 6*(12).

Kelly, G. E., McGrath, G., & More, S. J. (2010). Estimating the extent of spatial association of *Mycobacterium bovis* infection in badgers in Ireland. *Epidemiology and infection, 138*(02), 270-279.

Kéry, M., & Schaub, M. (2012). *Bayesian population analysis using WinBUGS: a hierarchical perspective*: Academic Press.

Klein, S. L. (2000). The effects of hormones on sex differences in infection: from genes to behavior. *Neuroscience and Biobehavioral Reviews, 24*(6), 627-638.

Klein, S. L. (2003). Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. *Physiology & Behavior, 79*(3), 441-449.

Knape, J., & de Valpine, P. (2011). Effects of weather and climate on the dynamics of animal population time series. *Proceedings of the Royal Society B-Biological Sciences, 278*(1708), 985-992.

Kneip, E., Van Vuren, D. H., Hostetler, J. A., & Oli, M. K. (2011). Influence of population density and climate on the demography of subalpine golden-mantled ground squirrels. *Journal of Mammalogy, 92*(2), 367-377.

Korpimaki, E., Brown, P. R., Jacob, J., & Pech, R. P. (2004). The puzzles of population cycles and outbreaks of small mammals solved? *Bioscience, 54*(12), 1071-1079.

Kowalczyk, R., Jedrzejewska, B., & Zalewski, A. (2003). Annual and circadian activity patterns of badgers (*Meles meles*) in Bialowieza Primeval Forest (eastern Poland) compared with other Palaearctic populations. *Journal of Biogeography, 30*(3), 463-472.

Kramer-Schadt, S., Fernandez, N., Eisinger, D., Grimm, V., & Thulke, H.-H. (2009). Individual variations in infectiousness explain long-term disease persistence in wildlife populations. *Oikos, 118*(2), 199-208.

Krebs, J. R., Anderson, R. M., Clutton-Brock, T., Morrison, I., Young, D., & Donnelly, C. (1997). *Bovine Tuberculosis in Cattle and Badgers*. MAFF Publications, London.

Kriger, K. M., Pereoglou, F., & HERO, J. (2007). Latitudinal variation in the prevalence and intensity of chytrid (*Batrachochytrium dendrobatidis*) infection in eastern Australia. *Conservation Biology, 21*(5), 1280-1290.

Kruuk, H. (1978). Spatial-organization and territorial behavior of European badger *Meles meles*. *Journal of Zoology, 184*(JAN), 1-19.

Kruuk, H., Parish, T., Brown, C. A. J., & Carrera, J. (1979). Use of pasture by the European badger (*Meles meles*). *Journal of Applied Ecology, 16*(2), 453-459.

Kullback, S., & Leibler, R. A. (1951). On information and sufficiency. *Annals of Mathematical Statistics, 22*(1), 79-86.

Laake, J. (2011). RMark: R code for MARK analysis: R package version 2.0.1.

Lachish, S., Jones, M., & McCallum, H. (2007). The impact of disease on the survival and population growth rate of the Tasmanian devil. *Journal of Animal Ecology, 76*(5), 926-936.

Langwig, K. E., Frick, W. F., Bried, J. T., Hicks, A. C., Kunz, T. H., & Marm Kilpatrick, A. (2012). Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology letters, 15*(9), 1050-1057.

Lebreton, J.-D., Nichols, J. D., Barker, R. J., Pradel, R., & Spendelow, J. A. (2009). Modeling individual animal histories with multistate capture-recapture models *Advances in Ecological Research, Vol 41* (Vol. 41, pp. 87-173).

Lebreton, J., & Cefe, R. P. (2002). Multistate recapture models: modelling incomplete individual histories. *Journal of Applied Statistics, 29*(1-4), 353-369.

Lebreton, J. D., Burnham, K. P., Clobert, J., & Anderson, D. R. (1992). Modeling survival and testing biological hypotheses using marked animals - a unified approach with case-studies. *Ecological monographs, 62*(1), 67-118.

Lloyd-Smith, J. O., Schreiber, S. J., Kopp, P. E., & Getz, W. M. (2005). Superspreading and the effect of individual variation on disease emergence. *Nature, 438*(7066), 355-359.

Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos, 88*(1), 87-98.

Loehle, C. (1995). Social barriers to pathogen transmission in wild animal populations. *Ecology*, 326-335.

Luis, A. D., Douglass, R. J., Mills, J. N., & Bjornstad, O. N. (2010). The effect of seasonality, density and climate on the population dynamics of Montana deer mice, important reservoir hosts for Sin Nombre hantavirus. *Journal of Animal Ecology, 79*(2), 462-470.

Lunn, D. J., Thomas, A., Best, N., & Spiegelhalter, D. (2000). WinBUGS - A Bayesian modelling framework: Concepts, structure, and extensibility. *Statistics and Computing, 10*(4), 325-337.

MacDonald, D. W., Harmsen, B. J., Johnson, P. J., & Newman, C. (2004). Increasing frequency of bite wounds with increasing population density in Eurasian badgers, *Meles meles*. *Animal Behaviour, 67*, 745-751.

Macdonald, D. W., & Newman, C. (2002). Population dynamics of badgers (*Meles meles*) in Oxfordshire, UK: numbers, density and cohort life histories, and a possible role of climate change in population growth. *Journal of Zoology, 256*, 121-138.

Macdonald, D. W., Newman, C., Buesching, C. D., & Johnson, P. J. (2008). Male-biased movement in a high-density population of the Eurasian badger (*Meles meles*). *Journal of Mammalogy, 89*(5), 1077-1086.

Macdonald, D. W., Newman, C., Buesching, C. D., & Nouvellet, P. (2010). Are badgers 'Under The Weather'? Direct and indirect impacts of climate variation on European badger (*Meles meles*) population dynamics. *Global Change Biology, 16*(11), 2913-2922.

Macdonald, D. W., Newman, C., Nouvellet, P. M., & Buesching, C. D. (2009). An analysis of Eurasian badger (*Meles meles*) population dynamics: implications for regulatory mechanisms. *Journal of Mammalogy, 90*(6), 1392-1403.

Macdonald, D. W., Newman, C., Stewart, P. D., Domingo-Roura, X., & Johnson, P. J. (2002). Density-dependent regulation of body mass and condition in badgers (*Meles meles*) from Wytham Woods. *Ecology, 83*(7), 2056-2061.

Markle, J., & Fish, E. (2013). SeXX matters in immunity. *Trends in immunology, 35*(3), 97-104.

McCallum, H., Barlow, N., & Hone, J. (2001). How should pathogen transmission be modelled? *Trends in Ecology & Evolution, 16*(6), 295-300.

McCallum, H., Tompkins, D. M., Jones, M., Lachish, S., Marvanek, S., Lazenby, B., Hocking, G., Wiersma, J., & Hawkins, C. E. (2007). Distribution and impacts of Tasmanian devil facial tumor disease. *EcoHealth, 4*(3), 318-325.

McClintock, B. T., Nichols, J. D., Bailey, L. L., MacKenzie, D. I., Kendall, W., & Franklin, A. B. (2010). Seeking a second opinion: uncertainty in disease ecology. *Ecology letters, 13*(6), 659-674.

McCrea, R. S., Morgan, B. J. T., Gimenez, O., Besbeas, P., Lebreton, J. D., & Bregnballe, T. (2010). Multi-site integrated population modelling. *Journal of Agricultural Biological and Environmental Statistics, 15*(4), 539-561.

McDonald, D. B., Fitzpatrick, J. W., & Woolfenden, G. E. (1996). Actuarial senescence and demographic heterogeneity in the Florida Scrub Jay. *Ecology, 77*(8), 2373-2381.

Michel, A. L., Bengis, R. G., Keet, D. F., Hofmeyr, M., de Klerk, L. M., Cross, P. C., Jolles, A. E., Cooper, D., Whyte, I. J., Buss, P., & Godfroid, J. (2006).

Wildlife tuberculosis in South African conservation areas: Implications and challenges. *Veterinary Microbiology, 112*(2-4), 91-100.

Mills, S. C., Grapputo, A., Jokinen, I., Koskela, E., Mappes, T., & Poikonen, T. (2010). Fitness trade-offs mediated by immunosuppression costs in a small mammal. *Evolution, 64*(1), 166-179.

Moore, S. L., & Wilson, K. (2002). Parasites as a viability cost of sexual selection in natural populations of mammals. *Science, 297*(5589), 2015-2018.

Murhead, R. H., & Burns, K. J. (1974). Tuberculosis in wild badgers in Gloucestershire - epidemiology. *Veterinary Record, 95*(24), 552-555.

Muths, E., Scherer, R. D., & Pilliod, D. S. (2011). Compensatory effects of recruitment and survival when amphibian populations are perturbed by disease. *Journal of Applied Ecology, 48*(4), 873-879.

Naranjo, V., Gortazar, C., Vicente, J., & de la Fuente, J. (2008). Evidence of the role of European wild boar as a reservoir of *Mycobacterium tuberculosis* complex. *Veterinary Microbiology, 127*(1-2), 1-9.

Nevoux, M., Forcada, J., Barbraud, C., Croxall, J., & Weimerskirch, H. (2010). Bet-hedging response to environmental variability, an intraspecific comparison. *Ecology, 91*(8), 2416-2427.

Nouvellet, P., Newman, C., Buesching, C. D., & Macdonald, D. W. (2013). A multi-metric approach to investigate the effects of weather conditions on the demographic of a terrestrial mammal, the European badger (*Meles meles*). *Plos One, 8*(7), e68116.

Péron, G., Crochet, P.-A., Doherty Jr, P. F., & Lebreton, J.-D. (2010). Studying dispersal at the landscape scale: efficient combination of population surveys and capture-recapture data. *Ecology, 91*(11), 3365-3375.

Peron, G., Nicolai, C. A., & Koons, D. N. (2012). Demographic response to perturbations: the role of compensatory density dependence in a North American duck under variable harvest regulations and changing habitat. *Journal of Animal Ecology, 81*(5), 960-969.

Pfister, C. A. (1998). Patterns of variance in stage-structured populations: Evolutionary predictions and ecological implications. *Proceedings of the*

National Academy of Sciences of the United States of America, 95(1), 213-218.

Plowright, R. K., Field, H. E., Smith, C., Divljan, A., Palmer, C., Tabor, G., Daszak, P., & Foley, J. E. (2008). Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (*Pteropus scapulatus*). *Proceedings of the Royal Society B: Biological Sciences, 275*(1636), 861-869.

Pollock, K. H. (1981). Capture-recapture models allowing for age-dependent survival and capture rates. *Biometrics, 37*(3), 521-529.

Pollock, K. H. (1982). A capture-recapture design robust to unequal probability of capture. *The Journal of Wildlife Management*, 752-757.

Pope, L. C., Butlin, R. K., Wilson, G. J., Woodroffe, R., Erven, K., Conyers, C. M., Franklin, T., Delahay, R. J., Cheeseman, C. L., & Burke, T. (2007). Genetic evidence that culling increases badger movement: implications for the spread of bovine tuberculosis. *Molecular Ecology, 16*(23), 4919-4929.

Potapov, A., Merrill, E., & Lewis, M. A. (2012). Wildlife disease elimination and density dependence. *Proceedings of the Royal Society B: Biological Sciences, 279*(1741), 3139-3145.

Pounds, J. A., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P., Foster, P. N., La Marca, E., Masters, K. L., Merino-Viteri, A., & Puschendorf, R. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature, 439*(7073), 161-167.

Pradel, R. (1996). Utilization of capture-mark-recapture for the study of recruitment and population growth rate. *Biometrics*, 703-709.

Promislow, D. E., & Harvey, P. H. (1990). Living fast and dying young: A comparative analysis of life-history variation among mammals. *Journal of Zoology, 220*(3), 417-437.

R Development Core Team. (2013). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

Redpath, S. M., Mougeot, F., Leckie, F. M., Elston, D. A., & Hudson, P. J. (2006). Testing the role of parasites in driving the cyclic population dynamics of a gamebird. *Ecology letters, 9*(4), 410-418.

Rodel, H. G., Bora, A., Kaetzke, P., Khaschei, M., Hutzelmeyer, H., & von Holst, D. (2004). Over-winter survival in subadult European rabbits: weather effects, density dependence, and the impact of individual characteristics. *Oecologia, 140*(4), 566-576.

Rogers, L. M., Cheeseman, C. L., Mallinson, P. J., & CliftonHadley, R. (1997). The demography of a high-density badger (*Meles meles*) population in the west of England. *Journal of Zoology, 242*, 705-728.

Rogers, L. M., Delahay, R., Cheeseman, C. L., Langton, S., Smith, G. C., & Clifton-Hadley, R. S. (1998). Movement of badgers (*Meles meles*) in a high-density population: individual, population and disease effects. *Proceedings of the Royal Society B-Biological Sciences, 265*(1403), 1269-1276.

Roper, T. J. (2010). *Badger*. London: HarperCollins.

Rosà, R., Bolzoni, L., Rosso, F., Pugliese, A., Hudson, P., & Rizzoli, A. (2011). Effect of *Ascaridia compar* infection on rock partridge population dynamics: empirical and theoretical investigations. *Oikos, 120*(10), 1557-1567.

Royle, J. A. (2008). Modeling individual effects in the Cormack-Jolly-Seber model: A state-space formulation. *Biometrics, 64*(2), 364-370.

Rushmore, J., Caillaud, D., Matamba, L., Stumpf, R. M., Borgatti, S. P., & Altizer, S. (2013). Social network analysis of wild chimpanzees provides insights for predicting infectious disease risk. *Journal of Animal Ecology, 82*(5), 976-986.

Ruxton, G. D. (1996). The effects of stochasticity and seasonality on model dynamics: Bovine tuberculosis in badgers. *Journal of Animal Ecology, 65*(4), 495-500.

Saether, B.-E., Coulson, T., Grotan, V., Engen, S., Altwegg, R., Armitage, K. B., Barbraud, C., Becker, P. H., Blumstein, D. T., Dobson, F. S., Festa-Bianchet, M., Gaillard, J.-M., Jenkins, A., Jones, C., Nicoll, M. A. C.,

Norris, K., Oli, M. K., Ozgul, A., & Weimerskirch, H. (2013). How life history influences population dynamics in fluctuating environments. *American Naturalist, 182*(6), 743-759.

Saino, N., Galeotti, P., Sacchi, R., & Moller, A. P. (1997). Song and immunological condition in male barn swallows (*Hirundo rustica*). *Behavioral Ecology, 8*(4), 364-371.

Schaub, M., & Abadi, F. (2011). Integrated population models: a novel analysis framework for deeper insights into population dynamics. *Journal of Ornithology, 152*(1), 227-237.

Schaub, M., Gimenez, O., Sierro, A., & Arlettaz, R. (2007). Use of integrated modeling to enhance estimates of population dynamics obtained from limited data. *Conservation Biology, 21*(4), 945-955.

Schloegel, L. M., Hero, J.-M., Berger, L., Speare, R., McDonald, K., & Daszak, P. (2006). The decline of the sharp-snouted day frog (*Taudactylus acutirostris*): the first documented case of extinction by infection in a free-ranging wildlife species? *EcoHealth, 3*(1), 35-40.

Schmitt, S. M., Fitzgerald, S. D., Cooley, T. M., BruningFann, C. S., Sullivan, L., Berry, D., Carlson, T., Minnis, R. B., Payeur, J. B., & Sikarskie, J. (1997). Bovine tuberculosis in free-ranging white-tailed deer from Michigan. *Journal of Wildlife Diseases, 33*(4), 749-758.

Schuurs, A., & Verheul, H. A. M. (1990). Effects of gender and sex steroids on the immune-response. *Journal of Steroid Biochemistry and Molecular Biology, 35*(2), 157-172.

Schwarz, C. J., & Arnason, A. N. (1996). A general methodology for the analysis of capture-recapture experiments in open populations. *Biometrics*, 860-873.

Schwarz, C. J., Schweigert, J. F., & Arnason, A. N. (1993). Estimating migration rates using tag-recovery data. *Biometrics, 49*(1), 177-193.

Seber, G. A. F. (1965). A note on multiple-recapture census. *Biometrika, 52*, 249-259.

Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution, 11*(8), 317-321.

Shirley, M. D. F., Rushtown, S. P., Smith, G. C., South, A. B., & Lurz, P. W. W. (2003). Investigating the spatial dynamics of bovine tuberculosis in badger populations: evaluating an individual-based simulation model. *Ecological Modelling, 167*(1-2), 139-157.

Smith, G., Cheeseman, C., & Clifton-Hadley, R. (1997). Modelling the control of bovine tuberculosis in badgers in England: culling and the release of lactating females. *Journal of Applied Ecology*, 1375-1386.

Smith, G. C., Bennett, R., Wilkinson, D., & Cooke, R. (2007). A cost-benefit analysis of culling badgers to control bovine tuberculosis. *Veterinary Journal, 173*(2), 302-310.

Smith, G. C., & Cheeseman, C. L. (2002). A mathematical model for the control of diseases in wildlife populations: culling, vaccination and fertility control. *Ecological Modelling, 150*(1-2), 45-53.

Smith, G. C., Cheeseman, C. L., Clifton-Hadley, R. S., & Wilkinson, D. (2001a). A model of bovine tuberculosis in the badger *Meles meles*: an evaluation of control strategies. *Journal of Applied Ecology, 38*(3), 509-519.

Smith, G. C., Cheeseman, C. L., Wilkinson, D., & Clifton-Hadley, R. S. (2001b). A model of bovine tuberculosis in the badger *Meles meles*: the inclusion of cattle and the use of a live test. *Journal of Applied Ecology, 38*(3), 520-535.

Smith, G. C., McDonald, R. A., & Wilkinson, D. (2012). Comparing badger (*Meles meles*) management strategies for reducing tuberculosis incidence in cattle. *Plos One, 7*(6).

Smith, G. C., Richards, M. S., CliftonHadley, R. S., & Cheeseman, C. L. (1995). Modelling bovine tuberculosis in badgers in England: Preliminary results. *Mammalia, 59*(4), 639-650.

Smith, K., Acevedo-Whitehouse, K., & Pedersen, A. (2009). The role of infectious diseases in biological conservation. *Animal Conservation, 12*(1), 1-12.

Spiegelhalter, D. J., Best, N. G., Carlin, B. P., & Van Der Linde, A. (2002). Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society: Series B (Statistical Methodology), 64*(4), 583-639.

Sturtz, S., Ligges, U., & Gelman, A. (2005). R2WinBUGS: A package for running WinBUGS from R. *Journal of Statistical software, 12*(3), 1-16.

Svensson, E., Sinervo, B., & Comendant, T. (2001). Density-dependent competition and selection on immune function in genetic lizard morphs. *Proceedings of the National Academy of Sciences of the United States of America, 98*(22), 12561-12565.

Thorns, C. J., Morris, J. A., & Little, T. W. A. (1982). A spectrum of immune-responses and pathological conditions between certain animal species to experimental *Mycobacterium-bovis* infection. *British Journal of Experimental Pathology, 63*(5), 562-572.

Tomlinson, A. J., Chambers, M. A., Wilson, G. J., McDonald, R. A., & Delahay, R. J. (2013). Sex-related heterogeneity in the life-history correlates of *Mycobacterium bovis* infection in European badgers (*Meles meles*). *Transboundary and emerging diseases, 60*, 37-45.

Tuyttens, F. A. M., Macdonald, D. W., Rogers, L. M., Cheeseman, C. L., & Roddam, A. W. (2000). Comparative study on the consequences of culling badgers (*Meles meles*) on biometrics, population dynamics and movement. *Journal of Animal Ecology, 69*(4), 567-580.

Vaupel, J. W., Manton, K. G., & Stallard, E. (1979). Impact of heterogeneity in individual frailty on the dynamics of mortality. *Demography, 16*(3), 439-454.

Verhulst, S., Dieleman, S. J., & Parmentier, H. K. (1999). A tradeoff between immunocompetence and sexual ornamentation in domestic fowl. *Proceedings of the National Academy of Sciences of the United States of America, 96*(8), 4478-4481.

Vicente, J., Delahay, R. J., Walker, N. J., & Cheeseman, C. L. (2007). Social organization and movement influence the incidence of bovine tuberculosis in an undisturbed high-density badger *Meles meles* population. *Journal of Animal Ecology, 76*(2), 348-360.

Vyas, A., Kim, S.-K., Giacomini, N., Boothroyd, J. C., & Sapolsky, R. M. (2007). Behavioral changes induced by Toxoplasma infection of rodents are highly specific to aversion of cat odors. *Proceedings of the National Academy of Sciences of the United States of America, 104*(15), 6442-6447.

Weber, N., Carter, S. P., Dall, S. R. X., Delahay, R. J., McDonald, J. L., Bearhop, S., & McDonald, R. A. (2013). Badger social networks correlate with tuberculosis infection. *Current Biology, 23*(20), R915-R916.

Wendland, L. D., Wooding, J., White, C. L., Demcovitz, D., Littell, R., Berish, J. D., Ozgul, A., Oli, M. K., Klein, P. A., & Christman, M. C. (2010). Social behavior drives the dynamics of respiratory disease in threatened tortoises. *Ecology, 91*(5), 1257-1262.

White, G. C., & Burnham, K. P. (1999). Program MARK: survival estimation from populations of marked animals. *Bird Study, 46*, 120-139.

White, P. C. L., & Harris, S. (1995). Bovine tuberculosis in badger (*Meles-meles*) populations in southwest England - the use of a spatial stochastic simulation-model to understand the dynamics of the disease. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, 349*(1330), 391-413.

White, P. C. L., Lewis, A. J. G., & Harris, S. (1997). Fertility control as a means of controlling bovine tuberculosis in badger (*Meles meles*) populations in south-west England: predictions from a spatial stochastic simulation model. *Proceedings of the Royal Society B-Biological Sciences, 264*(1389), 1737-1747.

Wilkinson, D., Bennett, R., McFarlane, I., Rushton, S., Shirley, M., & Smith, G. C. (2009). Cost-benefit analysis model of badger (*Meles meles*) culling to reduce cattle herd tuberculosis breakdowns in Britain, with particular reference to badger perturbation. *Journal of Wildlife Diseases, 45*(4), 1062-1088.

Wilkinson, D., Smith, G. C., Delahay, R. J., & Cheeseman, C. L. (2004). A model of bovine tuberculosis in the badger *Meles meles*: an evaluation of different vaccination strategies. *Journal of Applied Ecology, 41*(3), 492-501.

Wilkinson, D., Smith, G. C., Delahay, R. J., Rogers, L. M., Cheeseman, C. L., & Clifton-Hadley, R. S. (2000). The effects of bovine tuberculosis (*Mycobacterium bovis*) on mortality in a badger (*Meles meles*) population in England. *Journal of Zoology, 250*, 389-395.

Woodroffe, R., Donnelly, C. A., Cox, D. R., Bourne, F. J., Cheeseman, C. L., Delahay, R. J., Gettinby, G., McInerney, J. P., & Morrison, W. I. (2006a). Effects of culling on badger *Meles meles* spatial organization: implications for the control of bovine tuberculosis. *Journal of Applied Ecology, 43*(1), 1-10.

Woodroffe, R., Donnelly, C. A., Cox, D. R., Gilks, P., Jenkins, H. E., Johnston, W. T., Le Fevre, A. M., Gourne, F. J., Cheeseman, C. L., Clifton-Hadley, R. S., Gettinby, G., Hewinson, R. G., McInerney, J. P., Mitchel, A. P., Morrison, W. I., & Watkins, G. H. (2009a). Bovine tuberculosis in cattle and badgers in localized culling areas. *Journal of Wildlife Diseases, 45*(1), 128-143.

Woodroffe, R., Donnelly, C. A., Jenkins, H. E., Johnston, W. T., Cox, D. R., Bourne, F. J., Cheeseman, C. L., Delahay, R. J., Clifton-Hadley, R. S., Gettinby, G., Gilks, P., Hewinson, R. G., McInerney, J. P., & Morrison, W. I. (2006b). Culling and cattle controls influence tuberculosis risk for badgers. *Proceedings of the National Academy of Sciences of the United States of America, 103*(40), 14713-14717.

Woodroffe, R., Donnelly, C. A., Wei, G., Cox, D., Bourne, F. J., Burke, T., Butlin, R. K., Cheeseman, C., Gettinby, G., & Gilks, P. (2009b). Social group size affects *Mycobacterium bovis* infection in European badgers (*Meles meles*). *Journal of Animal Ecology, 78*(4), 818-827.

Woodroffe, R., & Macdonald, D. W. (1995). Female female competition in European badgers *Meles meles* - effects on breeding success. *Journal of Animal Ecology, 64*(1), 12-20.

Woolhouse, M. E. J., Dye, C., Etard, J. F., Smith, T., Charlwood, J. D., Garnett, G. P., Hagan, P., Hii, J. L. K., Ndhlovu, P. D., Quinnell, R. J., Watts, C. H., Chandiwana, S. K., & Anderson, R. M. (1997). Heterogeneities in the transmission of infectious agents: Implications for the design of control programs. *Proceedings of the National Academy of Sciences of the United States of America, 94*(1), 338-342.

Zuk, M., & McKean, K. A. (1996). Sex differences in parasite infections: Patterns and processes. *International Journal for Parasitology, 26*(10), 1009-1023.

Zuk, M., & Stoehr, A. M. (2002). Immune defense and host life history. *American Naturalist, 160*, S9-S22.