

ECOLOGICAL INVESTIGATIONS OF MYCOPLASMAL UPPER RESPIRATORY  
TRACT DISEASE IN NATURAL POPULATIONS OF THREATENED GOPHER  
TORTOISES: INSIGHTS FROM POPULATION ECOLOGY, MATHEMATICAL  
EPIDEMIOLOGY, AND BEHAVIORAL ECOLOGY

By

CAROLINA PEREZ-HEYDRICH

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL  
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2010

© 2010 Carolina Perez-Heydrich

To my mom

## ACKNOWLEDGMENTS

This research was made possible through grants provided by the National Institutes of Health and the National Science Foundation (DEB-0224953), and the Morris Animal Foundation (D07ZO-404). Additionally, the Florida Fish and Wildlife Conservation Commission provided incredible logistical support for data used in this dissertation. I would like to thank my committee, Mary Brown, Dan Brown, Don Forrester, Madan Oli, and Ben Bolker, for providing me with invaluable knowledge, advice, and support that have guided me throughout my graduate research. I am grateful to my major advisor, Mary Brown, for giving me the opportunity to join her lab and pursue a research career. And, I am indebted to Ben Bolker and Madan Oli for introducing me to the practice of ecological modeling, and for their relentless support in guiding me through its application to wildlife disease. Much thanks is also owed to all of the field technicians and researchers whose countless hours of hard work in the Florida heat contributed to the data used throughout this dissertation: J. Wooding, K. Miller, M. Clark, V. Kasper, S. James, M. Moyer, E. Langan, B. Hentges, and J. Coan. I would like to especially thank Katie Jackson for being my right hand throughout my years of data collection, and for bringing much laughter into what otherwise would have been grueling fieldwork. I would also like to thank Lori Wendland for teaching me the ropes of gopher tortoise research, and coordinating the massive NSF project that provided me with much-needed logistical help. Immense gratitude is also afforded to LeAnn White, my office mate, for helping me build confidence as a budding scientist, and more importantly, for her friendship. Lastly, I'd like to thank my family, but especially John, Lola, and Niley, for putting up with me, and providing me with many distractions throughout this process.

## TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS.....	4
LIST OF TABLES.....	9
LIST OF FIGURES.....	10
ABSTRACT .....	12
CHAPTER	
1 INTRODUCTION .....	14
Overview of Disease in Wildlife Populations.....	14
Interdisciplinary Study of Wildlife Disease.....	17
Use of Analytical Models in Wildlife Disease Systems .....	18
Importance of Host Behavior in Wildlife Disease Systems .....	20
Overview of Mycoplasmosis .....	20
Host Susceptibility Factors .....	23
Environmental Factors.....	24
Pathogen-Specific Virulence Factors .....	25
Mycoplasmosis in Wildlife.....	25
Study System.....	28
Characterization of <i>M. agassizii</i> Infection Within Hosts .....	29
Similarities of Mycoplasmal URTD and Other Respiratory Mycoplasmoses ....	32
Diagnostic Tests.....	33
Factors Affecting Tortoise Immunity .....	34
Respiratory Pathogens of Gopher Tortoises .....	35
Overview of Mycoplasmal URTD in Natural Gopher Tortoise Populations .....	38
Objectives.....	40
2 LONG-TERM IMPACTS OF RECURRING DISEASE ON POPULATION DYNAMICS AND PERSISTENCE OF A LONG-LIVED WILDLIFE HOST .....	43
Introduction .....	44
Methods.....	47
Study System .....	47
Model Formulation.....	49
Model assumptions .....	49
Definition of population states (Normal/Outbreak) .....	50
Parameter Estimation.....	52
Construction of Population Projection and Markovian Transition Matrices .....	54
Calculation of Stochastic Population Growth Rate ( $\lambda_S$ ) .....	56
Elasticity Analysis .....	57
Estimation of Quasi-Extinction Parameters .....	58

Results.....	59
Baseline Model.....	59
Outbreak Model.....	60
Discussion .....	63
<b>3 APPLICATION OF FORCE OF INFECTION MODELS TO ASSESS HYPOTHESES REGARDING STAGE-SPECIFIC DISEASE PROCESSES WITHIN A THREATENED WILDLIFE HOST POPULATION .....</b>	<b>79</b>
Introduction .....	80
Methods.....	84
Model Formulation.....	84
Assessment of Biological Realism.....	87
Data Collection .....	91
Results.....	93
Discussion .....	95
<b>4 THE EFFECT OF INDIVIDUAL MOVEMENT PATTERNS ON THE SUSCEPTIBILITY TO PATHOGEN EXPOSURE IN NATURAL POPULATIONS OF A THREATENED WILDLIFE HOST .....</b>	<b>108</b>
Introduction .....	109
Methods.....	112
Study Sites .....	112
Data Collection .....	113
Statistical Analysis.....	116
Results.....	119
Discussion .....	124
The Role of Mating System on Infection Dynamics .....	125
The Problem of Reverse Causation .....	127
Future Directions .....	130
<b>5 CONCLUSION.....</b>	<b>142</b>
Overview of Results.....	142
Caveats.....	144
Impacts of Recurring Outbreaks.....	145
A focus on mortality rather than morbidity.....	145
Long-term projections based on short-term data .....	145
Stage-Specific Disease Processes.....	146
Relationship Between Movement-Associated Behaviors and Exposure .....	147
Using movement indices as an indirect measure of contact .....	147
Reverse causation .....	148
Research Contributions and Gaps in Current Understanding of URTD Ecology ..	148
Exploring the Contributory Role of Other Factors on Disease Severity .....	150
Secondary infections.....	151
Environmental stress .....	153

Exploring the Relationship Between Morbidity and Impaired Biological Function .....	158
Impaired foraging abilities through damaged olfactory mucosa .....	159
Energetic costs of infection .....	159
Indirect effects of chronic disease on reduced survival.....	161
Exploring the Population-Level Effects of URTD .....	163
URTD-mediated die-offs .....	163
URTD-associated morbidity effects.....	165
Summary .....	167

## APPENDIX

A METHODS FOR THE ESTIMATION OF DEMOGRAPHIC PARAMETERS.....	169
Capture and Sampling of Tortoises .....	169
Parameter Estimation .....	170
Stage-Specific Survival Probabilities ( $\sigma_H$ , $\sigma_P$ , $\sigma_R$ ) .....	170
Pre-Reproductive Growth Probability ( $\gamma$ ) .....	171
Fecundity (m) .....	172
B A SUMMARY ESTIMATE OF GOPHER TORTOISE HATCHLING SURVIVAL FROM A META-ANALYSIS USING FIELD DATA AND PUBLISHED ESTIMATES .....	178
Background Information.....	179
Methods.....	182
Data Collection.....	182
Study site .....	182
Location of nests and documentation of hatching success .....	182
Hatchling collection and tracking.....	183
Statistical Analysis.....	184
Definition of event types.....	184
Hatchling survival.....	185
Meta-analysis.....	187
Results.....	188
Nest Location and Hatching Success.....	188
Hatchling Predation .....	188
Hatchling Survival.....	189
Meta-Analysis.....	190
Discussion .....	191
C METHODS FOR THE CALCULATION OF STOCHASTIC GROWTH RATES USING THE MATRIX SIMULATION APPROACH.....	203
D METHODS FOR THE CALCULATION OF STOCHASTIC AND DETERMINISTIC ELASTICITIES .....	204

E	COMPLETE LISTING OF ESTIMATED STOCHASTIC GROWTH RATES AND MEDIAN QUASI-EXTINCTION TIMES ACROSS ALL OUTBREAK SCENARIOS.....	207
F	TRANSLATION OF MODEL PARAMETER ESTIMATES FROM CARAPACE GROWTH RATES ( $MM^{-1}$ ) TO ANNUAL RATES ( $YR^{-1}$ ) .....	210
	LIST OF REFERENCES .....	211
	BIOGRAPHICAL SKETCH.....	233

## LIST OF TABLES

<u>Table</u>		<u>page</u>
2-1	Estimates of demographic parameters used for the parameterization of a baseline population projection matrix .....	71
3-1	Relative fits of models ranked according to AICc values.....	102
3-2	Parameter estimates and 95% confidence intervals from models best supported by the data.....	103
4-1	Summary statistics for movement-associated predictor variables .....	133
4-2	Results from the principal components analysis of movement variables.....	134
4-3	Maximum likelihood parameter estimates and odds ratios with 95% confidence intervals from the full logistic regression model.....	135
A-1	Stage-specific apparent survival estimates from a 4-year mark-recapture study of gopher tortoises from historically URTD-free populations.....	174
B-1	Estimated coefficients from the logistic regression model .....	194
B-2	Selection of a parametric model to provide continuous estimates of annual gopher tortoise hatchling survival .....	194
B-3	Group-specific annual survival probabilities, median survival time in days, and annual per-capita mortality rates for hatchlings. ....	195
E-1	Stochastic growth rates and 95% confidence intervals from 54 outbreak scenarios using Tuljapurkar’s small noise approximation.....	207
E-2	Stochastic growth rates and 95% confidence intervals from 54 outbreak scenarios using a matrix simulation approach.....	208
E-3	Median quasi-extinction times and ranges from 54 outbreak scenarios. ....	209

## LIST OF FIGURES

<u>Figure</u>	<u>page</u>
2-1	Conceptual models of (A) demographic processes, and (B) Markovian transitions for outbreak processes..... 72
2-2	Outbreak scenarios simulated from two-state Markov chain models..... 73
2-3	Contour plots of stochastic growth rates ( $\lambda_s$ ), calculated using Tuljapurkar's small noise approximation for autocorrelated environments..... 74
2-4	Elasticity of $\lambda_s$ to changes in outbreak- and disease-associated parameters ( $f$ , $\rho$ , $\mu$ , and $\phi$ ) calculated using the vec-permutation matrix approach..... 75
2-5	Mean cumulative distribution functions for quasi-extinction times ..... 76
2-6	Contour plots of median quasi-extinction times ( $T_q$ ) ..... 77
2-7	Relative effects of autocorrelation ( $W_2$ ) over interannual variability ( $W_1$ ) obtained through Tuljapurkar's small noise approximation..... 78
3-1	Conceptual model for metapopulation dynamics used to address the biological realism of best-fit models..... 104
3-2	Barplot of annual captures at the CF site by year..... 105
3-3	Age-seroprevalence curves resulting from force of infection models..... 106
3-4	Cumulative distributions of quasi-extinction probabilities for metapopulation models parameterized by the best-fitting force of infection models. .... 107
4-1	Attachment of a fluorescent powder dye pack to an adult tortoise..... 136
4-2	Sample map of individual movement patterns..... 137
4-3	Mosaic plot of captured tortoises at the three study sites ..... 138
4-4	Violin plots of first principal component scores ..... 139
4-5	Scatter plot of first principal scores in relation to <i>M. agassizii</i> infection. .... 140
4-6	Example of trails of multiple males overlapping with trails of a single female... 141
A-1	Location of study populations used for data collection for this dissertation. .... 175
A-2	The von Bertalanffy growth curve of gopher tortoises from historically URTD-free populations ..... 176

A-3	Forest plots of (A) nesting and (B) hatching success probability using results from published literature .....	177
B-1	Locations of nests in 2004 (triangles), 2005 (squares), and 2006 (pentagons) from which hatchlings were taken for this telemetry study.....	196
B-2	Construction of nest protection cages .....	197
B-3	Number of hatchlings included in the telemetry study each year stratified by month of hatching and month of release. ....	198
B-4	Observed predation of a hatchling gopher tortoise in 2004 by an Eastern Coachwhip ( <i>Masticophis flagellum flagellum</i> ).....	199
B-5	Temporal patterns of hatchling mortality.....	200
B-6	Survival curves for gopher tortoise hatchlings generated from field data .....	201
B-7	Forest plot of hatchling survival using results from published literature along with results from field data.....	202
F-1	Estimation of annual stage-specific growth in carapace length (mm) through the fitting of a von Bertalanffy growth model.....	210

Abstract of Dissertation Presented to the Graduate School  
of the University of Florida in Partial Fulfillment of the  
Requirements for the Degree of Doctor of Philosophy

ECOLOGICAL INVESTIGATIONS OF MYCOPLASMAL UPPER RESPIRATORY  
TRACT DISEASE IN NATURAL POPULATIONS OF THREATENED GOPHER  
TORTOISES: INSIGHTS FROM POPULATION ECOLOGY, MATHEMATICAL  
EPIDEMIOLOGY, AND BEHAVIORAL ECOLOGY

By

Carolina Perez-Heydrich

May 2010

Chair: Mary Brown  
Major: Veterinary Medical Sciences

Disease is a major conservation concern for imperiled wildlife populations. While a comprehensive understanding of wildlife disease in natural systems requires cross-disciplinary insights (i.e., microbiology, veterinary medicine, epidemiology, population ecology, and behavioral ecology, among others), I focus here on ecological and epidemiological methods. Using mycoplasmal upper respiratory tract disease (URTD) in natural gopher tortoise (*Gopherus polyphemus*) populations as a study system, the specific objectives of this dissertation were to (1) elucidate potential long-term impacts of URTD on host population dynamics, (2) identify and quantify mechanisms driving observed stage-specific seroprevalence patterns in exposed populations, and (3) describe how movement-associated behavior can contribute to infection susceptibility at the individual-level. Specifically, through the implementation of commonly used matrix models from population ecology, I projected the potential effects of recurring mycoplasmal URTD epizootics on the long-term dynamics of gopher tortoise populations. I solely focused on the impacts of URTD-mediated mortality events, although morbidity effects can also be important in other chronic respiratory

mycoplasmal infections. I determined that host demographic factors (i.e., survival, growth, and fecundity) were more important than disease-associated factors (i.e., force of infection, disease-induced mortality, outbreak duration, and outbreak frequency) on the long-term dynamics of exposed host populations, and that the largest impacts of disease were associated with the overall frequency of outbreaks and disease-induced mortality rates. Additionally, by fitting force of infection models to age-seroprevalence data, I determined that the most biologically plausible explanation for observed seroprevalence patterns involved stage-specific force of infection rates and negligible disease-induced mortality. This is consistent with other chronic respiratory mycoplasmal infections that are characterized by low mortality but high morbidity. Lastly, through the use of regression analyses and observed individual movement patterns, I attempted to quantify the risk of infection associated with individual behaviors. Movement-associated behavior, along with sex and site classification, was found to be significantly associated with individual infection status; however, this association was most likely a retro-causal one, whereby infected tortoises were more likely to exhibit foraging and basking behavior than mate-seeking behavior. Overall, the three studies comprising this dissertation provided insights into the epizootiology of mycoplasmal URTD in natural gopher tortoise populations, and its potential impacts on long-term host population dynamics.

## CHAPTER 1 INTRODUCTION

### **Overview of Disease in Wildlife Populations**

Wildlife epizootiology has emerged as a “crisis discipline,” integrating ecology, epidemiology, and pathobiology, among other fields, to investigate the effects of disease on host population processes such as long-term growth and persistence (Ostfeld et al., 2002). Managing wildlife populations within the context of disease has become increasingly important in light of current population declines resulting from anthropogenic activities such as exploitation, exotic species introductions, and habitat encroachment (McCallum and Dobson, 2002). Although pathogens alone are rarely the primary cause of species extinctions (de Castro and Bolker, 2005; Smith et al., 2006a), interactions with other factors such as habitat degradation or climate change can stress hosts and increase susceptibility for disease-mediated population declines (Lafferty and Holt, 2003).

Disease, therefore, is of major conservation concern for already-imperiled wildlife populations, which are more vulnerable to stochastic events that could affect the survival and/or fecundity of individuals within populations (Melbourne and Hastings, 2008; Smith et al., 2009). For a comprehensive review on pathogen-mediated host species extinctions refer to Smith et al. (2006a). Population-level impacts of disease may be mediated through either mortality or morbidity-associated effects. For example, through disease-induced mortality an infectious tumor disease has resulted in significant population declines (>60%) of the Tasmanian devil throughout its range and led to its listing as a threatened species (Lachish et al., 2007). Chytridiomycosis has led to significant population declines of amphibians worldwide (Cunningham et al. 2008). The

transmission of Ebola virus within and between critically endangered Lowland gorilla social groups has resulted in the disproportionate decline of females and juveniles, and threatened the resilience of exposed populations to local extinctions (Caillaud et al., 2006). Regarding disease-induced morbidity, bat white-nose syndrome has most recently been associated with drastic population declines (>75%) in the northeastern U.S., and is expected to have broad ecological impacts due to the critical functions that bats perform in pollination, seed dispersal, and insect predation (Blehert et al., 2009). Sarcoptic mange has mediated significant (>90%) declines of threatened and endangered wild mammals globally through indirect effects such as increasing host susceptibility to secondary infections, starvation, and hypothermia (Bornstein et al., 2001; Pence and Ueckermann, 2002).

### **Disease as a Context-Dependent Phenomenon**

Disease is a context-dependent phenomenon in which tissue damage results from interactions between the host and pathogen. For example, immune responses stimulated by interactions with a pathogen can lead to tissue damage within a host through inflammatory processes associated with pathogen clearance. In this sense, pathogenesis and virulence can be linked to host responses as well as pathogen activity (Casadevall and Pirofski, 2001; Casadevall and Pirofski, 2003b). Host responses, in turn, are influenced by a variety of factors including: genetic background, age, and gender. For instance, differences in the clinical severity of epizootic hemorrhagic disease are directly associated with differences in innate resistance between subspecies of white-tailed deer (Gaydos et al., 2002). Therefore, even within the same

species, bluetongue virus is not consistently virulent, and disease occurs only within the context of a host without the proper innate resistance.

Environmental factors can also influence the physiological and immunological conditions of hosts, and further contribute to the context-dependent nature of disease (Lafferty and Holt, 2003; Padgett and Glaser, 2003). Environmental conditions have been found to hasten immunosenescence of Soay sheep to parasite infections. In this system, environmental factors weaken hosts through time, and individuals exposed to the highest levels of environmental stress throughout their lifetimes also demonstrate the largest increases in parasite burden with age (Hayward et al., 2009). Weather, habitat quality, host population density, predator density, and the presence of other potential pathogens, among other factors can all serve as additional stressors to adversely impact the immunocompetence of hosts. Disease, therefore, is the combination of host, pathogen, and environmental factors that together influence the susceptibility and severity of host tissue damage, or abnormal function.

Several reviews have commented on the role of pathogens within host wildlife populations (May, 1988; Scott, 1988; Nettles, 1992; Grenfell and Gulland, 1995; McCallum and Dobson, 1995; Daszak et al., 2000; Cleveland et al., 2002; Gillin et al., 2002; McCallum and Dobson, 2002; Ostfeld et al., 2002; Tompkins et al., 2002; Smith et al., 2006a; Smith et al., 2009), and central to all of these was the notion of interacting phenomena driving the emergence and proliferation of disease within wildlife populations. Wildlife disease can therefore be viewed as a context-dependent phenomenon that is defined by interactions among host, pathogen, and environmental

factors (Wobeser, 2006). Indeed, this is not a concept unique to wildlife disease, but is common to infectious diseases in general.

### **Interdisciplinary Study of Wildlife Disease**

Because of the complex interplay among the pathogen, host, and environment, epizootiological studies can best reflect this complex network of interactions through a cross-disciplinary approach. Personnel within the fields of veterinary medicine and microbiology provide the first insights into the characterization of an epizootic through a description of disease etiology at the individual host level. Typically, this involves the identification of the causative agent, along with a description of host tissue damage associated with infection. Diagnostic tests may also be developed through which future disease detection and surveillance is made possible within natural populations. Furthermore, experimental transmission studies may define the pathogenicity associated with a given disease agent, and how it may translate into observable clinical signs.

Building on knowledge gained from pathological findings and diagnostic assay development, individuals within the fields of epidemiology and ecology can provide further insights into natural transmission processes, risk factors, and population-level effects. Observing wildlife hosts in their natural environments allows for a more realistic depiction of disease processes that would not be available through controlled experimental transmission studies alone. By incorporating knowledge of the ecology of host species, a more comprehensive understanding of disease within natural systems can be attained. Specifically, observational studies aimed at describing social structure and mixing patterns of individuals within an exposed population can help define contact network structures and quantify the potential rate of spread of an infectious pathogen

throughout exposed populations. Furthermore, by taking into account the demographic dynamics of host populations, population ecologists can project how disease may impact the long-term viability of host populations, and thus provide a more applied perspective of how disease would fit into the conservation goals for a given species.

Given that a comprehensive understanding of wildlife disease requires insights across various disciplines (i.e., microbiology, veterinary medicine, epidemiology, population ecology, and behavioral ecology, among others), with this dissertation I have focused on evaluating the impacts of disease from the perspective of the host population using ecological and epidemiological approaches. Namely, through the use of mathematical epidemiology, population ecology, and behavioral ecology, I have aimed to provide insights into disease processes and their potential long-term impacts on threatened wildlife host populations.

### **Use of Analytical Models in Wildlife Disease Systems**

Mathematical epidemiology has been useful in describing the ecology of several wildlife diseases, including rabies and phocine distemper virus (Swinton et al., 1998; Haydon et al., 2002; Russell et al., 2005), and has greatly contributed to the control of epidemics and epizootics such as SARS, malaria, and foot-and-mouth disease (Kao, 2002; McKenzie and Samba, 2004; Matthews and Woolhouse, 2005). Mathematical models are especially useful tools for describing the potential outcomes of events under hypothetical scenarios when data is incomplete, as is common for wildlife disease studies. For example, Markov chain models were used to assess the potential impacts of chronic carriers on the transmission of contagious bovine pleuropneumonia in cattle across a range of values describing the infectiousness of latent hosts, an unknown parameter (Lesnoff et al., 2004). A host-pathogen metapopulation model helped to

elucidate potential factors affecting disease dynamics and long-term persistence of coral exposed to white plague type II using a wide range of plausible values for unknown parameters such as transmission probability and disease-associated local extinction rates (Sokolow et al., 2009). Additionally, disease dynamics can also be incorporated into population models to project the long-term impacts of disease on the viability of host populations (Haydon et al., 2002; Briggs et al., 2005). Through heuristic explorations, models can help identify epizootiological parameters that can greatly influence host population and/or pathogen transmission dynamics, and subsequently drive future data collection and research efforts in order to attain a more thorough understanding of the role of disease within a host wildlife population.

Researchers have also used models to elucidate cryptic disease processes that are difficult to measure in wild populations, such as transmission dynamics. For example, by fitting models that represented competing hypotheses on routes of prion transmission, indirect environmental transmission was found to be a relatively more important form of transmission over direct infectious contacts in mule deer populations (Miller et al., 2006). Likewise, an assessment of several potential modes of *Mycobacterium bovis* infection in feral ferrets found that ingestion of infected matter was the most likely means of acquiring infection (Caley and Hone, 2002). Besides providing an understanding of underlying mechanisms of disease dynamics, these models can also serve to provide estimates of cryptic processes such as disease-induced mortality and force of infection rates, which are difficult to directly estimate in natural populations (Heisey et al., 2006; Gauthier et al., 2008).

## **Importance of Host Behavior in Wildlife Disease Systems**

In addition to the formulation and assessment of models by population ecologists and epidemiologists, documentation of individual host behavior and social structure can also shed light into contact structures and potential host-mediated mechanisms driving disease proliferation across exposed populations. For example, seasonal changes in host movement patterns were found to be associated with increases in the prevalence of mycoplasmal conjunctivitis in house finches (Hosseini et al., 2006). Another study, which focused on the movements of individual skunks, identified potential locations that most likely facilitated both inter- and intra-specific rabies transmission (Weissinger et al., 2009). Moreover, based on the observed movement behavior of white-tailed deer, researchers proposed the harvesting of yearling males as a control measure for chronic wasting disease due to higher dispersal rates that could advance transmission across a habitat (Skuldt et al., 2008). Studies on the behavioral ecology of host species, therefore, can provide valuable information on host processes that contribute to disease spread across populations.

## **Overview of Mycoplasmosis**

The focus of this dissertation pertains to a wildlife disease of mycoplasmal origin. General characteristics of mycoplasmoses include chronic and slowly progressive disease exacerbated by host immune responses, intermittent clinical signs, and the existence of a clinically silent carrier state (Razin et al., 1998; Minion, 2002). Most mycoplasmas that have been isolated from animals are considered commensals, while those that do cause disease have variable clinical presentations. Lesions, however, are more similar and are characterized primarily by lymphoid hyperplasia and chronic inflammation, often accompanied by loss of normal epithelial architecture.

Mycoplasmoses in animal hosts are generally chronic diseases in which morbidity is common, but mortality is rare. Infected animals that are asymptomatic serve as silent carriers, and promote the persistence of mycoplasmal disease within exposed populations (Frey, 2002). Furthermore, variability in the severity of mycoplasmal disease within hosts can be influenced by a variety of factors, including host factors (e.g., genetic background, gender, age), environmental factors (e.g., host population density, toxin exposure), and strain-specific pathogenicity factors (Simecka et al., 1992).

### **Host-Mediated Pathogenesis**

Mycoplasma infections initiate through adherence to ciliated mucosal epithelial surfaces of susceptible hosts (Razin et al., 1998). As with any pathogen, colonization of host surfaces elicits an innate immune response that begins with inflammation. Specifically, chemical factors released by injured cells attract phagocytes, such as macrophages and neutrophils (or heterophils in reptiles), which then initiate the movement of leukocytes and lymphocytes into the site of infection. As part of the non-specific innate immune response to microbial infection, the complement system is stimulated to trigger a cascade of events that will help clear infection. For example, the complement cascade triggers the recruitment of inflammatory cells, coats (or opsonizes) pathogen surfaces, leads to cytolysis of infected cells, and gets rid of antibody-antigen complexes (which neutralize the pathogen of interest). The innate immune response then triggers the adaptive immune response through the presentation of specific antigens by antigen-presenting cells, such as macrophages and dendritic cells, to T-cells. T-cells then stimulate B-cells to produce specific antibodies to the pathogen at hand. Following the resolution of this initial infection event, activated T- and B-cells

(which are committed to recognizing specific antigens of this pathogen) form circulating memory cells that are reactivated upon future encounters with these specific antigens (Janeway, 2001). Although specific immune responses elicited by mycoplasma invasion are necessary for host resistance and protection against infection, these reactions also contribute substantially to lesion development and disease progression within hosts (Razin et al., 1998).

Mycoplasmas have the capacity to manipulate the immune response and contribute to host-mediated disease exacerbation. Specifically, mycoplasmas can modulate the expression of host cytokines that can lead to non-specific polyclonal activation of lymphocytes, and consequently increased tissue damage. Through the modulation of host cellular immune responses (i.e., cytokine and chemokine expression), invading mycoplasmas are responsible for disease pathogenesis. For instance, *Mycoplasma pulmonis* induces T cell-mediated expression of cytokines within murine hosts resulting in severe pulmonary tissue damage (Cartner et al., 1998). Additionally, severe tracheal lesions within avian hosts have been attributed to up-regulation of cytokine mRNA by *Mycoplasma gallisepticum* (Mohammed et al., 2007). *Mycoplasma alligatoris* can induce host cell death by the activation of a host-mediated signal transduction cascade resulting in apoptosis of host cells by the expression of sialidase and hyaluronidase enzymes (Hunt and Brown, 2007).

The regional immune response associated with mycoplasma infection can also contribute to pathogenesis within hosts. Many mycoplasma infections can be characterized by focal accumulations of monocytes, such as lymphocytes and macrophages, and both antibody and T-cell responses can be associated with host-

mediated tissue damage (Jones and Simecka, 2003). For example, the antibody-induced formation of immune complexes can directly result in tissue damage by stimulating a neutrophilic inflammatory response and subsequent vasculitis (Simecka, 2005). The immune response, therefore, is a critical component of mycoplasma pathogenesis within hosts and is a major determining factor in the lesion severity (Simecka, 2003).

In general, the typical lesions associated with mycoplasma infection often include: ciliostasis, destruction of mucosal epithelial structure, infiltration of neutrophils and other inflammatory cells into the respiratory tract, mucosal hyperplasia, and a predisposition to secondary infections. Many respiratory mycoplasma infections may also disseminate and cause disease in areas beyond the respiratory tract. Additionally, several factors can contribute to the severity of mycoplasmoses within hosts. These include: host genetic background, host gender, host age, environmental factors, and strain-specific virulence factors.

### **Host Susceptibility Factors**

Genetic background, gender, and age have been shown to influence the severity of mycoplasmal disease within hosts (Simecka et al., 1992). The influence of genetic differences among hosts on mycoplasmosis has been observed through experimental transmission studies in a mouse model of *Mycoplasma pneumoniae* where the severity of lung lesions and disease was markedly strain-specific (Chu et al., 2006). The effect of host gender on disease susceptibility has also been noted in several studies. For example, in experimental transmission studies of *Mycoplasma pulmonis* in mice, males suffered from more severe respiratory disease than females. Interestingly, in one particular mouse strain, females were more likely to develop a chronic wasting

syndrome associated with high morbidity but low mortality, while males displayed a fatal shock-like syndrome in which mortality occurred rapidly after infection (Yancey et al., 2001). Likewise, in house finches (*Carpodacus mexicanus*) infected with *Mycoplasma gallisepticum*, males suffered from disproportionately more severe disease than females (Nolan et al., 1998). In addition to host genetic background and gender, age has also been determined to contribute to the susceptibility and severity of mycoplasmosis within hosts. Mycoplasmal pneumonia is more severe among elderly individuals than individuals in younger age groups (Mittermayer, 1998). In American alligators, increased mortality due to mycoplasmal disease was positively associated with host age (Clippinger et al., 2000). Interestingly, however, in Spanish Ibex (*Capra pyrenaica*) young females suffered from more severe mycoplasmosis than adults or males (Verbisck-Bucker et al., 2008)

### **Environmental Factors**

Environmental conditions can also influence the progression of mycoplasmal disease. Pathogenesis of murine respiratory mycoplasmosis has been linked to environmental exposure to ammonia and nitrogen dioxide (Broderick et al., 1976; Parker et al., 1989). Moreover, host density can increase susceptibility to mycoplasma infection in exposed populations, as has been noted for enzootic pneumonia caused by *Mycoplasma hyopneumonia* in swine populations (Zellweger et al., 2004), and mycoplasmal conjunctivitis in house finch populations (Hochachka and Dhondt, 2000), among others.

Synergistic interactions between mycoplasmas and other pathogens commonly exacerbate respiratory disease in hosts. For example, interactions between Sendai virus and *Mycoplasma pulmonis* lead to more severe lesions within the respiratory tract

of mice (Saito et al., 1981). Enzootic pneumonia is a disease complex of swine that involves interactions between *Mycoplasma hyopneumoniae* and other viruses (Iglesias Sahagun and Trujano Castillo, 2000). Additionally, mixed mycoplasma infections and co-infection with viruses can increase the severity of respiratory disease in poultry (Simecka et al., 1992).

### **Pathogen-Specific Virulence Factors**

In addition to host-specific and environmental factors, virulence associated with mycoplasmosis is also influenced by pathogen-specific factors. For example, two closely related mycoplasma species, *Mycoplasma alligatoris* and *Mycoplasma crocodyli*, differ greatly in their ability to cause disease in alligators due to differences in sialidase activity between the two species (Brown et al., 2004b). Within the same mycoplasma species, different strains of *Mycoplasma pulmonis* result in varying degrees of virulence within hosts (Davidson et al., 1988). Among *Mycoplasma synoviae* strains, differential sialidase activity was associated with strain-specific variability in virulence within avian hosts (May et al., 2007). Moreover, of roughly 36 different strains of *Mycoplasma conjunctivae* isolated from free-ranging alpine chamois (*Rupicapra rupicapra rupicapra*), only a few have been associated with outbreaks of infectious keratoconjunctivitis in natural host populations (Zimmermann et al., 2008). Therefore, not only do differences between mycoplasma species affect the severity of disease within hosts, but differences among strains within the same mycoplasma species can also lead to variable disease outcomes within hosts.

### **Mycoplasmosis in Wildlife**

Mycoplasma infections have been documented across a wide range of wildlife hosts (Brown, 2002; Brown et al., 2005). Mycoplasma-associated morbidity is more

commonly observed in wildlife populations than high rates of disease-induced mortality. Although generally a rare occurrence, mycoplasma-associated mortality has been observed in a few wildlife hosts. For example, an acute epizootic of mycoplasmosis in American alligators (*Alligator mississippiensis*), associated with pneumonia, pericarditis, and arthritis in infected hosts, was characterized by rapid mortality following infection, most frequently before the development of an immune response from the hosts (Clippinger et al., 2000). Factors such as age, population density, and mycoplasma virulence (i.e. sialidase and hyaluronidase activity), are thought to contribute to the severity of disease within infected alligators (Brown et al., 2001a; Brown et al., 2004b; Hunt and Brown, 2007). Another example of severe wildlife mycoplasmosis is contagious caprine pleuropneumonia (CCPP) caused by *Mycoplasma capricolum capripneumoniae*, which leads to acute respiratory disease and high mortality rates in wild ungulates (Arif et al., 2007). Additionally, contagious bovine pleuropneumonia caused by *Mycoplasma mycoides mycoides* can be a fatal disease of wild ungulates and is of grave economic importance for domestic livestock (Kock et al., 2002).

In some wildlife hosts, however, there is difficulty in delineating whether mycoplasmosis is associated with increased host mortality, due to the chronic nature of this disease. Moreover, morbidity associated with mycoplasmal disease may increase host susceptibility to predation, and indirectly contribute to increased mortality rates of infected individuals. For instance, in bighorn sheep with infectious keratoconjunctivitis caused by *Mycoplasma conjunctivae*, mortality of diseased individuals most commonly occurred as a direct consequence of predation or starvation, rather than disease pathogenesis (Jansen et al., 2007). Mycoplasmal disease has also been a concern for

desert tortoise (*Gopherus agassizii*) populations in the southwestern U.S. (Brown et al., 1999a), with population declines associated with pathogen introduction and high seroprevalence, although the direct impact on host populations remains undefined (Brown et al., 2002).

Probably the best-characterized wildlife mycoplasmosis has been mycoplasmal conjunctivitis in passeriforms, particularly house finches (Ley et al., 1996; Dhondt et al., 2005). *Mycoplasma gallisepticum* infection causes conjunctivitis in several passerine species including the American goldfinch (*Carduelis tristis*), house sparrow (*Passer domesticus*), and house finch, and varies in severity across host species (Dhondt et al., 2008). This wildlife disease system provides an excellent example demonstrating how research across several disciplines (i.e., veterinary medicine, microbiology, ecology, and mathematical epidemiology) can contribute to a more comprehensive understanding of disease within naturally occurring populations. Experimental transmission studies determined that, under controlled settings, *M. gallisepticum* was associated with high morbidity and low mortality (Kollias et al., 2004). However, in natural populations, seasonal population declines during the winter months have been attributed to seasonal increases in aggregative patterns (Hosseini et al., 2004) and attenuated host responses due to physiological stress caused by mating behaviors and/or weather conditions (Lindstrom et al., 2005). Host factors influencing the susceptibility and severity of mycoplasmal conjunctivitis in house finches include genetic background (Hawley et al., 2005), social status (Hawley et al., 2007b), and sex (Nolan et al., 1998). Additionally, re-exposure of infected finches to *M. gallisepticum*

results in more rapid and severe development of conjunctivitis (Sydenstricker et al., 2006).

In addition to defining host and environmental factors that influence disease progression within house finches, population-level impacts and disease dynamics associated with mycoplasmal conjunctivitis have been evaluated through the use of ecological and epidemiological models. Particularly, mechanisms driving seasonal epizootics (Hosseini et al., 2004), impacts on host demography (Hochachka and Dhondt, 2000) and population dynamics (Hurtado, 2008), epidemiological parameters (Faustino et al., 2004), and risk factors associated with infection (Altizer et al., 2004a) have been elucidated using quantitative ecological and epidemiological methodology. Furthermore, research regarding the social structure and behavior of naturally occurring house finches has provided insights into factors influencing host susceptibility to conjunctivitis, and pathogen transmission (Hawley et al., 2007a; Hawley et al., 2007b).

### **Study System**

Mycoplasmal upper respiratory tract disease (URTD) within gopher tortoise (*Gopherus polyphemus*) populations provides a good study system to apply and interdisciplinary framework to assess the dynamics and population-level impacts of disease within a species of conservation concern. Previous research on URTD identified an etiologic agent (Brown et al., 1999b), described the pathological lesions associated with infection (Klein et al., 1995; McLaughlin, 1997; McLaughlin et al., 2000), and developed diagnostic assays to detect exposure (Schumacher et al., 1993; Wendland et al., 2007; Brown et al., 2008) and active infection (Brown et al., 1995) in live animals.

## Characterization of *M. agassizii* Infection Within Hosts

*Mycoplasma agassizii* infection in both gopher and desert tortoises involves (1) adherence to and colonization of the upper respiratory tract, (2) intermittent expression of clinical disease, with nasal discharge as the most common clinical manifestation, (3) development of a humoral immune response 6-8 wks after initial exposure to an infectious dose, (4) tissue damage to the ciliated respiratory and olfactory epithelium that persists even during clinically silent infection periods, and (5) subsequent transition to a clinically silent, chronic disease state in which recrudescence of clinical disease and active shedding of mycoplasma occurs sporadically (Brown et al., 1999b; Wendland, 2007). Furthermore, experimental studies demonstrated that transmission of *M. agassizii* occurred via direct contact between tortoises, confirmed the intermittent shedding of the microbe, and suggested that vertical and/or environmental transmission was unlikely (McLaughlin, 1997; Schumacher et al., 1999).

Nasal discharge, ranging in severity from serous to mucopurulent, is the most significant clinical expression of mycoplasmal URTD (Schumacher et al., 1997; Wendland, 2007). Additionally, palpebral edema, conjunctivitis, and ocular discharge are common clinical signs of URTD in tortoises (Brown et al., 1999b). Clinical disease, however, is a cryptic occurrence in natural populations. For instance, only 12% of tortoises from populations with high *M. agassizii* seroprevalence exhibited a nasal discharge during annual sampling periods (Wendland, 2007), thus illustrating the intermittent nature of clinical disease expression in tortoises with chronic URTD. This is not surprising since even experimentally infected tortoises showed variation in clinical disease expression (Brown et al., 1999b; McLaughlin et al., 2000)

*Mycoplasma agassizii* infections, like most mycoplasmal respiratory infections, are rarely cleared by the host (Brown et al., 1999a; Brown et al., 2005; Wendland, 2007); however tortoises may remain asymptomatic, or free of clinical signs, for long periods of time during which mycoplasma abundances within hosts may decline beyond levels detectable by diagnostic assays (Wendland, 2007). Even in experimentally infected tortoises, *M. agassizii* may not be detected by PCR or culture in animals that do not present with a nasal discharge (Wendland, 2007). The pathogen can be isolated at necropsy, so the inability to detect *M. agassizii* in lavages from clinically silent tortoises is most likely due to a decreased microbial load and/or inability to obtain an adequate nasal lavage. The presence of specific antibodies to *M. agassizii* is highly correlated with clinical signs of URTD, histological lesions, and detection of the pathogen (Schumacher et al., 1997; McLaughlin et al., 2000; Wendland, 2007).

Tortoises suffering from mycoplasmal URTD may have an increased susceptibility to secondary infections, which can serve to further debilitate chronically-infected hosts (Jacobson et al., 1991; Jacobson et al., 1995; McLaughlin et al., 2000; Brown et al., 2005; Ordorica et al., 2008). Other microbes, including viral (i.e., herpesvirus, ranavirus, and iridovirus), and fungal pathogens have been isolated from the respiratory tracts of tortoises exhibiting clinical signs overlapping with those of URTD (Origi and Jacobson, 2000; Johnson et al., 2006; Ordorica et al., 2008); however, only experimental transmission studies using *M. agassizii* and *M. testudineum* have fulfilled Koch's postulates for URTD causation in gopher tortoises (Brown et al., 1999b; Brown et al., 2004a).

The destruction of normal respiratory epithelial surfaces, as in other mycoplasmal infections, is likely a key component of the morbidity associated with *M. agassizii* infection. Adherence of *M. agassizii* to ciliated mucosal cells leads to structural and functional changes in the upper respiratory tract (i.e., erosion of the ciliated epithelium) that ultimately result in the production of a nasal discharge composed of dead epithelial cells, infectious mycoplasma, and phagocytes, among other inflammatory cells (Jacobson et al., 1991; McLaughlin et al., 2000; Brown et al., 2001b). The loss of olfactory ciliated epithelium could adversely impact biological functions such as foraging that depend on the sense of smell.

Rates and causes of recrudescence from latent *M. agassizii* infection to active clinical infection are currently undefined. However, for other mycoplasma species, recrudescence of clinical disease has been associated with physiological stress (Simecka et al., 1992). Moreover, experimental reinoculation of *M. agassizii* within individuals previously exposed to the pathogen elicited a stronger immune response, and more acute clinical disease (Schumacher et al., 1993; McLaughlin, 1997). This is similar to the reinfection studies of finches with *M. gallisepticum* (Sydenstricker et al., 2006). In other words, serum antibody production against *M. agassizii* does not confer immunity, and re-exposure to the pathogen results in greater disease severity. Based on the immunopathologic nature of most mycoplasmal infections (Minion, 2002; Jones and Simecka, 2003; Simecka, 2005), this increased severity likely is a result of the cellular immune response. The active contribution of host immune responses to disease severity is a common attribute among mycoplasmal infections of animal hosts

(Simecka et al., 1992); however, specific understanding of the role of tortoise immunology on URTD pathogenesis is limited (Brown, 2002).

### **Similarities of Mycoplasmal URTD and Other Respiratory Mycoplasmoses**

Mycoplasmal URTD in tortoises is similar to other respiratory animal mycoplasmoses in that pathological lesions include ciliostasis, the focal loss of ciliated epithelium, mucosal hyperplasia, and the infiltration of leukocytes and phagocytic cells (e.g., heterophils). There have also been reports indicating a higher prevalence of secondary bacterial infections along the nasal cavity of *M. agassizii*-infected tortoises (Jacobson et al., 1991; McLaughlin et al., 2000). Dissemination from the upper respiratory tract (nasal cavity and olfactory mucosa) to the lower respiratory tract (trachea and lungs), however, is uncommon.

In addition to sharing general histopathological features with other respiratory mycoplasmoses of animals, *M. agassizii* infection is also associated with other common epidemiological features of mycoplasma infections. For instance, as with other mycoplasmal infections, *M. agassizii*-infected tortoises can become asymptomatic (clinically silent) carriers of infection (Schumacher et al., 1997). Mycoplasmal URTD can also be characterized as a chronic and slowly progressive disease in which the host immune response may contribute to pathogenesis (e.g., re-inoculation of *M. agassizii* increases disease severity in previously exposed individuals; McLaughlin, 1997). Disease may also be exacerbated with environmental stress (e.g., higher prevalence of clinical disease associated with URTD following drought years in desert tortoises; Christopher et al., 2003). And, finally strain-specific differences in virulence may account for variable clinical disease across exposed populations (Wendland, 2007).

## Diagnostic Tests

In general, mycoplasmal infections are diagnosed by direct detection of the pathogen by culture or polymerase chain reaction (PCR), presence of specific antibody through an enzyme linked immunosorbent assay (ELISA), or demonstration of histological lesions compatible with mycoplasmosis, preferably with immunohistochemical demonstration of the microbe at the site of the lesion. In some cases, electron microscopy is used. Additionally, an ELISA-based evanescent-wave biosensor has been evaluated for direct testing of tortoise sera in the field (Brown et al., 2008).

The ELISA detects the presence of anti-*M. agassizii* antibodies in tortoise plasma and detects exposure to the pathogen. The current enzyme linked immunosorbent assay (ELISA) has been validated by experimental infection studies, correlated with histological lesions by necropsy of experimentally and naturally infected tortoises, and with cultural and PCR detection of the pathogen (Brown et al., 1994; Klein et al., 1995; Schumacher et al., 1997; Homer et al., 1998; Brown et al., 1999b; McLaughlin et al., 2000; Wendland et al., 2007). The ELISA protocol used in this study was designed for *M. agassizii* identification in gopher and desert tortoises to minimize the incidence of false negatives. The ELISA exhibits a sensitivity of 98%, specificity of 99%, and positive and negative predictive values greater than 90% when seroprevalence within populations is between 9% and 85%. A cut-off value of  $\geq 64$  was used to define a seropositive titer because it results in the highest combined sensitivity and specificity, and thus the most accurate diagnosis of individuals (Wendland et al., 2007).

Although both direct culture of mycoplasma from nasal exudates and detection of *M. agassizii*-specific chromosomal DNA by polymerase chain reaction (PCR) are

indicative of the presence of the microbe, both tests have limited usefulness for routine diagnostics of living tortoises because of the long culture times required, fastidious growth requirements, difficulty in obtaining adequate lavages, and documented inability to detect *M. agassizii* in the absence of clinical signs. Thus, like mycoplasmal infections in swine and poultry, serology is currently the best diagnostic method available. The studies described in my dissertation used culture, PCR, and presence of specific antibody in conjunction with clinical presentation to characterize mycoplasma infection in sampled tortoises.

### **Factors Affecting Tortoise Immunity**

Because host immune responses can contribute to mycoplasma pathogenesis in other animal hosts, certain aspects of reptile and tortoise immunology are important to keep in mind in relation to URTD in gopher tortoises. Specifically, temperature, season, and hormones can influence antibody production in reptiles. For example, reptiles can undergo reversible thymic involution in a seasonal manner (linked to hibernation) that results in the depletion of lymphoid cells. Additionally, this thymic involution progresses with age, and season after season the lymphoid population decreases, never to reach the density present in young individuals. This suggests that older animals are subject to immunosenescence and may be less immunocompetent than young individuals. Reptiles do not have the intrinsic capability to regulate their body temperature, and therefore rely on behavioral means of thermoregulation (e.g., basking). Interestingly, antibody production in reptiles is lower at extreme temperatures. Additionally, seasonal changes in antibody production of reptiles have been correlated with systemic hormonal variations, with females generally eliciting stronger antibody responses than males (Origgi, 2007).

## Respiratory Pathogens of Gopher Tortoises

Other microbes may act synergistically with mycoplasmas to cause more severe respiratory disease in hosts; therefore, it is important to characterize and monitor other potential respiratory pathogens of tortoises that could impact URTD pathogenesis.

Currently, only two pathogens have undergone rigorous experimental trials and have been defined as etiologic agents of URTD through the fulfillment of Koch's postulates: *M. agassizii* (Brown et al., 1999b) and *Mycoplasma testudineum* (Brown et al., 2004a). Several case reports have described the isolation of other pathogens from tortoises exhibiting clinical signs consistent with URTD; however, a lack of rigorous experimental trials for these pathogens have failed to define their etiologic role in URTD.

In one case report iridovirus infection was identified in a gopher tortoise that presented with clinical signs of URTD (i.e., ocular/nasal discharge), lethargy, listlessness, and mild dehydration. At necropsy, this tortoise was found to have lesions in the lower respiratory tract (i.e., trachea and lung), and virions morphologically consistent with iridovirus (Westhouse et al., 1996b). Interestingly, the regional area from which this iridovirus case was obtained (Sanibel Island, FL) experienced a mass mortality event several years prior to the discovery of this iridovirus case (McLaughlin, 1997). Evaluation of tortoise plasma samples with a recently developed diagnostic assay indicated that the overall prevalence of iridovirus infections in gopher tortoises is very low. However, because this pathogen has been known to cause acute mortality in other turtle species (i.e., box turtles, *Terrapene carolina carolina*), its potential to do so in gopher tortoises should not be overlooked (Johnson et al., 2009).

Herpesvirus infections have also been identified in tortoises exhibiting clinical signs of respiratory disease. Pathological findings associated with herpesvirus infection

include lesions in the tongue, oral cavity, and lower respiratory tract (Harper et al., 1982; Pettan-Brewer et al., 1996). Herpesviruses can induce persistent and latent infections with intermittent clinical disease and infectious periods (like *M. agassizii*); however, experimental studies have not been conducted to determine its etiologic role in URTD in *Gopherus* tortoises. Interestingly, a survey of diseases in free-ranging desert tortoises found that tortoises with oral lesions (a characteristic sign of herpesvirus infection) were more likely than tortoises without lesions to be infected with *M. agassizii*, which may suggest the potential for synergistic interactions between herpesvirus and *M. agassizii* (Christopher et al., 2003). A more recent serological survey of *M. agassizii* and herpesvirus in captive tortoises found that clinical signs of URTD were more strongly correlated with *M. agassizii* infection than herpesvirus infection, and that the prevalence of herpesvirus was lower (27%) than that of *M. agassizii*. (87%) in captive tortoises. Additionally, individual exposure to both pathogens was observed, but the clinical disease observed in these co-exposed individuals was not severe (Johnson et al., 2006). However, because this study relied solely on serological evidence of past exposure to herpesvirus and *M. agassizii*, it failed to detect any potential clinical abnormalities associated with active co-infection. Therefore, the potential for synergistic interactions between herpesvirus and *M. agassizii* to result in greater URTD severity within hosts was not adequately addressed, and should not be disregarded based on these findings.

*Pasteurella testudinis* was also isolated from tortoises showing signs of URTD (Jacobson et al., 1991), but experimental transmission studies suggested that it had a relatively insignificant role in URTD pathogenesis and severity. Specifically, animals

experimentally infected with *P. testudinis* alone did not develop disease, and it was only when *P. testudinis* was administered along with *M. agassizii* that disease was induced. Furthermore, disease resulting from co-infection with both *P. testudinis* and *M. agassizii* was no more severe than that caused by *M. agassizii* alone. From this experiment, therefore, *P. testudinis* was considered to be a relatively benign secondary pathogen associated with *M. agassizii* infection (Brown et al., 1994).

Most recently, an *Acholeplasma* sp. was isolated from gopher tortoises exhibiting clinical signs of URTD; however, an experimental transmission study involving this potential pathogen found that the resulting clinical disease was mild, and that neither the expression of clinical signs nor histopathological lesions were consistent among infected tortoises. Therefore, this *Acholeplasma* sp. was considered a nonpathogenic commensal species of gopher tortoises (Wendland, 2007).

Additionally, other potentially pathogenic bacteria have been isolated from the nasal cavities and choanae of tortoises with clinical signs of URTD. These include: *Aeromonas hydrophila*, *Klebsiella oxytoca*, and *Psuedomonas* spp.. However, these bacteria have also been isolated from healthy tortoises (Dickinson et al., 2001). Thus, their pathogenic potential and etiologic associations with URTD are unlikely to be significant.

Finally, fungal pneumonia has also been diagnosed in tortoises presenting with clinical signs consistent with URTD. This was thought to be associated with secondary dual infections of *Aspergillus* and *Candida* spp. in immunocompromised hosts (Homer et al., 1998). As with many of the other respiratory pathogens of tortoises, the observation of this unknown fungal pathogen in a wild tortoise provided merely an

anecdotal account of its potential role as an etiologic agent of URTD, as no experimental trials have been conducted to more rigorously define it as an important pathogen of tortoises.

### **Overview of Mycoplasmal URTD in Natural Gopher Tortoise Populations**

Habitat loss has led to the decline of gopher tortoise populations across its range (Auffenberg and Franz, 1982; McCoy et al., 2006). The species is currently listed as threatened by the Florida Fish and Wildlife Conservation Commission (FFWCC 2007). Furthermore, its role as a keystone species raises concerns about the effects its decline could have on upland communities throughout its range (Mengak and Castleberry, 2004). Between 350-400 commensal species rely on the gopher tortoise for habitat construction, including rare and/or threatened species such as the gopher frog (*Rana capito*), eastern indigo snake (*Drymarchon couperi*), Florida pine snake (*Pituophis melanoleucus mugitus*), and Florida mouse (*Podomys floridanus*) (Cox et al., 1987; Jackson and Milstrey, 1989; Kent et al., 1997; Pike and Grosse, 2006). An extrinsic factor, such as disease, which could further accelerate population declines of this keystone species could also have debilitating repercussions on the future health of sandhill ecosystems.

The introduction of *M. agassizii*, an etiologic agent of upper respiratory tract disease (URTD), has been suggested as a more recent factor involved in gopher tortoise declines (Gates et al., 2002; Seigel et al., 2003). These studies, however, are primarily anecdotal. Research that could more rigorously evaluate claims of URTD-mediated population declines, such as studies addressing the interactions between host immune responses and *M. agassizii*, or the epizootiology of mycoplasmal URTD in

natural populations, are currently limited for this wildlife disease (McCoy, 2008; Sandmeier et al., 2009).

Serological surveillance for exposure to *M. agassizii* has been extensive throughout gopher tortoise populations in Florida (Deimer Berish et al., 2000; Thomas and Blankenship, 2002; Zipser and Ashton, 2003; Epperson, 2005; McCoy et al., 2007; Wendland, 2007; Karlin, 2008). Of roughly 7000 blood samples obtained across the state, 22% tested positive for *M. agassizii* through ELISA. Moreover, of the 41 counties from which samples were obtained, only seven were completely free of *M. agassizii* exposure (Wendland, 2007). However, the overall seroprevalence in most populations was <20%. Interestingly, populations and areas with seroprevalence >60% have been associated with clinical disease outbreaks and/or mortality events (McLaughlin, 1997; Wendland, 2007).

Additionally, notable stage-specific disparities in seroprevalence have been identified (Wendland, 2007; Karlin, 2008). Specifically, the prevalence of *M. agassizii* infection within the pre-reproductive subadult stage class is markedly lower than that of the reproductive adult stage class; however, mechanisms responsible for this variation have yet to be evaluated, and could correspond with behavioral differences between the two stages (Wendland et al., *in press*).

An extensive field study, which monitored 11 populations throughout Florida, attributed low-level increases in the recovery of dead tortoises to underlying effects of *M. agassizii*. Specifically, in three study populations experiencing different epizootic phases of URTD, increases in seroprevalence to *M. agassizii* were associated with increases in morbidity and mortality. Necropsies performed on tortoises from these

populations provided evidence of mycoplasmal lesions in the upper respiratory tract, in the absence of other significant pathogens (Wendland, 2007). Additionally, the annual survival of adult tortoises in populations with high *M. agassizii* seroprevalence has been estimated as roughly four percent lower than that of tortoises in populations with low seroprevalence (Ozgul et al., 2009). In this study, *M. agassizii* seroprevalence also had a slight positive association with the number of tortoise skeletal remains collected from a population (Ozgul et al., 2009). In other words, the number of dead tortoises recovered from the field increased with site seroprevalence. Importantly, this is the first study to quantify a low-level effect of URTD on tortoise survival.

Despite the extensive serosurveillance of *M. agassizii* in the wild, and the intriguing association between seroprevalence and an increase in mortality, little is known regarding the ecology of URTD within, or its long-term effects on, natural gopher tortoise populations. This dissertation served to address these gaps in the current knowledge of URTD in natural systems; in particular aspects associated with host susceptibility and population-level impacts were addressed using applications from population ecology, mathematical epidemiology, and behavioral ecology.

### **Objectives**

This project focused on elucidating the impacts of mycoplasmal URTD on gopher tortoise population health. Although extensive research has defined the pathology of URTD within individual hosts, little is known regarding how the host damage associated with URTD affects host biological functions (i.e., reproduction and/or survival) and how these individual-level effects translate into long-term impacts for exposed gopher tortoise populations (Brown et al., 2002). Specific goals of this project were: (1) to describe the potential effects of recurring URTD outbreaks on long-term gopher tortoise

population dynamics, (2) to evaluate potential mechanisms driving observed stage-specific disparities in seroprevalence within exposed populations, and (3) to determine how movement-associated factors contribute to the exposure and spread of *M. agassizii* across individuals within a population. The multifaceted nature of this project reflects the complex framework of interactions involved in the study of wildlife disease. This dissertation is organized into five chapters: a general introduction chapter (Chapter 1), three manuscript chapters (Chapters 2-4), and a general conclusion chapter (Chapter 5).

In Chapter 2, a framework is developed with which to evaluate the potential effects of recurring outbreaks on long-term host population dynamics using mycoplasmal URTD as a case study. Specifically, the influence of chronic recurring disease outbreaks on host population dynamics and persistence was investigated using matrix population and Markov chain models for temporally autocorrelated environments. By treating disease outbreaks as a form of environmental stochasticity, host population dynamics were evaluated across varying levels of outbreak duration, outbreak recurrence, and disease-induced mortality. This chapter served to provide a heuristic description of long-term URTD impacts within natural populations, when parameters associated with disease-dynamics (e.g. outbreak duration and recurrence frequency) and host-pathogen interactions (e.g. disease-induced mortality) are unknown.

With Chapter 3, force of infection models were applied to age-seroprevalence data to evaluate competing hypotheses pertaining to stage-specific disease processes (i.e., disease-induced mortality and force of infection) in natural populations. Several models, each corresponding to a specific hypothesis, were assessed to determine

whether the low observed seroprevalence of pre-reproductive tortoises, compared to reproductive tortoises, was likely due to stage-specific differences in URTD-induced mortality and/or differences in exposure to *M. agassizii*. This chapter served to address the likelihood of URTD-induced mortality given the serological profile of an exposed population, as well as determine whether significant exposure heterogeneities are likely to exist across stage-classes in a population.

In Chapter 4 the movement patterns of individual tortoises were described in relation to infection status in order to determine how specific movement-associated factors (i.e., daily distance traveled, number of burrows visited daily, home range area, number of burrows within a home range) may increase the risk of *M. agassizii* infection. A multiple logistic regression model was used in conjunction with a principal components analysis to determine whether disparities in the movement behavior of tortoises can increase their probability of becoming infected, and whether disparities in movement behavior existed across stage classes (i.e., pre-reproductive and reproductive) and/or between sexes, that may also contribute to the exposure heterogeneities addressed in the previous chapter.

Finally, Chapter 5 serves as a conclusion chapter. It synthesizes the findings from the previous three chapters in an effort to frame them within the context of past studies that have addressed the threat of URTD in tortoise populations. Additionally, future directions of study are proposed in light of caveats specific to this project, as well as gaps in our current understanding of URTD-mediated population impacts.

## CHAPTER 2

### LONG-TERM IMPACTS OF RECURRING DISEASE ON POPULATION DYNAMICS AND PERSISTENCE OF A LONG-LIVED WILDLIFE HOST

Despite a heightened interest regarding the role of infectious diseases in wildlife conservation, few studies have explicitly addressed the impacts of chronic, persistent diseases on long-term host population dynamics. With this study we explored how recurring epizootics can influence the long-term growth and persistence of threatened wildlife populations. Using mycoplasmal upper respiratory tract disease (URTD) within natural gopher tortoise (*Gopherus polyphemus*) populations as a case study, we investigated the influence of chronic recurring disease epizootics on host population dynamics and persistence using matrix population and Markov chain models for temporally autocorrelated environments. By treating epizootics as a form of environmental stochasticity, we evaluated host population dynamics across varying levels of outbreak duration ( $\rho$ ), outbreak recurrence ( $f$ ), and disease-induced mortality ( $\mu$ ). Baseline results indicated a declining growth rate ( $\lambda$ ) for unexposed populations ( $\lambda_{\text{Normal}} = 0.903$ , 95% CI: 0.765 – 1.04), and a median time to quasi-extinction of 23 years (range: 14-34 years). Upon the introduction of recurring epizootics, stochastic growth rates ranged between 0.838-0.902, and median quasi-extinction times ranged between 14-23 years, with both metrics decreasing as a function  $f$  and  $\mu$ . Overall, baseline conditions had a greater impact on  $\lambda$  than epizootic conditions, and demographic vital rate parameters were more proportionately influential on  $\lambda$  than disease- or outbreak-associated parameters. Lower-level elasticities revealed that among disease- and outbreak-associated parameters, increases in  $\mu$ , force of infection ( $\phi$ ), and  $f$  negatively influenced  $\lambda$ , while increases in  $\rho$  had slightly positive impacts.

When little is known regarding the influence of diseases on host wildlife populations, heuristic exercises like the one presented in this study can provide a baseline understanding of potential impacts of disease on host population dynamics, and help guide management actions. The modeling framework presented in this paper could be widely applied to a range of wildlife disease systems in which hosts suffer from persistent recurring diseases.

### **Introduction**

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

earlier than less virulent pathogens, and therefore have a less pronounced effect on long-term dynamics of host populations (Claessen and deRoos, 1995). Because chronic, or persistent, infections could potentially have greater long-term impacts than acute infections on host population dynamics, their assessment within wildlife populations of conservation concern warrants attention.

The dynamics of chronic disease in host populations are heavily influenced by the duration of host morbidity and infectiousness, and the rate of disease recrudescence. For the purpose of this study we define disease chronicity as the duration of morbidity, which generally corresponds to the amount of time an infected individual experiences active and overt clinical disease. For chronic diseases, tissue damage and/or altered host function resulting from this clinical disease state can be considered lifelong. Clinical disease is usually accompanied by host infectiousness, in which adequate numbers of infectious microbes are shed to transmit infection to susceptible contacts (Casadevall and Pirofski, 2002). Transition from an active (infectious) clinical disease state to a clinically silent and asymptomatic carrier state occurs after the infectious period, in which the pathogen is sequestered within hosts in the form of a latent infection. Recrudescence of active clinical disease from a latent subclinical state, then can be triggered by several factors, such as: (1) exposure to other infectious individuals, which stimulates a rapid memory immune response and possibly more severe clinical disease, (2) superinfection with a secondary pathogen (Casadevall and Pirofski, 2003a), or (3) physiological stress caused by environmental or anthropogenic factors (Padgett and Glaser, 2003).

We can apply these general concepts of chronic disease natural history from the host individual-level to the host population-level. For example, population-level recrudescence would manifest itself as a recurring epizootic. The rate of epizootic recurrence or re-emergence would be defined as the rate at which a critical threshold of individuals within an exposed population becomes clinically ill, and either spreads infection or elicits reactivation of chronically infected individuals from a latent/subclinical state to a clinical disease state. In disease ecology terms, this would represent conditions under which the basic reproductive number ( $R_0$ ) of a pathogen is greater than one, and infection (and subsequent disease) propagates throughout a population (Swinton et al., 2002). Recurring outbreaks of disease occur in many wildlife populations, and are often associated with seasonal environmental factors (Altizer et al., 2006). Following from the definition of infectiousness/morbidity duration at the individual-level, outbreak duration (at the population-level) could be defined as the epizootic time span, or the number of consecutive years in which a disease outbreak directly affects demographic processes such as survival, fecundity, or growth.

From the perspective of population dynamics, outbreak duration and recurrence could be represented using a Markov chain model. Under this framework, the emergence and re-emergence of an outbreak within a population at a specific time is probabilistically dependent on the temporal autocorrelation term  $\rho$ , which represents the length of outbreak duration, and the overall frequency term  $f$ , which defines the overall frequency of outbreak recurrence. This approach has been used to study the effects of fire and hurricane disturbance on the population dynamics of plant species (Pascarella and Horvitz, 1998; Caswell and Kaye, 2001). Under temporally autocorrelated

environments vital rates (i.e., survival, fecundity, and/or growth) display a memory of past conditions, which can have a profound impact on population dynamics (Tuljapurkar and Haridas, 2006). In other words, repeated consecutive exposure to adverse environmental conditions is expected to produce different results than repeated sporadic exposure in relation to long-term population dynamics. By modeling disease outbreaks as a form of environmental stochasticity, in which the probability of outbreak occurrence is temporally autocorrelated, the effects of outbreak duration and recurrence on the long-term dynamics and persistence of wildlife populations can be assessed.

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

## **Methods**

### **Study System**

Mycoplasmal respiratory infections in virtually all hosts are generally chronic and clinically silent, with low mortality but nearly 100% morbidity (Simecka et al., 1992;

Minion, 2002). Overt clinical signs are observed in early infections, and may be exacerbated in periods of stress or as the age of the individual increases. However, pathogenic mycoplasmal species can often cause gross and histological lesions, primarily in the respiratory tract, in the absence of overt clinical signs (Simecka et al., 1992).

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

major epizootiological importance when attempting to elucidate how a chronic disease could alter the long-term dynamics of wildlife populations.

## **Model Formulation**

### **Model assumptions**

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

**Demographic effects of disease:** We assumed that only the reproductive adult stage class would suffer significant effects of disease in the form of disease-induced mortality. Past serological surveys of gopher tortoise populations have indicated a strong discrepancy in stage-specific seroprevalence, whereby only a marginal percentage of pre-reproductive individuals tested positive for *M. agassizii* exposure (Wendland, 2007). We therefore assumed that the force of infection ( $\phi$ ) of the pre-reproductive class was negligible; thus, disease outbreaks did not influence the survival of pre-reproductive tortoises. Additionally, we assumed that disease had no effect on fecundity. In a previous study White (*unpubl. data*) found no association between *M. agassizii* infection and reproductive parameters (i.e., clutch size and gravidity) over a four-year period. Therefore, we made the conservative assumption that disease had no

effect on fecundity. Moreover, the most pressing concern regarding URTD in tortoise populations involves its speculated role in tortoise die-off events (Berry, 1997; Seigel et al., 2003). Therefore, we decided to focus our efforts on quantifying potential impacts of recurring mortality events on long-term host population dynamics by limiting the effects of URTD to depressed survival.

**Disease-induced mortality ( $\mu$ ):** We assumed that disease-induced mortality ( $\mu$ ) acts only on clinically ill individuals, and that the mortality of subclinically infected individuals during normal states is not significantly different from the baseline mortality rates of uninfected reproductive adults. We also assumed that disease-induced mortality acted randomly on clinically ill individuals (i.e. individuals actively presenting with clinical signs of disease) across all scenarios of outbreak duration and recurrence. Virulence and pathogenicity were assumed to remain constant over time, and therefore neither the duration nor frequency of outbreaks affected the rate at which individuals died from clinical disease. In other words, disease-induced mortality was assumed to be independent of outbreak duration and recurrence frequency, and at each time step the survival rate of clinically infected individuals was reduced by a constant amount  $\mu$  regardless of the epizootic state of the host population at the previous time step.

#### **Definition of population states (Normal/Outbreak)**

Disease outbreaks were modeled as a form of environmental stochasticity, in which populations were subjected to normal and outbreak conditions according to a probabilities defined by a Markov Chain model (Caswell, 2001; Tuljapurkar and Haridas, 2006). The normal state was defined as the baseline condition of a population in which the incidence of clinical disease is negligible, and disease-induced mortality does not

occur. Thus, this normal state also corresponds with an enzootic state of disease within a population. We defined an outbreak state as a condition in which a threshold level of clinically ill and infectious individuals is surpassed, and an increased incidence of clinical disease is associated with an increase in mortality due to disease ( $\mu$ ). As stated earlier, we assumed that under outbreak conditions subclinical individuals with latent infection recrudescence to a clinical disease state at a rate defined by  $\phi$ . Under normal conditions following outbreak events, previously infected individuals within a population may remain subclinically infected, and consequently serve to maintain seroprevalence at generally constant levels through time. The term  $\mu$  was applied to adult survival only during outbreak conditions, as the incidence of clinical disease (and subsequently the force of infection) during normal conditions was assumed to be negligible.

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

A stochastic population model based on the assumption of independent and identically distributed states (Caswell, 2001; in this case normal v. outbreak states) would fail to capture autocorrelation structure between disease states through time (e.g., under chronic conditions, the disease state at time  $t$  is dependent on its state at time  $t-1$ ). Because we were interested in assessing the potential impacts of URTD outbreak recurrence and duration on population dynamics, we incorporated these terms into a stochastic modeling framework using a Markov chain model (Silva et al., 1991; Caswell, 2001).

### **Parameter Estimation**

To parameterize a population projection matrix model with three demographic stages (hatchling, pre-reproductive juvenile, and reproductive adult) and two disease states (uninfected/subclinically infected, and clinically infected), the following parameters were needed: stage-specific annual survival probabilities ( $\sigma_H$ ,  $\sigma_{PR}$ ,  $\sigma_R$ ), annual growth rate of pre-reproductive individuals to the reproductive stage ( $\gamma$ ), annual fecundity ( $m$ ), and force of infection ( $\phi$ ). A brief overview of methods used to estimate these parameters follows (see Appendix A for additional methodological details).

Estimates of demographic and disease parameters used in this study are presented in Table 2-1.

**Stage-specific survival probabilities ( $\sigma_H$ ,  $\sigma_{PR}$ ,  $\sigma_R$ ):** Annual hatchling survival probability was defined as the summary estimate of a meta-analysis of five gopher tortoise hatchling survival studies (Appendix B). Survival probabilities for pre-reproductive juvenile and reproductive adult survival rates were estimated using a capture-mark-recapture analysis of four years of data collected from three populations

that had previously been described as healthy and URTD-free (Fig. A-1; Appendix A; Wendland, 2007).

**Pre-reproductive growth probability ( $\gamma$ ):** The probability that pre-reproductive tortoises survive and grow to become reproductive adults was inestimable from the 4-year mark-recapture data due to the slow-growing nature of this long-lived species. Therefore, we estimated  $\gamma$  using the fixed stage duration method described by Caswell (2001; Appendix A).

**Fecundity ( $m$ ):** The annual fecundity was estimated as the product of one-half times clutch size ( $cs$ ), proportion of females gravid ( $pg$ ), nesting success probability ( $ns$ ) and hatching success rate ( $hs$ ) (i.e.,  $m = (cs \times pg \times ns \times hs)/2$ ). Mean clutch size and proportion of females gravid were obtained from radiographs of adult females sampled from the pooled study populations prior to oviposition. Nest and hatch success were defined as the summary estimates of meta-analyses conducted using findings from published studies (Appendix A; Fig. A-3).

**Force of infection ( $\phi$ ):** The force of infection represents the per-capita annual rate at which susceptible individuals become infected (Swinton et al., 2002). Ozgul et al. (2008) estimated annual force of infection rates for *M. agassizii* from a 4-year CMR study of gopher tortoise populations with high ( $\geq 25\%$ ) and low ( $< 25\%$ ) seroprevalence. Their estimate of force of infection under high seroprevalence conditions ( $\phi = 0.22 \pm SE 0.04$ ) was used to quantify the transition from an uninfected (or subclinically infected) state to a clinically infected state among reproductive adult tortoises.

## Construction of Population Projection and Markovian Transition Matrices

We used matrix population models with four stages (hatchling, pre-reproductive juvenile, uninfected/subclinically infected reproductive adult, and clinically infected reproductive adult) to describe demography of gopher tortoises, and two-state Markovian transition matrices to model the transition of normal population conditions to outbreak states (Fig. 2-1). Population projection matrices were parameterized using the demographic rates presented in Table 2-1, and followed the form:

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

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

Nine Markovian transition matrices ( $P_i$ ) were generated to describe acute, intermediate, and chronic outbreak conditions under low, intermediate, and high

frequencies of recurrence. The autocorrelation term  $\rho$  was used to quantify the chronicity, or duration, of outbreak states (*i.e.*,  $\rho_{acute} = -0.10$ ,  $\rho_{intermediate} = 0.40$ ,  $\rho_{chronic} = 0.73$ ). When  $\rho < 0$ , normal and outbreak states tend to alternate so that when a population experiences an outbreak, it transitions quickly from an outbreak state to a normal state; however, when  $\rho > 0$ , long sequences of normal and outbreak states are generated so that the transitions between normal and outbreak states are prolonged (Fig. 2-2; Caswell and Kaye, 2001). The outbreak frequencies ( $f$ ) used to parameterize the Markovian transition matrices (*i.e.*,  $f_{low} = 0.1$ ,  $f_{intermediate} = 0.2$ ,  $f_{high} = 0.3$ ) represented how often populations experienced recurring outbreaks. Because the true recrudescence rate of URTD is unknown, we used a range of values of  $f$  to explore how outbreak recurrence may affect population dynamics. Using all possible combinations of  $\rho$  and  $f$ , values of  $p$  and  $q$  were calculated for the Markovian transition matrices using the following equations adapted from Caswell (2001):

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

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

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

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

## Calculation of Stochastic Population Growth Rate ( $\lambda_S$ )

In order to address the potential impacts of recurring outbreaks on long-term population growth, we compared the stochastic population growth rate ( $\lambda_S$ ) across different outbreak scenarios to the population growth rate of a normal population ( $\lambda_{\text{Normal}}$ ) under baseline conditions. Two approaches were used to quantify the long-term growth of gopher tortoise populations hypothetically exposed to *M. agassizii*: Tuljapurkar's small noise approximation (TSNA; Tuljapurkar and Haridas, 2006) and matrix simulation (MS; Caswell, 2001). Methodology used for the calculation of  $\lambda_S$  through the matrix simulation approach is described in detail in Appendix C. Fifty-four different stochastic growth rates, each corresponding to a different combination of normal-outbreak state grouping and Markov chain model (Fig. 2-2), were calculated using Tuljapurkar's approximation for autocorrelated environments (Tuljapurkar and Haridas, 2006). Through this approach  $\log \lambda_S \approx \log \lambda_0 + W_1 - W_2$ , where  $W_1$  is the effect of interannual variability on  $\lambda_S$ , and  $W_2$  is the effect due to autocorrelation. Using this formulation, we were able to assess the relative effect of temporal autocorrelation ( $W_2$ ) over the effects of interannual variability ( $W_1$ ) in order to address the impact of outbreak duration on host population dynamics. For details regarding the numerical computation of the terms  $W_1$  and  $W_2$  please refer to Tuljapurkar and Haridas (2006). The variance of  $\lambda_S$  ( $\sigma^2$ ) was estimated under the assumption of asymptotic lognormality of the weighted sum of stage-specific abundances (Caswell, 2001). Ninety-five percent confidence intervals were then generated as  $\exp(\log \lambda_S \pm 1.96 \sqrt{\sigma^2})$ . Additionally, we calculated the deterministic asymptotic growth rate of a normal (i.e., unexposed) population for comparison of population dynamics under baseline conditions and under conditions of

stochastically occurring disease outbreaks. Standard errors for the deterministic  $\lambda$  were calculated through series approximation using the sensitivities of  $\lambda$  to lower-level parameters (Caswell, 2001).

### **Elasticity Analysis**

In order to quantify the overall importance of model parameters (i.e., demographic vital rates, disease- and outbreak-associated parameters) to the long-term growth rate of exposed populations, we employed elasticity analyses. Specifically, elasticity analyses quantify the changes in  $\lambda$  resulting from a proportional change in a given model parameter (de Kroon et al., 2000). For instance, if the elasticity of  $\lambda$  to a given model parameter is 0.15, this means that a proportional change in that model parameter would result in a 15% increase in  $\lambda$  when all other model parameters are held constant. In relation to this study, the elasticities of  $\lambda$  to parameters such as  $\rho$  and  $f$  would quantify the proportional influence of outbreak duration and frequency on the long-term growth rate of a host population. This could therefore illustrate the importance of disease- and outbreak-associated parameters relative to that of baseline vital rates, and serve as another metric to quantify how disease could impact host population dynamics.

We calculated elasticities of  $\lambda$  to matrix elements, as well as lower-level elasticities of  $\lambda$  to vital rates using the chain rule for the baseline population projection matrix (population experiencing normal rather than outbreak conditions) through standard methods (Caswell, 2001). For each of 54 disease scenarios we calculated stochastic elasticities through the simulation of 50,000 time steps (Caswell, 2001). Additionally, we calculated lower-level elasticities of long-term population growth to vital rates, and disease- and outbreak-associated parameters (i.e.,  $\phi$ ,  $\mu$ ,  $f$ , and  $\rho$ ) using the vec

permutation approach for matrix metapopulation models (Hunter and Caswell, 2005). Using this framework, we defined “patches” as the normal and outbreak population states, and the transition between these patches as the Markov chain model described above (see Appendix D for details). We used product-moment correlations between stochastic elasticities and deterministic elasticities calculated from the vec-permutation approach for each outbreak scenario to examine the correlation between the elasticities calculated using both methods (stochastic simulation v. matrix metapopulation analysis).

### **Estimation of Quasi-Extinction Parameters**

The final metric used to investigate the population-level effects of recurring outbreaks was population persistence, or the length of time a population is projected to survive in the environment. Here we define persistence in terms of quasi-extinction parameters, and estimated the length of time a population was expected to persist before the total population size fell below a predefined quasi-extinction threshold. Quasi-extinction parameters for outbreak scenarios were estimated through stochastic simulation (Caswell, 2001; Morris and Doak, 2002). An initial population size of 500 tortoises was used to assess quasi-extinction times. The quasi-extinction threshold was set to 10% of the initial population size, or 50 individuals. Censuses across Florida have shown that only 31% of conservation lands harbor gopher tortoise populations with  $\geq 500$  tortoises (Miller, 2001), and that most populations contain between 100 and 500 individuals (Smith et al., 2006b). For this reason, an initial size of 500 tortoises was considered appropriate for our analyses. The initial population size was then multiplied by the stable stage distribution from the baseline normal population projection matrix in order to obtain an initial stage-structured population vector. For each simulation, a

sequence of environments corresponding to a specific Markov chain model was generated and used to assign a projection matrix at each time step. Population size with stage structure was then projected over time for each simulation, and the proportion of simulations that reached the quasi-extinction threshold at or before a given time step provided the cumulative probability of quasi-extinction (Morris and Doak, 2002). Median quasi-extinction time ( $T_{q50}$ ) was defined as the number of years a population would persist before the probability of quasi-extinction surpassed 0.50. The cumulative distributions of quasi-extinction probabilities over a 50-year time period were calculated based on 5000 simulations and 10 separate replicate runs. This process was repeated for each outbreak scenario ( $n = 54$  scenarios). For the normal (i.e., unexposed) population state, quasi-extinction parameters were estimated through diffusion approximation using the values of  $\lambda$  and  $\sigma^2$  estimated from the baseline population projection matrix (Caswell, 2001).

## Results

### Baseline Model

Under normal conditions and in the absence of clinical disease, the tortoise population was projected to be declining by approximately 9.7% each year ( $\lambda_{\text{normal}} = 0.903$ , 95% CI: 0.765 – 1.04). Matrix entry elasticities revealed that  $\lambda$  was proportionately most sensitive to survival of reproductive adults ( $e(a_{33}) = 0.9997$ ). Likewise, lower-level elasticities indicated that reproductive adult survival was proportionately the most influential vital rate on  $\lambda$  ( $e(\sigma_R) = 0.780$ ), followed by fecundity ( $e(m) = 0.000085$ ). The probability of quasi-extinction within a 50-yr time frame was 1.0.

The median time to quasi-extinction was 23 years and approximately ranged between 14-34 years.

### **Outbreak Model**

Stochastic growth rates ( $\lambda_S$ ) calculated using Tuljapurkar's small noise approximation ranged between 0.838-0.902, and decreased as a function  $f$  (outbreak recurrence) and  $\mu$  (disease-induced mortality; Fig. 2-3). The  $\lambda_S$ 's were inversely related to  $f$  and  $\mu$ , but increased marginally with an increase in  $\rho$ . (outbreak duration) Through their interaction,  $f$  and  $\mu$  reduced  $\lambda_S$  by as much as 7% (Fig. 2-3). Both Tuljapurkar's small-noise approximation and matrix simulation approaches provided comparable estimates of  $\lambda_S$  under all scenarios. Values of  $\lambda_S$  obtained through both approaches for various scenarios are presented in Table E-1 (Appendix E).

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

Overall, elasticity of  $\lambda$  to entries of the demographic block-diagonal matrix **B** (Appendix D) indicated that the survival of reproductive adults had the largest proportional influence on  $\lambda$ . The probability of survival of clinically ill reproductive adults was the most influential matrix element on  $\lambda$  under outbreak scenarios in which  $\mu \leq 0.20$  ( $\sum e(b_{44}^{(k)}) > 0.692$ ). However, under outbreak scenarios in which  $\mu > 0.20$ , the largest proportional impact on  $\lambda$  was made by the matrix element corresponding to the

probability of reproductive adults surviving and remaining in the uninfected or subclinical state ( $\sum e(b_{33}^{(k)}) > 0.935$ ). Elasticities of  $\lambda$  to elements of the dispersal (i.e., Markovian outbreak transition) block-diagonal matrix  $\mathbf{M}$  (Appendix D) indicated that among transitions between normal and outbreak states, the probability of remaining under normal conditions had the greatest proportional impact on  $\lambda$  across all outbreak scenarios ( $\sum e(m_{11}^{(k)}) > 0.472$ ). This result was further corroborated by the finding that population dynamics under normal (i.e., no outbreak) conditions made a higher proportional contribution to  $\lambda$  ( $\sum e(B_{normal}): 0.702-0.968$ ) than outbreak population dynamics ( $\sum e(B_{outbreak}): 0.032-0.298$ ) across all outbreak scenarios. Moreover, summed elasticities for each stage-specific matrix within  $\mathbf{M}$  indicated that the outbreak transition matrix for clinically ill adults ( $M_{R+}$ ) made the highest proportional contribution to  $\lambda$  when  $\mu \leq 0.20$  ( $\sum e(M_{R+}) > 0.692$ ); however, when  $\mu > 0.20$  the highest contribution to  $\lambda$  was made by dispersal parameters of uninfected (or subclinical) adults ( $\sum e(M_{R-}) > 0.935$ ) across all outbreak scenarios.

Lower-level elasticities indicated that, overall, demographic parameters were proportionately more influential on  $\lambda$  than disease- or outbreak-associated parameters. Among demographic parameters, survival of reproductive adults had the largest lower-level elasticity across all outbreak scenarios ( $e(\sigma_R) > 0.998$ ). Among disease- and outbreak-associated parameters,  $\mu$  had the largest overall proportional impact on  $\lambda$  ( $e(\mu): -0.188$  to  $-0.0099$ ), followed by  $f$  ( $e(f): -0.0642$  to  $-0.000979$ ; Fig. 2-4), under outbreak scenarios in which  $\mu \leq 0.20$ . However, under outbreak scenarios in which  $\mu > 0.20$ ,  $\phi$  had the largest overall proportional impact on  $\lambda$  ( $e(\phi): -0.233$  to  $-0.202$ ) among disease- and outbreak-associated parameters, followed by  $f$  ( $e(f): -0.0713$  to  $-0.0145$ ;

Fig. 2-4). Lower-level elasticities for disease-associated parameters indicated that increases in  $f$ ,  $\mu$ , and  $\phi$  would result in declines in  $\lambda$ , whereas increases in  $\rho$  would increase  $\lambda$  (Fig. 2-4). Additionally, with increases in  $\mu$ , elasticities of  $\lambda$  to  $\phi$  became more negative, indicating a greater negative effect on  $\lambda$ , whereas those for  $\mu$  became less negative and quickly approached 0 when  $\mu > 0.20$ .

The probability of quasi-extinction within a 50-year time period was 1.0 for all outbreak scenarios. Median quasi-extinction times ranged between 13-23 years across outbreak scenarios, and were also inversely related to  $f$  and  $\mu$ . Interestingly, despite its negligible effect on  $\lambda_S$ , increasing values of  $\rho$  slightly improved quasi-extinction probabilities and times (Figs. 2-5 and 2-6). Estimates of  $\lambda_S$  and median quasi-extinction time ( $T_{q50}$ ) for all values of  $\rho$ ,  $f$ , and  $\mu$  are presented in Table E-2 (Appendix E).

Varying the value of  $\rho$  had little effect on  $\lambda_S$ . Similarly, under Tuljapurkar's small noise approximation for autocorrelated environments, the effect of autocorrelation on  $\lambda_S$  ( $W_2$ ) was very small relative to the effect of interannual variability, or fluctuations in disease states ( $W_1$ ; Fig. 2-7). The largest relative effects of temporal autocorrelation over temporal variability across levels of  $f$  and  $\mu$  were observed at the disease-induced mortality rate of 0.20 and overall outbreak frequency of 0.30 for acute and intermediate outbreak duration values. Under chronic outbreak duration scenarios ( $\rho = 0.73$ ) the largest relative effects of temporal autocorrelation followed the same trend, with added increased effects at low overall outbreak frequencies and the disease-induced mortality rate of 0.01. However, at most,  $W_2$  was only about 0.0058 times the magnitude of  $W_1$  across all scenarios.

## Discussion

Disease can pose a serious threat to the viability of wildlife populations through mechanisms such as reductions in genetic diversity of hosts and contributions to demographic stochasticity within fragmented or small host populations (de Castro and Bolker, 2005). The study of wildlife diseases has emerged as a “crisis discipline,” integrating diverse fields such as ecology, epidemiology, and pathobiology for the management of wildlife populations. With an increase in the rate of wildlife population declines due to adverse anthropogenic effects (i.e., habitat degradation and fragmentation, the introduction of exotic species, and exploitation), adaptive strategies that focus on the interactions between biodiversity and animal health become increasingly important for the management of population health in order to preserve ecosystem function (Ostfeld et al., 2002). Unfortunately, logistical difficulties and cryptic disease processes often make the study of wildlife diseases within natural populations a complicated venture, and the population-level consequences of disease remain poorly understood. Thus, proper management strategies are difficult to formulate and implement based on field studies alone, and development of models that can be used as heuristic and predictive tools can be helpful.

Of particular interest to this study was the effect of recurrent disease outbreaks on long-term host population dynamics. Recurrent disease outbreaks have been commonly observed as seasonal phenomena associated with changes in host social behavior, immune function, and birth and death pulses within wildlife populations (Altizer et al., 2006). In house finches, for example, recurring outbreaks of mycoplasmal conjunctivitis have been linked to seasonal changes in host aggregative patterns and reproduction (Altizer et al., 2004b). In field voles, seasonal patterns of cowpox virus

were associated with the recruitment of susceptible hosts through birth pulses (Begon et al., 2009). Higher levels of parasite infestation were associated with compromised immune defenses of males during the mating season in red grouse populations (Mougeot et al., 2006). In these examples disease outbreaks are fairly predictable from seasonal host attributes. In other cases, however, mechanisms for disease recurrence are largely unknown, and heuristic models can be used to understand the effects of recurring disease outbreaks on host population dynamics.

The framework we present in this paper allows for the incorporation of chronic and recurring disease dynamics into an assessment of host population viability in a relatively simple and straightforward manner. The potential effects of chronic and recurring epizootics on host wildlife populations could be modeled as a form of environmental stochasticity. However, the common assumption of independent and identically distributed states would fail to properly describe the non-random transitions between normal and outbreak states experienced by exposed populations. By imposing an autocorrelation structure to potential disease states through time with the use of a Markov chain, we were able to determine, in a straightforward manner, how factors such as outbreak duration and recurrence could influence the growth and persistence of host populations exposed to chronic and endemic infections. Additionally, we have shown that matrix metapopulation models can adequately describe the dynamics of a population transitioning between normal and outbreak conditions, and provide a simple approach that allows one to quantify the proportional influence of disease- and outbreak-associated parameters on long-term population growth.

The results of our case study indicated that even an unexposed population was at substantial risk of extinction. When exposed to *M. agassizii*, the annual population growth rate declined by up to 16%, and median persistence time declined by up to 10 years. Increases in the force of infection, outbreak frequency, and disease-induced mortality all had negative effects on the long-term growth and persistence of exposed populations. The long-term growth rate of populations exposed to recurring epizootics was relatively unaffected by outbreak chronicity or duration; however, the overall persistence of populations was slightly improved with increasing chronicity values. According to the Markov chain model used to describe the transitions between outbreak and normal conditions, long times spans of outbreak conditions were coupled with long time spans of normal conditions. Therefore, while populations experienced longer outbreak periods, they also had more time to recover from these outbreaks. With increases in outbreak frequency, however, these recovery periods were shortened. Thus, the long-term growth and persistence of populations under the threat of frequent outbreaks was compromised due to reduced recovery periods between outbreaks. In a natural gopher tortoise population, the frequency of outbreaks is likely to increase through the exposure of subclinical or uninfected tortoises to clinically diseased animals who are actively shedding mycoplasma, as might occur through relocation events. Normal biological parameters and behaviors might also contribute to recrudescence. As males become reproductively active, increased conspecific aggression might lead to both increased stress and increased exposure risks. Time of exposure might also be an inherent risk factor, especially with respect to individual nutritional status or the stage of female reproductive cycle at the time of exposure. Recrudescence might also be

triggered by secondary bacterial or viral infections as well as by extrinsic stress factors, such as those associated with adverse environmental events (i.e., drought, hurricanes, or habitat degradation).

Using a metapopulation framework we assessed how population growth ( $\lambda$ ) was influenced by demographic, disease- and outbreak-associated parameters. Through elasticity analyses we found that population dynamics under normal conditions (i.e., baseline values of demographic parameters) had the greatest impact on the long-term growth rate of a population. Regardless of the level of disease-induced mortality, outbreak duration, or outbreak frequency, it was the baseline condition of an exposed population that was most influential to the long-term population growth rate. Overall, we also found that demographic parameters (i.e., survival, fecundity, and growth) were proportionately more influential on  $\lambda$  than disease- and outbreak-associated parameters. The survival of reproductive adults proved to be the most important lower-level parameter to  $\lambda$ , consistent with life history theory and previous analyses of turtle population dynamics (Crouse et al., 1987; Crowder et al., 1994; Doak et al., 1994; Heppell, 1998). The relative importance of host demographic processes over disease-related processes may have been attributed to declining growth rates ( $\lambda < 1.0$ ) at baseline conditions, and perhaps disease-related processes would have had a greater impact on population dynamics under baseline conditions of increasing or constant growth ( $\lambda \geq 1.0$ )

Although under most scenarios assessed  $\mu$  had the greatest effect on  $\lambda$  among disease- and outbreak-associated parameters, the proportional influence of  $\mu$  was surpassed by that of  $\phi$  under scenarios of high disease-induced mortality ( $\mu = 0.30$ ).

According to results from theoretical studies, a population threshold exists below which transmission would not be sustainable and the disease would ultimately disappear (Anderson, 1991). For example, when infectious contacts with susceptible hosts declined, phocine distemper virus faded out from a population of North Sea harbor seals (Swinton et al., 1998). The proportional influence of disease-associated parameters on long-term population growth, therefore, is contingent on disease persistence. In our study, at the threshold point in which more than 20% of clinically infected individuals die from disease, the relative effect of disease-induced mortality on the long-term population growth rate decreased and was surpassed by the force of infection.

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

The purpose of this study was not to deterministically predict how populations will be affected by recurring outbreaks, but to project how populations under current conditions may potentially be affected by recurring disease outbreaks. It is important to note that the current case study assessing the potential impacts of URTD in natural

gopher tortoise populations is completely heuristic in nature. Evidence for URTD-associated mortality is based primarily on associations between die-off events and seroprevalence, and no direct link has been established between URTD infection and gopher tortoise mortality (Sandmeier et al., 2009). However, such a link has always been notoriously difficult to confirm in wildlife disease systems (Plowright et al., 2008) and may be particularly challenging in long-lived species. Additionally, the mechanisms for URTD recrudescence in latently infected gopher tortoises remain undefined.

When little is known regarding the disease ecology of a host wildlife system, heuristic exercises like the one presented in this paper can provide a baseline understanding of outcomes arising from potential scenarios of disease dynamics, and help guide management actions. For example, the finding that population dynamics under normal conditions had a higher influence on  $\lambda$  than dynamics under outbreak conditions indicates that strategies aimed at improving demographic parameters, particularly survival rates, would promote the long-term growth and persistence of gopher tortoise populations regardless of disease emergence. Therefore, specific management strategies need not be specific to outbreak conditions. Additionally, given the relative importance of outbreak frequency on long-term population dynamics, educational programs that inform the public about the risks associated with the unauthorized (albeit well-intentioned) release of tortoises into established gopher tortoise populations are likely to be effective management actions to mitigate pathogen introductions and spread.

Our study served to provide a baseline description of potential outcomes arising from different scenarios of disease dynamics. The study could be refined by allowing

the force of infection to be density- or frequency-dependent, in which disease fade-out would be a more naturally governed process (Wilcox and Elder, 2003; Lloyd-Smith et al., 2005a). Theoretical and empirical studies addressing how environmental (i.e., hurricanes, drought) and anthropogenic factors (i.e., unauthorized relocations) can affect disease recrudescence at the individual level would improve our understanding of outbreak dynamics within natural populations. These data could help develop a more refined mechanistic explanation of recurring disease dynamics into host population viability analyses (Altizer et al., 2006).

Long-term surveillance will continue to be instrumental in measuring disease impacts on host demographic parameters such as survival and fecundity. In this study, we assumed that only mortality was affected by *M. agassizii* infection, and did not consider the impacts that long-term, chronic morbidity might have on populations. In fact, most mycoplasmal respiratory infections exhibit a high morbidity, low mortality profile and affect weight gain, ability to thrive, and in some cases, reproduction (Simecka et al., 1992; Minion, 2002). Although mortality is the most severe consequence of infectious diseases, it is often the most rare event; morbidity with substantive tissue damage is far more common but also more difficult to quantify (Wobeser, 2006). For example, the presence of antibody to *M. agassizii* is highly correlated with histological lesions and destruction of the normal respiratory architecture (Jacobson et al., 1991; McLaughlin et al., 2000), but the impacts of this level of tissue damage on critical biological functions are unknown.

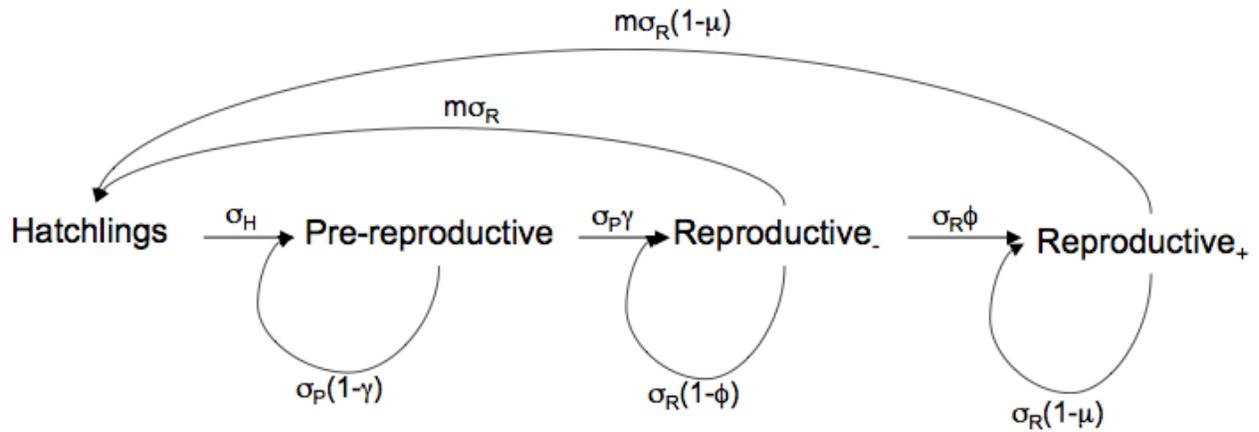
In this study we showed that the rate of outbreak recurrence in exposed populations and the level of disease-induced mortality of infected individuals could

adversely affect the rate of population decline and time to quasi-extinction, which warrants concern for the long-term persistence of gopher tortoise populations under the threat of recurrent disease outbreaks. Using mycoplasmal URTD within natural gopher tortoise populations as a case wildlife disease system, we demonstrated how chronic recurring disease outbreaks could be heuristically modeled within a host wildlife population using matrix population models and Markov chain models for temporally autocorrelated environments. The relative ease of implementation and straightforward interpretation of matrix population models (Caswell, 2001) have resulted in their extensive use for guiding management decisions for populations in peril. The approach we presented in this paper could be widely applied to a range of wildlife disease systems in which hosts suffer from persistent recurring diseases such as those caused by multi-host pathogens, pathogens that can maintain themselves subclinically within hosts but cause intermittent bouts of recrudescing clinical disease, or pathogens that can persist in the environment and cause re-infection of hosts. Clearly, further empirical and modeling studies on the long-term impacts of chronic diseases in natural populations are needed, especially in light of the increasing threat of disease in already imperiled host wildlife populations.

Table 2-1. Estimates of demographic parameters used for the parameterization of a baseline population projection matrix. Please refer to Appendix A for more details on the estimation of  $\sigma_P$ ,  $\sigma_R$ ,  $\gamma$ , and  $m$ .

Parameter	Symbol	Estimate	Variance
Hatchling survival	$\sigma_H$	0.127646	0.001425
Fecundity	$m$	0.495455	0.087356
Pre-reproductive survival	$\sigma_P$	0.388274	0.117469
Pre-reproductive growth	$\gamma$	0.001614	0.000076
Reproductive survival	$\sigma_R$	0.902545	0.005943
Force of infection	$\phi$	0.220000	0.001600

A



B

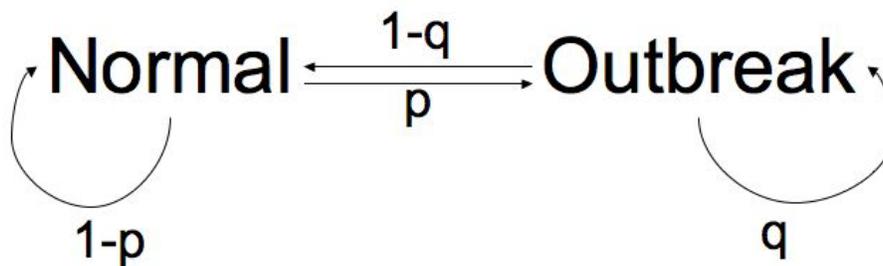


Figure 2-1. Conceptual models of (A) demographic processes, and (B) Markovian transitions for outbreak processes. A) Transition rates along the loops are defined according to parameters listed in Table 2-1. B) The Markov chain model parameters  $p$  and  $q$  are defined according to varying levels of outbreak duration and overall recrudescence.

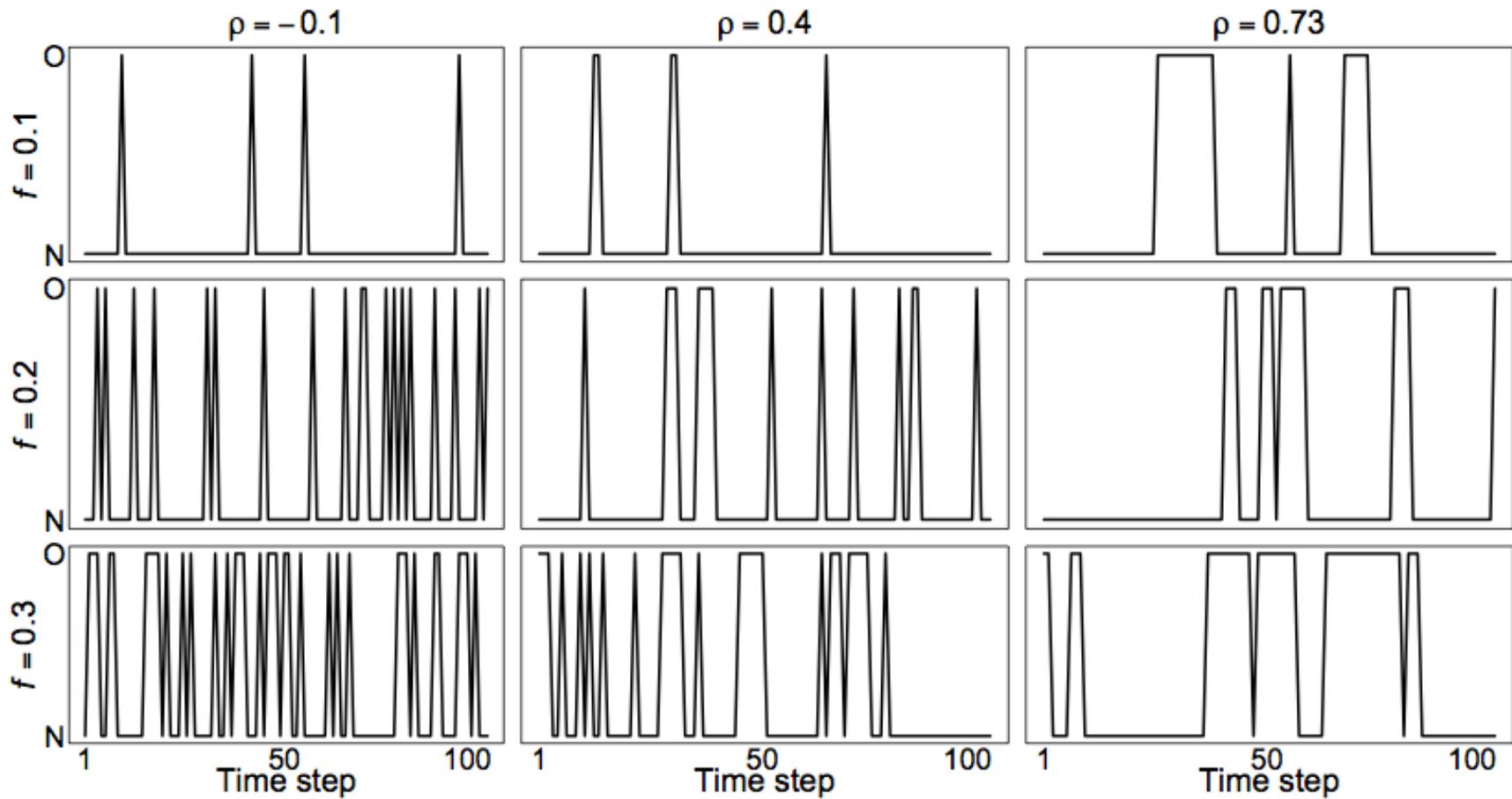


Figure 2-2. Outbreak scenarios simulated from two-state Markov chain models. Low intermediate, and high outbreak recrudescence scenarios were simulated in conjunction with low, intermediate, and high chronicity scenarios for 50,000 time steps; however, only the first 100 are displayed here. The two disease states, normal and outbreak, are labeled along the y-axis.

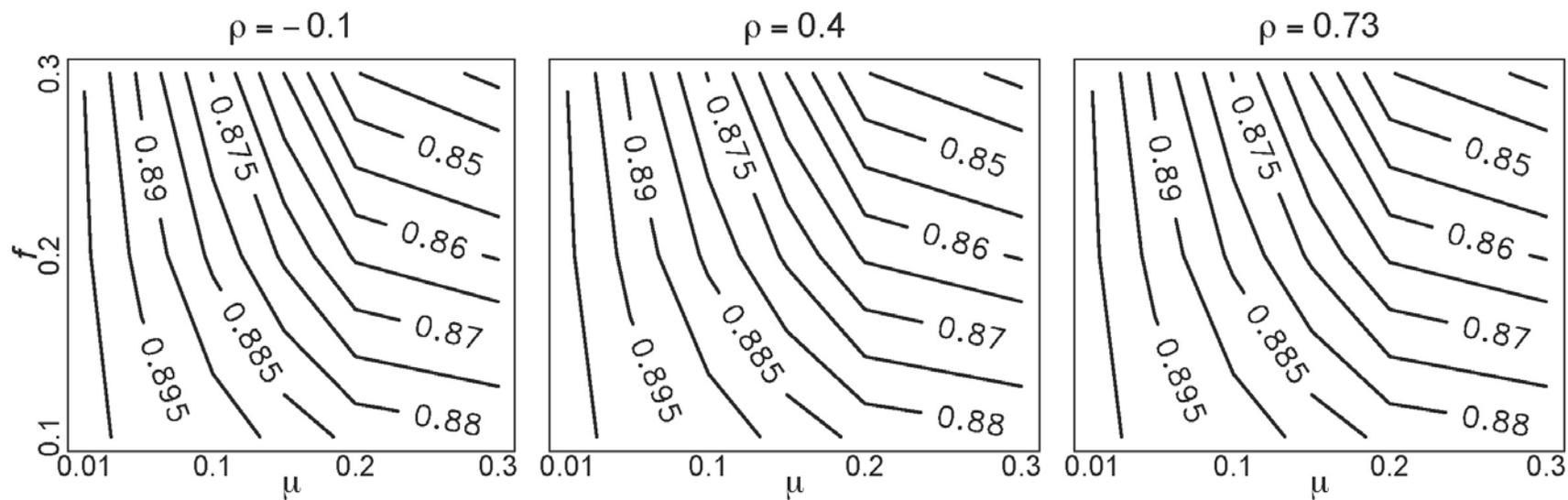


Figure 2-3. Contour plots of stochastic growth rates ( $\lambda_s$ ), calculated using Tuljapurkar's small noise approximation for autocorrelated environments, as a function of disease-induced mortality ( $\mu$ ) and outbreak recurrence ( $f$ ) at the three levels of outbreak duration ( $\rho$ ) considered in our study. Increases in  $\mu$  and  $f$  resulted in reduced levels of  $\lambda_s$ , whereas changes in  $\rho$  had little effect.

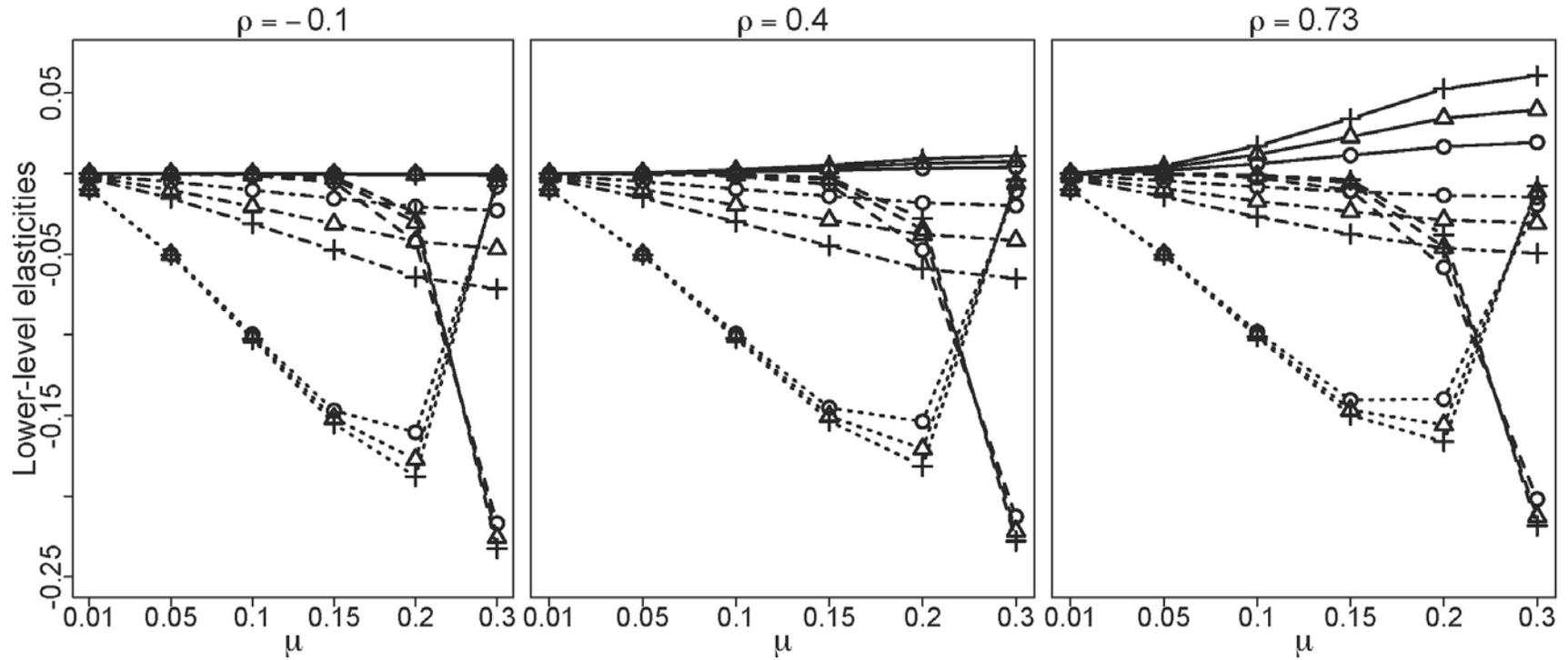


Figure 2-4. Elasticity of  $\lambda_S$  to changes in outbreak- and disease-associated parameters ( $f$ ,  $\rho$ ,  $\mu$ , and  $\phi$ ) calculated using the vec-permutation matrix approach. The three levels of  $f$  are represented through different symbols (circle:  $f = 0.1$ , triangle:  $f = 0.2$ , cross:  $f = 0.3$ ), and the four disease-associated parameters are represented by different line types (solid:  $\rho$ , dot-dash:  $f$ , dashed:  $\phi$ , dotted:  $\mu$ ). Across all levels of  $\rho$ , the parameters  $f$ ,  $\mu$ , and  $\phi$  exhibited negative effects on  $\lambda_S$ , while  $\rho$  provided for increasing positive effects.

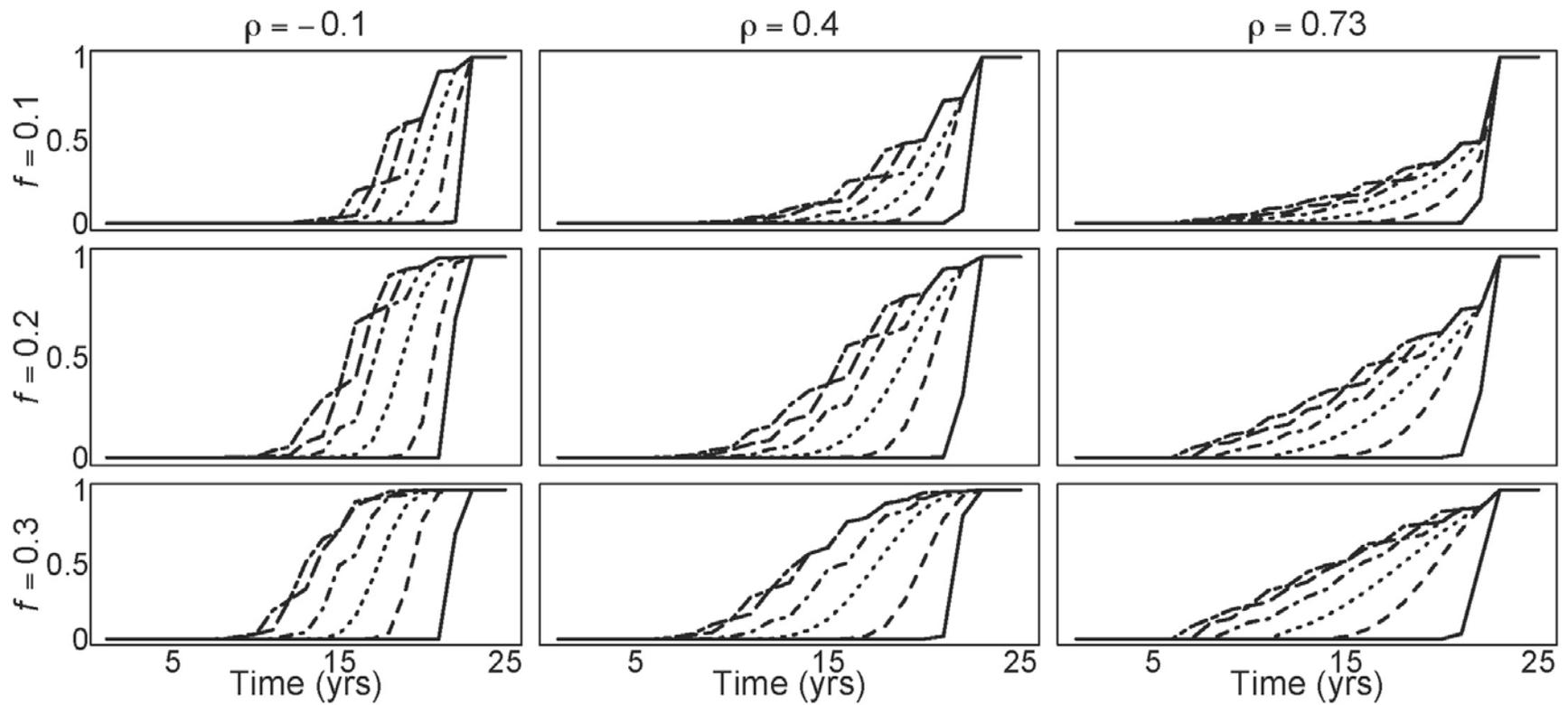


Figure 2-5. Mean cumulative distribution functions for quasi-extinction times at varying levels of outbreak duration ( $\rho$ ), overall outbreak frequency ( $f$ ), and disease-induced mortality ( $\mu$ ). Y-axes for all plots represent the probability of quasi-extinction. Different levels of disease-induced mortality are portrayed through the different line types within each plot (solid:  $\mu = 0.01$ , dashed:  $\mu = 0.05$ , dotted:  $\mu = 0.10$ , dot-dash:  $\mu = 0.15$ , long dash:  $\mu = 0.20$ , two-dash:  $\mu = 0.30$ ). Overall, quasi-extinction times declined as  $\mu$  and  $f$  increased, but slightly increased with  $\rho$ .

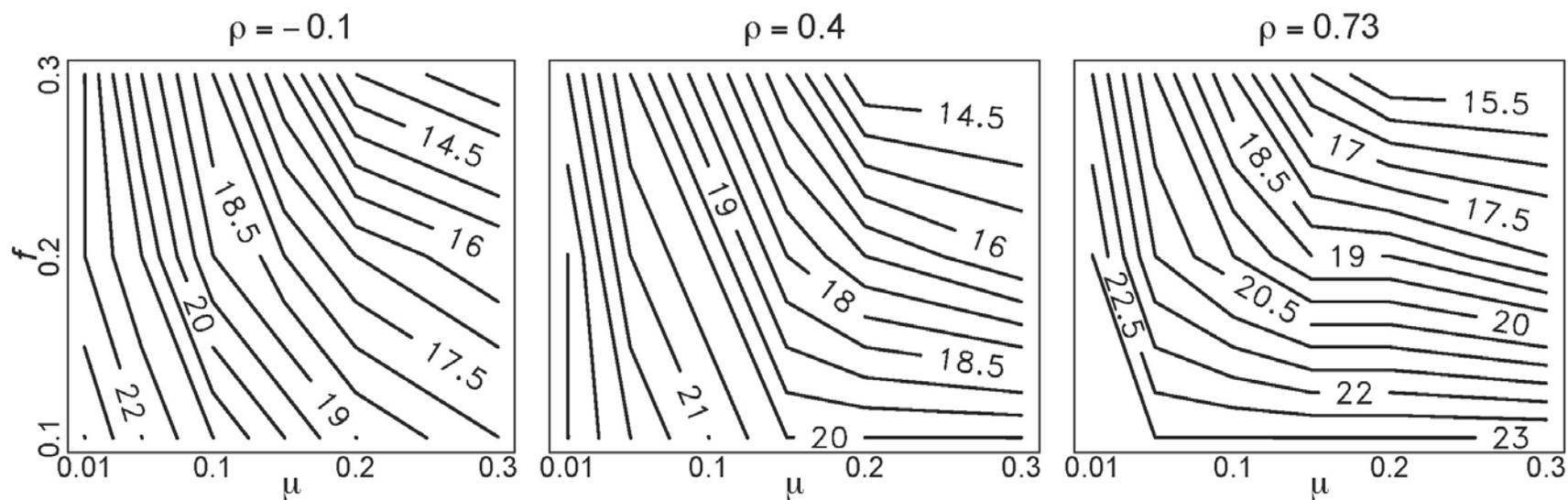


Figure 2-6. Contour plots of median quasi-extinction times ( $T_q$ ) as a function of disease-induced mortality ( $\mu$ ) and outbreak recurrence ( $f$ ) at the three levels of outbreak duration ( $\rho$ ) assessed. Increases in  $\mu$  and  $f$  reduced  $T_q$ , while increases in  $\rho$  improved  $T_q$ .

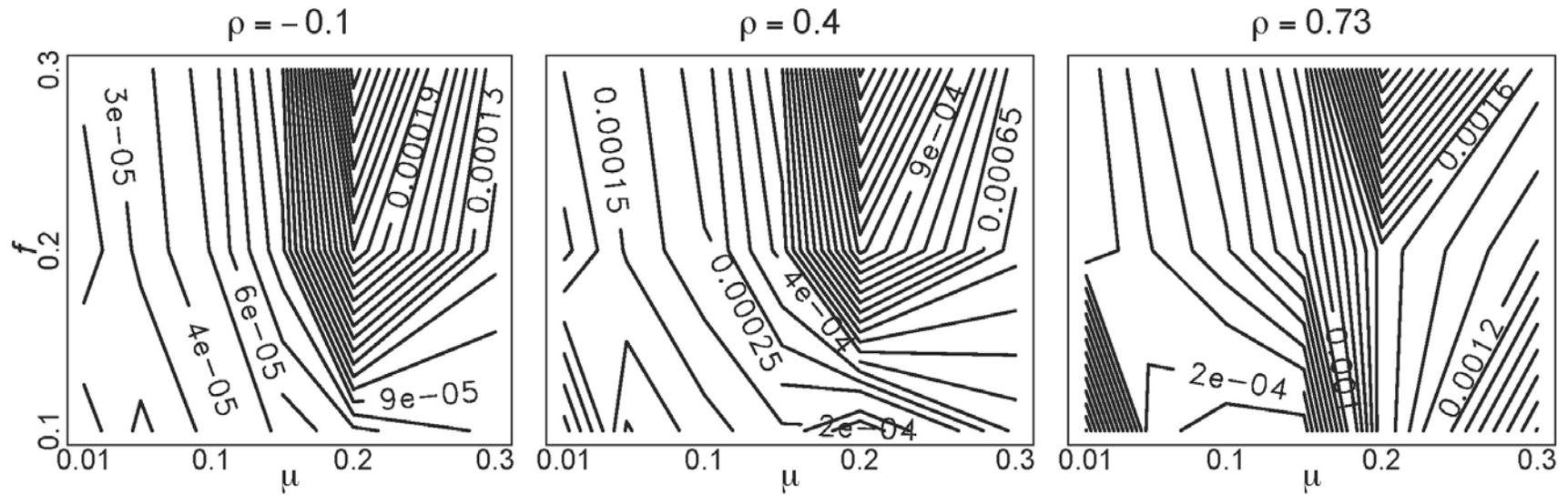


Figure 2-7. Relative effects of autocorrelation ( $W_2$ ) over interannual variability ( $W_1$ ) obtained through Tuljapurkar's small noise approximation. At most,  $W_2$  is only about 0.0058 times the magnitude of  $W_1$ . These very low relative effects support the finding that disease chronicity had little impact on the long-term population dynamics.

### CHAPTER 3

#### APPLICATION OF FORCE OF INFECTION MODELS TO ASSESS HYPOTHESES REGARDING STAGE-SPECIFIC DISEASE PROCESSES WITHIN A THREATENED WILDLIFE HOST POPULATION

Few wildlife disease studies have used seroprevalence data to test hypotheses related to disease dynamics. Using mycoplasmal upper respiratory tract disease within gopher tortoise populations as a study system, we applied force of infection models to age-seroprevalence data to evaluate competing hypotheses regarding stage-specific disease processes in natural host populations. Seven hypotheses, which differed in their assumptions of stage-specific processes of force of infection and disease-induced mortality, were generated to define potential mechanisms for observed disparities in seroprevalence between juveniles and adults. These hypotheses were expressed as a series of force of infection models, and assessed via Akaike's Information criterion corrected for small sample sizes (AICc) to determine which hypothesis was best supported by the data. The four models that included terms for stage-specific force of infection ( $\phi$ ) and/or disease-induced mortality ( $\mu$ ) were adequately supported by the data according to AICc metrics ( $\Delta\text{AICc} \leq 2.2$ ). These four best-fit models were further evaluated for biological plausibility by using model-derived estimates of  $\mu$  and  $\phi$  to project long-term population growth and persistence. Best-fit models that included terms for disease-induced mortality generated implausible estimates of population growth ( $\lambda$ ) and median time to quasi-extinction ( $T_{q50}$ , a measure of population persistence), and described a population collapse ( $\lambda = 0.467$ ,  $T_{q50} = 4$  yrs). Projected population dynamics associated with the model depicting negligible disease-induced mortality and stage-specific force of infection, however, were more realistic ( $\lambda = 0.903$ ,

$T_{q50} = 23$  yrs). Although this model (stage-specific  $\phi$ ,  $\mu = 0$ ) had the lowest AICc rank among best-fit force of infection models, it was associated with the highest degree of biological relevance and validity because inferences made from parameter estimates were most realistic and plausible. Therefore, after incorporating measures of biological realism into our assessment of competing hypotheses, we found that stage-specific disparities in seroprevalence were best explained by differences in incidence rather than disease-induced mortality. This study demonstrates and promotes the utility of an information-theoretic approach for elucidation of cryptic processes in wildlife disease research where age-seroprevalence data are available.

### **Introduction**

Serological surveillance studies are essential for providing baseline descriptions of pathogen exposure within host populations; however, more involved analyses are required to elucidate the mechanisms driving disease processes within a host wildlife system. Unfortunately, quantitative measures of disease processes such as force of infection and disease-induced mortality are notoriously difficult to estimate directly from field data, and preferably require the implementation of time-intensive longitudinal studies to quantify (Martin et al., 1987). Such prospective studies are generally carried out within the framework of capture-mark-recapture studies in wildlife systems. Although some studies have been successful at defining epizootiological processes such as the force of infection through the use of capture-mark-recapture (CMR) methods (Senar and Conroy, 2004; Briggs et al., 2005; Lachish et al., 2007; Ozgul et al., 2009), the logistic feasibility of intensive longitudinal surveillance programs compromises their application within many natural wildlife systems. Additionally, other

disease-associated processes such as the rate of disease-induced mortality often remain undetectable due to factors that preclude the analysis of fresh carcasses, such as removal by predators or decomposition between sampling periods. Thus, the impact of disease-induced mortality remains poorly quantified in many wildlife systems.

Information-theoretic approaches have been used extensively in ecological studies as a means of quantitatively assessing the support of competing hypotheses given observed patterns from field data (Hilborn and Mangel, 1997; Burnham and Anderson, 2002). Their application within the field of wildlife disease, however, has been more limited. Plausible mechanisms of disease processes may be evaluated through the use of an information-theoretic approach in which several hypotheses that can potentially explain observed disease patterns within a population are formulated and then evaluated in terms of their relative fits to the data. The hypothesis associated with the model which best fits the data, according to some predefined model selection criterion, is then given the highest degree of support among candidate models, and thus competing hypotheses. This approach is especially useful within the framework of wildlife disease research, where observational studies within natural systems are common and unobservable mechanisms of disease dynamics are of interest to researchers.

Although a longitudinal framework involving time to seroconversion data is the preferred means of quantifying force of infection within epidemiological studies, many important inferences may still be drawn from cross-sectional age-specific seroprevalence data. Recent studies have developed methods to estimate cryptic disease processes such as force of infection and disease-induced mortality using age-

specific seroprevalence data. Force of infection models may be fit to age-seroprevalence data in order to estimate epidemiological parameters of interest (Woolhouse, 1989; Hudson and Dobson, 1997; Caley and Hone, 2002), or to test hypotheses regarding disease dynamics within host populations (Caley and Hone 2002). Therefore, in addition to providing a static description of pathogen exposure within host wildlife populations, age-specific seroprevalence data can also be used to quantitatively estimate dynamic disease processes and to elucidate potential mechanisms driving observed seroprevalence patterns. As many wildlife disease studies are likely to collect this type of data, this approach would prove to be useful when little is known about cryptic processes such as disease-induced mortality within natural host populations. With the present study we demonstrate the utility of such an approach by applying force of infection models to age-seroprevalence data to test hypotheses pertaining to stage-specific force of infection and disease-induced mortality in threatened gopher tortoise populations exposed to *Mycoplasma agassizii*.

*Mycoplasma agassizii* was first discovered as an etiologic agent of upper respiratory tract disease (URTD) in free-ranging desert tortoises (*Gopherus agassizii*) (Jacobson et al., 1991; Brown et al., 1994). Most commonly, mycoplasmas are associated with clinically silent infections (i.e. limited overt signs of disease) but the microbes are pathogenic and cause lesions and tissue damage throughout the upper respiratory tract (Simecka et al., 1992; Minion, 2002). Overt clinical signs associated with mycoplasmal URTD include ocular and nasal discharge and palpebral edema (Schumacher et al., 1997), however, many tortoises remain subclinically and chronically

infected and recrudescence to a clinical disease state under situations of elevated stress or through re-exposure by infectious contacts (Brown et al., 1994).

Little is known regarding the effects of URTD caused by *M. agassizii* on gopher tortoise populations (Florida Fish and Wildlife Conservation Commission 2007). Despite the lack of a direct link between mycoplasmal URTD and mortality, several observational studies have implicated URTD as a serious threat to tortoise survival. A mass mortality event discovered outside of Brooksville, FL (Oldenburg Mitigation Park) was speculated to be associated with URTD because exposure to *M. agassizii* had been documented at that population, and moribund tortoises were observed exhibiting clinical signs of disease upon discovery of the die-off event (J. Berish, *pers. comm.*). Seigel et al. (2003) attributed another high mortality event near Cape Canaveral, FL to URTD due to the increase in presentation of clinical disease that occurred concurrently with the increased detection of dead tortoises. Surveillance of a northern Florida population found that the number of skeletal remains and dead tortoises increased following exposure to *M. agassizii* (Wendland, 2007). Moreover, Ozgul et al. (2008) found that the apparent survival of adult tortoises in sites with high seroprevalence of *M. agassizii* was lower than that of tortoises in low seroprevalence sites, and that the number of carcasses recovered within a given population increased as a function of seroprevalence.

Preliminary analysis of seroprevalence data has demonstrated significant stage-specific differences in *M. agassizii* prevalence between pre-reproductive juveniles and reproductive adults. Apparent exposure (i.e., seropositivity) to *M. agassizii* has primarily involved sexually mature adults, whereas the prevalence of *M. agassizii* infections in

pre-reproductive individuals is more limited (Wendland, 2007). With this study we test several hypotheses that can explain the observed phenomenon: (H1) Juveniles die upon infection, (H2) Juveniles become infected at a slower rate than adults and disease-induced mortality is negligible, and (H3) Juveniles simply do not become infected. The purpose of this study was to quantitatively express the three hypotheses above through the use of force of infection models, and determine which hypothesis best describes the observed patterns of low juvenile infection rates. Using mycoplasmal upper respiratory tract disease within gopher tortoise populations as a study system, we demonstrate how age-seroprevalence models can be used to evaluate competing hypotheses pertaining to stage-specific disease processes in natural populations.

## Methods

### Model Formulation

Models were generated from hypotheses explaining observed seroprevalence patterns, and compared to each other to determine which fit the observed patterns best (Anderson et al., 2001). It is important to note that all models are likely to have different inconsistencies with the data. Therefore, rather than defining an absolute true representation of a given study system, competing models provide alternative representations with varying degrees of support (Hilborn and Mangel, 1997). In other words, candidate models are assessed relative to one another. For the present study, force of infection models were formulated to describe apparent prevalence for each hypothesis under question.

**Hypothesis 1: Juveniles die upon *M. agassizii* infection.** Limited data are available on the effect of *M. agassizii* infection on survival, or whether disease-induced mortality is a significant force acting on gopher tortoise populations. This hypothesis

states that infected juveniles experience acute disease-induced mortality. Specifically, it predicts that infected juveniles are not observed because they die upon infection.

Therefore, the apparent seroprevalence of the pre-reproductive stage class would be biased towards seronegative individuals, as most seropositive individuals would have died acutely after infection (Heisey et al., 2006). We define disease-induced mortality as the increase in mortality that results from *M. agassizii* infection relative to the baseline mortality rate of tortoises that remain unexposed. The model used to describe this hypothesis was adapted from Heisey et al. (2006):

$$v(t) = 1 - \frac{S_{\phi}(0, t)}{S_{\phi}(0, t) + \int_0^t f_{\phi}(0, w) S_{\mu}(w, t) dw}, \quad (3-1)$$

where apparent size-specific prevalence ( $v(t)$ ) is defined by size-specific hazard functions for infection ( $\phi$ ) and disease-induced mortality ( $\mu$ ). Specifically, the numerator of the above model represents the probability of surviving to carapace length  $t$  and remaining negative, and the denominator represents the probability of becoming infected between stages  $t_{i-1}$  and  $t_i$ , and surviving to carapace length  $t$ .  $S_{\phi}(0, t)$  represents the survival model corresponding to an underlying size-specific force of infection function from birth to carapace length  $t$ ,  $f_{\phi}(0, w)$  represents the probability density function of the carapace length at infection ( $w$ ), and  $S_{\mu}(w, t)$  represents the survival model corresponding to an underlying disease-induced mortality hazard function from carapace length at infection  $w$  to carapace length  $t$ . For more details on the survival functions and corresponding hazard functions, see Heisey et al. (2006).

Four sub-models were used to represent the importance of disease-induced mortality to the observed seroprevalence patterns (H1a-H1d). H1a assumes a constant

force of infection for adults and juveniles ( $\phi_A = \phi_J$ ), with the discrepancy in seroprevalence related only to an increased susceptibility of disease-induced mortality for juveniles ( $\mu_J \neq \mu_A$ ). H1b assumes that both disease-induced mortality and force of infection are stage-specific ( $\mu_J \neq \mu_A$  and  $\phi_A \neq \phi_J$ ). H1c assumes that both disease-induced mortality and force of infection are constant across stage classes ( $\mu_J = \mu_A$  and  $\phi_A = \phi_J$ ). H1d assumes that while disease-induced mortality is constant across stage classes, force of infection is stage-specific ( $\mu_J = \mu_A$  and  $\phi_A \neq \phi_J$ ). In order to compare the fits of H1a-H1d, these models were re-parameterized to include difference terms for  $\phi$  and  $\mu$  between juveniles and adults. In other words, instead of obtaining maximum likelihood estimates for  $\mu_A$  and  $\phi_A$ , the parameters  $\Delta\mu$  and  $\Delta\phi$  were estimated, where  $\Delta\phi = \phi_A - \phi_J$ , and  $\Delta\mu = \mu_A - \mu_J$ . Through this parameterization, adult-specific rates of force of infection ( $\phi_A$ ) and disease-induced mortality ( $\mu_A$ ) were estimated relative to the corresponding juvenile-specific rates. For H1a,  $\Delta\phi$  was fixed at 0. For H1c, both  $\Delta\phi$  and  $\Delta\mu$  were fixed at 0. For H1d,  $\Delta\mu$  was fixed at 0.

**Hypothesis 2: Juveniles become infected at a lower rate than adults.** This hypothesis (H2) states that reproductive adults become infected at a higher rate than juveniles, possibly through some mechanism associated with behavior. The model expressing this hypothesis assumes a stage-specific force of infection for juveniles and adults ( $\phi_A \neq \phi_J$ ), and is therefore expressed as a 2-stage exponential model:

$$v(t) = \begin{cases} 1 - e^{-\phi_J t}, & t < A \\ 1 - e^{-(\phi_J - \phi_A)A - \phi_A t}, & t \geq A \end{cases}, \quad (3-2)$$

where  $t$  represents a vector of carapace lengths from captured tortoises,  $\phi_i$  is the force of infection for stage  $i$  to be estimated by maximum likelihood methods, and  $A$  is

the size at sexual maturity which separates pre-reproductive juveniles from reproductive adults (220 mm). This model assumes an inconsequential effect of disease-induced mortality on both juveniles and adults ( $\mu_J = \mu_A = 0$ ).

**Hypothesis 3: The rate at which juveniles acquire infection is negligible.** This hypothesis (H3) predicts that pre-reproductive juveniles simply do not encounter infectious individuals, and thus have no opportunity to be infected. Therefore, the model representing this hypothesis generates a curve that reaches nonzero force of infection values at sexual maturity or 220 mm carapace length ( $\phi_J = 0$ , and  $\phi_A > 0$ ). The model for this hypothesis is the same 2-stage catalytic model used in H2 (Eq. 3-2), with  $\phi_J$  fixed at 0.

Additionally, a model describing constant force of infection with no disease-induced mortality (H0:  $1 - e^{-\phi t}$ ) was fit to the data. The fit of the above force of infection models to the observed age-seroprevalence data was assessed through Akaike's Information Criteria corrected for small sample sizes (Burnham and Anderson, 2002). Model parameters were estimated through maximum likelihood methods using the *bbmle* package in R programming language (Bolker 2007). Standard errors of model parameters were obtained by inverting the Hessian matrix at maximum likelihood parameter values, and 95% confidence intervals were calculated by quadratic approximation.

### **Assessment of Biological Realism**

It is important to note that none of the models evaluated represents the one true explanation behind the observed age-seroprevalence data; however, credibility lies with “which models are more consistent and which ones meet the challenges of new

experiments and new data better” (Hilborn and Mangel 1997). For this reason, models that received high degrees of support (according to AICc ranks) were further evaluated according to the biological validity of respective model inferences.

Parameters estimated from the above models represented rates based on the time required to grow 1 mm in carapace length. For slow-growing species such as the gopher tortoise, the implications of such rates provide limited insights. Thus, translation of these model-derived parameter estimates into annual probabilities would better illustrate the implications of model predictions than incidence and mortality rates based on a time equivalent of 1 mm carapace growth. Model parameters were converted to annual probabilities using the equation  $1 - \exp(-\beta_i t_i)$ , where  $\beta_i$  represents a stage-specific rate (i.e.,  $\mu_i$  or  $\phi_i$ ) obtained from a force of infection model, and  $t_i$  represents the average stage-specific change in carapace length that occurs in one year. The value for  $t_i$  was obtained by fitting a von Bertalanffy growth model to age-size data from the study population, with plastral scute annuli used to represent the age of an individual in years (Germano, 1988; Mushinsky et al., 1994; Berry, 2002; Wilson and Tracy, 2003; see Appendix F). The average instantaneous annual growth rates for each stage class were used to define  $t_i$ .

Furthermore, in order to address the biological plausibility of the best-fit models, we applied their parameter estimates (converted from rates to annual probabilities) to a metapopulation projection model (Hunter and Caswell, 2005). Under this metapopulation framework, the population was divided into seronegative and seropositive sub-populations (or “patches”). Individuals moved between patches, which corresponded to seronegative (i.e., unexposed to *M. agassizii*) and seropositive (i.e.,

exposed to *M. agassizii*) states (Fig. 3-1). Within each patch (i.e., seronegative and seropositive), each subpopulation grew according to distinct population dynamics governed by stage-specific survival, growth, and fecundity rates. Demography within each patch was described by population projection matrices specific to seronegative and seropositive demographic dynamics, respectively:

$$\mathbf{B} = \begin{bmatrix} B_{Seronegative} & \\ & B_{Seropositive} \end{bmatrix},$$

where each diagonal matrix of  $\mathbf{B}$  represented a 3-stage population projection matrix of the form:

$$B_i = \begin{bmatrix} 0 & 0 & \sigma_A(1-\mu_A)m \\ \sigma_H & \sigma_J(1-\mu_J)(1-\gamma) & 0 \\ 0 & \sigma_J(1-\mu_J)\gamma & \sigma_A(1-\mu_A) \end{bmatrix},$$

in which  $\sigma_i$  represents stage-specific survival probabilities,  $\gamma$  represents the probability of transition from the pre-reproductive juvenile stage class to the reproductive adult stage class,  $m$  represents fecundity, and  $\mu_i$  represents a stage-specific disease-induced mortality probability obtained from a force of infection model. The diagonal matrices of  $\mathbf{B}$ , which represented the demographic dynamics of seropositive and seronegative sub-populations, differed only by the term  $\mu_i$ , which was fixed at 0 for  $B_{Seronegative}$ . In other words, the population dynamics of the seronegative sub-population was not influenced by disease-induced mortality. Only seropositive sub-populations experienced lowered survival due to disease-induced mortality. Estimates for  $\sigma_i$  were obtained from a previous capture-mark-recapture field study. An estimate of  $m$  was obtained from field-based radiographs of adult females, and an estimate of  $\gamma$  was

obtained by fitting a von Bertalanffy growth curve to age and carapace length data (Appendix A)<sup>1</sup>.  $\mu_i$  was estimated from competing force of infection models.

Dispersal between patches (i.e., seroconversion) was defined according to transition matrices parameterized by the stage-specific infection probabilities obtained from best-fit models.

$$\mathbf{M} = \begin{bmatrix} M_H & 0 & 0 \\ 0 & M_J & 0 \\ 0 & 0 & M_A \end{bmatrix},$$

where each diagonal matrix of  $\mathbf{M}$  represented a 2-state transition matrix of the form:

$$M_i = \begin{bmatrix} 1-\phi & 0 \\ \phi & 1 \end{bmatrix},$$

in which  $\phi_i$  represents a stage-specific infection probability obtained from a force of infection model. Note that according to this seroconversion matrix, once an individual seroconverts from negative to positive status, it remains in the seropositive state (i.e., the probability of a seropositive individual remaining within the seropositive state is 1.0). Additionally, because the hatchling stage class was not included within the study population in the assessment of force of infection models, we assumed  $\phi_H = 0$ .

Demography was assumed to occur after dispersal (i.e., seroconversion) so that the stage-specific infection probability ( $\phi$ ) and stage-specific disease-induced mortality probability ( $\mu$ ) would dictate demographic processes such as stage-specific survival and growth. The metapopulation projection matrix was then calculated as  $\mathbf{A} = \mathbf{B}\mathbf{V}'\mathbf{M}\mathbf{V}'$ ,

---

<sup>1</sup>In this chapter we refer to the pre-reproductive and reproductive stage classes as juvenile and adult, respectively. Therefore, upon referencing the parameterization of  $\mathbf{B}$ , note that the terms  $\sigma_A$  and  $\sigma_J$ , used in Chapter 3, are equivalent to  $\sigma_R$  and  $\sigma_P$  in Appendix A, respectively.

where **B** represents the block-diagonal demography matrix, **M** is the block-diagonal dispersal matrix, and **V** is the vec-permutation matrix (Hunter and Caswell, 2005). Long-term population growth rate ( $\lambda$ ) was calculated as the dominant eigenvalue of the metapopulation projection matrix **A**. Time to quasi-extinction ( $T_q$ ) was calculated using a population threshold of 0.1 (or 10% the initial population size) through diffusion approximation (Caswell, 2001).

### **Data Collection**

Data were collected from May-October between 2003-2006 at Branan Field Mitigation Park (CF) along the outskirts of Jacksonville, FL. The study population was selected for its documented history of *M. agassizii* exposure (Wendland et al., in press). Historical data of URTD within the study population were available since 1997, at which point the population was considered “healthy” and unexposed (Epperson 1997). In 2000, concomitant with the development and occupancy of neighboring residential plots, the unauthorized release of tortoises onto the site is suspected to have introduced *M. agassizii* into the population. The population, which had previously shown no clinical signs associated with URTD, exhibited higher levels of morbidity and mortality after the introduction of *M. agassizii*.

Animal capture and handling protocols were fully approved by the University of Florida Institutional Animal Care and Use Committee. Bucket traps were placed at the mouth of burrows randomly selected according to size class and activity status, and traps were checked daily for captures. Holes were drilled in all buckets for water drainage, and shade covers made out of vinyl siding were placed over the traps to protect the tortoises from overheating. Blood samples and nasal flushes were collected

from each captured tortoise for determination of infection status by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR; Schumacher et al., 1993; Wendland et al., 2007). Morphometric measurements and complete health assessments were performed on each tortoise (Berry and Christopher, 2001). Tortoises were sexed according to degree of plastral concavity, and aged according to the count of plastral rings when discernible (Germano, 1988; Mushinsky et al., 1994). The ELISA protocol used in this study was designed and validated for detection of specific antibody to *M. agassizii* in gopher and desert tortoises. The ELISA exhibits a sensitivity of 98%, and a specificity of 99%. A cut-off value of  $\geq 64$  was used to define a seropositive titer because it results in the highest combined sensitivity and specificity, and thus the most accurate diagnosis of individuals (Wendland et al., 2007).

For the purposes of this study, carapace length was used as a surrogate for age, since the accurate aging of tortoises through plastral ring counts was made difficult in many cases where rings were completely worn and indiscernable (Germano, 1988; Germano, 1992; Mushinsky et al., 1994). Tortoises were categorized into two stage-classes: juveniles (pre-reproductive) and adults (reproductive), based on carapace length. Juveniles were defined by carapace lengths  $< 220$  mm, and adults were defined by carapace lengths  $\geq 220$  mm. This cut-off point was obtained from fecundity data collected from 11 gopher tortoise populations throughout Florida between 2003 and 2006. The smallest gravid female captured throughout these sampling periods was 222 mm in carapace length (Wendland 2007); therefore, 220 mm was selected as the cut-off point for sexual maturity in this analysis to minimize the misclassification of adults.

## Results

A total of 279 tortoise blood samples was collected between 2003 and 2006. Only 250 samples were used for this analysis because diagnostic tests for samples that provided suspect results (Ab titer = 32; n = 29) were considered inconclusive and were omitted from the analysis. Of the 250 samples included in the study, 73.4% were ELISA-positive for *M. agassizii*. Approximately 20% of the total sample population consisted of juveniles, 34.4% were adult females, and 44.8% were adult males. Seroprevalence fluctuated among years, and most notably between 2004 and 2005 ( $\Delta = -15\%$ ); however the lower seroprevalence of *M. agassizii* within the study population during the 2005 (68%) and 2006 (73%) sampling periods is likely due to the more intensive sampling of pre-reproductive juveniles throughout those years, as stage-specific prevalence patterns remained stable across all years (Fig. 3-2). Censuses conducted during 2004-2006 using total burrow counts demonstrated that the population structure and density have remained fairly stable at the site. During the 2004-2006 sampling periods approximately 40% of the population consisted of juveniles, as indicated by active burrow width measurements, with adults comprising the remaining 60% (Wooding, unpublished data).

Two models were substantially supported by the data. H1a (stage-specific disease-induced mortality with constant force of infection) and H1d (constant disease-induced mortality with stage-specific force of infection) had the lowest AICc of all models fitted (AICc = 207.3; Table 3-1). The two were identical to each other regarding their fits to the data, as indicated by model weights and  $\Delta$ AICc values (Table 3-1, Fig. 3-3; evidence ratio = 1.0). H1b and H2 had adequate support ( $\Delta$ AICc = 2.1 and 2.2,

respectively), while all other models (H0, H3, and H1c) fit the data poorly ( $\Delta\text{AICc} > 10$ ; Table 3-1). Because H1a and H1d provided the overall best fits to the data, and were virtually indistinguishable according to AICc values and model weights, model-averaged estimates of parameter values were generated (Burnham and Anderson, 2002). These model-averaged (H1ad) parameter estimates are listed along with the parameter estimates from the four best fit models (H1a, H1b, H1d, and H2) in Table 3-2.

Force of infection ( $\phi$ ) and disease-induced mortality ( $\mu$ ) rates obtained from H1 models were higher than expected. The survival ratios, given by  $e^{-\mu}$ , for seropositive individuals relative to seronegative individuals were 0.591 and 0.828 for juveniles and adults under H1ad. This equates to a 41% decrease in survival due to *M. agassizii* infection for juveniles, and a 17% decrease for adults.

Upon fitting a von Bertalanffy growth model to scute annuli and carapace length data, the average growth rate for juvenile pre-reproductive tortoises (CL < 220 mm) was approximately 15.4 mm CL/year (95%CI: 10.3 – 23.1). The average growth rate of reproductive adults (CL  $\geq$  220 mm) was approximately 3.70 mm CL/year (95%CI: 1.57 – 8.69). The annual probabilities of infection for juveniles and adults under H1ad were 0.893 (95%CI: 0.695 – 1.00) and 0.483 (95%CI: 0.293 – 0.795), respectively. Under H2, the annual probabilities of infection for juveniles and adults were 0.040 (95%CI: 0.0392 – 0.142) and 0.156 (95%CI: 0.142 – 0.171), respectively. Additionally, under H1ad the annual probabilities of disease-induced mortality were 1.00 (95%CI: 0.433 – 1.00) and 0.503 (95%CI: 0.0605 – 1.00) for juveniles and adults, respectively.

The long-term metapopulation growth rate ( $\lambda$ ) generated by H1ad parameters was 0.467 (95%CI: 0.129 – 1.684), and the median time to quasi-extinction was 4 years

(range: 1 – 19). H2 parameters, which omitted terms for disease-induced mortality, estimated  $\lambda$  at 0.903 (95%CI: 0.764 – 1.07), and median quasi-extinction time at 23 years (range: 11 – 48; Fig. 3-4).

## Discussion

Our analyses suggest that stage-specific processes are driving observed seroprevalence patterns within exposed populations. The null models (H1c and H0), which assume that disease-induced mortality ( $\mu$ ) and/or force of infection ( $\phi$ ) are constant across stages, were very poor fits to the data. Omission of  $\mu$  from models resulted in lower  $\phi$  estimates, with H1 models providing much higher  $\phi$  estimates than H2. Caley and Hone (2002) noted that the effect of ignoring  $\mu$  was to lower  $\phi$  significantly. Results from this analysis corroborate this finding, with models assuming negligible  $\mu$  (H2, H3, and H0) providing much lower estimates of force of infection than models assuming sizeable  $\mu$ .

The AICc ranks and combined model weights of H1a, H1d, and H1b support the hypotheses that include  $\mu$  as an important component of the observed patterns of seroprevalence, and imply that stage-specific  $\phi$  alone is not sufficient to describe underlying disease processes associated with this observed pattern. In other words, the highest degree of support was provided to models that represented the general hypothesis of disease-induced mortality as an important force acting on a population exposed to *M. agassizii*. Of the models that assumed stage-specific  $\phi$  (H1b, H1d, H2), only H1b provided maximum likelihood estimates of  $\phi$  in which juveniles acquire infection at a faster rate than adults. However, it is important to note that the precision of parameter estimates associated with H1b was poor, and therefore that the reliability

and validity of these estimates was questionable. Interestingly, of the models that assumed stage-specific  $\mu$  (H1a, H1b), both provided estimates of  $\mu$  in which juveniles consistently died from disease at a faster rate than adults. These findings imply that if indeed stage-specific processes are acting on both,  $\phi$  and  $\mu$ , then it is most likely that juveniles acquire infection at a slower rate than adults but die from disease at much higher rates. A higher susceptibility to disease-induced mortality has been previously observed in exposed desert tortoise hatchlings (*Gopherus agassizii*), whereby infected hatchlings suffer from severe URTD and consequential disease-induced mortality (Ofstedal et al., 1996).

In addition to using AICc values to rank the support of competing models according to observed seroprevalence data, we also assessed the biological realism of top-ranked models as a further means of challenging their validity. Although the inclusion of a term for  $\mu$  led to better model fits and was most highly supported by AICc metrics, the biological relevance of these model outputs was suspect. Standard errors for H1 parameter estimates were much larger than those for H2. In fact, we discounted H1b altogether as a plausible mechanism to describe observed seroprevalence patterns due to the low reliability of such large and non-informative standard errors associated with the parameter estimates of this model. Additionally, estimated rates of  $\mu$  from H1ad imply that juveniles die from disease at about 4 times the rate at which they acquire infection. Given that H1ad predicted that susceptible juveniles have a high annual probability of becoming infected (0.798, 95CI: 0.683, 0.932), this translates into a 100% chance of dying once infected (1.00, 95CI: 0.433 – 1.00). This seems excessively high for the system under study, and describes a population on the brink of

local extinction. A long-term host population growth rate ( $\lambda$ ) of 0.467, as projected by H1ad, implies a 57% annual population decline, and is unreasonably low for the study population. Likewise, H1ad projected a median time to quasi-extinction ( $T_{q50}$ ) of 4 years, which also represents an unreasonably short time frame within which the population is expected to persist before declining to one-tenth its initial size. Population projections associated with H1ad most certainly describe the impending collapse of the study population, but this is unlikely to occur within natural populations and has not been corroborated by annual population surveys.

A direct estimate of disease-induced mortality is difficult to obtain from field studies, and although many skeletal remains have been collected from the study population since the introduction of *M. agassizii* in 2000 ( $n = 24$ ), population size and structure have remained fairly stable across years of sampling (Wooding, unpublished data). According to burrow composition data obtained from population estimates, the size/age structure of the population has not changed significantly between 2004 and 2006. Roughly 40% of the burrows surveyed throughout the sample periods belonged to juveniles, and the collapse of the juvenile subpopulation does not appear to be imminent. Furthermore, when disease-induced mortality is considered to be a sizeable force acting on a host population, a decline in seroprevalence through sample periods can be attributed to the death of infected individuals (Gauthier, Latour et al. 2008); however, stage-specific seroprevalence did not decline across sampling years in our study population. According to top-ranked force of infection models, in order for disease-induced mortality to be considered a plausible mechanism driving the observed stage-specific disparities in exposure, the rates at which individuals are expected to

acquire infection and die from disease would be too high to allow for the persistence of exposed populations. The lack of biological plausibility associated with this assumption, therefore, reduces the support and validity of these models as accurate representations of host population processes.

Age-seroprevalence data provided adequate support, according to AICc values, for the hypothesis that juveniles acquire infection at a slower rate than adults and death from disease is negligible (H2). Additionally the degree of support for H2 as the most plausible mechanism for observed seroprevalence patterns was advanced by the validity of model inferences associated with H2.  $\lambda$  and  $T_{q50}$  were much more realistic using  $\phi$  estimated from H2, and assuming negligible  $\mu$ . Under these estimates an exposed population was expected to decline by approximately 10% annually, and hit its quasi-extinction threshold in roughly 23 years. Although these metrics still describe a population in decline, the rate of decline is more plausible than that associated with H1ad. Currently from this study, therefore, H2 provided the most biologically relevant and plausible description of epizootiological processes experienced by an exposed population.

Although more credibility was assigned to the hypothesis that disease-induced mortality was negligible and that juveniles simply acquire infection at a lower rate than adults, the potential for low rates of disease-induced mortality should not be overlooked. In a previous study that sought to estimate the annual survivorship of adults, researchers found that the apparent survival of individuals within high seroprevalence sites was roughly 5% lower than that of individuals within low seroprevalence sites. Likewise the incidence of recovered carcasses also increased as a function of site

seroprevalence (Ozgul et al., 2009). Interestingly, results provided by Ozgul et al. (2009) are more consistent with the implications of H2 than with H1ad. Specifically, Ozgul et al. (2009) estimated the annual infection probability for adults within exposed populations at 0.22 (95CI: 0.17 – 0.25), a value similar to that estimated from H2 (0.16; 95CI: 0.043 – 0.57). H1ad, however, provided an estimate of this same infection probability at 0.48 (95CI: 0.29 – 0.80). Additionally, the assumption of negligible disease-induced mortality according to H2 better corresponded with the 5% reduction in survival due to disease discussed by Ozgul et al. (2009) than the inflated probability for disease-induced mortality provided by H1ad (50%). Although H2 provided the most plausible explanation of observed seroprevalence patterns among the set of candidate models tested, the potential for disease-induced mortality to exist within exposed populations should not be disregarded completely given past research findings (Ozgul et al., 2009). The greater validity of H2 among other candidate models merely suggests that if disease-induced mortality is a true force acting on a population, it is more likely to occur at a low rate, more comparable to 0 than the high rates postulated by H1 models.

In a wildlife disease system where high levels of seroprevalence through time indicate the presence of an endemic chronic disease in which mortality is a cryptic process, the use of cross-sectional age-seroprevalence data can be used to estimate disease processes that are difficult to observe, such as the force of infection and disease-induced mortality rates (Heisey et al., 2006; Gauthier et al., 2008).

Furthermore these models may then be used to test hypotheses related to disease dynamics within exposed populations to gain a better understanding of how host populations may be affected by a given disease of interest. With the present study we

tested several hypotheses pertaining to disease-induced mortality and force of infection rates within an exposed wildlife host population by fitting force of infection models to age-seroprevalence data. We further assessed the biological realism associated with model inferences within the framework of host population dynamics. The incorporation of model-derived parameter estimates into population projection models provided a concrete description of how hypothesized mechanisms of unobservable disease processes related to host population processes, and thereby ascribed a higher (or lower) degree of support to a given hypothesis based on its implications at the host population level. Through this methodology we demonstrated the importance of challenging models with not only data, but an assessment of the biological plausibility of implications associated with candidate models, and showed how incorporating the population biology of the host system contributes to a more realistic interpretation of model validity compared to analyses that rely solely on model selection criteria.

The results from this study provide a good basis for further investigation of disease ecology within our case study system, specifically regarding the low-level impacts of disease-induced mortality in exposed populations. From this study we infer that juveniles are simply acquiring infection at a much slower rate than adults, and that disease-induced mortality is very low for both juveniles and adults, if it exists. However, further research pertaining to host and pathogen ecology would help to substantiate a hypothesis that describes URTD as a negligible force of mortality in gopher tortoises. For chronic infections, morbidity likely plays a more important role than frank mortality. Although morbidity effects are difficult to assess, development of models that begin to

incorporate these more common effects of chronic infectious disease would be valuable to improve our understanding of host-pathogen interactions.

Table 3-1. Relative fits of models ranked according to AICc values. Model parameters are listed below as  $\mu$  and  $\phi$ , corresponding to disease-induced mortality and force of infection, respectively. Subscripts indicate stage-specific rates for juveniles (J) and adults (A).

Model	Biological Hypothesis	Parameters	$\Delta$ AICc	weight	-Log(L)
H1a	Juveniles acquire infection at the same rate as adults, but die from disease at a different rate	$\phi, \mu_J, \mu_A$	0	0.372	100.6
H1d	Juveniles acquire infection at a different rate than adults, but die from disease at the same rate	$\phi_J, \phi_A, \mu$	0	0.372	100.6
H1b	Juveniles acquire infection and die from disease at a different rate than adults	$\phi_J, \phi_A, \mu_J, \mu_A$	2.1	0.132	100.6
H2	Juveniles acquire infection at a different rate than adults, and disease-induced mortality is negligible	$\phi_J, \phi_A$	2.2	0.124	102.7
H0	Juveniles acquire infection at the same rate as adults, and disease-induced mortality is negligible	$\phi$	33.0	<0.001	119.1
H1c	Juveniles acquire infection and die from disease at the same rate as adults	$\phi, \mu$	81.2	<0.001	142.2
H3	Juveniles are not infected, and disease-induced mortality is negligible	$\phi_A$	388.5	<0.001	296.9

Table 3-2. Parameter estimates and 95% confidence intervals from models best supported by the data. Rate parameters were obtained directly from maximum likelihood estimates provided by fitting force of infection models to age-seroprevalence data, and were subsequently converted to annual probabilities, listed below, to assess the biological realism of competing models in terms of projected population dynamics.

		<b>H1a</b>	<b>H1b</b>	<b>H1d</b>	<b>H1ad*</b>	<b>H2</b>
$\mu_J$	Rate	1.28 (-0.164, 2.72)	2.58 (-458, 462)	0.375 (-0.0493, 0.800)	0.525 (-0.311, 1.36)	NA
	Pr	1.00 (0.236, 1.00)	1.00 (0.00, 1.00)	0.997 (0.651, 1.00)	1.00 (0.433, 1.00)	NA
$\mu_A$	Rate	0.375 (-2.13, 2.88)	0.375 (-920, 920)	0.375 (-0.0493, 0.800)	0.189 (-0.875, 1.25)	NA
	Pr	0.750 (0.0688, 1.00)	0.750 (0.00, 1.00)	0.750 (0.426, 1.00)	0.503 (0.0605, 1.00)	NA
$\phi_J$	Rate	0.354 (-0.0125, 2.88)	0.715 (-127, 128)	0.104 (-0.0200, 0.228)	0.145 (-0.0784, 0.369)	0.00267 (0.00166, 0.00367)
	Pr	0.996 (0.689, 1.00)	0.798 (0.00, 1.00)	0.798 (0.683, 0.932)	0.893 (0.695, 1.00)	0.0402 (0.0392, 0.142)
$\phi_A$	Rate	0.354 (-0.0125, 2.88)	0.354 (-254, 255)	0.354 (-0.0243, 0.732)	0.178 (-0.0626, 0.420)	0.0458 (0.0314, 0.0601)
	Pr	0.730 (0.442, 1.00)	0.730 (0.00, 1.00)	0.730 (0.495, 1.00)	0.483 (0.293, 0.795)	0.156 (0.142, 0.171)

\*Corresponds to model-averaged estimates generated from H1a and H1d

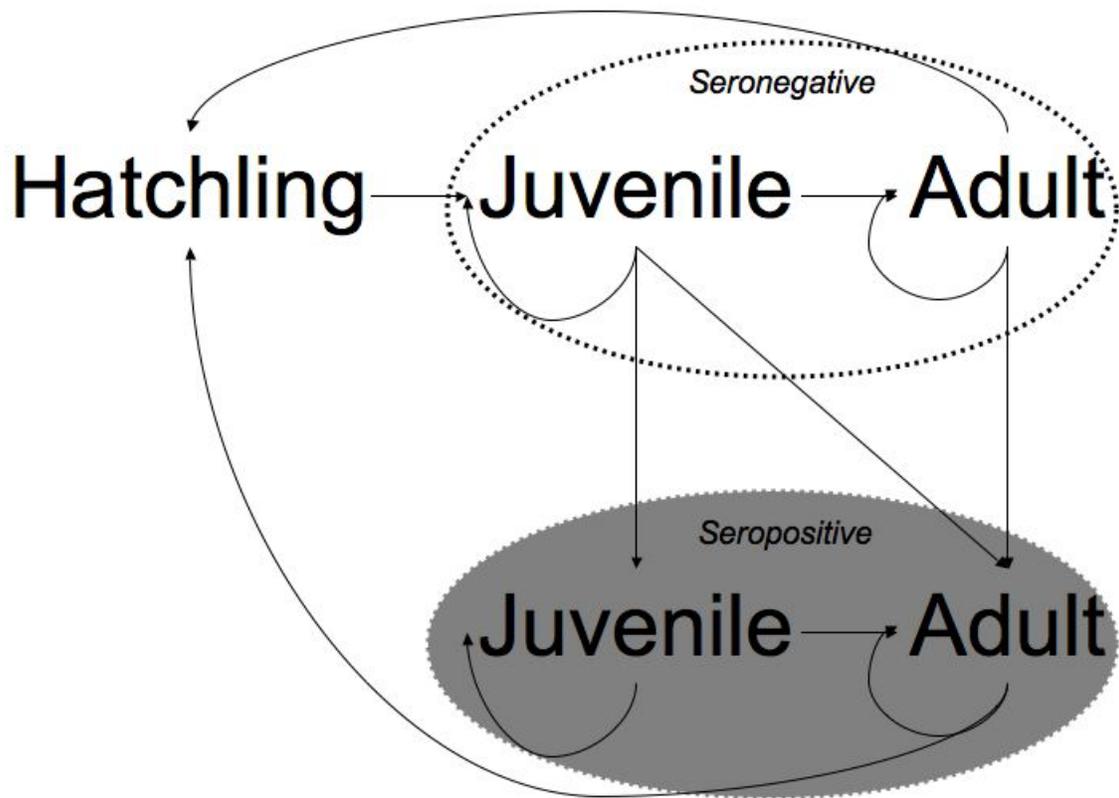


Figure 3-1. Conceptual model for metapopulation dynamics used to address the biological realism of best-fit models through the demonstration of population-level implications of model-derived parameters. Arrows indicate transitions between stages and patches (or exposure states).

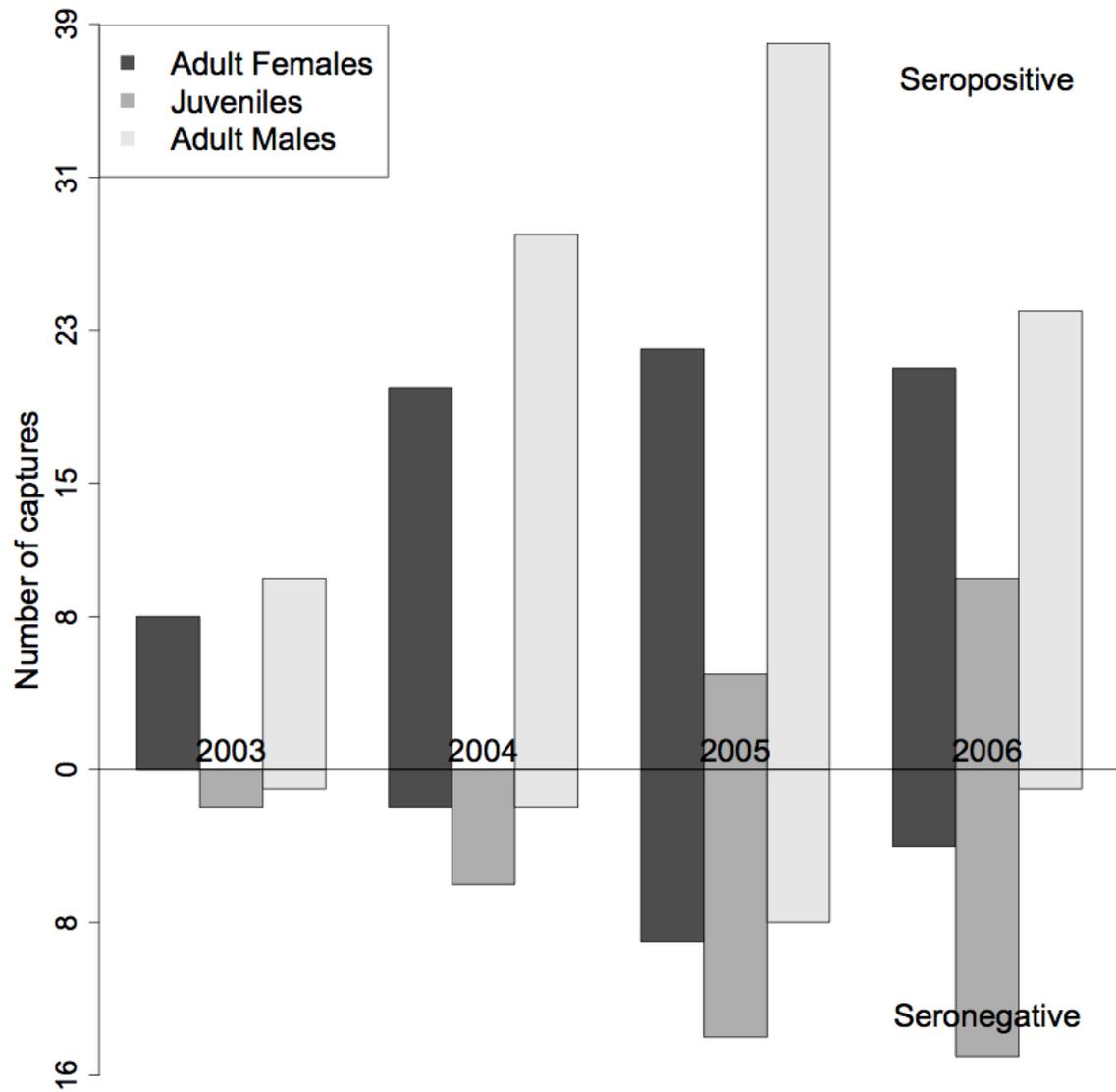


Figure 3-2. Barplot of annual captures at the CF site by year. Bars above and below the 0 mark represent the abundance of seropositive and seronegative individuals, respectively.

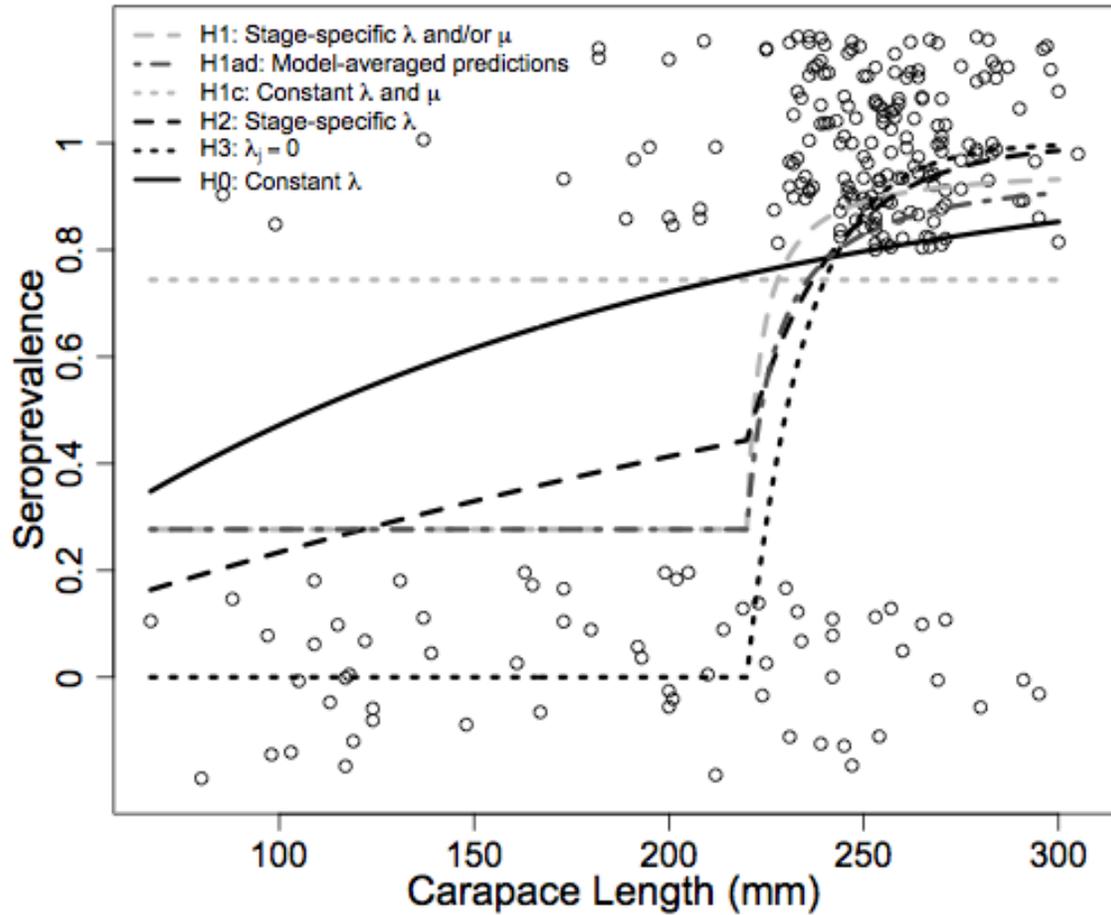


Figure 3-3. Age-seroprevalence curves resulting from force of infection models for hypotheses describing potential mechanisms driving stage-specific disparities in *M. agassizii* exposure. H1a, H1b, and H1d did not differ in their resulting curves, and are all depicted by the H1 curve.

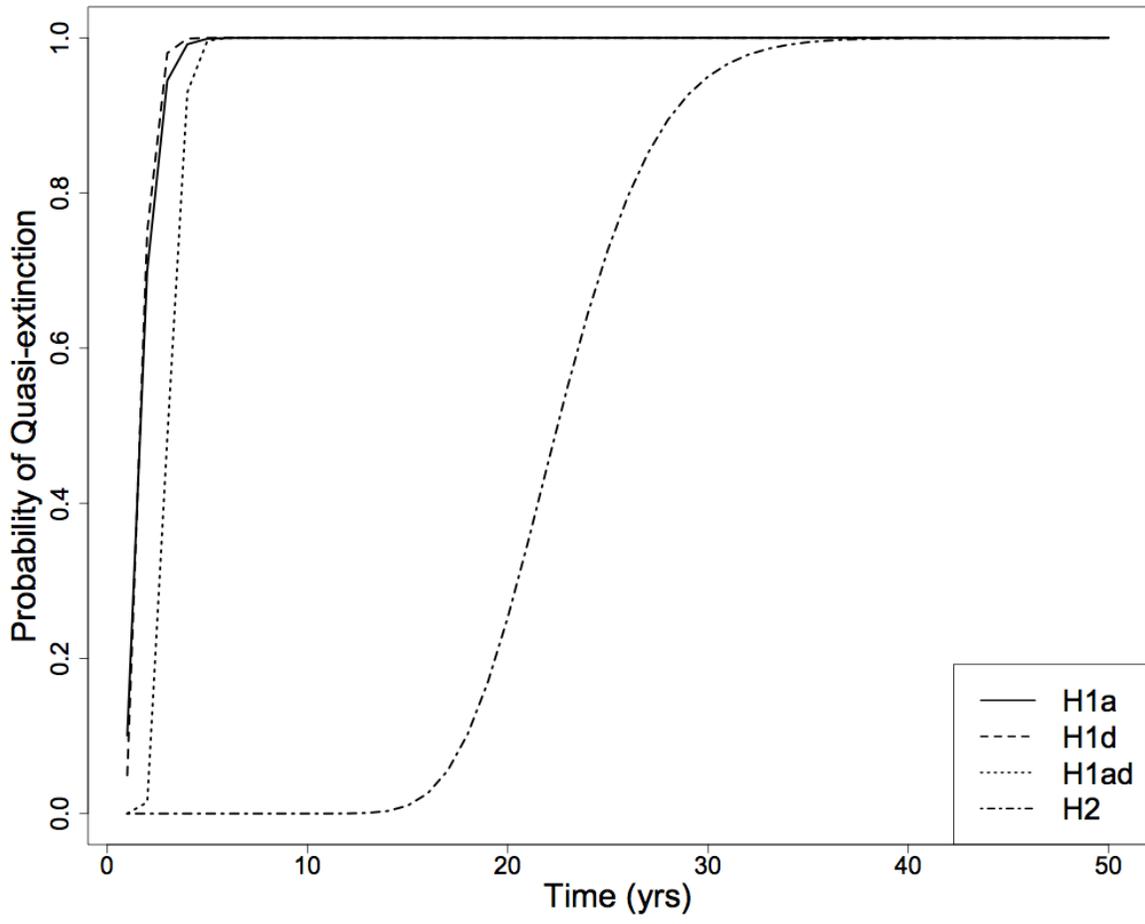


Figure 3-4. Cumulative distributions of quasi-extinction probabilities for metapopulation models parameterized by the best-fitting force of infection models.

CHAPTER 4  
THE EFFECT OF INDIVIDUAL MOVEMENT PATTERNS ON THE SUSCEPTIBILITY  
TO PATHOGEN EXPOSURE IN NATURAL POPULATIONS OF A THREATENED  
WILDLIFE HOST

The behavioral ecology of a host species can serve to elucidate factors underlying disease processes in natural populations. The purpose of this study was to describe movement patterns of individual hosts as they relate to pathogen exposure, and determine how specific movement patterns may increase the risk of infection in gopher tortoise populations exposed to *Mycoplasma agassizii*, an etiologic agent of upper respiratory tract disease (URTD). One hundred fifty-two gopher tortoises were sampled for *M. agassizii* infection, and tracked through the use of a fluorescent powder dye throughout the active season (May-October) between 2004-2006. Continuous daily movement patterns of sampled tortoises were documented through ArcPad Mobile GIS, and the relationship between *M. agassizii* infection and movement-associated factors (i.e., distance traveled, number of burrows visited, home range area, home range burrow density, and frequency of burrow emergence) were assessed through multivariate logistic regression. A principal components analysis differentiated between two types of movement-associated behavior: basking and foraging behavior, and mate-seeking behavior. Movement-associated behavior, along with sex and site classification, was found to be significantly associated with individual infection status; however, this association was most likely a retro-causal one, whereby infected tortoises were more likely to exhibit foraging and basking behavior than mate-seeking behavior. Future work that addresses this study question through a longitudinal framework, and that more specifically analyzes the social network structure of contacts among tortoises

may help to better delineate the effect of individual movement patterns on *M. agassizii* transmission dynamics in wild gopher tortoise populations.

### **Introduction**

The social ecology of host species is an important, although rarely studied factor associated with disease dynamics in wildlife populations. Social structure and grouping behavior have been implicated in seasonal outbreaks of several wildlife diseases (Altizer et al., 2006), such as rabies in skunks (Greenwood et al., 1997), phocine distemper virus in seals (Swinton et al., 1998), mycoplasmal conjunctivitis in house finches (Altizer et al., 2004b), chronic wasting disease in mule deer (Farnsworth et al., 2006), and hemipteran ectoparasite infestation in Cliff Swallows (Brown and Brown, 2004). These studies and others that have directed their efforts towards linking sociality with pathogen prevalence have primarily been concerned with group-level dynamics (i.e., social aggregations) rather than individual-level heterogeneities in host social behavior (Altizer et al., 2003; Ezenwa, 2004). An understanding of group-level dynamics is important for the clarification of disease processes in gregarious species. However, in species where social aggregations are uncommon or nonexistent, studies that link individual host behavior to pathogen prevalence serve better to elucidate disease dynamics.

Through this study, we sought to bridge the gap between ecology and epidemiology in our assessment of individual risk factors associated with upper respiratory tract disease (URTD) in wild gopher tortoises (*Gopherus polyphemus*). URTD has been implicated as a factor associated with several gopher tortoise mass mortality events (Epperson, 1997; McLaughlin, 1997; Smith et al., 1998; Deimer Berish et al., 2000; Seigel et al., 2003; Wendland, 2007), although a direct causal relationship

has yet to be established. *Mycoplasma agassizii* was first implicated as an etiologic agent of URTD in free-ranging desert tortoises (*Gopherus agassizii*) in Nevada, with the fulfillment of Koch's postulates through experimental transmission studies (Jacobson et al., 1991; Brown et al., 1994). First reports of URTD emergence in gopher tortoises were made in 1989 in Sanibel Island, FL (Westhouse et al., 1996a). Experimental studies have indicated transmission of *M. agassizii* through direct contact, and that the potential for environmental exposure of this pathogen is relatively unlikely (McLaughlin 1997; Schumacher, Hardenbrook et al. 1997). The clinical manifestations of URTD include the occurrence of nasal discharge, ocular discharge, palpebral edema, and conjunctivitis. Nasal discharge is the most commonly observed clinical sign. Clinical signs are intermittently expressed, and tortoises maintain subclinical chronic infections in which recurrence of acute disease is possible under situations of elevated stress, or through re-exposure resulting from contact with an infectious individual (Brown et al., 1994; McLaughlin, 1997).

Because they remain in their subterranean burrows 90% of the time, the furtive nature of gopher tortoises makes them difficult subjects of behavioral ecology (Wilson et al., 1994). Nevertheless, results from past telemetry studies have consistently demonstrated that adults move more extensively than juveniles, and that among adults, males move more expansively than females. More specifically, males tend to traverse larger home ranges than females and juveniles, and travel farther distances between burrows (McRae et al., 1981). This behavior has been associated with scramble competition polygyny, a mating system in which males strive to outrace competitors to

females that are too sparsely dispersed to be defended within a territory (Boglioli et al., 2003).

Mate-seeking behavior has been implicated as a putative factor for male-biased infection across several species (Poulin, 1996; Zuk and McKean, 1996; Skorping and Jensen, 2004; Cowan et al., 2007). Higher levels of parasite exposure in male yellow-necked mice (*Apodemus flavicollis*), which exhibit the same trends in movement as gopher tortoise males (i.e., larger territories, greater overall movement), were attributed to mate-seeking behavior. Interestingly, females harbored similar levels of parasites as males, despite their behavioral differences. The authors of this study found that a reduction in parasite intensity of males through treatment coincided with a decline in helminth prevalence among females; however, treatment of females did not result in the same decreased prevalence among males. From this, investigators concluded that males were primarily responsible for disease transmission and persistence in the host population, and that females did not contribute significantly to the dynamics of helminth infections in yellow-necked mice populations (Ferrari et al., 2004). Moreover, investigators determined that these mate-seeking adult males comprised a functional group, made up of ~20% of the population, that was responsible for ~80% of tick-borne infections within an exposed population (Perkins et al., 2003). Adult male yellow-necked mice could therefore be considered “superspreaders” (Galvani and May, 2005) because they produced more infections relative to other infected individuals in the population.

A previous study on age-specific prevalence of *M. agassizii* suggested that the low seroprevalence in the juvenile subpopulation could be attributed to a lower rate of contacts with infectious individuals in comparison to adults (Chapter 3). Less overall

movement by juveniles, relative to adults, is speculated as being one possible mechanism for this lower contact rate. According to this hypothesis, lower levels of movement should therefore correspond with lower probabilities of infection.

The purpose of this study was to determine whether individual movement-related factors are associated with increased risk of *M. agassizii* infection in gopher tortoises, with the broader goal of corroborating findings from a previous study implicating movement as the explanation for observed heterogeneities in *M. agassizii* exposure. Home range area, number of burrows encompassed by a home range, proportion of days an individual emerged from its burrow during the sampling period, distance traveled daily, and number of burrows visited daily were all expected to reflect the potential for a tortoise to encounter an infected tortoise, and therefore increase an individual's risk of infection. Additionally, movement patterns of adult males were expected to be more extensive than those of adult females and juveniles, which were also expected to increase their risk of becoming exposed to *M. agassizii*, relative to adult females and juveniles.

## **Methods**

### **Study Sites**

Data from 2004 and 2005 were collected from only one study site (CF; Fig. A-1). In 2006, two more sites (CE and OR; Fig. A-1) were added to the study in order to increase the number of uninfected tortoises in the total sample population. Preliminary data of the populations from the three study sites existed from an extensive serological surveillance program established by researchers at the University of Florida through a multi-year grant from the National Science Foundation. The CF site (Branan Field Mitigation Park), located outside of Jacksonville, FL (N30.20349<sup>o</sup>, W81.86611<sup>o</sup>), was a

42.9 hectare (ha) sandhill habitat predominantly composed of slash/longleaf pine overstory and wiregrass/saw palmetto ground cover. During the 2004-2006 sampling periods approximately 40% of the population consisted of juveniles, as indicated by burrow width measurements, with adults comprising the remaining 60%. Additionally, population density was approximately 4-5 tortoises/ha (Wooding, unpublished data). Seroprevalence of *M. agassizii* was historically high in this population ( $\geq 62\%$ ) since 2000. The CE site, located outside of Kissimmee, FL (N28.41573°, W81.61827°), was a 6.7 ha hilltop surrounded by wetlands with very little canopy cover, and ground cover predominantly composed of natal grass and Mexican clover; however, only 3.1 ha of the total area was used for the movement study. Juveniles comprised approximately 65% of the population. Tortoise density was extremely high at this site, estimated at 29.1 tortoises/ha, but seroprevalence was historically low ( $\leq 25\%$ ; Wooding, unpublished data). The OR site, located in Melrose, FL (N29.69323°, W82.00454°) was a 63.5 ha longleaf pine-turkey oak-wiregrass sandhill habitat; however, only 17.8 ha of the habitat was used for trapping tortoises for the movement study. Tortoise density was estimated at a low 0.37 tortoises/ha, and seroprevalence was also historically low ( $\leq 9\%$ ). Adults made up about 83% of the population structure (Wooding, unpublished data).

## **Data Collection**

**Capture and sampling methodology:** Total population censuses at each study site were conducted using strip transects immediately prior to the study periods. Burrows were mapped with a GPS, and flagged according to size class, as determined from burrow width and depth measurements. In order to maximize the capture of animals with overlapping ranges, bucket traps were placed at the mouth of burrows in the area within the study site that had the highest population density. Traps were

checked daily for captures. The trapping methodology utilized was contingent on the size class of the burrow being trapped, with 1 gallon buckets used for juveniles and hatchlings (burrow width  $\leq 155$  mm in diameter), 5 gallon buckets used for subadult and adult burrows ( $155 \text{ mm} < \text{burrow width} < 320 \text{ mm}$ ), and 10 gallon buckets for large adult burrows (burrow width  $\geq 320$  mm). Holes were drilled in all buckets for water drainage, and shade covers made out of vinyl siding were placed over the traps to protect the tortoises from overheating.

Blood samples and nasal flushes were collected from each captured tortoise for determination of infection status through ELISA and PCR tests. Specific antibody to *M. agassizii* was detected using a validated monoclonal antibody-based ELISA protocol (Wendland et al., 2007). Sera obtained from blood samples were tested to determine serological status of each individual captured. Additionally, a PCR test with subsequent restriction fragment length polymorphism analysis of the PCR product (Brown et al., 2002) was used to identify the species of mycoplasma obtained from individual tortoise nasal lavages. Infection status of captured individuals was established at the end of the field season season after completion of all laboratory diagnostics, and was unknown to investigators at the time of data collection.

Morphometric measurements and complete health assessments were performed on each tortoise. Tortoises were sexed according to degree of plastral concavity, and aged according to the count of plastral rings. Clinical signs of interest include nasal discharge, ocular discharge, palpebral edema, periocular edema, and conjunctivitis. These indicators were ranked from 0-3 according to severity of clinical sign

presentation, with 0 reflecting absence of clinical sign and 3 describing the highest level of severity.

**Documentation of individual movement patterns:** In addition to the collection of morphometric and disease data, movement patterns of individual tortoises were documented in order to describe how they influenced the potential for *M. agassizii* exposure among individuals. In order to capture the highest potential amount of overlap among tortoises at each study site, 20 tortoises from the area with the highest population density were sequentially enrolled as subjects for the movement study during each sampling period. After disease and morphometric data were collected, dye packs were attached to tortoises on one of the drilled holes located on the rear carapacial scutes used for identification. Each dye pack was constructed by scooping approximately 15 g of fluorescent dye into a stretched piece of nylon panty hose (Blankenship et al., 1990). Using a small drill bit, the ends of the nylon were pushed through the drilled hole at the posterior end of the tortoise. Once the nylon was through, the dye pack was pulled close enough to the tortoise so that when the tortoise was picked up, the bottom of the pouch fell level to its back legs. Allowing too much or too little distance between the trailing pouch and tortoise could hinder movement by either increasing the chances that the pouch get caught on vegetation or obstructing the actual mechanics of walking through direct contact (Fig. 4-1). Tortoises were then returned to the burrow from which they were captured.

Because dye trails were most visible in complete darkness with the use of a UV light, tracking of tortoise movement commenced approximately 45 minutes after sunset the day after the tortoise was caught. A handheld UV light (Versalume) exposed the

fluorescent dye trail left by each tortoise during each day of movement. A sample size of 20 tortoises was selected for this aspect of the project because it was the maximum amount of tortoises to feasibly track between 9 pm and 6 am during each sampling period at each study site. Burrows entered along each trail were marked, and daily trails were mapped using ArcPad mobile GIS software on a handheld PC (HP iPAQ hx2110; Fig. 4-2). Number of burrows entered and total distance traveled each day were also documented. Each tracking period lasted between 6-10 days, depending on the visibility of the dye after the sixth day. Observed habitat utilization areas were calculated on ArcMap (ArcView GIS, ESRI) using the minimum convex polygon method from Hawth's Tools (Beyer, 2004) in order to capture the entire range encompassed by individual tortoise movements. In 2004 and 2005, dye tracking was performed at three different periods on the CF site alone. In 2006, dye tracking was performed at two different periods (May-June, and July-September) for each of the three sites.

### **Statistical Analysis**

**Definition of infection status:** The purpose of this study was to determine how individual movement patterns may increase the risk of infection in gopher tortoise populations exposed to *M. agassizii*. The sample population consisted of tortoises that were captured and tracked throughout the active seasons (May- October) between 2004 and 2006. Infected tortoises were defined as individuals from the total sample population that tested positive either by ELISA (antibody titer  $\geq 64$ ), or PCR for *M. agassizii* infection, or exhibited nasal discharge. Infected tortoises, therefore, included tortoises that were previously exposed to *M. agassizii* but were not actively shedding mycoplasma through nasal discharge, as well as tortoises that were clinically infected

and actively shedding mycoplasma but may not have produced antibody levels to the pathogen at the time the serum sample was taken. Uninfected tortoises were defined as individuals from this same sample population that were both, ELISA-negative (antibody titer < 32) and PCR-negative for *M. agassizii* infection. Individual tortoises that were both, ELISA-suspect (antibody titer of 32) and PCR-negative, were excluded from the study because exposure of these tortoises to *M. agassizii* could not be determined with certainty, and the small sample size of this group (n=4) prevented their inclusion as a third category.

**Movement-associated predictor variables:** Movement patterns, specifically daily distance traveled, number of burrows visited daily, observed habitat utilization area (m<sup>2</sup>), and total number of burrows within the observed habitat utilization area were compared between uninfected and infected tortoises. With the data collected from dye tracking, specific movement-associated risk factors were identified and quantified through the implementation of a multiple logistic regression model. Proportion of days a tortoise emerged from its burrow during a sampling period was also included as a movement parameter to represent the general activity level of a tortoise. A tortoise that came out of its burrow many days during the sampling period was considered highly active, whereas a tortoise that seldom emerged from its burrow during the sampling period was considered less active. Proportions were used in lieu of absolute number of days a tortoise emerged during the sampling period in order to standardize this variable and account for variability in tracking effort due to lost dye packs or poor trail visibility before the end of the 10-day sampling period. Additionally, adult female tortoises at the CF site were tracked for longer periods as part of a separate collaborative study with the

Florida Fish and Wildlife Conservation Commission pertaining to a head-starting program. Kruskal-Wallis tests were used to compare movement parameters between uninfected and infected tortoises, and juvenile and adult tortoises. Wilcoxon Rank Sum tests were used to compare movement parameters among adult females, juveniles, and adult males, and among the three study sites. P-values  $\leq 0.10$  were considered significant.

**Principal components analysis:** In order to address the potential problem of multicollinearity among movement-associated predictor variables, movement indices were generated through the use of a Principal Component Analysis (PCA). Proportion of days emerged during the sampling period, home range area ( $m^2$ ), number of burrows within the home range, median daily distance (m), and median number of burrows visited daily were all included in the PCA.

**Regression analysis:** The following generalized linear model was used for the analysis:  $P(\text{infection}) = [1 + \exp(-X\beta)]^{-1}$ , where  $P(\text{infection}) \sim \text{Binomial}([1 + \exp(-X\beta)]^{-1})$ ,  $X$  was a matrix with 152 rows of observations and  $p$  columns, each representing a different predictor variable with the first column consisting of ones.  $\beta$  was a vector of estimated regression coefficients of length  $p$ , and  $P(\text{infection})$  represented the probability of infection based on model parameters. All regression parameters were estimated assuming success = 1 (i.e., infection). Specifically, the infection status of individuals (no infection = 0 and infection = 1) was plotted as a function of principal component scores, sex classification (Juvenile/Adult Male/Adult Female), site (CF/CE/OR), and capture period (May-October, 2004-2006). Additionally, a sex:site interaction term was also included in the model. Because predictor variables differed in

their units of scale, estimated slope parameters were rescaled by dividing each by two times its standard deviation using the *arm* package in R 2.9.1 (Gelman and Hill, 2007). Standardization of regression coefficients thereby facilitated the comparability of each variable's relative influence on infection status. Overall goodness-of-fit diagnostics were assessed through Hosmer-le Cessie omnibus tests for binary data (Hosmer et al., 1997).

The risk of mycoplasma infection associated with movement patterns was quantified through odds ratios. Odds ratios were calculated using the equation:  $\exp(b)$ , where  $b$  is a vector of estimated slope parameters in their original (i.e., non-standardized) form. Odds ratios for sex and site variables were calculated using adult males and the CF site as the reference levels, respectively. A stepwise AIC selection process was implemented to determine which combination of parameters resulted in the best relative fit to the data (Kutner et al., 2005; Murtaugh, 2009).

Model parameters were estimated through the method of iteratively reweighted least squares (IRLS), and 95% confidence intervals were generated through profile likelihoods. Statistical analysis was performed through the use of the *MASS*, *Design*, and *stats* packages in R programming language (Venables and Ripley, 2002; Harrell, 2007; R Development Core Team, 2007). Calculation of individual home ranges was performed through the use of Hawth's tools for animal movement in ArcView (Beyer, 2004).

## Results

One hundred fifty-two tortoises were sampled and tracked using the fluorescent powder dye technique. Tortoises were tracked an average of  $8.2 \pm 5.0$  days. The number of days tracked ranged between 1 day (due to loss of a dye pack,  $n = 9$ ), and

32 days ( $n = 1$ ). A total number of 755 tortoise days was documented between May-October 2004-2006. Approximately, 65% of all adult males and females captured were categorized as infected ( $n_M = 58$ ;  $n_F = 62$ ). Roughly 18% of all captured juveniles were categorized as infected ( $n_J = 32$ ). Figure 4-3 summarizes the infection status and sex classification of tortoises captured from each study site.

Proportion of days a tortoise emerged from its burrow during the sampling period differed by site ( $p$ -value =  $4.9 \times 10^{-6}$ ) and infection status ( $p$ -value =  $3.1 \times 10^{-2}$ ), with CF tortoises ( $Mdn_{CF} = 0.667$ ,  $IQR_{CF} = 0.349$ ) emerging more often from their burrows than CE ( $Mdn_{CE} = 0.619$ ,  $IQR_{CE} = 0.311$ ) or OR tortoises ( $Mdn_{OR} = 0.400$ ,  $IQR_{OR} = 0.167$ ), and infected tortoises emerging from their burrows more often than uninfected tortoises. Home range area differed by sex ( $p$ -value =  $4.0 \times 10^{-7}$ ), stage ( $p$ -value =  $1.8 \times 10^{-2}$ ), site ( $p$ -value =  $6.4 \times 10^{-2}$ ), and disease status ( $p$ -value =  $2.0 \times 10^{-5}$ ). Juveniles had the smallest home ranges ( $Mdn_{JUV} = 488 \text{ m}^2$ ,  $IQR_{JUV} = 833$ ), followed by adult females ( $Mdn_F = 1030 \text{ m}^2$ ,  $IQR_F = 3260$ ). CE tortoises ( $Mdn_{CE} = 436 \text{ m}^2$ ,  $IQR_{CE} = 1590$ ) had the smallest home ranges among the three sites ( $Mdn_{CF} = 1240 \text{ m}^2$ ,  $IQR_{CF} = 3210$ ;  $Mdn_{OR} = 1740 \text{ m}^2$ ,  $IQR_{OR} = 7600$ ), and uninfected tortoises ( $Mdn_{UI} = 969 \text{ m}^2$ ,  $IQR_{UI} = 2070$ ) encompassed smaller ranges than infected tortoises ( $Mdn_I = 1520 \text{ m}^2$ ,  $IQR_I = 3590$ ). Home range burrows differed by sex ( $p$ -value =  $2.2 \times 10^{-4}$ ) and stage classes ( $p$ -value =  $4.1 \times 10^{-2}$ ). The home ranges of adult males encompassed the most burrows ( $Mdn_M = 7.50$ ,  $IQR_M = 13.0$ ), followed by those of adult females ( $Mdn_F = 3.00$ ,  $IQR_F = 4.00$ ). Daily distance traveled differed by site ( $p$ -value =  $4.8 \times 10^{-6}$ ), sex ( $p$ -value =  $6.4 \times 10^{-4}$ ), and stage classes ( $p$ -value =  $2.4 \times 10^{-5}$ ), and number of burrows visited daily differed by

sex ( $p$ -value =  $1.4 \times 10^{-4}$ ). Table 4-1 depicts the median and interquartile ranges associated with movement variables stratified by sex, site, stage, and infection status.

The purpose of the principal components analysis was to reduce the dimensionality of movement and activity patterns, which were comprised of proportion of days emerged during a sampling period, home range area, home range burrows, daily distance, and daily burrows visited, to a single index value. In other words, rather than having to use five separate variables to describe tortoise movement patterns, the PCA generated a single summary value of movement based on the relationships among individual movement-associated variables. The first and second principal components explained approximately 72% of the variation among movement variables (Table 4-2). The remaining principal components (PC3-PC5) accounted for <18% of the variation. When principal components were incorporated into the logistic regression model in lieu of individual movement parameters, the score from the first principal component (PC1) was found to be an important predictor along with sex and site, according to stepwise AIC parameter selection procedures (AIC = 123.4).

The first principal component reflected an index of overall movement and activity, in which activity level (as defined by burrow emergence) was inversely related to overall movement (as defined by daily distance traveled, median number of burrows visited daily, home range area, and number of burrows within a home range; Table 4-2). Specifically, individuals that emerged often from their burrows, generally traveled short distances, visited few burrows on a daily basis, and traversed small home ranges that encompassed few burrows (Table 4-2). Likewise, individuals that emerged less often from their burrows tended to travel greater distances and visit more burrows on a daily

basis, as well as traverse larger home ranges that encompassed many burrows. In order to facilitate the interpretation of PC1 in terms of overall movement, the original signs of PC1 loadings and scores were reversed so that low PC1 scores would relate to restricted movement coupled with frequent emergence, and high PC1 scores would relate to extensive overall movement coupled with infrequent emergence. These loadings are listed under Table 4-2.

First principal component scores differed by sex ( $p$ -value =  $2.75 \times 10^{-7}$ ), site ( $p$ -value =  $6.08 \times 10^{-2}$ ), and stage class ( $p$ -value =  $2.43 \times 10^{-4}$ ), with adult males exhibiting higher values ( $Mdn_M = 0.270$ ,  $IQR_M = 1.88$ ) than adult females ( $Mdn_F = -0.576$ ,  $IQR_F = 0.784$ ) and juveniles ( $Mdn_{JUV} = -0.925$ ,  $IQR_{JUV} = 0.674$ ), and tortoises from OR demonstrating higher values ( $Mdn_{OR} = -0.0548$ ,  $IQR_{OR} = 2.13$ ) than those from CF ( $Mdn_{CF} = -0.440$ ,  $IQR_{CF} = 0.966$ ) and CE ( $Mdn_{CE} = -0.792$ ,  $IQR_{CE} = 1.62$ ). First principal component scores of infected tortoises were not significantly different from those of uninfected tortoises ( $p$ -value = 0.93). Uninfected tortoises, adult males, and tortoises from the OR site exhibited the highest amount of variability in their movement patterns, as described by the distribution of PC1 scores. Additionally, these groups also provided for the highest PC1 scores (Fig. 4-4).

Movement patterns, along with site and sex classification, were significantly associated with *M. agassizii* infection status, with site and sex classification exerting the greatest impact on infection status (Table 4-3). From the full model, first principal component score, sex, and site were significantly associated with infection status according to z-test statistics (*not shown*). The importance of these parameters was further corroborated by a stepwise AIC model selection procedure, which demonstrated

that the reduced model containing only these same three variables fit the data best (Table 4-3).

According to the logistic regression, the odds of being infected decreased by 41% for each unit increase in PC1, while adjusting for sex and site classification (Table 4-3). Recalling that an increase in PC1 score indicates higher overall movement, this finding suggests that individuals who demonstrate more extensive movement patterns are less likely to be infected than individuals who are more restricted in their movement patterns. Additionally, adult females were as likely as adult males to be infected with *M. agassizii*, but juveniles were 99% less likely to be infected than adult males, when accounting for site and movement pattern differences. Additionally, tortoises within the CE and OR sites were also much less likely to have been exposed to *M. agassizii* than tortoises in CF, which had the greatest seroprevalence among the three sites (75.7%; Fig. 4-3). Figure 4-5 displays the PC1 scores of individual tortoises as they relate to infection status, site, and sex classification.

Hosmer-le Cessie omnibus tests indicated that the model was not a particularly good fit to the data ( $Z = -1.91$ ,  $p\text{-value} = 0.06$ ). The fit was heavily influenced by several outlying observations. However, when influential observations were identified and removed, the logistic model was found to be adequate ( $Z = 1.44$ ,  $p\text{-value} = 0.15$ ). Despite the better fit of the model without outliers, influential points were not discarded because there was no valid biological reason to omit these observations. Additionally, regardless of whether influential points were retained or removed, the overall interpretations remained the same. In other words, the same model parameters were found to be significant by stepwise AIC selection in both cases, with and without

outlying observations, and the trends describing the inverse relationship between *M. agassizii* infection and movement were maintained.

### **Discussion**

Gopher tortoise movement patterns described in this study were consistent with findings from previous studies. Like McRae et al. (1981) and Wilson et al. (1994), we found that adults tend to move farther distances and use larger home ranges that encompass more burrows than juvenile tortoises, and that adult males tend to move more extensively than adult females. Additionally, we observed considerable overlap between breeding ranges of adult males and foraging ranges of adult females, as McRae et al. (1981) reported. In one aspect, however, observations from our study differed from findings of previous research. The level of activity we defined by burrow emergence, was vastly greater than that reported by Wilson et al. (1994), who stated that tortoises were inactive about 90% of the time. The tortoises sampled for this study were inactive only about 33% of the time. This is most probably due to differences in sampling design, whereby Wilson et al. (1994) tracked tortoises through radio telemetry for a continuous year, while we tracked tortoises only during the active season (May-October) across three years. This should have skewed our measures of emergence to reflect higher activity levels.

Upon assessment of the relationship between movement and exposure susceptibility, first principal component scores were considered important predictors of *M. agassizii* infection according to AIC stepwise selection procedures. Furthermore, the PCA defined two types of movement patterns, namely restricted movement with frequent emergence and extensive movement with less frequent emergence, which could relate to foraging/basking behavior, and mate-seeking behavior, respectively.

According to these terms, the PC1 scores of juveniles and adult females correspond with movement associated with basking and foraging, whereas those of adult males correspond with mate-seeking behavior. The extensive movement of males documented in this study may correspond with prolonged periods of search for receptive females, as discussed by Boglioli et al. (2003), in relation to scramble competition polygyny.

We expected mate-seeking behavior to be riskier than foraging behavior in relation to pathogen exposure. In other words, individuals that travel farther distances and visit more burrows on a daily basis were expected to have a higher probability of encountering an infectious tortoise than individuals who are more sedentary and do not move extensively. Contrary to expected results, however, the present analysis suggests that the probability of being seropositive to *M. agassizii* actually decreases with greater overall movement. The negative relationship between PC1 scores and infection status indicated that that lower PC1 scores were actually associated with increased exposure susceptibility. In other words, there was a higher probability of infection associated with basking and foraging behavior than there was with mate-seeking behavior. We highlight two potential mechanisms to explain this inverse relationship between movement patterns and *M. agassizii* exposure: (1) an increased potential for infectious contacts through a polyandrous mating system, and (2) reverse causation.

### **The Role of Mating System on Infection Dynamics**

In a theoretical study investigating the effects of polygynous mating systems on the spread of sexually transmitted diseases, Thrall et al. (2000) determined that females were primarily responsible for disease persistence. Specifically, when a pathogen was

introduced through males, the ensuing disease failed to establish itself in a population. However, when introduced through females, the rate of infection always increased. The investigators found that with high variance in male mating success, an infected male had a high probability of not encountering any mates, and therefore not infecting other susceptible hosts. Females, however, were more likely to encounter mate-seeking males, and would therefore serve to propagate infection within a population. Likewise, Ezenwa (2000) noted that the isolation brought about through male territoriality would otherwise limit their exposure to pathogens, but that association with females greatly increases their probability of infection as females are likely to have been exposed to pathogens outside of a male's territory.

Implications of the results from these two studies correspond with those of our present study. Although *M. agassizii* is not sexually transmitted, its transmission dynamics resemble that of sexually transmitted diseases due to the social structure of the host species. Because of their solitary nature, contacts between gopher tortoises ~~will~~ most likely ensue as a result of mate-seeking behavior, which includes male-female encounters, and aggressive male-male or male-subadult territorial disputes (Douglass, 1976; Landers et al., 1980). In our present study, adult males were more variable than females and juveniles in all movement parameters except burrow emergence and home range area. This high variability in movement, like high variability in mating success, could therefore reduce the potential for *M. agassizii* exposure according to Thrall et al. (2000). This would suggest that the spatiotemporal density of potentially infectious contacts is higher for individuals that frequent small ranges compared to individuals that traverse larger areas, as the latter are more likely to have sporadic rather than

consistent encounters with other tortoises. Moreover, the results of this study indicate that contacts between females and males may not be symmetric, and a single female may contact more males than a single male will contact females and/or other males (Fig. 4-6). Therefore, in addition to corresponding with a polygynous mating system, as stated by McRae et al. (1981), our observations suggest that gopher tortoises may also subscribe to a polyandrous mating system, whereby individual females mate with multiple males. Support for polyandrous behavior has been corroborated through the documentation of multiple paternity within single clutches of gopher tortoise females (Moon et al., 2006). Interestingly, through their contacts with multiple males, adult females can play a pivotal role in the persistence of mycoplasmal URTD in natural gopher tortoise populations.

### **The Problem of Reverse Causation**

Adjusted odds ratios indicated that sex and site had a great influence on the infection status of an individual, and that juveniles and adult females (given a particular site and level of movement) were much less likely to be infected than adult males. This is consistent with a 5-year study of 11 free-ranging populations of gopher tortoises that found male tortoises within seven URTD-positive populations were more likely to be seropositive than female tortoises. Further, a greater proportion of males seroconverted at a smaller size than did females, suggesting that younger males may be particularly vulnerable as they attempt to establish their place in the social hierarchy (Wendland et al., in press).

In our current study, for a given site and sex classification, increased foraging behavior was directly associated with *M. agassizii* infection, whereas increased mate-seeking behavior depicted an inverse relationship with infection. Despite interesting

implications arising from the potential role of polyandry in *M. agassizii* infection dynamics, a simpler explanation of our results is that the observed relationship between infection status and movement was not a causal association, but rather a retro-causal one, in which the outcome of interest (i.e., infection status) occurred before the hypothesized cause (i.e., movement behavior). In other words, rather than having foraging behavior lead to an increased risk of infection, infection could have led to increased foraging behavior. For example, it is possible that infected tortoises tended to spend more time foraging than uninfected tortoises, possibly due to a need to meet higher energetic demands associated with immune function.

An observational study on the behavior of house finches in relation to their status of *M. gallisepticum* infection demonstrated that infected birds tended to spend more time at feeders than uninfected birds (Hawley et al., 2007a). Moreover, the feeding efficiency and weight gain of swine and poultry infected with mycoplasma species are poorer than that of uninfected animals (Rautiainen et al., 2000; Regula et al., 2000; Saif, 2003), suggesting that the energetic costs of animals with mycoplasma infections are much higher than those of healthy animals. Thus, infected animals may compensate for this increased energetic demand by allocating more time to feeding/foraging.

Additionally, the increased activity (i.e., more frequent burrow emergence) of gopher tortoises infected with *M. agassizii* has been corroborated by a previous study (Ozgul et al., 2009), which estimated a higher capture probability for infected tortoises compared to uninfected tortoises. Ozgul et al. (2009) attributed this higher rate of capture to an increased need for infected individuals to behaviorally induce a fever response. Because of differences in their immune systems compared to mammals, fish

and reptiles induce a fever response to infection through behavioral rather than metabolic means (Brown, 2002). Basking, for instance, may serve to increase the core body temperature of an infected tortoise, and thereby produce a more hostile environment for pathogens that helps a tortoise clear infection. Therefore, increased burrow emergence may have been another potential outcome of *M. agassizii* infection, rather than a risk factor.

From this current study, we found that movement related to basking and foraging behavior was positively associated with infection status, whereas movement related to mate-seeking behavior was inversely associated. Given past research on respiratory mycoplasmoses of animals, it is likely that the results from our study reflect a greater energetic cost of infection that is compensated by increased nutritional requirements. This, in turn, would equate to infected tortoises spending a greater amount of time foraging. Although this explanation provides less insight into how the behavioral ecology of gopher tortoises impacts *M. agassizii* infection dynamics, it does provide for a simpler explanation of observed results, and one that is more easily supported by the data we collected for this study. It is also important to note that our study period occurred after the major period (2000 to 2004) of pathogen transmission had occurred on the CF site. During that time, the seroprevalence increased from <10% to 70%. It is possible that during the initial introduction of *M. agassizii* to a naïve population, mate-seeking movement patterns may have been a key component of transmission. Therefore, we cannot exclude the possibility that mate-seeking behaviors might be a significant contributor to disease transmission in the initial stages of pathogen introduction.

## Future Directions

A description of the relationship between movement patterns and infection status provides a preliminary basis from which to address the contribution of individual host social behavior to pathogen exposure. However, the link between movement patterns and pathogen exposure requires insight into the temporal scale of infection at the individual-level (i.e., seroconversion rates), as well as the social network structure of hosts within exposed populations, which is not captured by an analysis of movement patterns that is independent of spatial configuration. For instance, we framed this study around a case-control study design; however, a prospective longitudinal study design would provide more conclusive results linking the potential role of individual behaviors on infection and transmission dynamics in exposed gopher tortoise populations. Ideally, a longitudinal study would involve a naïve population that experienced the introduction of the pathogen during the study period. In a longitudinal study, susceptible (i.e., uninfected) individuals could be followed through time to determine how their movement patterns contribute to infection risk. Similarly, newly infected animals could be followed to determine their risk potential to transmit the pathogen to contacts. Such a study would control for the confounding effect of reverse causation (as it would capture the transition from an uninfected to an infected state), and better define individual-level behavioral risk factors associated with infection.

Although juveniles exhibited similar movement patterns as adult females in the present study, the observed disparities between the two regarding *M. agassizii* infection may be better described through their vastly different social network structures. Females may be situated in areas of high tortoise density, in which their home ranges can extensively overlap with those of other potentially infectious tortoises, whereas

juveniles may inhabit areas of lower density along the edges of adult territories, and consequently be less susceptible to making infectious contacts. Social network structure could be defined according to spatially explicit parameters that take into account factors such as the centrality and connectedness of individuals to others within a population (Cross et al., 2004; May, 2006; Bansal et al., 2007; Perkins et al., 2009). Using information such as the number of overlapping ranges within an individual's home range, or the mean distance between the center of an individual's home range and that of others within a population, could potentially provide insights into individual social network structures that could more directly define how movement patterns could propagate infection throughout exposed populations.

Examples of empirical studies that describe individual movement patterns or behavioral characteristics in relation to disease status are limited (Yaremych et al., 2004), whereas much theoretical work has been published on the effect of heterogeneous contact rates on transmission dynamics (Galvani and May, 2005). For example, Lloyd-Smith et al. (2005b) introduced the concept of the individual reproductive number, which represents the expected number of secondary infections caused by a particular individual. With this value, the ability of certain individuals to be more "infectious" than others through a more connected network of social contacts is captured. Its application can be useful for epidemiological studies of sexually transmitted diseases, where a small number of infected individuals may be responsible for a large number of infections in an exposed population (Lloyd-Smith et al., 2004). Although theoretical work points to the importance of individual heterogeneities in pathogen transmission, the correspondence between empirically measured behavioral

data and analytically derived model parameters could be greatly improved upon. According to Altizer et al. (2003), this can only be achieved through the increased collaboration of behavioral ecologists and epidemiologists.

In review, this study implemented the use of logistic regression models to address the relationship between movement patterns and *M. agassizii* infection, with the aim of defining movement-related risk factors associated with pathogen exposure. Movement-associated behavior, along with sex and site classification, was found to be significantly associated with individual infection status; however, this association was most likely a retro-causal one, whereby infected tortoises were more likely to exhibit foraging and basking behavior than mate-seeking behavior. Future work that approaches this study question through a longitudinal framework, and that more specifically addresses the social network structure of contacts among tortoises may help to better delineate the effect of individual movement patterns on *M. agassizii* transmission dynamics in wild gopher tortoise populations.

Table 4-1. Summary statistics for movement-associated predictor variables. The median and interquartile ranges of movement parameters stratified by sex, site, stage, and infection classification.

Classification	Home Range Area (m <sup>2</sup> )	No. of Home Range Burrows	Median Daily Distance (m)	Median No. of Daily Burrow Visits	Proportion of Days Emerged	First Principal Component Scores
Adult Male	2490 (1200, 6440)	7.5 (3, 16)	86.2 (50.3, 148)	0.25 (0, 1)	0.667 (0.5, 0.746)	0.270 (-0.478, 1.40)
Adult Female	1030 (263, 3530)	3 (2, 6)	56.7 (43.9, 86.1)	0 (0, 0)	0.667 (0.451, 0.8)	-0.576 (-0.902, -0.118)
Juvenile	488 (157, 991)	3 (2, 4.25)	36.2 (22.4, 51.6)	0 (0, 0.125)	0.592 (0.5, 0.839)	-0.925 (-1.04, -0.368)
CF	1240 (433, 3640)	4 (2, 10)	61 (45, 96)	0 (0, 0.5)	0.667 (0.571, 0.852)	-0.44 (-0.871, 0.0948)
CE	436 (127, 1720)	4 (2, 9.25)	33.5 (18.5, 57.4)	0 (0, 1)	0.619 (0.489, 0.8)	-0.792 (-1.06, 0.560)
OR	1740 (507, 8110)	3 (1, 9)	81 (53.5, 168)	0 (0, 1)	0.4 (0.333, 0.5)	-0.0548 (-0.728, 1.40)
Adult	1540 (446, 4320)	4.5 (2, 10)	64.5 (47, 114)	0 (0, 0.5)	0.667 (0.451, 0.796)	-0.343 (-0.769, 0.658)
Juvenile	488 (165, 1030)	3 (2, 4.75)	40.2 (23.2, 54.8)	0 (0, 0)	0.592 (0.5, 0.804)	-0.925 (-1.03, -0.336)
Infected	1520 (431, 4020)	4 (2, 9)	59.5 (45, 98)	0 (0, 0.5)	0.667 (0.571, 0.833)	-0.455 (-0.861, 0.178)
Uninfected	969 (259, 2330)	3 (2, 9.5)	53.5 (33.4, 100)	0 (0, 1)	0.5 (0.4, 0.75)	-0.482 (-0.947, 0.585)

Table 4-2. Results from the principal components analysis of movement variables. Only PC1 scores were deemed significant according to regression analyses. Signs of PC1 loadings indicate a trade-off between movement and activity level. Larger PC1 scores represented the coupling of extensive movement and infrequent emergence, and lower PC1 scores represented the inverse.

		PC1	PC2	PC3	PC4	PC5
Loadings	Home Range Area	0.433	-0.503	-0.263	-0.667	0.215
	Home Range Burrows	0.411	-0.547	-0.162	0.69	-0.168
	Median Daily Distance	0.571	0.337	0.216	-0.191	-0.691
	Median Daily Burrows	0.551	0.359	0.291	0.188	0.67
	Proportion of Days Emerged	-0.117	-0.453	0.879	NA	NA
Importance of Components	Standard deviation	1.462	1.2	0.928	0.602	0.407
	Proportion of Variance	0.43	0.29	0.173	0.073	0.033
	Cumulative Proportion	0.43	0.72	0.894	0.967	1.00

Table 4-3. Maximum likelihood parameter estimates and odds ratios with 95% confidence intervals from the full logistic regression model. Odds ratios were calculated using original, rather than standardized parameter estimates. Only PC1 score, sex classification, and site classification were considered significant predictors (\*) according to both p-values and stepwise AIC model selection methods.

Parameter	Standardized Estimate (95% CI)	Odds Ratio <sup>a</sup> (95% CI)	
		Crude	Adjusted
PC1 score*	-1.55 (-3.10, -0.143)	0.89 (0.71, 1.11)	0.59 (0.35, 0.95)
Adult Female*	-2.12 (-4.70, -0.0150)	1.03 (0.48, 2.19)	0.12 (0.01, 0.98)
Juvenile*	-4.76 (-7.40, -2.73)	0.12 (0.04, 0.33)	0.01 (0.00, 0.07)
CE site*	-3.65 (-6.40, -1.45)	0.07 (0.02, 0.19)	0.03 (0.00, 0.23)
OR site*	-5.52 (-9.00, -3.02)	0.03 (0.01, 0.13)	0.00 (0.00, 0.05)
Adult Female:CE site	-0.64 (-4.30, 2.57)	NA	0.53 (0.01, 13.1)
Juvenile:CE site	2.76 (-0.86, 6.07)	NA	0.16 (0.42, 434)
Adult Female:OR site	2.41 (-1.50, 6.43)	NA	11.1 (0.22, 618)
Juvenile:OR site	-12.3 (-2474, 413)	NA	NA
Early 2005 season	-14.7 (-1128, 151)	0.00 (0.00, Inf)	0.00 (0.00, Inf)
Early 2006 season	-15.5 (-1100, 155)	0.00 (0.00, Inf)	0.00 (0.00, Inf)
Late 2004 season	-0.70 (-32230, 430)	1.00 (0.00, Inf)	0.50 (0.00, Inf)
Late 2005 season	-16.7 (-1056, 163)	0.00 (0.00, Inf)	0.00 (0.00, Inf)
Late 2006 season	-16.0 (NA, 190)	0.00 (0.00, Inf)	0.00 (0.00, Inf)

<sup>a</sup> Odds ratios calculated for sex- and site-level factors used adult males and the CF site as reference levels, respectively



Figure 4-1. Attachment of a fluorescent powder dye pack to an adult tortoise. Dye packs were attached to tortoises through one of the drilled holes located on the rear carapacial scutes used for identification. The dye pack was pulled close enough to the tortoise to minimize potential impediments to tortoise movement.

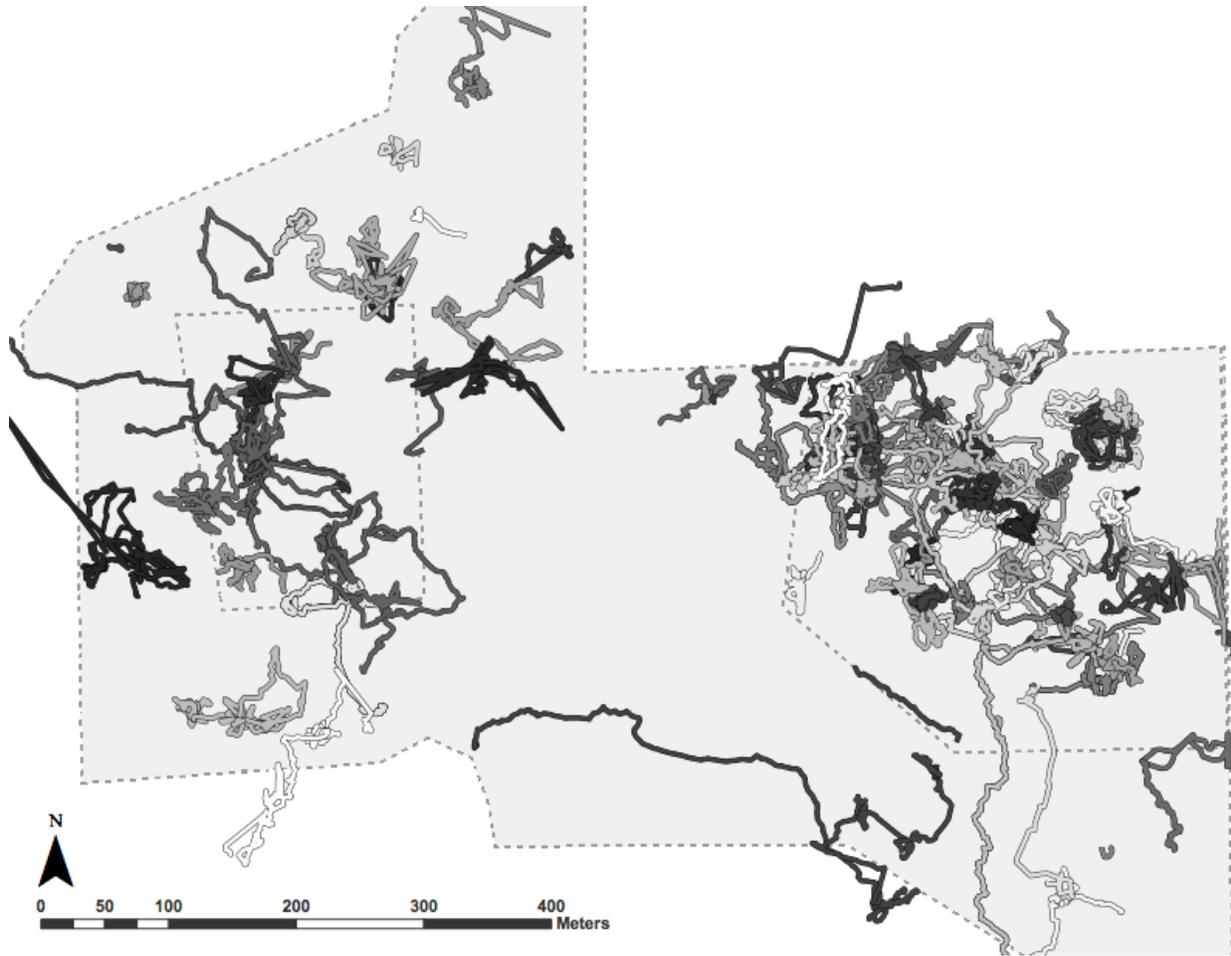


Figure 4-2. Sample map of individual movement patterns. This map depicts trails used by individual tortoises (represented by different color shades) from the CF site throughout the study period. Dashed lines indicate study site boundaries and trapping areas.

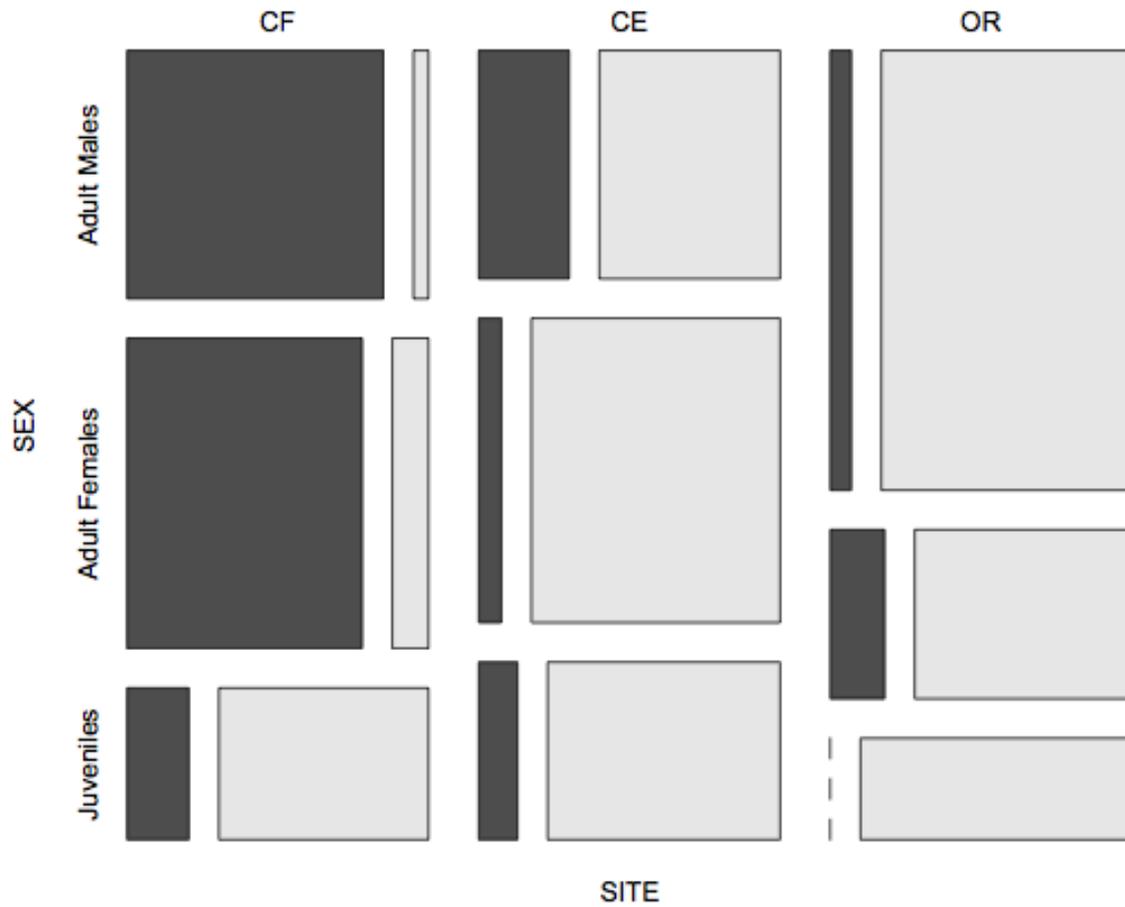


Figure 4-3. Mosaic plot of captured tortoises at the three study sites (CF, CE, OR). A total of 152 tortoises were sampled and tracked between May-October from 2004-2006. The dark grey and light grey blocks represent the proportion of infected and uninfected captures, respectively, from each site.

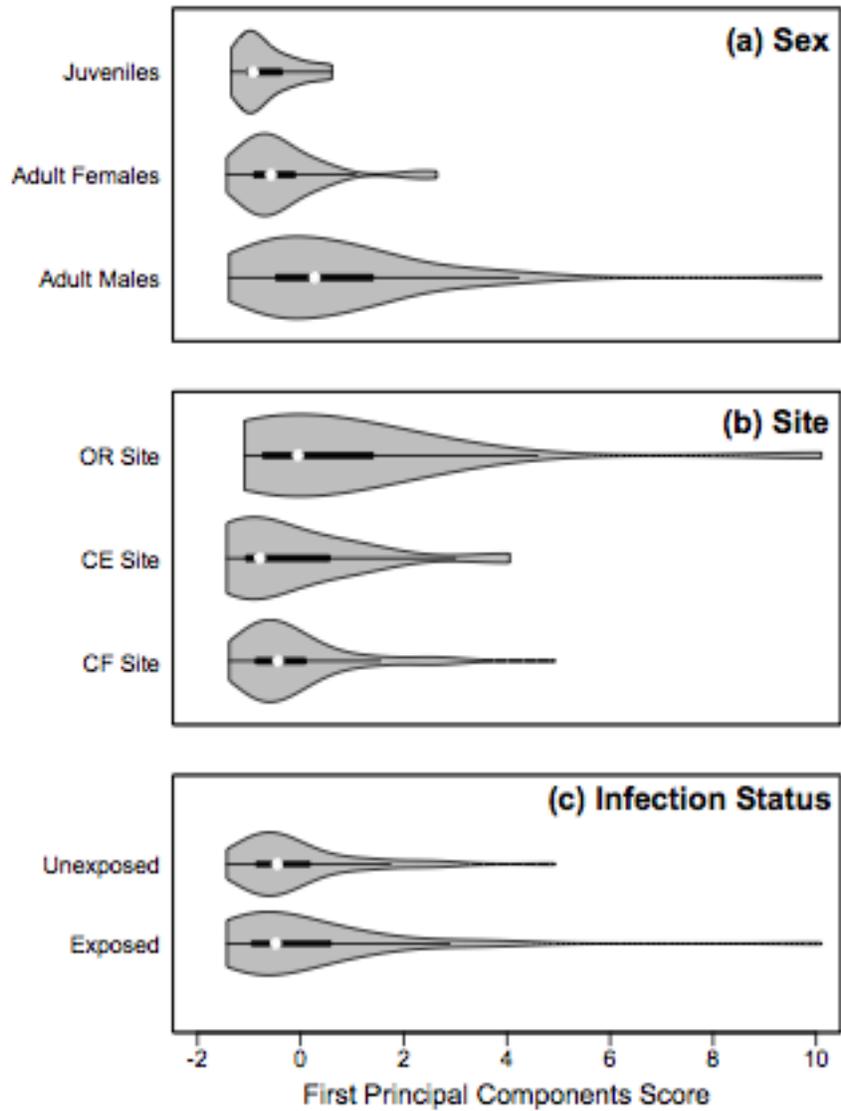


Figure 4-4. Violin plots of first principal component scores. Plots indicate the distribution of PC1 scores across different strata: A) Sex, B) Site, and C) Infection status. Scores were found to be significantly different among sex and site classes, but not infection status, according to Kruskal Wallis and Wilcoxon Rank Sum tests ( $p \leq 0.10$ ).

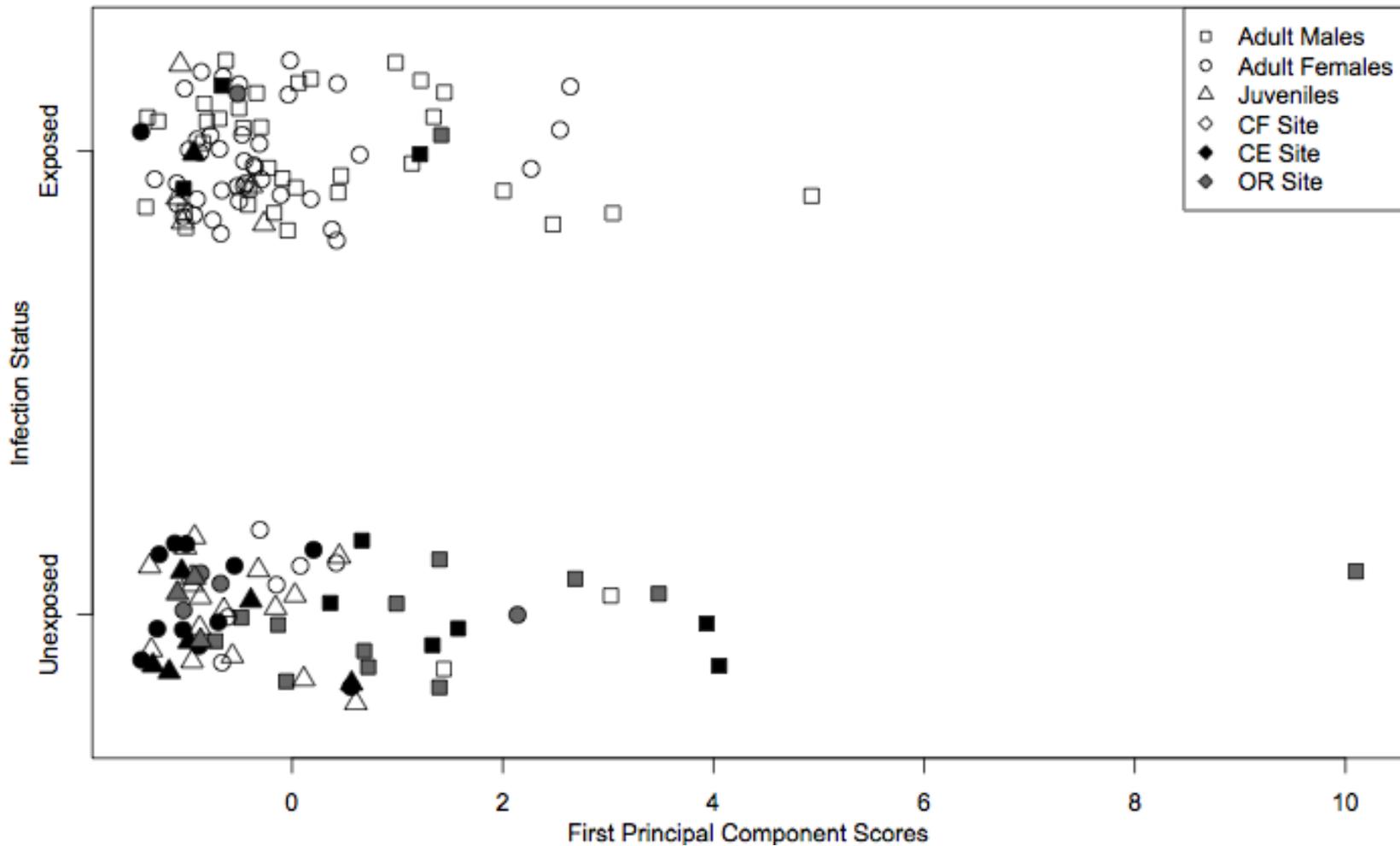


Figure 4-5. Scatter plot of first principal scores in relation to *M. agassizii* infection. Results for adult males (squares), females (circles), and juveniles (triangles) at CF (white), CE (black), and OR (gray) are shown. Adult males across all sites were consistently extensive movers, whereas adult females and juveniles were more restricted in their movements.



Figure 4-6. Example of trails of multiple males overlapping with trails of a single female. Adult males tend to move more extensively than adult females; however, the more sedentary nature of adult females (striped line) may increase their likelihood of making infectious contacts, relative to itinerant males (solid lines), through a polyandrous mating system.

## CHAPTER 5 CONCLUSION

### Overview of Results

Due to the complex interplay among host, pathogen, and environmental factors, an interdisciplinary approach to the study of emerging wildlife diseases is essential. In addition to individual-level studies of the host-pathogen relationship involving disciplines like microbiology, immunology, and pathology, valuable insights into the description of dynamics and host population-level impacts of disease in wildlife populations also can be provided by epizootiological and ecological studies. Past studies have reported on the pathological aspects of mycoplasmal URTD and the general surveillance of *M. agassizii* throughout gopher tortoise populations. The aim of this dissertation was to address gaps in knowledge associated with the ecology of mycoplasmal URTD in natural populations. With this dissertation, I applied methods from population ecology, mathematical epidemiology, and behavioral ecology to (1) elucidate potential long-term impacts of URTD on host population dynamics, (2) quantify and determine the likelihood of certain stage-specific disease processes, and (3) describe how individual behavior can contribute to URTD spread within exposed populations.

In Chapter 2, the long-term impacts of chronic and persistent epizootics were heuristically evaluated to determine how disease-induced mortality, outbreak duration, and outbreak recurrence frequency could potentially impact natural populations. I used matrix population and Markov chain models to depict host population dynamics under the threat of recurring disease outbreaks. The analyses indicated that host demographic factors (i.e., survival, growth, and fecundity) were more important than disease-associated factors (i.e., force of infection, disease-induced mortality, outbreak

duration, and outbreak frequency) on the long-term dynamics of exposed host populations, and that managing populations to maintain or improve upon host vital rates would better serve to promote persistence and viability than reactively managing populations during outbreaks. However, regarding disease-associated factors, the frequency of outbreak recurrence and the level of disease-induced mortality were most influential on long-term population dynamics. The greatest impact of disease, therefore, resulted from how often a population underwent an epizootic state (rather than how long an epizootic remained in effect), and by how much host survival was reduced through disease.

In Chapter 3, competing hypotheses of stage-specific disease processes in natural populations were evaluated by fitting force of infection models to age-seroprevalence data. Implications of the best-fit models, which included terms for both stage-specific disease-induced mortality and force of infection, predicted the imminent decline and local quasi-extinction of the study population within four years time, and were therefore biologically implausible. However, the next best-fit model, which had adequate support according to AICc metrics, provided more realistic model outputs. This model implied that disease-induced mortality was likely a negligible or low-level force acting on populations exposed to *M. agassizii*, and that force of infection was a stage-specific process. These results therefore support the hypothesis that pre-reproductive subadults become exposed to *M. agassizii* at much lower rates than reproductive adults, possibly due to differences in social behavior, and mortality associated with infection of both adults and juveniles is likely to be limited or negligible.

In Chapter 4, the movement patterns of individuals within an exposed population were evaluated to determine whether differences in exposure to *M. agassizii* could be attributed to differences in behavior. There were notable behavioral differences between pre-reproductive subadults and reproductive adults; however, these differences did not directly reflect increased exposure probabilities. Interestingly, the results from this study were suggestive of a retro-causal effect of infection on altered host behavior. Infected tortoises were more likely to participate in basking and foraging behavior than mate-seeking behavior. Because the temporal relationship between *M. agassizii* exposure and observed movement patterns could not be properly defined with this case-control study design, the study failed to provide insights regarding how individual behaviors contributed to infection susceptibility. However, the significant association between infection and basking/foraging behavior provides preliminary evidence of altered host behavior resulting from *M. agassizii* infection in tortoises.

### **Caveats**

This dissertation serves to provide a baseline quantitative assessment of mycoplasmal URTD in naturally occurring gopher tortoise populations. I assessed how recurring URTD outbreaks could influence the long-term dynamics and persistence of host populations. I addressed potential mechanisms that may be driving disparate exposure rates between pre-reproductive subadults and reproductive adults. Finally, I found that *M. agassizii* infection could potentially alter host behavior. As with any mathematical models, those presented here only reflect an approximation of the true host wildlife system and are subject to inaccuracies brought about through sampling efforts and simplifying model assumptions.

## **Impacts of Recurring Outbreaks**

### **A focus on mortality rather than morbidity**

Most respiratory mycoplasmoses of animals are characterized by high morbidity with low mortality (Simecka et al., 1992). In this study (Chapter 2), however, I focused on heuristically describing how recurring acute mortality events could potentially affect the long-term dynamics and viability of a gopher tortoise population exposed to *M. agassizii*. Morbidity could impact host demographic factors, basic biological function, and behavior. However, while morbidity is likely a component of the chronic stage of disease, such effects are notoriously difficult to quantitate and were not addressed by my models. Although any chronic disease effects associated with repressed growth or reduced fecundity were disregarded, elasticity analyses indicated that changes in these parameters would have little overall effect on long-term population growth. Additionally, a preliminary study that addressed the effects of *M. agassizii* exposure on fecundity found no significant reduction in clutch size or gravidity in infected female gopher tortoises (White, *unpubl. data*). However, because the long-term effects of disease on reduced fecundity and/or growth could have led to different projected dynamics, the results from this study may provide a conservative representation of potential population-level outcomes arising from URTD presence.

### **Long-term projections based on short-term data**

In general, empirical models are only as good as the data used to define them. The long-term projections presented in this study were based on demographic conditions representative of only a short time frame, and are subject to errors associated with imperfect vital rate estimates. Moreover, such projections are influenced by the precision of vital rate estimates. The data used to estimate these

rates were based on a short, four-year sample period and were therefore very limited in their representation of demographic processes of such a long-lived species.

Additionally, information obtained from a short time scale that is used to project future behavior/dynamics over a longer time scale is subject to bias, as the events observed during the short-term sampling period may not be representative of average long-term host population behavior/dynamics. Continued surveillance that would provide long-term ecological data related to demographic processes (i.e., survival, growth, and fecundity), would therefore improve the precision of vital rate estimates as well as the reliability of model projections.

### **Stage-Specific Disease Processes**

The cross-sectional age-seroprevalence data used in this study represented a snapshot in time of *M. agassizii* exposure within a population experiencing an endemic phase of URTD. Historical serosurveillance of this study population has demonstrated that after an initial spike in *M. agassizii* incidence (<10% prior to 2001, and going from 20% in 2001 to 77% in 2003), the seroprevalence of *M. agassizii* has remained relatively high and stable since 2003 (roughly 75% between 2003 and 2006; Wendland, 2007). Therefore, it appears likely that mycoplasmal URTD shifted from an acute epizootic phase between 2001-2003, to an endemic phase after 2004 when clinical disease and shedding levels decreased. The age-seroprevalence data used in this study were collected between 2003-2006, and therefore most likely represented the population under an endemic phase of URTD. It is possible that if data were collected from a population undergoing an acute epizootic phase of URTD, results may have differed. Specifically the force of infection during the initial acute stage of an epizootic is expected to be higher than the force of infection during the more stable endemic phase,

as the density of naïve and susceptible individuals declines through time (Martin et al., 1987). Likewise, the rate of disease-induced mortality could also vary among different epizootic phases. Therefore, inferences based on the results of this study should only be interpreted within the context of endemic URTD, as estimates of disease-induced mortality and force of infection from a population undergoing an acute epizootic phase may differ from those presented here.

## **Relationship Between Movement-Associated Behaviors and Exposure**

### **Using movement indices as an indirect measure of contact**

Given that the primary route of *M. agassizii* transmission is through direct contact (i.e., nose-to-nose; McLaughlin, 1997), the relationship between movement patterns and *M. agassizii* exposure was expected to reflect how the behaviors of individuals contributed to their susceptibility to participate in infectious encounters with other tortoises. In other words, movement patterns were expected to act as a surrogate index for contact, and reflect an overall tendency or potential to make infectious contacts, rather than define the true rate of contact among tortoises. However, because movement patterns alone were devoid of any spatial components of tortoise activity, they were unreliable indicators of potential contact rates. Therefore, I proposed further analyses (see *Chapter 4: Discussion*) that would address the social networks of tortoise contacts based on spatially explicit habitat utilization data available from this study (e.g., proximity to other infected tortoises, centrality regarding observed tortoise activity patterns, and connectedness to other individuals). Although the best method to elucidate *M. agassizii* transmission dynamics would involve an intensive longitudinal study design that could capture the seroconversion rates of naïve animals in an exposed population, and a social network analysis that would use cross-sectional data

to provide a preliminary understanding of how host behaviors can contribute to URTD spread within exposed populations.

### **Reverse causation**

In a case-control study design, the temporal relationship between exposure and outcome is difficult to establish. Thus, the results of such studies are prone to misinterpretation through the mechanism of reverse causation (Dohoo et al., 2003). With a retro-causal effect, the outcome of interest (e.g., *M. agassizii* exposure in Chapter 4) precedes, rather than follows, the exposure factor under question (e.g., movement-associated behaviors in Chapter 4). In relation to the analysis conducted in Chapter 4, although there was a significant association between movement-associated behavior and serological status, it was most likely that *M. agassizii* infection resulted in (rather than from) behavioral changes in the host that increased basking and foraging. Although results from this analysis were unexpected, the retro-causal effect of disease on host behavior was an interesting finding, and one that may provide an example as to how chronic mycoplasmal infection can influence host biological function (*see below*).

### **Research Contributions and Gaps in Current Understanding of URTD Ecology**

Across the literature, URTD is commonly implicated as a significant factor contributing to tortoise population declines. The most rigorous scientific undertakings addressing URTD in tortoises have involved experimental clinical studies focused on defining the etiology and pathogenesis of URTD. From these studies we learned that: (1) *M. agassizii* can cause URTD (Brown et al., 1994; Brown et al., 1999b), (2) the pathogenesis of *M. agassizii* infection involves ciliostasis, the focal loss of ciliated epithelium, mucosal hyperplasia, and the infiltration of leukocytes and phagocytic cells (e.g., heterophils) (Jacobson et al., 1991; Klein et al., 1995; Homer et al., 1998;

McLaughlin et al., 2000), (3) dissemination of infection from the upper to the lower respiratory tract is uncommon, (4) disease associated with *M. agassizii* is chronic and slowly progressive, (5) clinical signs can occur intermittently, and most commonly include nasal discharge, palpebral edema, and conjunctivitis (Schumacher et al., 1997), and (6) *M. agassizii* infection can increase host susceptibility to secondary infections (McLaughlin, 1997; McLaughlin et al., 2000). Additionally, field studies have monitored the prevalence of *M. agassizii* in natural tortoise populations (Lederle et al., 1997; Deimer Berish et al., 2000; Zipser and Ashton, 2003; McCoy et al., 2007; Karlin, 2008), and several have reported on occurrences of increased mortality associated with increased morbidity in populations previously exposed to *M. agassizii* (Berry, 1997; Seigel et al., 2003; Epperson, 2005; Wendland, 2007).

Although field reports of URTD-mediated die-offs are inconsistent across tortoise populations, with some reports of exposed populations remaining stable through time (Lederle et al., 1997; McCoy et al., 2007; Karlin, 2008), the scientific foundation for the hypothesis of URTD-mediated population declines likely involves case reports from the field describing increased mortality concurrent with high seroprevalence of *M. agassizii* and/or high occurrence of clinical disease. In each of these case studies, however, the specific cause of mortality has never been established. For most chronic diseases, cause of death is frequently secondary to the initial predisposing event and is often difficult to determine. Therefore, more evidence is needed to substantiate this hypothesis of URTD-mediated population declines.

The fulfillment of Koch's postulates has adequately defined *M. agassizii* as an etiologic agent of URTD; however, the mechanisms linking mortality to URTD-

associated morbidity remain vague and undefined. It is common within wildlife systems for cause-specific mortality to be a challenging process to define. Further, unless associated with specific toxins, few infectious diseases cause frank mortality. With this in mind, however, more care should be ascribed in referring to URTD as a “significant factor associated with population declines.” To effectively define the role of URTD on tortoise population viability, future studies need to adequately address: (1) the contributory role other factors (i.e., other pathogens, environmental factors) on disease severity, (2) the mechanisms through which morbidity associated with URTD relates to impaired biological function, and finally (3) how this impaired biological function could subsequently impact long-term population dynamics. With this dissertation, I attempted to address specific aspects of the research gaps listed above (specifically #2 and #3) in order to advance our understanding of URTD in natural gopher tortoise populations.

### **Exploring the Contributory Role of Other Factors on Disease Severity**

Severe respiratory mycoplasmoses can result in reduced fecundity, low weight gain, and even mortality in some animals. However, one of the major differences between mycoplasmal URTD in tortoises and severe respiratory mycoplasmoses of other animal species is that infection (and disease) in the former is restricted to the upper respiratory tract, whereas in most severe respiratory mycoplasmoses, infection (and disease) disseminates into the lower respiratory tract. In general, lower respiratory tract infections result in more severe disease than upper respiratory tract infections. For instance, chronic bovine pleuropneumonia caused by *Mycoplasma mycoides subsp. mycoides* is characterized by chronic lung lesions and can result in up to a 50% case-fatality rate in exposed herds of cattle (Simecka et al., 1992). Endemic calf pneumonia caused by *Mycoplasma bovis* can also result in high mortality through secondary

mycoplasmal invasions of the lower respiratory tract (Simecka et al., 1992). Lower respiratory tract disease resulting in enzootic pneumonia, although less severe than endemic calf pneumonia and chronic bovine pleuropneumonia, results in lowered feeding efficiency and poor weight gain in infected swine (Rautiainen et al., 2000; Regula et al., 2000). Additionally, complications of the lower respiratory tract (i.e., pneumonia and air sacculitis) caused by *Mycoplasma gallisepticum* infection can result in weight loss and depressed reproductive output in poultry (Jordan, 1975). Mice and rats infected with *Mycoplasma pulmonis* develop both upper respiratory tract manifestations as well as a chronic, life-long pneumonia (Simecka et al., 1992; Cartner et al., 1995) with significant immune-mediated airway pathology. Additionally, the severity of the disease is influenced by both environmental and host genetic factors (Broderson et al., 1976). Based on experimental infection studies and necropsies of naturally infected animals, *Mycoplasma agassizii* infection is restricted to causing URTD. Because dissemination of *M. agassizii* into the lower respiratory tract has not been reported from pathological findings, any severe effects of infection would likely involve other factors that may act synergistically with *M. agassizii* to elicit a more severe response within hosts.

### **Secondary infections**

Several studies have previously identified other respiratory pathogens of gopher tortoises, and were reviewed in Chapter 1 (see *Respiratory pathogens of gopher tortoises*). Of these potential respiratory pathogens, the surveillance of and interactions among *M. agassizii*, iridovirus, and herpesvirus have been most commonly addressed through observational studies (Johnson et al., 2006; Johnson et al., 2009). Given that these pathogens are associated with lesions throughout the respiratory tract, including

lungs and trachea (components of the lower respiratory tract), they may have a greater potential to cause significant morbidity that would result in depressed host functions, such as those observed in respiratory mycoplasmoses of cattle, rodents, swine, and poultry. Therefore, mycoplasma infection could result in significant respiratory disease through mechanisms that predispose hosts to secondary infections by herpes- and/or iridovirus.

Damage to the ciliated epithelium in the nasal cavity resulting from *M. agassizii* infection could potentially make it easier for secondary pathogens to invade and colonize the respiratory tract of infected tortoises. This is supported by research studies that have found increased abundances of gram-negative bacteria in the nasal passages of *M. agassizii*-infected tortoises compared to uninfected tortoises (Jacobson et al., 1991; McLaughlin et al., 2000). Additionally, a survey of diseases in free-ranging desert tortoises found that tortoises with oral lesions (a characteristic sign of herpesvirus infection) were more likely than tortoises without lesions to be infected with *M. agassizii* (Christopher et al., 2003), which may suggest the potential for synergistic interactions between herpesvirus and *M. agassizii*.

Further studies that employ controlled trials and/or more rigorous experimental designs are needed to demonstrate if and how other respiratory pathogens can act synergistically with *M. agassizii* to increase the severity of URTD in tortoises. In addition to increasing the level of surveillance of these pathogens in natural populations (i.e., through recently developed diagnostic assays), experimental trials are needed to address the pathogenic roles of herpesvirus and iridovirus infections in gopher tortoises. Experimental trials can also address how co-infection may contribute to the

pathogenesis and severity of URTD, and thus elucidate potential mechanisms through which *M. agassizii* may predispose a host to significantly debilitating secondary infections. Given the precarious status of the natural hosts, the ability to perform experimental trials may be impossible, or greatly limited at best. Therefore, development of surrogate animal models may be required if these questions are ever to be completely understood.

### **Environmental stress**

One of the most challenging aspects of wildlife disease research is that the interactions among host, pathogen, and environment usually result in highly variable dynamics of disease occurrence and severity. Often when attempting to elucidate the significance of a particular disease in a wildlife population, it becomes difficult to define the isolated effects of different factors on overall population health. For this reason, disease is considered to be a context-dependent phenomenon that relies on factors involving host susceptibility, pathogen virulence, and environmental conditions.

For example, even though *M. agassizii* causes URTD in both desert and gopher tortoise populations, the severity of disease and potential contribution to die-off events may be greater in desert tortoises because these animals experience more extreme environmental conditions. Depressed fecundity has not been observed in gopher tortoises with *M. agassizii* infection (White, *unpubl. data*); however, in desert tortoises an infection-associated decline in gravidity has been detected (Rostal et al., 1994). Additionally, although, the lesions associated with *M. agassizii* infection are similar between gopher tortoises and desert tortoises (Brown et al., 1999b), the recurrence and severity of die-off events are seemingly higher in desert tortoise populations compared to gopher tortoise populations.

Drought has been implicated as a potential factor associated with population declines of the desert tortoise (Peterson, 1994), and criticisms have been raised regarding the disregard of environmental conditions as a potential contributory factor in speculated URTD-mediated die-off events (Lederle et al., 1997; Sandmeier et al., 2009). For instance, Lederle et al. (1997) found that despite high seroprevalence to *M. agassizii*, populations appeared stable, and suggested that the claim of URTD-mediated declines was confounded by environmental conditions preceding die-off events. Interestingly, our analyses (see Chapter 2) would suggest that the frequency of drought events could potentiate more frequent recrudescence events and thereby increase adverse URTD-mediated events. Drought is a common feature associated with high adult mortality, and significant increases in mortality between normal rainfall and drought years have been previously reported (Turner et al., 1987). A longitudinal telemetry study by Peterson (1994), which included information on the physiological status of tortoises between 1988 and 1990, corroborated the significance of droughts in driving episodic mortality events in desert tortoises. In this study, although both study sites suffered from elevated mortality events, the patterns of mortality differed. Tortoises along the eastern Mojave Desert (i.e., Ivanpah Valley -- IV) were physiologically stressed (i.e., high plasma osmolality and blood urea nitrogen values) prior to death, and thus mortality at this site was most commonly attributed to drought-imposed physiological stress. Tortoises along the western Mojave Desert (i.e., Desert Tortoise Natural Area -- DTNA), however, were not physiologically stressed, and the majority of mortality events at this site were attributed to coyote predation. Interestingly, mortality events at DTNA during this time period had also been attributed to URTD

(Berry, 1997), however, in his study, Peterson (1994) found that coyote predation was the most common proximate cause of mortality at this site. If desert tortoises, like gopher tortoises, exhibit aberrant basking/foraging behaviors as sequelae to URTD (see Chapter 3), then they may be at increased risk for predation.

Although both the IV and DTNA populations experienced drought conditions concurrently during the study, the forage availability at the IV site was considerably more limited than that at DTNA, thus potentially accounting for differences in the physiological conditions of tortoises at both sites. Additionally, drought conditions were suspected to have indirectly contributed to the observed predation rates at DTNA through the limitation of prey availability, which consequently increased predation of tortoises by coyotes (Peterson, 1994). Therefore, not only can drought confound the effects of disease on tortoise mortality, but limited resources during drought periods could also increase predation pressure and thus mortality associated with predation. When the presence of infectious disease is superimposed on these extrinsic environmental conditions, a full understanding of the complexity of the natural systems and underlying predisposing cause of mortality is clearly challenging.

Mortality is considered to be an episodic process in desert tortoises that peaks during periods of drought, with host physiological stress likely a factor involved in these mortality events. In a survey of disease in desert tortoise populations, azotemia (an indicator of dehydration) was the most common form of laboratory abnormality (Christopher et al., 2003). Moreover, a five-year study that addressed physiologic changes across ranging environmental conditions (i.e., rainfall and drought years) in desert tortoises reported that rainfall patterns significantly affected laboratory values for

several analytes (Christopher et al., 1999). These included decreased levels of measures associated with forage availability (i.e., phosphorus, glucose, uric acid, iron, and triglycerides) and increased measures of dehydration (i.e., BUN, osmolality, electrolytes). Additionally, tortoises from the site that experienced the most severe environmental conditions in relation to drought (i.e., Goffs/Fenner Valley) also experienced the most severe physiologic alterations. Interestingly, another study found that clinical disease occurred most often following drought years, and that dehydrated tortoises had a greater prevalence of oral lesions and positive nasal cultures for *M. agassizii* (Christopher et al., 2003). This same study found that mortality of adult females was highest at the Goffs/Fenner Valley study site, in which severe shell disease, active *M. agassizii* infection, and oral lesions occurred (Christopher et al., 2003). The most challenging environmental conditions among the study sites was also observed at the Goffs/Fenner Valley study site (Christopher et al., 1999); therefore, it is likely that environmental stress contributed to the severity and prevalence of disease observed there. Additionally, when comparing biochemical profiles of desert tortoises with and without URTD, blood urea nitrogen (BUN) levels were significantly higher in tortoises with URTD (Jacobson et al., 1991), which suggests a potential association between disease and dehydration. Given that a nasal exudate is a primary clinical sign of URTD, it is reasonable to suggest that the nasal discharge may have a negative impact on water balance in these animals. Based on our study, the frequency of recrudescence adversely impacted population growth and viability (Chapter 2); therefore frequent droughts and other stressors could potentially act synergistically with URTD to increase both morbidity and mortality.

Oxalosis has also been associated with respiratory disease in tortoises. This condition stems from nutritional rather than biotic factors and is indicative of renal failure, whereby excess oxalate accumulates in the blood and is not excreted through urine. In one study, comparisons between healthy and diseased tortoises indicated there was no significant difference in the presence of oxalates between groups, and that oxalosis in tortoises with respiratory disease was merely incidental (Jacobson et al., 2009). Although no causal association was observed between oxalosis and respiratory disease, this environmentally-mediated disease could still serve to depress host condition and provides another example depicting how desert tortoises may face greater threats to their physiologic condition compared to gopher tortoises.

Addressing the mechanisms through which *M. agassizii* infection and environmental stress could alter the physiological condition of hosts would provide preliminary evidence of the individual and interactive effects of environment and pathogen on host population health. Biochemical profiles of gopher tortoises showed no significant differences in the blood chemistry of infected and uninfected tortoises (White, *unpubl. data*); however, assignment of specific indicators that may better reflect a measure of physiological stress could more accurately define this relationship. Furthermore, understanding the direct effects of URTD on tortoise populations would involve reducing the environmental “noise” associated with host body condition, disease state (i.e., active, chronic, or recrudescant) and overall population health. This would involve extensive, long-term surveillance of several populations that differ according to *M. agassizii* exposure, and would help answer questions such as: (1) do exposed populations suffer population declines at different times/rates than unexposed

populations, and (2) are population declines more correlated with *M. agassizii* exposure than environmental conditions.

It is possible that differences in the severity of population impacts associated with disease may also be attributed to environmental conditions. Interactions between disease and environmental factors make it difficult to differentiate between the direct effects of disease and those of environmental conditions. Indeed, these effects likely act synergistically to determine long term outcomes. Because gopher tortoises are probably faced with less challenging environmental situations that are less likely to compromise the physiologic condition of hosts, it is likely that the effects of URTD would probably play a more significant role in desert tortoise populations that are inherently more physiologically compromised.

### **Exploring the Relationship Between Morbidity and Impaired Biological Function**

Experimental pathological studies of *M. agassizii* infection in gopher tortoises have been restricted to short time frames of study, namely to several months post-exposure. Additionally, although necropsies have been conducted on naturally infected tortoises from wild populations, it has been difficult to ascertain how pathological lesions associated with chronic infection differ from those associated with acute infection since the time of initial infection cannot be readily determined in wild-caught animals. Experimental infection studies have demonstrated that re-exposure to *M. agassizii* can result in more severe clinical disease within a host; however, the long-term implications of re-exposure and chronic infection on URTD pathogenesis remain undefined. In addition to predisposing hosts to secondary infections, perhaps there are some aspects of chronic URTD pathogenesis that would result in reduced fecundity, growth, or survival. For example, the effect of URTD on fecundity might be a function of the stage

of reproduction when the female exhibits clinical disease. Although directed research is needed to define how pathological lesions associated with *M. agassizii* infection relate to impaired host biological functions, several mechanisms may be postulated.

### **Impaired foraging abilities through damaged olfactory mucosa**

Structural changes to the olfactory mucosa resulting from extensive damage to the ciliated epithelium could impair olfactory function involved in foraging activities.

Behavioral research on the desert tortoise has highlighted the importance of sniffing actions on daily tortoise activities such as selective foraging (Ruby and Niblick, 1994). Moreover, another study demonstrated that desert tortoises preferentially select forage that is lower in potassium, most probably due to a need to minimize the loss of nitrogen through excretion of potassium urates (Oftedal and Allen, 1996). An impaired sense of smell would likely harm this ability to selectively forage, and thus result in the consumption of lower quality vegetation, which could subsequently lead to the depressed nutritional status of infected hosts. There are no experimental data addressing this possibility, thus these are highly speculative inferences to draw from the observation of mycoplasma-induced lesions in the olfactory mucosa, and merely provide a potential connection between URTD pathology and impaired host function. In order to substantiate (and quantify) this potential effect of URTD on host function, further studies that address the impact of *M. agassizii* infection status on olfactory function would prove useful.

### **Energetic costs of infection**

Several respiratory mycoplasmoses of animals have been associated with lowered feeding efficiency and weight gain (Simecka et al., 1992), and may relate to increased energetic costs associated with infection. Energetic costs associated with immune

responses may in turn result in depressed reproductive output and/or growth (Lochmiller and Deerenberg, 2000). Because gopher tortoises commit to egg production in the fall prior to the spring in which the clutch is laid, the timing of clinical disease expression could be critical to any role in reproduction. White (*unpubl. data*) found that female gopher tortoises with lower body condition scores and lower plasma phosphorus levels were less likely to have eggs than were females with higher body condition scores. Unfortunately, the clinical status of animals in the previous fall was not available for most females in the study. More comprehensive studies over several reproductive cycles will be required to determine if URTD, nutritional demands, and fecundity interact significantly.

It is possible that increased energetic costs of infection may manifest themselves behaviorally in hosts through increased feeding activity. For instance, house finches with mycoplasmal conjunctivitis tend to spend more time at bird feeders than finches without conjunctivitis (Hawley et al., 2007a). Likewise, I found that infected gopher tortoises tended to spend more time basking and foraging compared to uninfected tortoises (Chapter 4). Abnormal basking and foraging patterns could also increase risk of predation. The research findings I presented in Chapter 4, therefore, provide preliminary support that URTD may compromise host biological function through increased energetic costs of infection; however, the mechanism through which *M. agassizii* infection increases the energetic demands of hosts remains unclear. In general, infections lead to a hyper-metabolic state within hosts in order to up-regulate immune responses. Moderate infections can increase rates of gluconeogenesis within hosts by 150-200%, which can often lead to severe wasting of lean tissue if infection

becomes chronic (Lochmiller and Deerenberg, 2000). Necropsies conducted on moribund desert tortoises demonstrated that *M. agassizii*-infected tortoises had less body fat than uninfected tortoises (Jacobson et al., 1991). This would support a hypothesis stating that mycoplasmal URTD could increase the caloric requirements of infected hosts through an elevated metabolic state established through infection. Although increased foraging behavior may relate to elevated caloric requirements of infected hosts, further research would need to define the metabolic differences between uninfected and infected tortoises. Moreover, the significance of increased energetic demands and altered host behavior on population viability would also need further evaluation if this effect is considered to be a significant impact of URTD on host populations.

#### **Indirect effects of chronic disease on reduced survival**

Disease may also indirectly influence survival by increasing host susceptibility to predation. For instance, Peterson (Peterson, 1994) attributed most of the desert tortoise mortality he observed at one of his study sites to coyote predation, but acknowledged that because URTD was also prevalent, it was possible that *M. agassizii* infection could have increased the predation susceptibility of hosts. In Chapter 4, I showed that *M. agassizii* infection could lead to aberrant basking and foraging behavior, which could subsequently lead to increased predation risk. The mechanism through which infection contributed to the modification of host behavior was not addressed with this observational study, but, may involve increased caloric requirements, and thermoregulatory behaviors associated with infection. Ozgul et al. (2009) noted that tortoises seropositive to *M. agassizii* were more likely to emerge from their burrows and thus have higher capture probabilities than seronegative tortoises. This higher rate of

emergence was attributed to a greater need for infected tortoises to behaviorally induce a fever response through basking activity (Brown, 2002). The increased emergence of infected tortoises in my study (Chapter 4) most likely corresponded with this same thermoregulatory mechanism.

Commonly, moribund tortoises have been characterized as appearing cachectic and lethargic (Jacobson et al., 1991). Therefore increased above-ground activity associated with foraging and basking under a lethargic state could make tortoises easier targets for predators. Moreover, if these activities were to occur aberrantly during normal periods of tortoise inactivity (i.e., hibernation), tortoises may also be susceptible to greater environmental stress associated with limited forage availability and/or cold stress. Finally, if tortoises rely on olfactory cues to find food and burrows, then the damage to the olfactory epithelium could also impact these biological functions.

Although the objective of Chapter 4 was to quantify the risk associated with the movement-associated behavior of individuals within an exposed population, the results obtained were suggestive of a potential effect of *M. agassizii* infection and chronic URTD on host behavior resulting in increased basking and foraging activities that could potentially increase host predation risk. The results from my study provide preliminary support regarding the potential for *M. agassizii* to modify host activity patterns. However, in order to adequately address the difference in predation risk associated with these modified activity patterns between infected and uninfected tortoises, researchers would need to carry out a longitudinal telemetry study of both chronically infected and uninfected tortoises to define the role of behavioral differences on predation rates.

## Exploring the Population-Level Effects of URTD

### URTD-mediated die-offs

Because of the difficulty involved in defining cause-specific patterns of mortality in wildlife systems, I heuristically addressed how sporadic and recurring bursts of URTD-induced mortality could potentially impact the long-term growth and persistence of gopher tortoise populations (Chapter 2). Even though the relationship between URTD and mass mortality events has not been adequately substantiated, I attempted to hypothetically determine how these speculative events could affect the long-term viability of tortoise populations. This heuristic exercise, therefore, provided a rough projection of the long-term population-level effects of recurring URTD-associated die-off events.

Results from this exercise indicated a relatively low impact of URTD-associated parameters (i.e., force of infection, disease-induced mortality, outbreak duration, and frequency of outbreak recurrence) on long-term population growth compared to the influence of demographic parameters (i.e., survival, growth, and fecundity). These results were based on elasticity analyses of population projection models, which quantified the proportional change in long-term population growth arising from proportional changes in each of these parameters. In other words, an increase in the force of *M. agassizii* infection would result in a very small decrease in  $\lambda$ , relative to the increase in  $\lambda$  brought about through an increase in adult survival. Results from these elasticity analyses do not suggest that URTD has no effect on long-term population dynamics, but rather that the effects are small relative to baseline demographic processes.

Moreover, by comparing the long-term growth and persistence times of populations experiencing recurring outbreaks with elevated disease-induced mortality across a range of values for outbreak duration, frequency of recurrence, and disease-induced mortality, the potential effects of URTD on population growth and persistence were quantified. URTD outbreaks associated with acute mortality events (as has been commonly reported in field studies implicating *M. agassizii* as a causal factor of sporadic die-off events), resulted in estimates of long-term population growth and persistence times that overlapped with the 95% confidence region of estimates obtained from an unperturbed population (i.e., a population which never experienced outbreak conditions). Given the variance associated with the estimated demographic rates used in this study, the sporadic recurrence of URTD-associated mortality events was not projected to result in significant differences in long-term population growth rates and persistence times between baseline and outbreak conditions. Additionally, although hypothetical increases in the rate of disease-induced mortality and the frequency of recurring outbreaks lowered the projected population growth rate and persistence times of gopher tortoise populations, these lowered rates and times were still within the normal range of values projected from an unperturbed normal population. In other words, the precision of  $\lambda$  associated with a normal baseline population was wide enough to encompass estimates derived from outbreak scenarios. Therefore, the population-level effects of recurring outbreaks still fell within the normal range of the dynamics associated with a normal (unperturbed) population.

In the above study, I only considered situations in which disease-induced mortality was at or below 30%. It is possible that under conditions of even higher disease-

induced mortality, the effects of URTD on projected population growth would significantly differ from that of a normal baseline population. However, results from the study described in Chapter 3 failed to support such high rates of disease-induced mortality. In Chapter 3, I addressed whether URTD-associated mortality was a likely force reducing the survival of infected tortoises, and found that disease-induced mortality was most likely occurring at a negligible rate within populations experiencing endemic URTD. Although the results obtained from this study may not be consistent across populations experiencing different epizootic phases of URTD (see *Caveats*), they more broadly support the hypothesis that URTD most likely exhibits a low-level influence, rather than a dramatic impact on exposed populations.

#### **URTD-associated morbidity effects**

As stated earlier (see *Caveats*), the study described in Chapter 2 only addressed the potential impacts of repeated die-off events, and disregarded any other potential effects of chronic URTD on other demographic processes such as fecundity or growth. In addition to elevated mortality, however, it is important to consider other mechanisms through which disease may lead to long-term population declines, especially in light of a chronic pathogen such as *M. agassizii*. Specifically, if morbidity associated with URTD is found to contribute to depressed growth, such as may result as a consequence of the energetic costs of infection, then tortoises may take longer to reach sexual maturity, and age at first reproduction would be delayed. A delay in age at first reproduction would result in a lower lifetime reproductive output, and consequently shorter persistence times for local populations. Additionally, if clutch sizes were significantly reduced by the same mechanism (i.e., high energetic cost of infection), lifetime reproductive output would also decline, and the long-term viability and persistence of exposed populations

would be compromised. However, these population-level effects of URTD-associated morbidity are currently unknown, as the energetic costs of *M. agassizii* infection have not been adequately defined (see above *Depressed fecundity and/or growth resulting from energetic costs of infection*).

Although the studies in this dissertation did not address the population-level impacts of chronic URTD-associated morbidity, one way in which these impacts on long-term population viability could be addressed is through life table response experiments (LTRE). Population ecologists commonly use LTRE analyses to statistically compare the effects of different environmental conditions on long-term population growth (Caswell, 2001). For instance, through this ANOVA-like framework, observational studies can be used to compare the differences in projected population dynamics between populations that vary according to URTD status (i.e., unexposed, undergoing an acute epizootic, and/or undergoing an endemic phase of URTD). In relation to mycoplasmal URTD, an LTRE analysis would show how projected population growth would differ according to whether or not a population was exposed to *M. agassizii*, or according to the epizootic phase of URTD experienced by a population. The main caveat to this approach is a need to estimate vital rates (i.e., stage-specific survival, fecundity, and growth) for each of the population conditions of interest, which would involve intensive longitudinal studies with at least three years of annual mark-recapture data per population condition of interest. Further, populations would have to be identified at different stages of infection and with similar population structures and habitats. Ozgul et al. (2009) noted that a clear understanding of the long-term, low-level impacts of URTD on natural tortoise populations would require between 10-20 years of

surveillance. Despite the intensive data requirements, such a study design would directly quantify the population-level impacts of chronic disease processes on host populations. Ozgul et al. (2009) noted that a clear understanding of the long-term, low-level impacts of URTD on natural tortoise populations would require between 10-20 years of surveillance. Continuous long-term surveillance of populations with known exposure to *M. agassizii*, and at different epizootic phases of URTD, will therefore continue to provide invaluable data regarding the impacts of infection on the survival, growth, and reproduction of tortoise hosts, and consequently on the long-term population dynamics of exposed populations.

### **Summary**

The purpose of this dissertation was not to define the role of mycoplasmal URTD on host population viability, but rather to describe ecological aspects of URTD in natural host populations, with the specific aims of advancing our current knowledge regarding (1) how recurring URTD-associated mortality events could impact populations in the long-term, (2) how stage-specific patterns of incidence can be used to quantify URTD effects on reduced survival, and (3) how individual behavioral differences may contribute to transmission dynamics within exposed populations. The first two specific aims addressed the long-term implications and statistical likelihood of reduced survival associated with *M. agassizii* infection, and found that recurring URTD-mediated die-off events were unlikely to cause dramatic changes to the long-term dynamics of exposed populations, and disease-induced mortality was likely a negligible rate in populations with endemic URTD. Although, the third specific aim was not adequately addressed by the study design implemented, results from the study were suggestive of altered host behavior arising from infection, possibly related to higher nutritional requirements of

infected tortoises. Taken as a whole, results of the studies included in this dissertation should not be interpreted as definitive proof of a limited impact of URTD in gopher tortoise populations. Rather, the results merely fail to support the hypothesis of URTD-mediated population declines, and reiterate that the effects of disease are context-dependent. Although under the scenarios assessed in this dissertation, the potential effects of URTD on long-term population viability appeared to be minimal, under other circumstances the effects may be substantial. This may be especially true if synergistic effects occur with other environmental factors or if morbidity-associated effects are considered. Therefore, extrapolating results from only a few populations within a given time frame to all gopher tortoise populations would be disregarding the context-dependent nature of wildlife diseases that make them such complex and interesting systems to study. This work suggests that focusing on the morbidity-associated impacts of chronic infection with *M. agassizii* may be an important component to understanding the long-term impacts on tortoise population health. Future research should advance our understanding of the long-term population impacts of URTD by challenging (or supporting) the findings presented here through continued surveillance of exposed gopher tortoise populations.

APPENDIX A  
METHODS FOR THE ESTIMATION OF DEMOGRAPHIC PARAMETERS

**Capture and Sampling of Tortoises**

All protocols were fully approved by the University of Florida IACUC. Census surveys were conducted at the three study sites (BS, FE, OR; Fig. A-1) to locate active and inactive burrows. Burrows were mapped with a GPS device, and flagged according to size class, as determined from burrow width and depth measurements. Bucket traps were placed at the mouth of burrows randomly selected according to size class and activity status, and traps were checked daily for captures. The trapping methodology utilized depended on the size class of the burrow being trapped, with one gallon buckets used for juveniles and hatchlings (burrow width  $\leq 155$  mm in diameter), five gallon buckets used for subadult and adult burrows ( $155 < \text{burrow width} < 320$ ), and 10 gallon buckets for large adult burrows (burrow width  $\geq 320$  mm). Holes were drilled in all buckets for water drainage, and shade covers made out of vinyl siding were placed over the traps to protect the tortoises from overheating.

Blood samples and nasal flushes were collected from each captured tortoise for determination of *M. agassizii* infection status as indicated by ELISA and PCR results (Brown et al., 2002). Morphometric measurements and complete health assessments were performed on each tortoise. Tortoises were sexed according to degree of plastral concavity and aged according to the count of plastral rings (Germano, 1988). Radiographs were obtained from adult females to determine the presence of eggs and clutch size. After data collection, tortoises were returned to the burrow from which they were captured.

## Parameter Estimation

### Stage-Specific Survival Probabilities ( $\sigma_H$ , $\sigma_P$ , $\sigma_R$ )

Capture histories spanning the 2003-2006 sampling period were generated for each tortoise from each study site. A general regression approach to Cormack-Jolly-Seber models, described by Amstrup et al. (2005), was used to estimate annual survival and capture probabilities from the individual capture histories (McDonald, 2008). For all models, annual survival probability ( $s$ ) was set to vary only by stage class, whereas annual capture probability ( $c$ ) could vary by stage, year, or site, depending on the model. Stages were defined as “pre-reproductive” (carapace length < 220 mm) and “reproductive” (carapace length  $\geq$  220 mm). AICc values were compared among models to determine which provided the best representation of the study population. Because two models provided adequate fits to the data ( $\Delta\text{AICc} = 0.032$ ), while all others fit the data poorly ( $\Delta\text{AICc} > 10.0$ ), the model-averaged estimates of stage-specific survival were used to define stage-specific survivorship probabilities for the normal population projection matrix (Table A-1).

Model-averaged estimates were obtained through the following equation (Burnham and Anderson, 2002, p. 150):

$$\tilde{\theta} = \sum_{i=1}^R w_i \hat{\theta}_i,$$

where  $R$  represents the number of models being averaged across,  $w_i$  represents the model-specific weight, and  $\theta_i$  represents the model-specific parameter estimate.

The unconditional variance of the model-averaged estimate was calculated as (Burnham and Anderson, 2002, p. 162):

$$\overline{\text{var}}(\tilde{\theta}) = \sum_{i=1}^R w_i \sqrt{\text{var}(\hat{\theta}_i | g_i) + (\hat{\theta}_i - \tilde{\theta})^2},$$

where  $g_i$  represents the specific model used to estimate the parameter  $\hat{\theta}_i$ .

### Pre-Reproductive Growth Probability ( $\gamma$ )

The probability of growth from the pre-reproductive to the reproductive stage class was therefore estimated through the fixed stage duration approach described by Caswell (2001):

$$\gamma = \frac{\left(\frac{\sigma}{\lambda}\right)^T - \left(\frac{\sigma}{\lambda}\right)^{T-1}}{\left(\frac{\sigma}{\lambda}\right)^T - 1}. \quad (\text{A-1})$$

The fixed stage duration ( $T$ ) was obtained by first fitting a von Bertalanffy curve to age-size data from the pooled study populations, with plastral ring counts used to represent a tortoise's age in years (Germano, 1988; Mushinsky et al., 1994; Berry, 2002; Wilson and Tracy, 2003). Maximum likelihood parameter estimates from the von Bertalanffy growth curve were then used for the inverse prediction of age at 220 mm carapace length, since this was the size used to delineate pre-reproductive and reproductive stage classes (Fig. A-2). The age of a tortoise at 220 mm represents the length of time a tortoise spends in the pre-reproductive stage, and therefore the fixed stage duration ( $T$ ) of pre-reproductive tortoises. Using this value of  $T$  (7.278, 95CI: 2.982 – 11.573) along with the annual survival probability of pre-reproductive tortoises ( $\sigma_p$ ) obtained from the above mark-recapture analysis, the growth probability of this stage class ( $\gamma$ ) was calculated using Equation A-1 with  $\lambda = 1$ . This method has been used to estimate stage-specific growth probabilities for other turtle populations (Crouse et al., 1987).

## **Fecundity (m)**

Fecundity, a multi-factorial parameter comprised of clutch size ( $cs$ ), proportion of females gravid ( $pg$ ), nest success probability ( $ns$ ) and hatch success rate ( $hs$ ), required several steps to quantify. Mean clutch size and proportion of females gravid were obtained from radiographs of adult females sampled from the pooled study populations prior to oviposition. Estimates of nest and hatch success were derived from published studies because these parameters were not directly measured from the study populations. However, rather than adopting the results of a single study to quantify either parameter, two separate meta-analyses were conducted that pooled together the results of all available studies that measured nest and/or hatch success across different gopher tortoise populations.

The data for the meta-analyses were obtained through a literature review of the Web of Knowledge database for all years. For the nest success meta-analysis, a total of 12 published studies were identified through the search, but after reviewing titles and abstracts, only 5 were relevant. Of these five studies, none provided an actual estimate of nest depredation rates. The cited literature from these studies was then further investigated. Three studies that quantified nest success in natural populations were finally identified (Landers et al., 1980; Wright, 1982; Marshall, 1987). The same process was followed for hatch success data, which identified seven studies that provided data of utility for the hatch success meta-analysis (Brode, 1959; Landers et al., 1980; Smith, 1995; Butler and Hull, 1996; Epperson and Heise, 2003; Pike and Seigel, 2006).

Effect sizes for each study identified through the literature search were defined as proportions ( $p$ ), and standard errors of these proportions were calculated using the equation (Lipsey and Wilson, 2001):

$$\sqrt{\frac{p(1-p)}{n}}, \quad (\text{A-2})$$

where  $n$  represents the study-specific sample size. For the nest success meta-analysis  $n$  was the total number of nests monitored for each study, but for the hatch success meta-analysis  $n$  represented the total number of eggs monitored for each study. The summary estimates of nest and hatch success that resulted from these meta-analyses were expected to be less biased representations of these parameters in an average gopher tortoise population, as these parameters were found to vary greatly across studies and populations according to tests of heterogeneity (Fig. A-3;  $Q_{ns}=11.98$ ,  $df=2$ ,  $p=0.003$ ;  $Q_{hs}=521.86$ ,  $df=6$ ,  $p=0.000$ ). The composite parameter  $m$  was then taken as the product of its components ( $m = (cs \times pg \times ns \times hs)/2$ ). The variance of  $m$  was calculated using the delta method.

Table A-1. Stage-specific apparent survival estimates from a 4-year mark-recapture study of gopher tortoises from historically URTD-free populations (BS, FE, and OR). Values in parenthesis represent the 95% Wald confidence intervals for model-specific apparent survival estimates.

Model	$\Delta AICc$	Deviance	Pre-reproductive Survival	Reproductive Survival
$C_{stage}C_{year}C_{site}S_{stage}$	0.000	546.188	0.408 (0.016-0.967)	0.902 (0.624-0.981)
$C_{year}C_{site}S_{stage}$	0.032	546.221	0.368 (0.084-0.789)	0.903 (0.625-0.981)
$C_{site}S_{stage}$	10.521	546.417	0.377 (0.087-0.794)	0.910 (0.663-0.981)
$C_{stage}C_{site}S_{stage}$	12.591	546.384	0.418 (0.016-0.970)	0.910 (0.662-0.981)
$C_{stage}C_{year}S_{stage}$	23.175	569.363	0.730 (0.000-1.000)	0.881 (0.601-0.973)
$C_{year}S_{stage}$	24.430	570.619	0.336 (0.076-0.756)	0.886 (0.595-0.976)
$C. S_{stage}$	28.634	570.750	0.343 (0.081-0.756)	0.893 (0.697-0.968)
$C_{stage}S_{stage}$	29.423	569.480	0.740 (0.000-1.000)	0.888 (0.635-0.973)
Model-averaged estimates	NA	NA	0.388 (0.037-0.913)	0.902 (0.634-0.981)

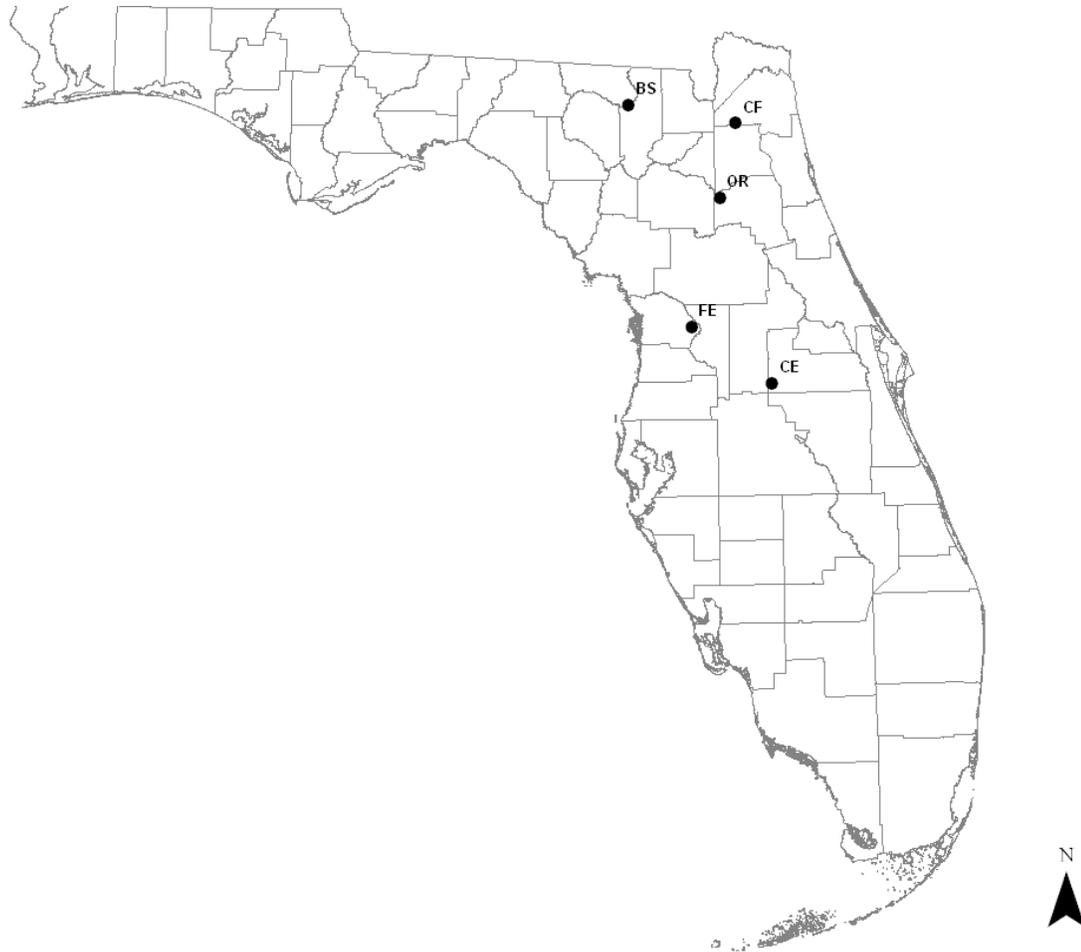


Figure A-1. Location of study populations used for data collection for this dissertation. The study sites are represented by initials and include: Big Shoals State Park (BS), Flying Eagle Wildlife Management Area (FE), Ordway-Swisher Biological Station (OR), Branan Field Mitigation Park (CF), and a privately-owned tract of land in central Florida (CE).

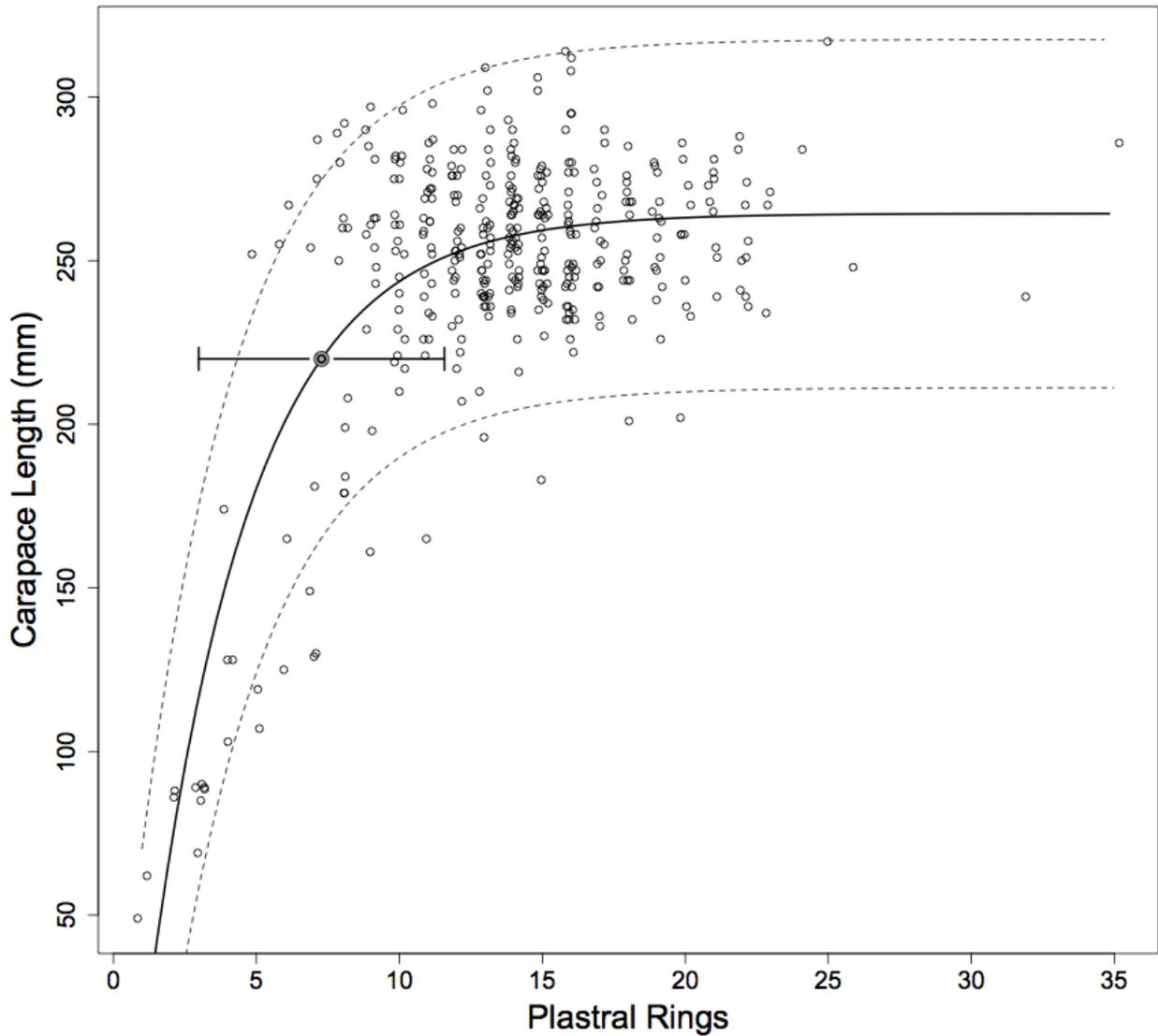
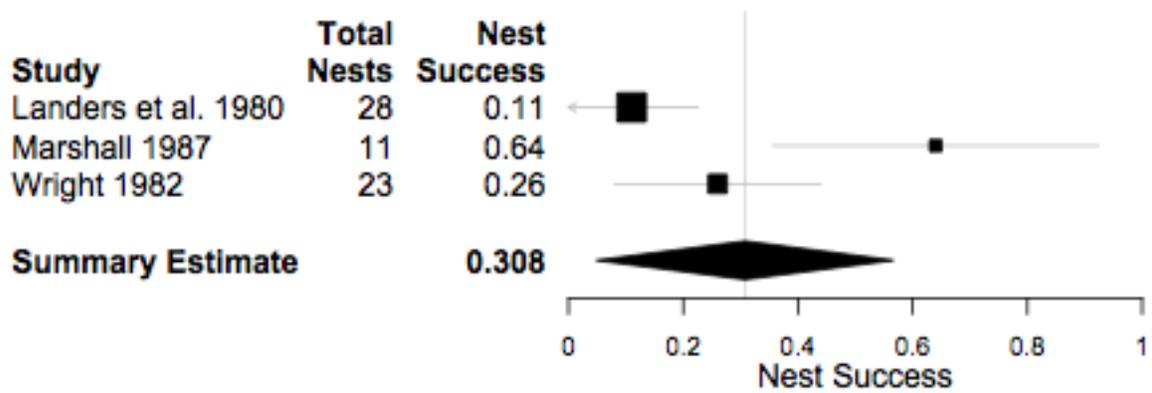


Figure A-2. The von Bertalanffy growth curve of gopher tortoises from historically URTD-free populations (BS, FE, and OR) with pointwise 95% confidence limits. The grey point on the plot represents the mean age of tortoises that measure 220 mm in carapace length (7.278; 95CI: 2.982 – 11.573), and represents the time a tortoise spends in the pre-reproductive stage ( $T$ ).

A



B

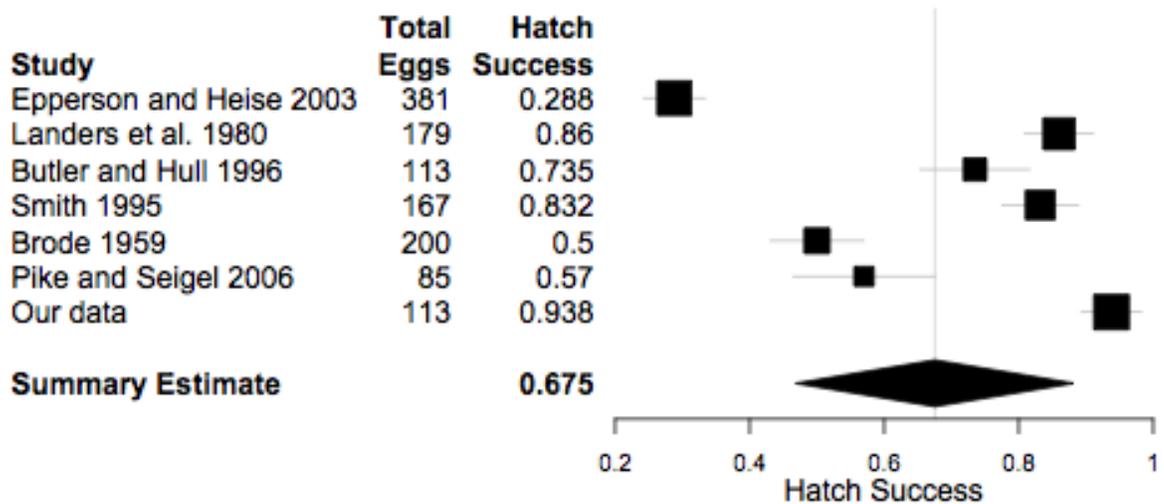


Figure A-3. Forest plots of (A) nesting and (B) hatching success probability using results from published literature. Summary estimates were generated through the use of random-effects models. Mean probability of nesting success across studies was estimated to be 0.308 (95CI: 0.049-0.566), and mean hatching success of eggs across studies was estimated to be 0.675 (95CI: 0. 0.469-0.881).

APPENDIX B  
A SUMMARY ESTIMATE OF GOPHER TORTOISE HATCHLING SURVIVAL  
FROM A META-ANALYSIS USING FIELD DATA AND PUBLISHED ESTIMATES

Meta-analysis is a particularly useful technique for integrating results of published studies that provide different estimates of a single parameter of interest. For example, estimates of vital rates (i.e., survival, growth, and fecundity) can vary across study populations due to differing habitat characteristics, predation pressures, or adverse anthropogenic impacts, among other factors. A summary value from multiple studies could therefore provide a more representative estimate of a vital rate across heterogeneous study populations, which can later be used to parameterize projection models of population dynamics. The purpose of this study was to generate a single summary estimate of gopher tortoise (*Gopherus polyphemus*) hatchling survivorship using published values along with field data from a three-year telemetry study. For the field study, parametric Weibull survival models were fit to weekly telemetry data and used to provide continuous estimates of survival through time, and test for differences in survival among years, hatching month, and release month. There were significant differences in survival among years ( $p = 0.0073$ ), between release months ( $p = 0.0022$ ), and between hatching months ( $p = 0.0053$ ). Hazard ratios (HR) indicated that hatchlings released in November were approximately 70% less likely to die within the first year of life than hatchlings released in September ( $HR_{Nov} = 0.285$ , 95CI: 0.148 – 0.549). Survival estimates specific to hatchlings released in September or 2006 were expected to be the least biased estimates of survival probability available from

this study because they were associated with the earliest age at release; however, because the 2006 sample size was very small ( $n = 9$ ), we considered the September release estimate to be the least biased. Using the least biased estimate of hatchling survival of the September releases from this study ( $n = 28$ ;  $\sigma_H = 0.108$ , 95CI: 0.008-0.207), a meta-analysis, which included values obtained from four other published studies, estimated overall hatchling survival to be 0.128 (95CI: 0.040-0.340) through the first year of life. We suggest the use of this summary estimate of hatchling survival for future parameterization of population projection models that aim to describe the long-term dynamics of natural gopher tortoise populations.

### **Background Information**

Meta-analysis is a statistical technique that has been used extensively in epidemiologic research to compile data from multiple sources for further analyses. Among its many uses, the synthesis of multiple results into a single summary value is of particular interest to ecologists concerned with defining overall or summary effects (Gurevitch et al., 2001). Briefly, the results of individual studies are summarized as effect sizes, which are then used to fit either a fixed effect or random effect model using the standard errors of effect sizes and study weights defined by the investigator. The meta-analysis then provides a summary measure of effect size across all studies among its output. This technique is especially useful for integrating results from multiple studies that provide different estimates of a parameter of interest.

The purpose of this study was to estimate hatchling survivorship from a natural population in North Florida using hatchling cohorts from 2004, 2005, and 2006, and to compile estimates from other studies in order to generate a single summary estimate of hatchling survivorship. This summary estimate can then be used in future studies to parameterize population projection matrices, and subsequently quantify the long-term population dynamics and persistence of gopher tortoise populations. The summary estimate of annual hatchling survival that resulted from this meta-analysis is expected to be a less biased representation of the true survivorship because of the high degree of heterogeneity across studies.

Briefly, the gopher tortoise is a long-lived species that reaches sexual maturity at 10-15 years of age (Landers et al., 1982; Deimer and Moore, 1986; Cox et al., 1987; Mushinsky et al., 1994). Adult female gopher tortoises produce annual clutches of 5-7 eggs; however in a typical ten-year period, the equivalent of only one clutch survives through the first year of life (Landers et al., 1980). Female gopher tortoises lay a single clutch of eggs annually between mid-May and mid-June (Iverson, 1980; Deimer and Moore, 1986; Cox et al., 1987; Smith, 1992). The incubation period varies geographically, ranging between 80-90 days, and nest depredation in gopher tortoise populations is estimated at 89% (Landers et al., 1980), with documented nest predators including the eastern coachwhip, eastern diamondback rattlesnake, indigo snake, pine snake, nine-banded armadillo, raccoon, gray fox, skunk, and the domestic dog (Douglass and Winegarner, 1977). The majority of documented nest and hatchling predators

are mammals, while snakes have been found to account for less than 11% of hatchling deaths in these studies (Butler and Sowell, 1996; Epperson and Heise, 2003; Pike and Seigel, 2006). Nest and hatchling predation are major factors contributing to the low recruitment rates of gopher tortoise populations, with mean hatchling mortality estimated at 90% (Landers et al., 1980).

Previous research has emphasized the importance of adult survivorship over hatchling survivorship on the long-term persistence of turtle populations (Crouse et al. 1987, Doak et al. 1994, Mills et al. 1999, Wisdom et al. 2000); however, in order to properly understand the long-term dynamics of threatened gopher tortoise populations, valid estimates of hatchling survival are required. Estimates of annual hatchling survival vary widely across studies. Generally, hatchling survival for the first year of life has been estimated to be between 5-10% (Landers et al., 1980; Butler and Sowell, 1996; Epperson and Heise, 2003). However, Pike and Seigel (2006) reported a 0% annual survival probability from their one-year study across three sites throughout Florida. In another study, Butler and Sowell (1996) monitored two cohorts of hatchlings throughout two different sampling periods. In one cohort ( $n = 14$ ), all hatchlings survived their first year, whereas none of the hatchlings in the second cohort ( $n = 6$ ) survived through year one, thereby providing an overall annual survival of 40%. Because many factors, such as habitat and predator composition, may influence and lead to heterogeneities in hatchling survival, the external validity and the degree to which the estimate is representative of most populations may be poor.

Therefore, the use of any one estimate from the above studies to describe hatchling survival would give rise to significantly biased results.

## **Methods**

### **Data Collection**

#### **Study site**

The hatchling survival field data was collected from a single population of gopher tortoises within Branan Field Wildlife Mitigation Park in Jacksonville, FL (CF; Fig. A-1). The 45.2 hectare study area consists of a mixed habitat composed primarily of pine flatwoods and sandhill. Prescribed burns in February 2006 and July 2006 by the Jacksonville Port Authority and the Florida Fish and Wildlife Conservation Commission in different parts of the study area did not affect the survival of either hatchlings or nests in the study.

#### **Location of nests and documentation of hatching success**

In 2004, nests were located through the use of wire probes (90 cm long wire inserted into the ground) along the aprons and surrounding pocket gopher mounds of 500 adult burrows (Smith, 1992). In 2005 and 2006, dye packs, consisting of one tablespoon of fluorescent dye (DayGlo) and constructed of nylon, were attached to one of the posterior scutes of all gravid females captured (i.e., radiographs indicated the presence of eggs), in order to facilitate the location of nesting sites. Trails left by the females were tracked nightly using a handheld UV lamp (Versalume), and documented through ArcPad mobile GIS (ESRI) on a handheld PC (Hp iPaq hx2110). Burrows visited by females were searched for eggs using a wire probe. Located nests (Fig. B-1) were protected through the use of a hardware cloth cage that was buried with the eggs at the

location of nest (Fig. B-2). Nests were checked daily for emerging hatchlings, in mid-late August of each year, as the incubation period for gopher tortoises in northern Florida is estimated to be between 80-90 days (Iverson, 1980).

Habitat characteristics of nest sites (i.e., canopy cover, ground cover, and basal cover) were also compared to those of non-nest sites through the use of a logistic regression model in order to determine whether females displayed any form of nest-site preference. In 2004, non-nest sites were randomly selected from the entire spectrum of adult burrows that were probed but did not yield nests ( $n_{2004} = 16$ ). In 2005 and 2006, burrows along which gravid females traveled but did not nest were randomly selected for habitat assessment of non-nested sites ( $n_{2005} = 13$ ;  $n_{2006} = 7$ ). Canopy cover surrounding the sites was measured with a densiometer, and basal cover was measured through the use of a prism. Percent ground cover was estimated using the quadrat method (Cox et al., 1987) and categorized according to vegetation type (i.e., woody plants, herbaceous plants, grasses, and litter).

### **Hatchling collection and tracking**

Three cohorts of hatchling tortoises were followed between 2004 and 2006, and provided a total sample size of 40 tortoises ( $n_{2004} = 19$ ,  $n_{2005} = 12$ ,  $n_{2006} = 9$ ). Upon emergence, morphometric measurements were recorded for each hatchling, and half of the members of each clutch were randomly assigned a radio transmitter (American Wildlife Enterprises, Inc. AWE-HG), which was attached to the carapace with epoxy (PC-7) according to methods discussed by Epperson and Heise (2003). Radio transmitters with the epoxy added approximated 2.5 g of additional weight to each tortoise, which amounted to less

than 10% of the body mass of each hatchling. Previous studies have demonstrated that there is no effect of transmitter weight on the survival of hatchlings (Epperson and Heise, 2003; Pike and Seigel, 2006).

In 2004 hatchling release was delayed due to the weather constraints posed by three hurricanes that passed through the North Central Florida region. The longest time span tortoises in this cohort were held in captivity after hatching was 32 days. Due to logistical difficulties, all hatchlings were released at the beginning of November 2005. In 2006 hatchlings were returned to their nests within 1-4 days of hatching depending on the success of radio transmitter attachment in the laboratory (Fig. B-3). Upon attachment of radio transmitters, hatchlings were tracked weekly until either a mortality event was documented or the transmitter signal was lost. Transmitters were replaced every 7-8 months, which coincided with the maximum battery life of the transmitters, according to the manufacturer. The 2006 cohort was tracked from September 2006 to March 2007.

## **Statistical Analysis**

### **Definition of event types**

Telemetry data was used to estimate the annual survival of hatchling gopher tortoises in this study. All observations except those that were suspected or confirmed to be mortality events were right-censored. In other words, upon exiting the study an individual was no longer considered in the analysis beyond that point in time, and its survival probability was assumed to be constant and equal to that at its last observation. For example, a lost telemetry signal arising from transmitter failure would result in a right-censored event at the time point at

which the signal was last observed; however, when transmitter failure was not the suspected cause of lost signals, conforming to this right-censoring assumption could have significantly confounded survival estimates (Hagen et al., 2006).

In order to reduce the potential confounding effects arising from this right-censored data, a hatchling mortality event was defined according to the following criteria: (1) tortoise remains were recovered alongside a radio transmitter (n = 5), (2) the signal was tracked to a snake until the transmitter was passed by the snake (n = 10), (3) the signal was lost with no prior indication of decreasing battery life, and tortoises were no longer found in or around the burrow hatchlings were known to inhabit (n = 8), (4) the transmitter with or without noticeable bite marks was found detached from the tortoise away from its known burrow without any signs of tortoise remains or problems with epoxy attachment (n = 6), or (5) mammalian digging tracks were apparent at the burrow they were known to inhabit at the time the signal was lost (n = 1). Otherwise, a right-censored event was defined when: (1) transmitter failure was the suspected cause of a lost signal (n = 7), (2) transmitter detachment was suspected from faulty epoxy attachment (n = 2), or (3) tortoises were still known to be alive at the end of the study (n = 1). The date of event occurrence was documented, along with the probable cause of mortality for suspected or known mortality events

### **Hatchling survival**

All analyses were performed using R programming language (R Development Core Team, 2007). Survival curves were generated through the *survival* package (Lumley and Therneau, 2008). Nonparametric survival

estimates were calculated through the Kaplan-Meier method in order to account for censored observations. Release age of each hatchling, rather than release date was used as the start time in the Kaplan-Meier analysis in order to account for the potential confounding effect age may have on survival estimates. Hatchling age on the day a hatchling was last tracked, before mortality or censorship, was taken as the end measurement in the analysis. Because the Kaplan-Meier estimator of the survivor function assumes constant survival probabilities between event times, several commonly used parametric survival models were also fit to the data to provide continuous estimates of survival through time. Specifically, survival models with exponential, weibull, logistic, log-normal, log-logistic, and Gaussian distributions were all fit to the data. According to AIC values, the parametric model best fit the data and was used to provide continuous survival estimates. Parametrically estimated survival probabilities were visually compared to non-parametric Kaplan-Meier estimates to determine whether the parametric model adequately described the censored survival data. Additionally, differences among cohort years (2004, 2005, 2006), hatching months (August, September, October), and release months (September, November) were evaluated through likelihood ratio tests using chi-square test statistics obtained from fitted parametric regression models that included cohort year, hatching month, and release month as covariates. Ninety-five percent confidence intervals around survival probabilities, median survival times, and hazard rates were estimated using a bivariate version of the delta method (Tableman and Kim, 2002).

## Meta-analysis

A literature search was conducted to compile all published estimates of annual hatchling survival in order to calculate a single summary value. The minimum criteria for inclusion into the meta-analysis were that studies had to: 1) quantitatively describe annual hatchling survival either as proportions or rates, and 2) provide either a sample size of hatchlings, for proportions, or standard errors for rates. A database search on the Web of Knowledge in May 2007 revealed 45 results, of which only nine were found to be relevant. Two of the nine relevant studies presented results repeated from previous studies so were excluded from the meta-analysis. The cited literature from those seven studies, which comprised an additional 243 studies, was then assessed. Four studies that provided adequate data on annual hatchling survival were identified. The total number of estimates used for the meta-analysis was five, including the present study.

The results, or effect sizes, for each study identified through the literature search were defined as proportions of hatchlings alive through the first year of life ( $p$ ), and standard errors of these proportions were calculated using Equation A-2, where  $n$  represents the study-specific sample size, which in this case is the total number of hatchlings monitored. For the meta-analysis, a random effects model was run using logit-transformed effect sizes and standard errors, with study weights defined by the inverse of their variance. The meta-analysis was run in R using the *rmeta* package (Lumley, 2008).

## Results

### Nest Location and Hatching Success

Thirty-one nests were located between 2004 and 2006. Nest site selection was not found to be preferentially associated with habitat characteristics. The logistic regression indicated that neither canopy cover (*cc*), basal cover (*bc*), nor ground cover (*gc*) were significant predictors of nest presence ( $p_{cc} = 0.838$ ,  $p_{bc} = 0.230$ , and  $p_{gc} = 0.088$ , respectively; Table B-1). Odds ratios of predictor variables also corroborated this conclusion, as 95% confidence intervals of the log odds of habitat parameters included the null value of 1.0 ( $OR_{pc} = 0.996$ , 95CI: 0.959-1.03;  $OR_{bc} = 0.84$ , 95CI: 0.64-1.11;  $OR_{gc} = 1.05$ , 95CI: 0.99-1.11).

Hatchlings emerged from protected nests between 16 August and 10 October. The 2004 cohort emerged earlier (16 August – 12 September) than 2005 and 2006 cohorts (9 September – 10 October). Mean clutch sizes of protected nests for 2004-2006 were  $5.33 \pm 1.50$ ,  $5.25 \pm 1.03$ , and  $4.40 \pm 1.67$  eggs, respectively. Hatching success, defined as the proportion of hatchlings that emerged successfully from a nest, was 85% for 2004 ( $n_{2004} = 48$ ), 90% for 2005 ( $n_{2005} = 42$ ), and 96% for 2006 ( $n_{2006} = 23$ ).

### Hatchling Predation

Twenty-nine predation events were documented during the hatchling tracking period. Of the 29 predation events, five were attributed to coachwhips (Fig. B-4), four to cottonmouths, one to an unknown snake, and nine to mammals. Twelve of the 29 predation events were attributed to unknown predators because neither the tortoise nor transmitter was ever found. Roughly 34% of all known predation events involved snakes, and 31% involved mammals.

## Hatchling Survival

Hatchling longevity exhibited a bimodal distribution, with peaks occurring between the ages of 25-50 days and 250-300 days, and with very low incidence of death occurring between these time periods (Fig. B-5a). Peak periods of hatchling mortality coincided with hatchling emergence and the onset of the tortoise active season at the end of May (Fig. B-5b). Age at death ranged between 1-381 days, with a median of 62 days. Median death ages differed among cohort years, hatching months, and release months; however, 95% confidence intervals for all groups overlapped. Estimated survival was significantly different among cohort years ( $\chi^2 = 9.85$ ;  $p = 0.0073$ ), release months ( $\chi^2 = 9.37$ ;  $p = 0.0022$ ), and hatching months ( $\chi^2 = 9.56$ ;  $p = 0.002$ ). In 2004, hatchling mortality events were most prevalent between mid-September and early October, whereas in 2005 and 2006, mortality events were more dispersed throughout the year.

The Weibull survival model provided the best fit to the data (Table B-2), and was therefore used to generate continuous estimates of annual hatchling survival. The annual survival of hatchlings was estimated to be 0.229 (95CI: 0.104 – 0.353) from the parametric survival model (Fig. B-6). Annual survival for 2004, 2005 and 2006 was estimated at 0.130 (95CI: 0.000 – 0.263), 0.529 (95CI: 0.258 – 0.799), and 0.065 (95CI: 0.000 – 0.187), respectively. Annual survival of hatchlings released in September and November was 0.108 (95CI: 0.008 – 0.207) and 0.530 (95CI: 0.260 – 0.800), respectively. Overall median survival time of hatchlings was 112 days (95CI: 47 – 177 days). Median survival time and

annual hazard rates of hatchlings stratified by release month and year are listed in Table B-3. Hazard ratios (HR) indicated that hatchlings released in November were approximately 70% less likely to die within the first year of life than hatchlings released in September ( $HR_{Nov} = 0.285$ , 95CI: 0.148 – 0.549). Additionally, hatchlings that emerged in 2005 and 2006 were 69% less likely and 34% more likely, respectively, to die within the year than hatchlings that emerged in 2004 ( $HR_{2005} = 0.313$ , 95CI: 0.169 – 0.577;  $HR_{2006} = 1.344$ , 95CI: 0.770 – 2.346). Likewise, age upon release was significantly correlated with hatchling follow-up time (0.404, 95CI: 0.105-0.635; p-value = 0.01).

### **Meta-Analysis**

The overall annual survival probability from the present study was confounded by the late release of hatchlings in November 2005, as these hatchlings were older and age at release significantly influenced annual survival probability. Survival estimates specific to hatchlings from the 2006 cohort or hatchlings released in September of either year were likely to be the least biased because they were associated with the earliest age at release, and were thus least affected by release dates. Because the sample size for the 2006 cohort was very small ( $n = 9$ ), the annual survival estimate obtained from hatchlings released in September across all years ( $n = 28$ ;  $\sigma_H = 0.108$ , 95CI: 0.008-0.207) was selected for inclusion into this meta-analysis. Heterogeneity among individual study estimates was found to be statistically significant ( $Q = 13.03$ ,  $df = 4$ ,  $p = 0.01$ ). Overall annual hatching survival was 0.128 (95CI: 0.040-0.340; Fig. B-7).

## Discussion

Canopy cover, basal cover, and ground cover were poor predictors of nest presence; however, the logistic regression analysis indicated negative effects of canopy and basal cover, and positive effects of the amount of available bare ground on nest presence (Table B-1). In other words, nests tended to be located in open sunny areas that were also unobstructed by ground vegetation, presumably to facilitate egg deposition. However, as these habitat characteristics did not differ among burrows without nests, it is likely that this combination of factors was more indicative of burrow location preference rather than nesting site selection.

Epperson and Heise (2003) estimated annual hatchling survival at approximately 0.05 through a Kaplan-Meier estimator. Pike and Siegel (2006) estimated annual survival to be approximately 0.04. Our estimate of annual hatchling survival probability was 0.229. Annual survival probability of the 2006 cohort (0.065, 95CI: 0 – 0.187) was closest to those reported by previous studies, corroborating that the first month of life is the most hazardous to hatchling survival. As evident from the survival of hatchlings released in November, protection of hatchlings for about 44 days post-emergence increased annual survival to approximately 53%. The pronounced effect of age at release on annual survival of hatchling gopher tortoises purports the early protection of hatchlings to be an effective strategy for head-starting programs that aim to increase the survival of these young age classes, although the long-term efficacy of such programs in relation to broader population impacts has been brought into question (Heppell et al., 1996).

In addition to the older release ages of 2004 and 2005 hatchlings, discrepancy from estimates of previous studies may also be due to geographic and individual variation (Deimer and Moore, 1986). Pike and Seigel (2006) suggested that differing predator compositions contributed to variability in hatchling mortality among sites in their study, so it is plausible that because a high rate of mortality events were snake-related compared to other studies, the difference in these survival estimates may have been attributed to dissimilar predator compositions. The high variation in hatchling survival between years could have been due to higher predator levels in 2004 compared to 2005 and 2006. Since snake predation was the predominant cause of hatchling mortality in this study, perhaps hatchlings that emerged later in the summer had reduced probabilities of encountering snakes, and thus had higher probabilities of surviving for a longer time period than hatchlings that emerged earlier when snake predators were more active. Because nests in 2005 and 2006 were located through the dye tracking of gravid females, the added stress of capture during the nesting period may have attributed to the later dates of oviposition and consequently, the later hatching dates of their offspring. The hypothesis of increased survival with delayed hatchling emergence is supported by the temporal distribution of mortality events (Fig. B-5), where mortality events in 2004 occurred with highest frequency between mid-September and early-October, but mortality events in 2005 and 2006 were more dispersed throughout the year. Overall, these results support findings from other studies that describe the low survival of hatchlings, although the point estimates for survival obtained from this

study are higher than those previously reported. The significant variability between these survival estimates and those of previous studies may have been associated with release age, predator composition, or geographic variation. Therefore, we suggest using the summary value of 0.128 (95CI: 0.040-0.340) obtained from this meta-analysis to parameterize annual hatchling survival in models of population dynamics for future studies.

Table B-1. Estimated coefficients from the logistic regression model, which assessed the ability of habitat variables, canopy cover, basal cover, and ground cover, to predict nest presence. None of the variables were significant predictors of nest presence/absence ( $\alpha < 0.05$ ), but basal and canopy cover had slightly negative effects, and bare ground had marginally positive effects on nest presence.

	Estimate	Standard Error	z value	Pr(> z )
(Intercept)	0.182	0.667	0.273	0.785
Canopy Cover	-0.00399	0.0195	-0.205	0.838
Basal Cover	-0.169	0.141	-1.201	0.230
Bare Ground	0.0467	0.0274	1.707	0.0879

Table B-2. Selection of a parametric model to provide continuous estimates of annual gopher tortoise hatchling survival. The Weibull survival probability distribution provided the best fit to the field data compared to other probability distributions, according to AICc metrics.

Model	df	Log-likelihood	$\Delta$ AICc
Weibull	2	-192.1	0.000
Log-normal	2	-192.5	0.7835
Log-logistic	2	-192.8	1.469
Exponential	1	-197.2	10.16
Logistic	2	-215.4	46.55
Gaussian	2	-217.3	50.43

Table B-3. Group-specific annual survival probabilities, median survival time in days, and annual per-capita mortality rates for hatchlings estimated from field data with 95% confidence intervals in parentheses. The annual survival estimate obtained from September releases ( $n = 28$ ;  $\sigma_H = 0.108$ , 95CI: 0.008-0.207) was selected for inclusion into a broader meta-analysis of previously published estimates of hatchling survival in order to generate an overall mean estimate of this parameter.

Group	Sample Size	Annual Survival	Median Survival Time	Annual Hazard Rate
Overall	40	0.229 (0.105-0.353)	112 (47.1-178)	0.00259 (0.00119-0.00399)
September Release	28	0.108 (0.008-0.207)	64.3 (22.3-106)	0.00411 (0.00175-0.00646)
November Release	12	0.530 (0.260-0.800)	416 (0.00-913)	0.00117 (0.000150-0.00219)
2004 Cohort	19	0.130 (0.000-0.263)	75.3 (16.2-134)	0.00382 (0.00143-0.00620)
2005 Cohort	12	0.529 (0.258-0.799)	413 (0.00-899)	0.00119 (0.000149-0.00224)
2006 Cohort	9	0.065 (0.000-0.187)	48.8 (0.60-97.0)	0.00513 (0.000838-0.00942)



Figure B-1. Locations of nests in 2004 (triangles), 2005 (squares), and 2006 (pentagons) from which hatchlings were taken for this telemetry study. The area outlined below depicts the study area within the CF site.

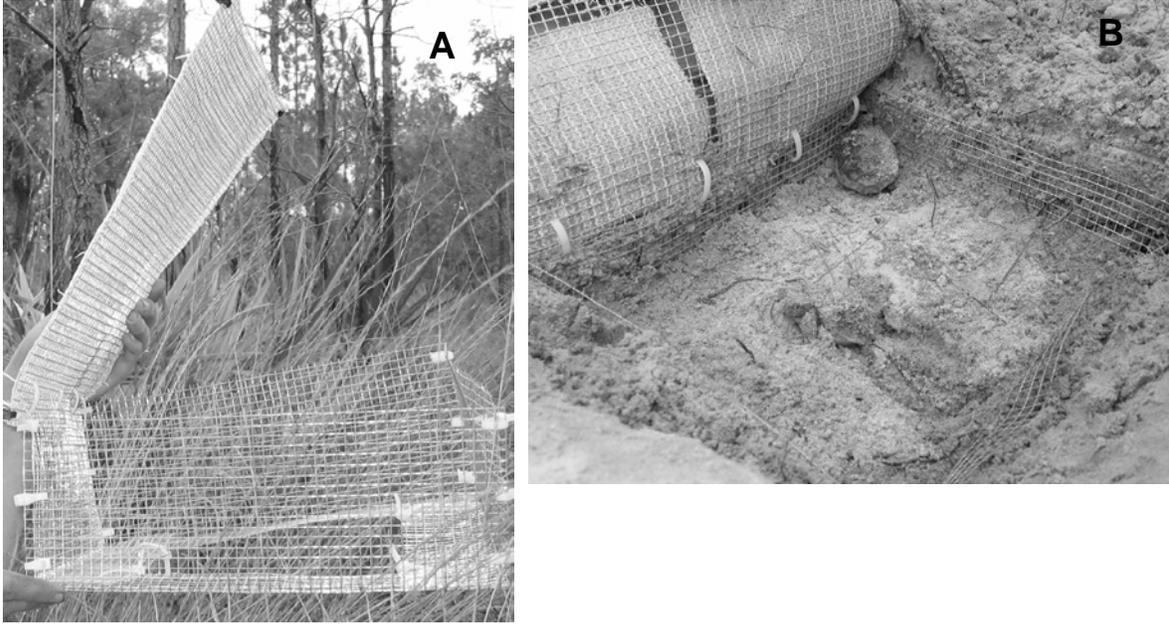


Figure B-2. Construction of nest protection cages. Nests were excavated and eggs removed for the placement of a buried nest cage to protect eggs from potential predation. (A) Nests cages were constructed from hardware cloth, and (B) buried at the mouth of burrows from which they were found.

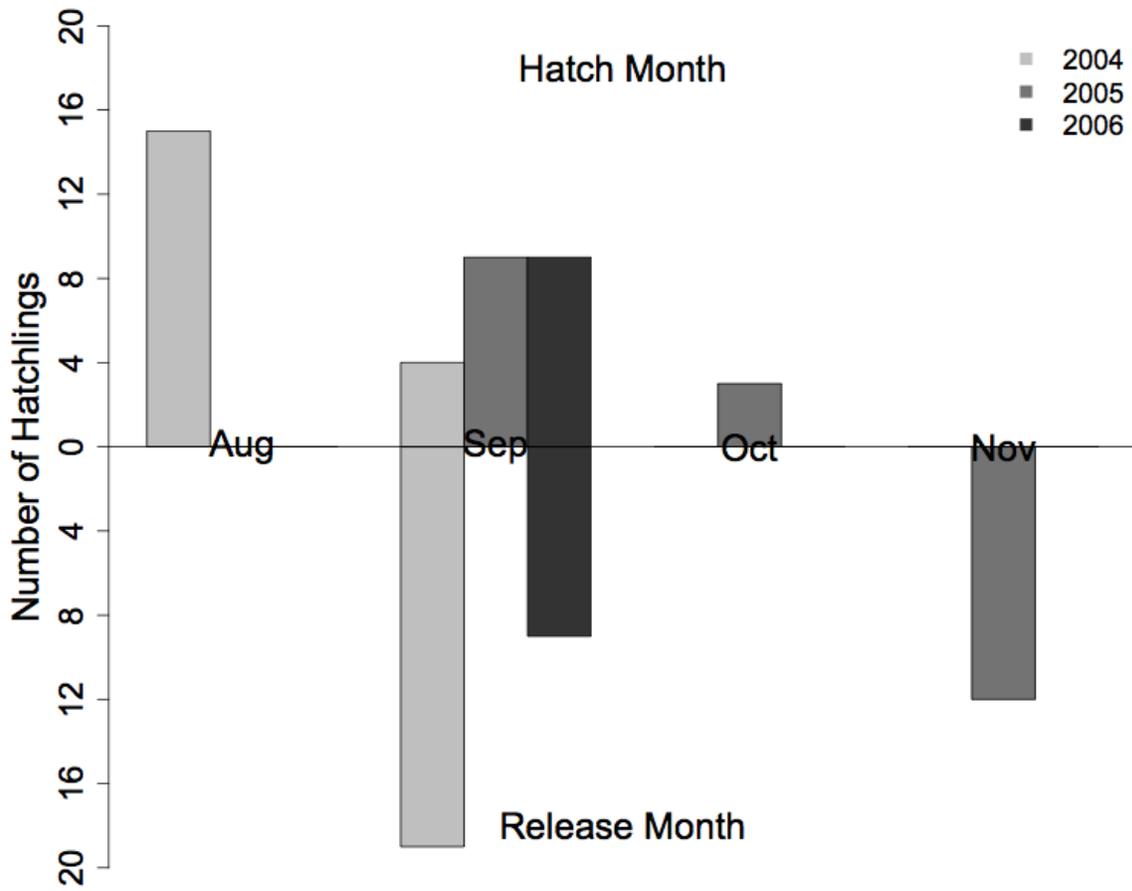


Figure B-3. Number of hatchlings included in the telemetry study each year stratified by month of hatching and month of release.



Figure B-4. Observed predation of a hatchling gopher tortoise in 2004 by an Eastern Coachwhip (*Masticophis flagellum flagellum*).

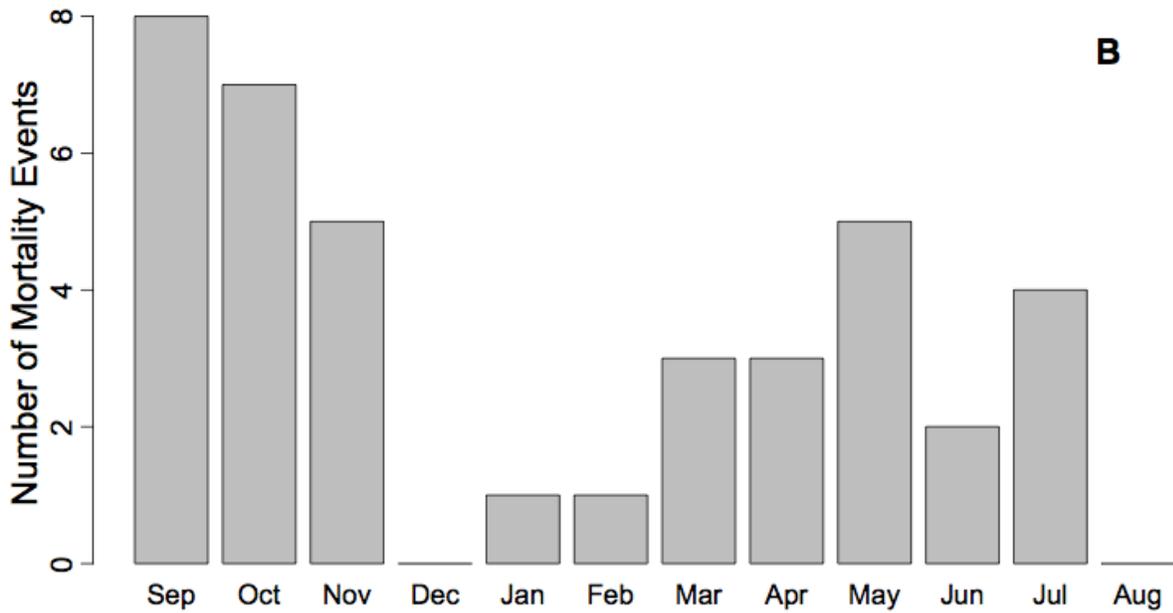
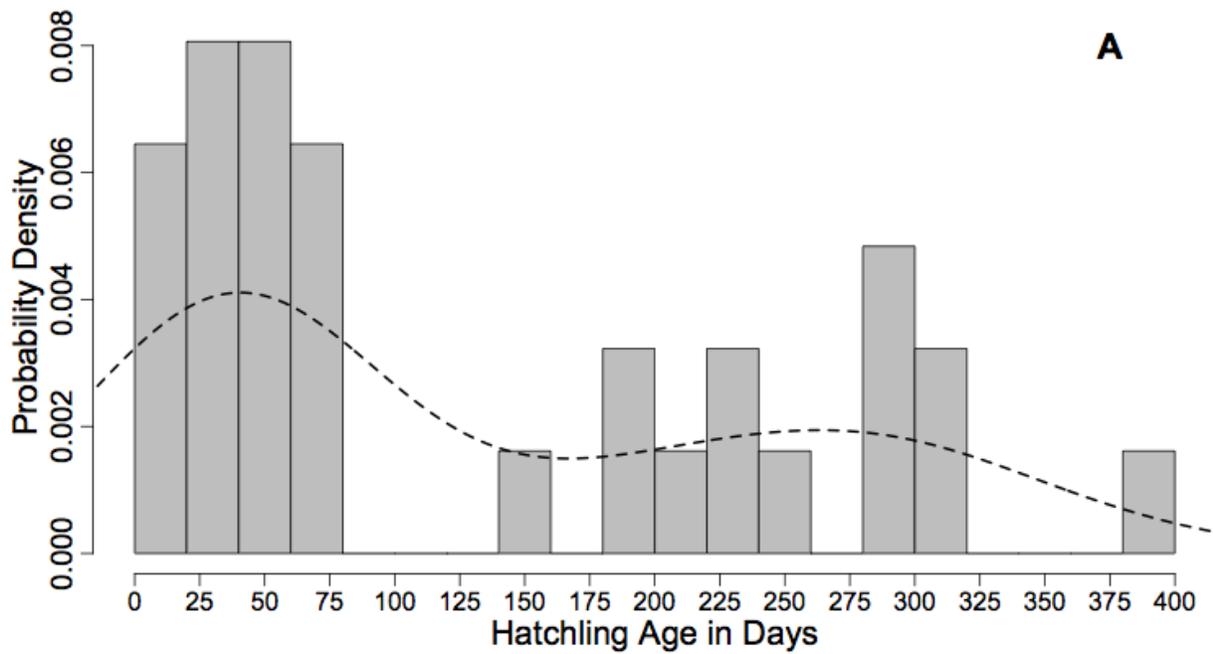


Figure B-5. Temporal patterns of hatchling mortality. A) The distribution of hatchling ages at death follows a bimodal pattern with peaks occurring between 25-50 days and 275-300 days, which corresponds with B) the distribution of mortality events across calendar years.

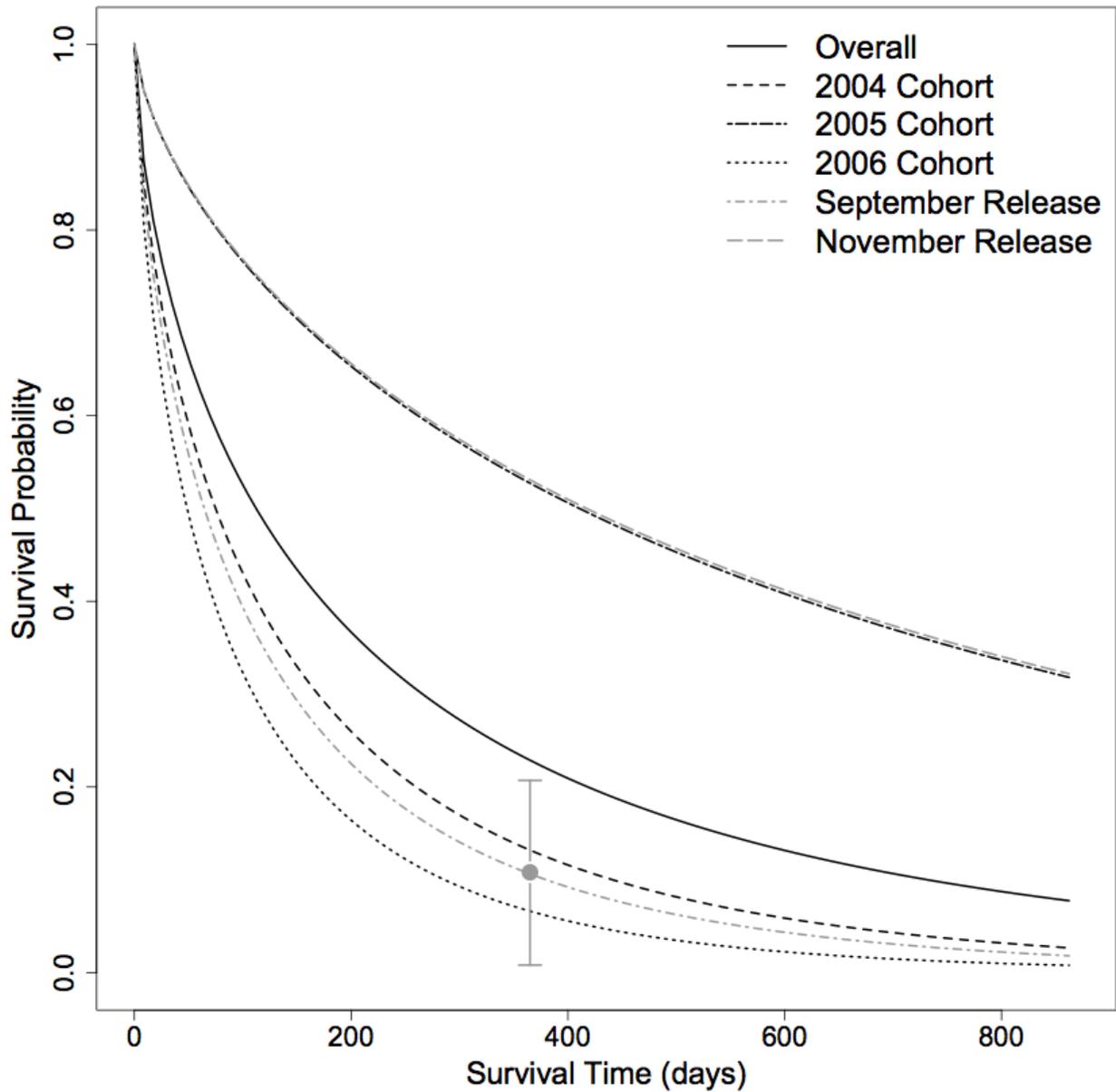


Figure B-6. Survival curves for gopher tortoise hatchlings generated from field data. Different curves represent the overall, year-specific, and release month-specific survival probabilities of hatchlings included in the study. The grey point represents the annual survival probability, with corresponding 95% confidence limits, used in the meta-analysis (0.108, 95CI: 0.008 – 0.207).

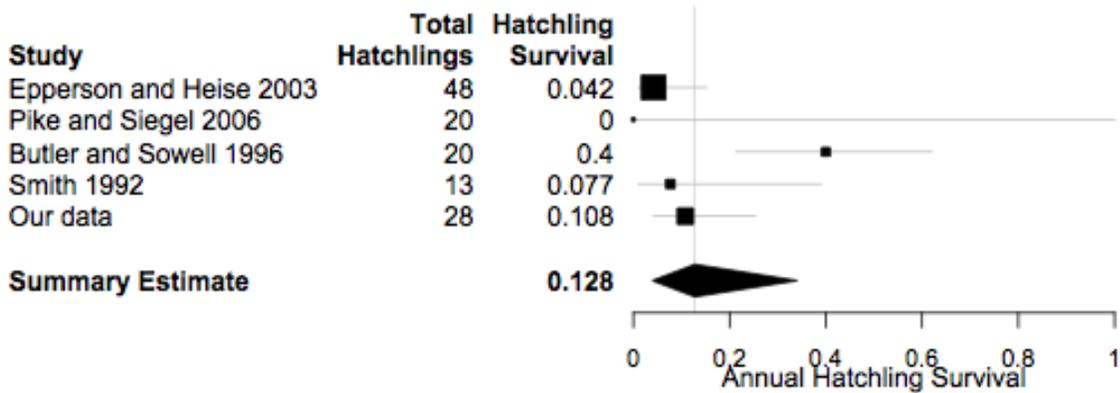


Figure B-7. Forest plot of hatchling survival using results from published literature along with results from field data. A summary estimate was generated through the use of a random-effects model. Mean annual hatchling survival across studies was estimated to be 0.128 (95CI: 0.040-0.340).

APPENDIX C  
METHODS FOR THE CALCULATION OF STOCHASTIC GROWTH RATES USING  
THE MATRIX SIMULATION APPROACH

Fifty-four different stochastic growth rates, each corresponding to a different combination of normal-outbreak state grouping and Markov chain model, were each estimated from the average population growth rate over 50,000 simulated time steps. For each disease scenario, a sequence of environments corresponding to a specific Markov chain model was generated and used to assign a projection matrix at each time step. The starting population size for these simulations was taken to be the stable stage distribution of the mean projection matrix. Using the projection matrix assigned by the Markov chain model, the population size was projected for one time step, and the log growth rate ( $r$ ) calculated for this one time step. After each simulated time step, the projected population size was renormalized to sum to 1 for computational efficiency. The simulated stochastic log growth rate ( $\overline{\log \lambda_s}$ ) was then calculated as the mean  $r$  over all time steps (Morris and Doak, 2002). Estimates presented in Table E-2 represent the exponentiated terms (i.e.,  $\lambda_s$  rather than  $\log \lambda_s$ ).

APPENDIX D  
METHODS FOR THE CALCULATION OF STOCHASTIC AND DETERMINISTIC  
ELASTICITIES

Elasticities from stochastic simulations were calculated using the following equation (Caswell, 2001):

$$\frac{\partial \log \lambda_s}{\partial \log a_{ij}} = \frac{1}{T} \sum_{t=0}^{T-1} \frac{v_{t+1} w_t' \circ A_t}{R_t v_{t+1}' w_{t+1}}$$

A sequence of  $T = 50,000$  disease states was generated according to probabilities assigned by a given Markovian transition matrix. From this simulated sequence of environments, a sequence of 50,000 population projection matrices ( $A_0, A_1, \dots, A_{49999}$ ) was then generated in which  $A_t$  corresponded to the simulated disease state at time  $t$ . Assuming an arbitrary non-negative initial population structure vector ( $w_0$ ) whose scalar product was 1, a sequence of  $w$  vectors and population growth rates ( $R_t$ ) was created

such that  $w_{t+1} = \frac{A_t w_t}{\|A_t w_t\|}$ , and  $R_t = \|A_t w_t\|$ . Likewise, assuming an arbitrary initial non-

negative reproductive value vector ( $v_0$ ) whose scalar product was also 1, another sequence of vectors was generated from the sequence of population projection

matrices, whereby  $v_{t+1}' = \frac{v_t' A_{t-1}}{\|v_t' A_{t-1}\|}$ .

Elasticities from deterministic conditions were calculated through the use of a metapopulation matrix model, as described by Hunter and Caswell (2005). The “patches” under this framework corresponded to normal and outbreak population states. Demography within each patch was described by the population projection matrix specific to normal and outbreak population dynamics, respectively:

$$\mathbf{B} = \begin{bmatrix} B_{normal} & \\ & B_{outbreak(i)} \end{bmatrix},$$

where  $i$  represents one of the six levels of disease-induced mortality  $\mu$  associated with a given outbreak condition. Dispersal between patches was held constant across all demographic stages, and was therefore described by the same Markov chain model ( $P_j$ ) for all 4 stages:

$$\mathbf{M} = \begin{bmatrix} M_H & 0 & 0 & 0 \\ 0 & M_P & 0 & 0 \\ 0 & 0 & M_{R-} & 0 \\ 0 & 0 & 0 & M_{R+} \end{bmatrix},$$

where  $M_H = M_P = M_{R-} = M_{R+} = P_j$ , and  $j$  here represents a specific combination of  $f$  and  $\rho$  values that generated each of the 9 Markov chain models used in the analysis.

Demography was assumed to occur after dispersal so that the force of infection ( $\phi$ ) and reproductive stage survival ( $\sigma_R$ ) would depend on whether the population was acting under normal conditions or had transitioned to outbreak conditions. The metapopulation projection matrix was then calculated as  $\mathbf{A} = \mathbf{B}\mathbf{V}'\mathbf{M}\mathbf{V}'$ , where  $\mathbf{B}$  represents the block-diagonal demography matrix,  $\mathbf{M}$  is the block-diagonal dispersal matrix, and  $\mathbf{V}$  is the vec-permutation matrix (Hunter and Caswell, 2005). The corresponding sensitivity matrices for demographic and dispersal transitions were calculated as  $\mathbf{S}_B = \mathbf{S}_A\mathbf{V}'\mathbf{M}'\mathbf{V}$  and  $\mathbf{S}_M = \mathbf{V}\mathbf{B}'\mathbf{S}_A\mathbf{V}'$ , respectively, where  $S_A$  represents the sensitivity matrix for the metapopulation projection matrix  $A$ . Elasticities of  $\lambda_A$  to  $\mathbf{B}$  and  $\mathbf{M}$  were calculated as  $E_B = \frac{1}{\lambda_A} B \circ S_B$  and  $E_M = \frac{1}{\lambda_A} M \circ S_M$ , respectively. In total, 54 metapopulation projection matrices were constructed to represent each type of outbreak scenario as defined by  $\rho$ ,  $f$ , and  $\mu$ .

For each metapopulation projection matrix, elasticities of  $\lambda$  to lower-level outbreak-associated parameters (i.e.,  $\rho$  and  $f$ ), disease-associated parameters (i.e.,  $\mu$  and  $\phi$ ) and demographic parameters (i.e.,  $\sigma_H$ ,  $\sigma_P$ ,  $\sigma_R$ ,  $\gamma$ , and  $m$ ) were calculated as follows:

$$e_\rho = \frac{\rho}{\lambda} \sum_{i,j} \frac{\partial \lambda}{\partial m_{ij}^{(k)}} \frac{\partial m_{ij}^{(k)}}{\partial \rho}$$

$$e_f = \frac{f}{\lambda} \sum_{i,j} \frac{\partial \lambda}{\partial m_{ij}^{(k)}} \frac{\partial m_{ij}^{(k)}}{\partial f}$$

$$e_\mu = \frac{\mu}{\lambda} \sum_{i,j} \frac{\partial \lambda}{\partial b_{ij}^{(k)}} \frac{\partial b_{ij}^{(k)}}{\partial \mu}$$

$$e_\phi = \frac{\phi}{\lambda} \sum_{i,j} \frac{\partial \lambda}{\partial b_{ij}^{(k)}} \frac{\partial b_{ij}^{(k)}}{\partial \phi}$$

$$e_{\sigma_H} = \frac{\sigma_H}{\lambda} \sum_{i,j} \frac{\partial \lambda}{\partial b_{ij}^{(k)}} \frac{\partial b_{ij}^{(k)}}{\partial \sigma_H}$$

$$e_{\sigma_P} = \frac{\sigma_P}{\lambda} \sum_{i,j} \frac{\partial \lambda}{\partial b_{ij}^{(k)}} \frac{\partial b_{ij}^{(k)}}{\partial \sigma_P}$$

$$e_{\sigma_R} = \frac{\sigma_R}{\lambda} \sum_{i,j} \frac{\partial \lambda}{\partial b_{ij}^{(k)}} \frac{\partial b_{ij}^{(k)}}{\partial \sigma_R}$$

$$e_\gamma = \frac{\gamma}{\lambda} \sum_{i,j} \frac{\partial \lambda}{\partial b_{ij}^{(k)}} \frac{\partial b_{ij}^{(k)}}{\partial \gamma}$$

$$e_m = \frac{m}{\lambda} \sum_{i,j} \frac{\partial \lambda}{\partial b_{ij}^{(k)}} \frac{\partial b_{ij}^{(k)}}{\partial m}$$

where  $m_{ij}^{(k)}$  and  $b_{ij}^{(k)}$  represent elements within the  $k^{th}$  block-diagonal matrix within **M** and **B**, respectively. Lower-level elasticities associated with  $\rho$  and  $f$  were summed across stages, while those associated with  $\mu$  and  $\phi$  were summed across patches, in order to show the overall effect of these parameters on  $\lambda$ .

APPENDIX E  
 COMPLETE LISTING OF ESTIMATED STOCHASTIC GROWTH RATES AND MEDIAN QUASI-EXTINCTION TIMES  
 ACROSS ALL OUTBREAK SCENARIOS

Table E-1. Stochastic growth rates and 95% confidence intervals from 54 outbreak scenarios using Tuljapurkar's small noise approximation.

$\rho$	f	$\mu$					
		0.01	0.05	0.1	0.15	0.2	0.3
-0.1	0.1	0.902 (0.895-0.908)	0.898 (0.865-0.931)	0.893 (0.827-0.96)	0.888 (0.788-0.989)	0.884 (0.749-1.018)	0.881 (0.731-1.031)
	0.2	0.901 (0.892-0.91)	0.893 (0.849-0.938)	0.884 (0.793-0.974)	0.874 (0.735-1.013)	0.864 (0.676-1.052)	0.859 (0.65-1.069)
	0.3	0.9 (0.89-0.91)	0.889 (0.838-0.94)	0.875 (0.769-0.98)	0.86 (0.696-1.024)	0.845 (0.62-1.07)	0.838 (0.586-1.09)
0.40	0.1	0.902 (0.895-0.908)	0.898 (0.865-0.931)	0.893 (0.827-0.96)	0.888 (0.788-0.989)	0.884 (0.749-1.018)	0.881 (0.731-1.031)
	0.2	0.901 (0.892-0.91)	0.893 (0.849-0.938)	0.884 (0.793-0.974)	0.874 (0.736-1.013)	0.864 (0.676-1.052)	0.86 (0.65-1.069)
	0.3	0.9 (0.89-0.91)	0.889 (0.838-0.94)	0.875 (0.769-0.98)	0.86 (0.696-1.024)	0.845 (0.62-1.07)	0.838 (0.586-1.09)
0.73	0.1	0.902 (0.895-0.908)	0.898 (0.865-0.931)	0.893 (0.827-0.96)	0.888 (0.788-0.989)	0.884 (0.749-1.018)	0.881 (0.731-1.031)
	0.2	0.901 (0.892-0.91)	0.893 (0.849-0.938)	0.884 (0.793-0.974)	0.874 (0.736-1.013)	0.864 (0.676-1.052)	0.86 (0.65-1.069)
	0.3	0.9 (0.89-0.91)	0.889 (0.838-0.94)	0.875 (0.769-0.98)	0.86 (0.696-1.024)	0.845 (0.62-1.07)	0.838 (0.587-1.09)

Table E-2. Stochastic growth rates and 95% confidence intervals from 54 outbreak scenarios using a matrix simulation approach.

$\rho$	f	$\mu$					
		0.01	0.05	0.1	0.15	0.2	0.3
-0.1		0.902	0.898	0.893	0.888	0.883	0.881
	0.1	(0.896-0.907)	(0.857-0.939)	(0.817-0.969)	(0.797-0.98)	(0.732-1.035)	(0.736-1.026)
	0.2	0.901	0.893	0.884	0.874	0.864	0.859
	0.3	(0.886-0.916)	(0.853-0.934)	(0.79-0.978)	(0.73-1.018)	(0.666-1.062)	(0.636-1.082)
0.40	0.9	0.889	0.889	0.875	0.86	0.845	0.839
	0.1	(NaN-NaN)	(0.833-0.944)	(0.776-0.973)	(0.705-1.016)	(0.618-1.072)	(0.602-1.076)
	0.2	0.902	0.898	0.893	0.889	0.884	0.881
	0.3	(0.89-0.913)	(0.843-0.953)	(0.815-0.971)	(0.818-0.96)	(0.757-1.011)	(0.728-1.034)
0.73	0.9	0.901	0.893	0.884	0.873	0.864	0.859
	0.1	(NaN-NaN)	(0.857-0.93)	(0.808-0.96)	(0.713-1.034)	(0.669-1.059)	(0.626-1.091)
	0.2	0.898	0.898	0.893	0.888	0.884	0.88
	0.3	(NaN-NaN)	(0.862-0.916)	(0.767-0.983)	(0.672-1.046)	(0.608-1.082)	(0.578-1.098)
	0.1	0.898	0.898	0.893	0.888	0.884	0.88
	0.2	(0.857-0.939)	(0.854-0.942)	(0.826-0.96)	(0.784-0.992)	(0.756-1.011)	(0.694-1.066)
	0.3	0.893	0.893	0.884	0.873	0.865	0.859
	0.1	(0.853-0.934)	(0.855-0.932)	(0.828-0.941)	(0.693-1.052)	(0.709-1.022)	(0.643-1.075)
	0.2	0.889	0.889	0.874	0.861	0.844	0.838
	0.3	(0.833-0.944)	(0.831-0.947)	(0.722-1.025)	(0.722-0.999)	(0.589-1.099)	(0.564-1.111)

Table E-3. Median quasi-extinction times and ranges from 54 outbreak scenarios.

$\rho$	f	$\mu$					
		0.01	0.05	0.1	0.15	0.2	0.3
-0.1	0.1	23 (22-23)	22 (20-23)	21 (16-23)	20 (14-23)	19 (12-23)	18 (11-23)
	0.2	22 (22-23)	21 (18-23)	19 (14-23)	18 (11-23)	17 (9-23)	16 (9-23)
	0.3	22 (22-23)	20 (17-23)	18 (13-23)	16 (10-23)	14 (8-23)	13 (7-23)
0.40	0.1	23 (21-23)	22 (17-23)	21 (12-23)	20 (9-23)	20 (8-23)	20 (7-23)
	0.2	23 (21-23)	21 (15-23)	20 (12-23)	18 (9-23)	17 (8-23)	16 (7-23)
	0.3	22 (21-23)	20 (16-23)	18 (12-23)	16 (9-23)	14 (8-23)	14 (7-23)
0.73	0.1	23 (21-23)	23 (16-23)	23 (12-23)	23 (9-23)	23 (8-23)	23 (7-23)
	0.2	23 (21-23)	21 (15-23)	20 (12-23)	19 (9-23)	19 (8-23)	18 (7-23)
	0.3	22 (21-23)	20 (15-23)	18 (12-23)	16 (9-23)	15 (8-23)	15 (7-23)

APPENDIX F  
TRANSLATION OF MODEL PARAMETER ESTIMATES FROM CARAPACE GROWTH  
RATES ( $\text{MM}^{-1}$ ) TO ANNUAL RATES ( $\text{YR}^{-1}$ )

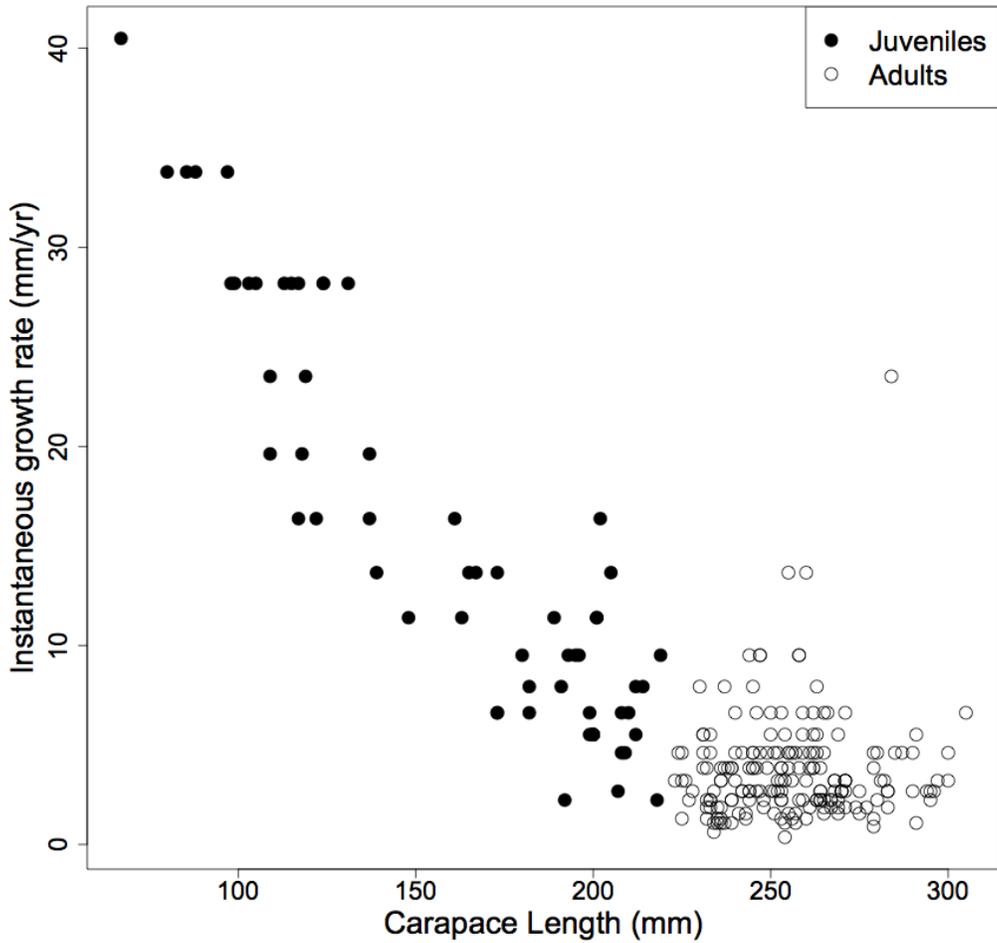


Figure F-1. Estimation of annual stage-specific growth in carapace length (mm) through the fitting of a von Bertalanffy growth model. Annual stage-specific growth rates were defined by the average instantaneous growth rates for juveniles ( $x < 220$ ) and adults ( $x \geq 220$ ), respectively.

## LIST OF REFERENCES

- Albon, S. D., Stein, A., Irvine, R. J., Langvatn, R., Ropstad, E. & Halvorsen, O. (1997) *The role of parasites in the dynamics of a reindeer population. Proceedings of the Royal Society of London Series B Biological Sciences*, **269**, 1625-1632.
- Altizer, S., Davis, A. K., Cook, K. C. & Cherry, J. J. (2004a) *Age, sex, and season affect the risk of mycoplasmal conjunctivitis in a southeastern house finch population. Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **82**, 755-763.
- Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M. & Rohani, P. (2006) *Seasonality and the dynamics of infectious diseases. Ecol Lett*, **9**, 467-484.
- Altizer, S., Hochachka, W. M. & Dhondt, A. A. (2004b) *Seasonal dynamics of mycoplasmal conjunctivitis in eastern North American house finches. Journal of Animal Ecology*, **73**, 309-322.
- Altizer, S., Nunn, C. L., Thrall, P. H., Gittleman, J., Antonovics, J., Cunningham, A. A., Dobson, A., Ezenwa, V., Jones, K. E., Pedersen, A. B., Poss, M. & Pulliam, J. R. C. (2003) *Social organization and parasite risk in mammals: Integrating theory and empirical studies. Annual Review of Ecology Evolution and Systematics*, **34**, 517-547.
- Amstrup, S. C., McDonald, T. L. & Manly, B. F. J. (2005) *Handbook of Capture-Recapture Analysis*. Princeton University Press, Princeton, N.J.
- Anderson, D. R., Link, W. A., Johnson, D. H. & Burnham, K. P. (2001) *Suggestions for presenting the results of data analyses. The Journal of Wildlife Management*, **65**, 373-378.
- Anderson, R. M. (1991) *Discussion: the Kermack-McKendrick epidemic threshold theorem. Bull Math Biol*, **53**, 3-32.
- Arif, A., Schulz, J., Thiaucourt, F., Taha, A. & Hammer, S. (2007) *Contagious caprine pleuropneumonia outbreak in captive wild ungulates at Al Wabra Wildlife Preservation, State of Qatar. Journal of Zoo and Wildlife Medicine*, **38**, 93-96.
- Auffenberg, W. & Franz, R. (1982) *The status and distribution of the gopher tortoise (Gopherus polyphemus). North American Tortoises: Conservation and Ecology* (eds R. B. Bury), pp. 95-126. U.S. Fish and Wildlife Service.
- Bansal, S., Grenfell, B. T. & Meyers, L. A. (2007) *When individual behaviour matters: homogeneous and network models in epidemiology. Journal of the Royal Society Interface*, **4**, 879-891.

- Begon, M., Telfer, S., Smith, M. J., Burthe, S., Paterson, S. & Lambin, X. (2009) *Seasonal host dynamics drive the timing of recurrent epidemics in a wildlife population. Proceedings of the Royal Society B-Biological Sciences*, **276**, 1603-1610.
- Berry, K. H. (1997) Demographic consequences of disease in two desert tortoise populations in California, USA. *Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles--An International Conference*, pp. 91-99. New York Turtle and Tortoise Society.
- Berry, K. H. (2002) *Using growth ring counts to age juvenile desert tortoises (Gopherus agassizii) in the wild. Chelonian Conservation and Biology*, **4**, 416-424.
- Berry, K. H. & Christopher, M. M. (2001) *Guidelines for the field evaluation of desert tortoise health and disease. Journal of Wildlife Diseases*, **37**.
- Beyer, H. L. (2004). Hawth's Analysis Tools for ArcGIS.
- Blankenship, E. L., Bryan, T. W. & Jacobsen, S. P. (1990) *A method for tracking tortoises using fluorescent powder. Herpetological Review*, **21**, 88-89.
- Blehert, D. S., Hicks, A. C., Behr, M., Meteyer, C. U., Berlowski-Zier, B. M., Buckles, E. L., Coleman, J. T. H., Darling, S. R., Gargas, A., Niver, R., Okoniewski, J. C., Rudd, R. J. & Stone, W. B. (2009) *Bat White-Nose Syndrome: An Emerging Fungal Pathogen? Science*, **323**, 227-227.
- Boglioli, M. D., Guyer, C. & Michener, W. K. (2003) *Mating opportunities of female Gopher Tortoises, Gopherus polyphemus, in relation to spatial isolation of females and their burrows. Copeia*, **2003**, 846-850.
- Bolker, B. (2007). *bbmle: Tools for general maximum likelihood estimation. R package version 0.8.1.*
- Boots, M., Greenman, J., Ross, D., Norman, R., Hails, R. & Sait, S. (2003) *The population dynamical implications of covert infections in host-microparasite interactions. Journal of Animal Ecology*, **72**, 1064-1072.
- Bornstein, S., Morner, S., Samuel, W. M., Pybus, M. J. & Kocan, A. A. (2001) *Sarcoptes scabiei and sarcoptic mange. Parasitic diseases of wild mammals. Second edition.*, 107-119.
- Briggs, C. J., Vredenburg, V. T., Knapp, R. A. & Rachowicz, L. J. (2005) *Investigating the population-level effects of chytridiomycosis: An emerging infectious disease of amphibians. Ecology*, **86**, 3149-3159.
- Brode, W. E. (1959) *Notes on behavior of Gopherus polyphemus. Herpetologica*, **15**, 101-102.

- Broderson, J. R., Lindsey, J. R. & Crawford, J. E. (1976) *Role of environmental ammonia in respiratory mycoplasmosis of rats. American Journal of Pathology*, **85**, 115-&.
- Brook, B. W. & Kikkawa, J. (1998) *Examining threats faced by island birds: a population viability analysis on the Capricorn silvereve using longterm data. Journal of Applied Ecology*, **35**, 491-503.
- Brown, C. R. & Brown, M. B. (2004) *Empirical measurement of parasite transmission between groups in a colonial bird. Ecology*, **85**, 1619-1626.
- Brown, D. R. (2002) *Mycoplasmosis and immunity of fish and reptiles. Frontiers in Bioscience*, **7**, 1338-1346.
- Brown, D. R., Crenshaw, B. C., McLaughlin, G. S., Schumacher, I. M., McKenna, C. E., Klein, P. A., Jacobson, E. R. & Brown, M. B. (1995) *Taxonomic analysis of the tortoise mycoplasmas *Mycoplasma agassizii* and *Mycoplasma testudinis* by 16S rRNA gene sequence comparison. International Journal of Systematic and Evolutionary Microbiology*, **45**, 348-350.
- Brown, D. R., Merritt, J. L., Jacobson, E. R., Klein, P. A., Tully, J. G. & Brown, M. B. (2004a) *Mycoplasma testudineum sp nov., from a desert tortoise (*Gopherus agassizii*) with upper respiratory tract disease. International Journal of Systematic and Evolutionary Microbiology*, **54**, 1527-1529.
- Brown, D. R., Nogueira, M. F., Schoeb, T. R., Vliet, K. A., Bennett, R. A., Pye, G. W. & Jacobson, E. R. (2001a) *Pathology of experimental mycoplasmosis in American alligators. Journal of Wildlife Diseases*, **37**, 671-679.
- Brown, D. R., Schumacher, I. M., McLaughlin, G. S., Wendland, L. D., Brown, M. B., Klein, P. A. & Jacobsen, E. R. (2002) *Application of diagnostic tests for mycoplasmal infections of desert and gopher tortoises with management recommendations. Chelonian Conservation and Biology*, **4**, 497-507.
- Brown, D. R., Wendland, L. D., Ortiz, G. J., Kramer, M. F., Lim, D. V., Brown, M. B. & Klein, P. A. (2008) *Evanescence-wave biosensor for field serodiagnosis of tortoise mycoplasmosis. Veterinary Immunology and Immunopathology*, **124**, 322-331.
- Brown, D. R., Zacher, L. A. & Farmerie, W. G. (2004b) *Spreading factors of *Mycoplasma alligatoris*, a flesh-eating mycoplasma. Journal of Bacteriology*, **186**, 3922-3927.
- Brown, D. R., Zacher, L. A., Wendland, L. D. & Brown, M. B. (2005) *Emerging Mycoplasmoses in Wildlife. Mycoplasmas: Molecular Biology, Pathogenicity, and Strategies for Control* (eds A. Blanchard & G. Browning), pp. 353-414. Horizon Bioscience, Norfolk.

- Brown, M. B., Berry, K. H., Schumacher, I. M., Nagy, K. A., Christopher, M. M. & Klein, P. A. (1999a) *Seroepidemiology of upper respiratory tract disease in the desert tortoise in the western Mojave Desert of California. Journal of Wildlife Diseases*, **35**, 716-727.
- Brown, M. B., Brown, D. R., Klein, P. A., McLaughlin, G. S., Schumacher, I. M., Jacobson, E. R., Adams, H. P. & Tully, J. G. (2001b) *Mycoplasma agassizii* sp nov., isolated from the upper respiratory tract of the desert tortoise (*Gopherus agassizii*) and the gopher tortoise (*Gopherus polyphemus*). *International Journal of Systematic and Evolutionary Microbiology*, **51**, 413-418.
- Brown, M. B., McLaughlin, G. S., Klein, P. A., Crenshaw, B. C., Schumacher, I. M., Brown, D. R. & Jacobsen, E. R. (1999b) *Upper respiratory tract disease in the gopher tortoise is caused by Mycoplasma agassizii. Journal of Clinical Microbiology*, **37**, 2262-2269.
- Brown, M. B., Schumacher, I. M., Klein, P. A., Harris, K., Correll, T. & Jacobsen, E. R. (1994) *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. *Infection and Immunity*, **62**, 4580-4586.
- Burnham, K. P. & Anderson, D. R. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edn. Springer, New York.
- Butler, J. A. & Hull, T. W. (1996) *Reproduction of the tortoise, Gopherus polyphemus, in northeastern Florida. Journal of Herpetology*, **30**, 14-18.
- Butler, J. A. & Sowell, S. (1996) *Survivorship and Predation of Hatchling and Yearling Gopher Tortoises, Gopherus polyphemus. Journal of Herpetology*, **30**, 455-458.
- Caillaud, D., Levrero, F., Cristescu, R., Gatti, S., Dewas, M., Douadi, M., Gautier-Hion, A., Raymond, M. & Menard, N. (2006) *Gorilla susceptibility to Ebola virus: The cost of sociality. Current Biology*, **16**, R489-R491.
- Caley, P. & Hone, J. (2002) *Estimating the force of infection: Mycobacterium bovis infection in feral ferrets Mustela furo in New Zealand. Journal of Animal Ecology*, **71**.
- Cartner, S. C., Lindsey, J. R., Gibbs-Erwin, J., Cassell, G. H. & Simecka, J. W. (1998) *Roles of innate and adaptive immunity in respiratory mycoplasmosis. Infection and Immunity*, **66**, 3485-3491.
- Cartner, S. C., Simecka, J. W., Lindsey, J. R., Cassell, G. H. & Davis, J. K. (1995) *Chronic respiratory mycoplasmosis in C3H/HEN and C57BL/6N mice - Lesion severity and antibody-response. Infection and Immunity*, **63**, 4138-4142.
- Casadevall, A. & Pirofski, L. (2001) *Host-Pathogen Interactions: The attributes of virulence. Journal of Infectious Diseases*, **184**, 337-344.

- Casadevall, A. & Pirofski, L. (2002) *What is a pathogen? Annals of Medicine*, **34**, 2-4.
- Casadevall, A. & Pirofski, L. (2003a) *Microbial virulence results from the interaction between host and microorganism. Trends in Microbiology*, **11**, 157-158.
- Casadevall, A. & Pirofski, L. A. (2003b) *The damage-response framework of microbial pathogenesis. Nature Reviews Microbiology*, **1**, 17-24.
- Caswell, H. (2001) *Matrix Population Models*, 2nd edn. Sinauer Associates, Sunderland.
- Caswell, H. & Kaye, T. N. (2001) *Stochastic demography and conservation of an endangered perennial plant (Lomatium bradshawii) in a dynamic fire regime. Advances in Ecological Research, Vol 32*, **32**, 1-51.
- Christopher, M. M., Berry, K. H., Henen, B. T. & Nagy, K. A. (2003) *Clinical disease and laboratory abnormalities in free-ranging desert tortoises in California (1990-1995). Journal of Wildlife Diseases*, **39**, 35-56.
- Christopher, M. M., Berry, K. H., Wallis, I. R., Nagy, K. A., Henen, B. T. & Peterson, C. C. (1999) *Reference intervals and physiologic alterations in hematologic and biochemical values of free-ranging desert tortoises in the Mojave Desert. Journal of Wildlife Diseases*, **35**, 212-235.
- Chu, H. W., Breed, R., Rino, J. G., Harbeck, R. J., Sills, M. R. & Martin, R. J. (2006) *Repeated respiratory Mycoplasma pneumoniae infections in mice: effect of host genetic background. Microbes and Infection*, **8**, 1764-1772.
- Claessen, D. & deRoos, A. M. (1995) *Evolution of virulence in a host-pathogen system with local pathogen transmission. Oikos*, **74**, 401-413.
- Cleveland, S., Hess, G., Dobson, A., Laurenson, M. K., McCallum, H., Roberts, M. G. & Woodroffe, R. (2002) The role of pathogens in biological conservation. *The Ecology of Wildlife Diseases* (eds P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek & A. P. Dobson), pp. 139-150. Oxford University Press, New York.
- Clippinger, T. L., Bennett, R. A., Johnson, C. M., Vliet, K. A., Deem, S. L., Oros, J., Jacobson, E. R., Schumacher, I. M., Brown, D. R. & Brown, M. B. (2000) *Morbidity and mortality associated with a new mycoplasma species from captive American alligators (Alligator mississippiensis). Journal of Zoo and Wildlife Medicine*, **31**, 303-314.
- Cowan, K. M., Shutler, D., Herman, T. B. & Stewart, D. T. (2007) *Extreme male-biased infections of masked shrews by bladder nematodes. Journal of Mammalogy*, **88**, 1539-1543.

- Cox, J., Inkley, D. & Kautz, R. (1987) *Ecology and Habitat Protection Needs of Gopher Tortoise (Gopherus polyphemus) Populations Found on Lands Slated for Large-Scale Development in Florida*. Florida Game and Fresh Water Fish Commission, Tallahassee, FL.
- Cross, P. C., Lloyd-Smith, J. O., Bowers, J. A., Hay, C. T., Hofmeyr, M. & Getz, W. M. (2004) *Integrating association data and disease dynamics in a social ungulate: bovine tuberculosis in African buffalo in the Kruger National Park*. *Annales Zoologici Fennici*, **41**, 879-892.
- Crouse, D. T., Crowder, L. B. & Caswell, H. (1987) *A stage-based population model for loggerhead sea turtles and implications for conservation*. *Ecology*, **68**, 1412-1423.
- Crowder, L. B., Crouse, D. T., Heppell, S. S. & Martin, T. H. (1994) *Predicting the impact of turtle excluder devices on loggerhead sea turtle populations*. *Ecological Applications*, **4**, 437-445.
- Daszak, P., Cunningham, A. A. & Hyatt, A. D. (2000) *Emerging Infectious Diseases of Wildlife--Threats to Biodiversity and Human Health*. *Science*, **287**.
- Davidson, M. K., Lindsey, J. R., Parker, R. F., Tully, J. G. & Cassell, G. H. (1988) *Differences in virulence for mice among strains of Mycoplasma pulmonis*. *Infection and Immunity*, **56**, 2156-2162.
- de Castro, F. & Bolker, B. (2005) *Mechanisms of disease-induced extinction*. *Ecology Letters*, **8**, 117-126.
- de Kroon, H., van Groenendael, J. & Ehrlén, J. (2000) *Elasticities: a review of methods and model limitations*. *Ecology (Washington D C)*, **81**.
- Deimer Berish, J. E., Wendland, L. D. & Gates, C. A. (2000) *Distribution and prevalence of upper respiratory tract disease in gopher tortoises in Florida*. *Journal of Herpetology*, **34**, 5-12.
- Deimer, J. E. & Moore, C. T. (1986) *Reproduction of gopher tortoises in north-central Florida*. (eds, pp. 129-137.
- Dhondt, A. A., Altizer, S., Cooch, E. G., Davis, A. K., Dobson, A., Driscoll, M. J., Hartup, B. K., Hawley, D. M., Hochachka, W. M., Hosseini, P. R., Jennelle, C. S., Kollias, G. V., Ley, D. H., Swarthout, E. C. & Sydenstricker, K. V. (2005) *Dynamics of a novel pathogen in an avian host: Mycoplasmal conjunctivitis in house finches*. *Acta Trop*, **94**, 77-93.
- Dhondt, A. A., Dhondt, K. V. & McCleery, B. V. (2008) *Comparative infectiousness of three passerine bird species after experimental inoculation with Mycoplasma gallisepticum*. *Avian Pathology*, **37**, 635-640.

- Dickinson, V. M., Duck, T., Schwalbe, C. R., Jarchow, J. L. & Trueblood, M. H. (2001) *Nasal and cloacal bacteria in free-ranging desert tortoises from the western United States. Journal of Wildlife Diseases*, **37**, 252-257.
- Doak, D., Kareiva, P. & Klepetka, B. (1994) *Modeling population viability for the desert tortoise in the western Mojave Desert. Ecological Applications*, **4**, 446-460.
- Dohoo, I., Martin, W. & Stryhn, H. (2003) *Veterinary Epidemiologic Research*. AVC Inc., Charlottetown, Prince Edward Island.
- Douglass, J. F. (1976) The mating system of the gopher tortoise, *Gopherus polyphemus*, in southern Florida MS, University of South Florida.
- Douglass, J. F. & Winegarner, C. E. (1977) *Predators of Eggs and Young of the Gopher Tortoise, Gopherus Polyphemus (Reptilia, Testudines, Testudinae) in Southern Florida. Journal of Herpetology*, **11**, 236-238.
- Epperson, D. M. (1997) Gopher tortoise (*Gopherus polyphemus*) populations: Activity patterns, upper respiratory tract disease, and management on a military installation in northeast Florida. University of Florida.
- Epperson, D. M. (2005) *Mycoplasma testing of Gopher Tortoises (Gopheras polyphemus) in relation to military training activities at Camp Shelby Training Site, Mississippi. Herpetological Review*, **36**, 12-15.
- Epperson, D. M. & Heise, C. D. (2003) *Nesting and hatchling ecology of the gopher tortoise. Journal of Herpetology*, **37**, 315-324.
- Ezenwa, V. O. (2004) *Host social behavior and parasitic infection: a multifactorial approach. Behav. Ecol.*, **15**, 446-454.
- Farnsworth, M. L., Hoeting, J. A., Hobbs, N. T. & Miller, M. W. (2006) *Linking chronic wasting disease to mule deer movement scales: A hierarchical bayesian approach. Ecological Applications*, **16**, 1026-1036.
- 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**, 651-669.
- Ferrari, N., Cattadori, I. M., Nespereira, J., Rizzoli, A. & Hudson, P. J. (2004) *The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse Apodemus flavicollis. Ecology Letters*, **7**, 88-94.
- Fieberg, J. & Ellner, S. P. (2001) *Stochastic matrix models for conservation and management: a comparative review of methods. Ecology Letters*, **4**, 244-266.

- Florida Fish and Wildlife Conservation Commission. (2007) Gopher Tortoise Management Plan. (eds, pp. 127 pp. Tallahassee, FL.
- Frey, J. (2002) Mycoplasmas of animals. *Molecular Biology and Pathogenicity of Mycoplasmas* (eds S. Razin & R. Herrmann), pp. 73-90. Kluwer Academic/Plenum Publishers, New York, New York.
- Galvani, A. P. & May, R. M. (2005) *Dimensions of superspreading Nature*, **438**, 293-295.
- Gates, C. A., Allen, M. J., Deimer Berish, J. E., Stillwaugh, D. M. & Shattler, S. R. (2002) *Characterization of a gopher tortoise mortality event in west-central Florida*. *Florida Scientist*, **65**, 159-184.
- Gauthier, D. T., Latour, R. J., Heisey, D. M., Bonzek, C. F., Gartland, J., Burge, E. J. & Vogelbein, W. K. (2008) *Mycobacteriosis-associated mortality in wild striped bass (Morone saxatilis) from Chesapeake Bay, USA*. *Ecological Applications*, **18**, 1718-1727.
- Gaydos, J. K., Davidson, W. R., Elvinger, F., Mead, D. G., Howerth, E. W. & Stallknecht, D. E. (2002) *Innate resistance to epizootic hemorrhagic disease in white-tailed deer*. *Journal of Wildlife Diseases*, **38**, 713-719.
- Gelman, A. & Hill, J. (2007) *Data Analysis Using Regression and Multilevel/Hierarchical Models*. Cambridge University Press, New York, NY.
- Gerber, L. R., McCallum, H., Lafferty, K. D., Sabo, J. L. & Dobson, A. (2005) *Exposing extinction risk analysis to pathogens: Is disease just another form of density dependence?* *Ecological Applications*, **15**, 1402-1414.
- Germano, D. J. (1988) *Age and Growth Histories of Desert Tortoises Using Scute Annuli*. *Copeia*, **1988**, 914-920.
- Germano, D. J. (1992) *Longevity and Age-Size Relationships of Populations of Desert Tortoises*. *Copeia*, **1992**, 367-374.
- Gillin, C. M., Tabor, G. M. & Aguirre, A. A. (2002) Ecological health and wildlife disease management in national parks. *Conservation Medicine: Ecological Health in Practice* (eds A. A. Aguirre, R. S. Ostfeld, G. M. Tabor, C. House & M. C. Pearl), pp. 130-143. Oxford University Press, Oxford.
- Greenwood, R., Newton, W., Pearson, G. & Schamber, G. (1997) *Population and movement characteristics of radio-collared striped skunks in North Dakota during an epizootic of rabies*. *J Wildl Dis*, **33**, 226-241.

- Grenfell, B. T., Amos, W., Arneberg, P., Bjornstad, O. N., Greenman, J., Harwood, J., Lanfranchi, P., McLean, A. R., Norman, R., Read, A. F. & Skorping, A. (2002) Visions for future research in wildlife epidemiology. *The Ecology of Wildlife Diseases* (eds P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek & A. P. Dobson), pp. 151-164. Oxford University Press, New York.
- Grenfell, B. T. & Gulland, F. M. D. (1995) *Introduction: Ecological impact of parasitism on wildlife host populations*. *Parasitology*, **111**, S3-S14.
- Gurevitch, J., Curtis, P. S. & Jones, M. H. (2001) *Meta-analysis in ecology*. *Advances in Ecological Research*, Vol 32, **32**, 199-247.
- Hagen, C. A., Sandercock, B. K., Pitman, J. C., Robel, R. J. & Applegate, R. D. (2006) *Radiotelemetry survival estimates of lesser prairie-chickens in Kansas: Are there transmitter biases?* *Wildlife Society Bulletin*, **34**, 1064-1069.
- Harper, P. A. W., Hammond, D. C. & Heuschele, W. P. (1982) *A Herpesvirus-like agent associated with pharyngeal abscess in a desert tortoise*. *Journal of Wildlife Diseases*, **18**, 491-494.
- Harrell, F. E. (2007). *Design: Design Package*. R package version 2.1-1.
- Hawley, D. M., Davis, A. K. & Dhondt, A. A. (2007a) *Transmission-relevant behaviours shift with pathogen infection in wild house finches (Carpodacus mexicanus)*. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **85**, 752-757.
- Hawley, D. M., Jennelle, C. S., Sydenstricker, K. V. & Dhondt, A. A. (2007b) *Pathogen resistance and immunocompetence covary with social status in house finches (Carpodacus mexicanus)*. *Functional Ecology*, **21**, 520-527.
- Hawley, D. M., Sydenstricker, K. V., Kollias, G. V. & Dhondt, A. A. (2005) *Genetic diversity predicts pathogen resistance and cell-mediated immunocompetence in house finches*. *Biology Letters*, **1**, 326-329.
- Haydon, D. T., Laurenson, M. K. & Sillero-Zubiri, C. (2002) *Integrating epidemiology into Population Viability Analysis: Managing the risk posed by rabies and canine distemper to the Ethiopian wolf*. *Conservation Biology*, **16**, 1372-1385.
- Hayward, A. D., Wilson, A. J., Pilkington, J. G., Pemberton, J. M. & Kruuk, L. E. B. (2009) *Ageing in a variable habitat: environmental stress affects senescence in parasite resistance in St Kilda Soay sheep*. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 3477-3485.
- Heisey, D. M., Joly, D. O. & Messier, F. (2006) *The fitting of general force of infection models to wildlife disease prevalence data*. *Ecology*, **87**, 2356-2365.
- Heppell, S. S. (1998) *Application of life-history theory and population model analysis to turtle conservation*. *Copeia*, 367-375.

- Heppell, S. S., Crowder, L. B. & Crouse, D. T. (1996) *Models to evaluate headstarting as a management tool for long-lived turtles. Ecological Applications*, **6**, 556-565.
- Hilborn, R. & Mangel, M. (1997) *The Ecological Detective: Confronting models with data*. Princeton University Press, Princeton, NJ.
- Hochachka, W. M. & Dhondt, A. A. (2000) *Density-dependent decline of host abundance resulting from a new infectious disease. Proceedings of the National Academy of Sciences of the United States of America*, **97**, 5303-5306.
- Holder, G., Allen, M., Berish, J., Hinkle, M., Kropp, A., McCann, R., Williams, A. & Yaun, M. (2007) *Gopher Tortoise Management Plan*. Florida Fish and Wildlife Conservation Commission.
- Homer, B. L., Berry, K. H., Brown, M. B., Ellis, G. & Jacobsen, E. R. (1998) *Pathology of diseases in wild desert tortoises from California. Journal of Wildlife Diseases*, **34**, 508-523.
- Hosmer, D. W., Hosmer, T., leCessie, S. & Lemeshow, S. (1997) *A comparison of goodness-of-fit tests for the logistic regression model. Statistics in Medicine*, **16**, 965-980.
- Hosseini, P. R., Dhondt, A. A. & Dobson, A. (2004) *Seasonality and wildlife disease: How seasonal birth, aggregation, and variation in immunity affect the dynamics of Mycoplasma gallisepticum in house finches. Proceedings of the Royal Society of London. Series B.*, **271**, 2569-2577.
- Hosseini, P. R., Dhondt, A. A. & Dobson, A. P. (2006) *Spatial spread of an emerging infectious disease: conjunctivitis in House Finches. Ecology*, **87**.
- Hudson, P. J. & Dobson, A. P. (1997) *Transmission dynamics and host-parasite interactions of Trichostrongylus tenuis in red grouse (Lagopus lagopus scoticus). Journal of Parasitology*, **83**, 194-202.
- Hunt, M. E. & Brown, D. R. (2007) *Role of sialidase in Mycoplasma alligatoris-induced pulmonary fibroblast apoptosis. Veterinary Microbiology*, **121**, 73-82.
- Hunter, C. M. & Caswell, H. (2005) *The use of the vec-permutation matrix in spatial matrix population models. Ecological Modelling*, **188**, 15-21.
- Hurtado, P. (2008) *The Potential Impact of Disease on the Migratory Structure of a Partially Migratory Passerine Population. Bulletin of Mathematical Biology*, **70**, 2264-2282.
- Iglesias Sahagun, G. & Trujano Castillo, M. (2000) *Patterns of microbial interactions in the porcine respiratory complex. Veterinaria - Mexico*, **31**, 59-65.

- Iverson, J. B. (1980) *The reproductive biology of Gopherus polyphemus*. *American Midland Naturalist*, **103**, 353-359.
- Jackson, D. R. & Milstrey, E. G. (1989) The fauna of gopher tortoise burrows. *Gopher Tortoise Relocation Symposium. FGFWFC Non-game Wildlife Program Technical Report 5* (eds J. E. Diemer, D. R. Jackson, J. L. Landers, J. N. Layne & D. A. Wood), pp. 86-98. FGFWFC, Tallahassee, FL.
- Jacobson, E. R., Berry, K. H., Stacy, B., Huzella, L. M., Kalasinsky, V. F., Fleetwood, M. L. & Mense, M. G. (2009) *Oxalosis in wild desert tortoises, Gopherus agassizii*. *Journal of Wildlife Diseases*, **45**, 982-988.
- Jacobson, E. R., Brown, M. B., Schumacher, I. M., Collins, B. R., Harris, R. K. & Klein, P. A. (1995) *Mycoplasmosis and the desert tortoise (Gopherus agassizii) in Las Vegas Valley, Nevada*. *Chelonian Conservation and Biology*, **1**, 279-284.
- Jacobson, E. R., Gaskin, J. M., Brown, M. B., Harris, R. K., Gardiner, C. H., Lapointe, J. L., Adams, H. P. & Reggiardo, C. (1991) *Chronic upper respiratory-tract disease of free-ranging desert tortoises (Xerobates agassizii)*. *Journal of Wildlife Diseases*, **27**, 296-316.
- Janeway, C. A. (2001) *Immunobiology: The immune system in health and disease*, 5th edn. Garland Publishing, New York.
- Jansen, B. D., Krausnlaln, P. R., Heffelfinger, J. R., Noon, T. H. & Devos, J. C. (2007) *Population dynamics and behavior of bighorn sheep with infectious keratoconjunctivitis*. *Journal of Wildlife Management*, **71**, 571-575.
- Johnson, A. J., Morafka, D. J. & Jacobson, E. R. (2006) *Seroprevalence of Mycoplasma agassizii and tortoise herpesvirus in captive desert tortoises (Gopherus agassizii) from the Greater Barstow Area, Mojave Desert, California*. *Journal of Arid Environments*, **67**, 192-201.
- Johnson, A. J., Wendland, L., Norton, T. M., Belzer, B. & Jacobson, E. R. (2009) *Development and use of an indirect enzyme-linked immunosorbent assay for detection of iridovirus exposure in gopher tortoises (Gopherus polyphemus) and eastern box turtles (Terrapene carolina carolina)*. *Veterinary Microbiology*.
- Jones, H. P. & Simecka, J. W. (2003) *T lymphocyte responses are critical determinants in the pathogenesis and resistance to mycoplasma respiratory disease*. *Frontiers in Bioscience*, **8**, D930-D945.
- Jordan, F. T. W. (1975) *Avian mycoplasma and pathogenicity - A Review*. *Avian Pathology*, **4**, 165-174.
- Kao, R. R. (2002) *The role of mathematical modelling in the control of the 2001 FMD epidemic in the UK*. *Trends in Microbiology*, **10**, 279-286.

- Karlin, M. L. (2008) *Distribution of Mycoplasma agassizi in a Gopher Tortoise population in south Florida. Southeastern Naturalist*, **7**, 145-158.
- Kent, D. M., Langston, M. A. & Hanf, D. W. (1997) *Observations of vertebrates associated with gopher tortoise burrows in Orange County, Florida. Florida Scientist*, **60**, 197-201.
- Klein, P. A., McKenna, C. E., Schumacher, I. M., Jacobson, E. R., McLaughlin, G. S., Brown, D. R. & Brown, M. B. (1995) *The etiology, pathology, and diagnosis of upper respiratory tract disease in the Florida gopher tortoise. FASEB Journal*, **9**, A145.
- Kock, M. D., Mullins, G. R. & Perkins, J. S. (2002) Wildlife health, ecosystems, and rural livelihoods in Botswana. *Conservation medicine: ecological health in practice.* (eds A. A. Aguirre, M. C. Pearl, R. S. Ostfeld, C. House & G. M. Tabor), pp. 265-275. Oxford University Press.
- Kollias, G. V., Sydenstricker, K. V., Kollias, H. W., Ley, D. H., Hosseini, P. R., Connolly, V. & Dhondt, A. A. (2004) *Experimental infection of house finches with Mycoplasma gallisepticum. Journal of Wildlife Diseases*, **40**, 79-86.
- Kutner, M. H., Nachtsheim, C. J., Neter, J. & Li, W. (2005) *Applied Linear Statistical Models, 5th edition*, 5th edn. McGraw Hill, Boston, MA.
- 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**, 926-936.
- Lafferty, K. D. & Gerber, L. R. (2002) *Good medicine for conservation biology: The intersection of epidemiology and conservation theory. Conservation Biology*, **16**, 593-604.
- Lafferty, K. D. & Holt, R. D. (2003) *How should environmental stress affect the population dynamics of disease? Ecology Letters*, **6**, 654-664.
- Landers, J. L., Garner, J. A. & McRae, W. A. (1980) *Reproduction of gopher tortoises (Gopherus polyphemus) in southwestern Georgia. Herpetologica*, **36**, 353-361.
- Landers, J. L., McRae, W. A. & Garner, J. A. (1982) *Growth and maturity of the gopher tortoise in southwest Georgia. Bulletin of the Florida State Museum Biological Sciences*, **27**.
- Lederle, P. E., Rautenstrauch, K. R., Rakestraw, D. L., Zander, K. K. & Boone, J. L. (1997) *Upper respiratory tract disease and mycoplasmosis in desert tortoises from Nevada. Journal of Wildlife Diseases*, **33**, 759-765.

- Lesnoff, M., Laval, G., Bonnet, P., Abdicho, S., Workalemahu, A., Kifle, D., Peyraud, A., Lancelot, R. & Thiaucourt, F. (2004) *Within-herd spread of contagious bovine pleuropneumonia in Ethiopian highlands. Preventive Veterinary Medicine*, **64**, 27-40.
- Ley, D. H., Berkhoff, J. E. & McLaren, J. M. (1996) *Mycoplasma gallisepticum isolated from house finches (Carpodacus mexicanus) with conjunctivitis. Avian Diseases*, **40**, 480-483.
- Lindstrom, K. M., Hawley, D. M., Davis, A. K. & Wikelski, M. (2005) *Stress responses and disease in three wintering house finch (Carpodacus mexicanus) populations along a latitudinal gradient. General and Comparative Endocrinology*, **143**, 231-239.
- Lipsey, M. W. & Wilson, D. B. (2001) *Practical Meta-Analysis*. Sage Publications Inc, Thousand Oaks, CA.
- Lloyd-Smith, J. O., Cross, P. C., Briggs, C. J., Daugherty, M., Getz, W. M., Latto, J., Sanchez, M. S., Smith, A. B. & Swei, A. (2005a) *Should we expect population thresholds for wildlife disease? Trends in Ecology & Evolution*, **20**, 511-519.
- Lloyd-Smith, J. O., Getz, W. M. & Westerhoff, H. V. (2004) *Frequency-dependent incidence in models of sexually transmitted diseases: portrayal of pair-based transmission and effects of illness on contact behavior. Proceedings of the Royal Society of London. Series B.*, **271**, 625-635.
- Lloyd-Smith, J. O., Schreiber, S. J., Kopp, P. E. & Getz, W. M. (2005b) *Superspreading and the effect of individual variation on disease emergence. Nature*, **438**, 355-359.
- Lochmiller, R. L. & Deerenberg, C. (2000) *Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos*, **88**, 87-98.
- Lumley, T. (2008). *rmeta: Meta-analysis*. R package version 2.32.
- Lumley, T. & Therneau, T. (2008). *survival: Survival analysis, including penalised likelihood*. R package version 2.32.
- Marshall, J. E. (1987) *The effects of nest predation on hatchling gopher tortoises (Gopherus polyphemus)*. Master of Science, University of South Alabama.
- Martin, S. W., Meek, A. H. & Willeberg, P. (1987) *Veterinary Epidemiology: Principles and Methods*. Iowa State University Press, Ames, IA.
- Matthews, L. & Woolhouse, M. (2005) *New approaches to quantifying the spread of infection. Nature Reviews Microbiology*, **3**, 529-536.

- May, M., Kleven, S. H. & Brown, D. R. (2007) *Sialidase Activity in Mycoplasma synoviae*. *Avian Diseases*, **51**, 829-833.
- May, R. M. (1988) *Conservation and Disease*. *Conservation Biology*, **2**, 28-30.
- May, R. M. (2006) *Network structure and the biology of populations*. *Trends in Ecology & Evolution*, **21**, 394-399.
- McCallum, H. & Dobson, A. (1995) *Detecting disease and parasite threats to endangered species and ecosystems*. *Trends in Ecology and Evolution*, **10**, 190-194.
- McCallum, H. & Dobson, A. (2002) *Disease, habitat fragmentation and conservation*. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **269**, 2041-2049.
- McCoy, E. D. (2008) *A Data Aggregation Problem in Studies of Upper Respiratory Tract Disease of the Gopher Tortoise*. *Herpetological Review*, **39**, 419-422.
- McCoy, E. D., Mushinsky, H. R. & Lindzey, J. (2006) *Declines of the gopher tortoise on protected lands*. *Biological Conservation*, **128**, 120-127.
- McCoy, E. D., Mushinsky, H. R. & Lindzey, J. (2007) *Conservation strategies and emergent diseases: The case of upper respiratory tract disease in the gopher tortoise*. *Chelonian Conservation and Biology*, **6**, 170-176.
- McDonald, T. (2008). mra: Analysis of Mark-Recapture data. R package version 1.3.
- McKenzie, F. E. & Samba, E. M. (2004) *The role of mathematical modeling in evidence-based malaria control*. *Am J Trop Med Hyg*, **71**, 94-96.
- McLaughlin, G. S. (1997) *Upper respiratory tract disease in gopher tortoises, Gopherus polyphemus : pathology, immune responses, transmission, and implications for conservation and management*. Dissertation, University of Florida.
- McLaughlin, G. S., Jacobsen, E. R., Brown, D. R., McKenna, C. E., Schumacher, I. M., Adams, H. P., Brown, M. B. & Klein, P. A. (2000) *Pathology of upper respiratory tract disease of gopher tortoises in Florida*. *Journal of Wildlife Diseases*, **36**, 272-283.
- McRae, W. A., Landers, J. L. & Garner, J. A. (1981) *Movement patterns and home range of the gopher tortoise*. *American Midland Naturalist*, **106**, 165-179.
- Melbourne, B. A. & Hastings, A. (2008) *Extinction risk depends strongly on factors contributing to stochasticity*. *Nature*, **454**, 100-103.

- Mengak, M. T. & Castleberry, S. B. (2004) Wildlife management issues and opportunities in slash pine forests. *Slash pine: still growing and growing! Proceedings of the Slash pine symposium, Jekyll Island, Georgia, USA, 23-25 April 2002*. (eds, pp. 79-83.
- Miller, M. W., Hobbs, N. T. & Tavener, S. J. (2006) *Dynamics of prion disease transmission in mule deer. Ecological Applications*, **16**, 2208-2214.
- Miller, P. S. (2001) Preliminary population viability assessment for the gopher tortoise (*Gopherus polyphemus*) in Florida. *Proceedings: PVA Workshop*.
- Minion, F. C. (2002) *Molecular pathogenesis of mycoplasma animal respiratory pathogens. Frontiers in Bioscience*, **7**, 1410-1422.
- Mittermayer, H. (1998) *Pneumonia in elderly people. Atemwegs- und Lungenkrankheiten*, **24**, S63-S66.
- Mohammed, J., Frasca, S., Cecchini, K., Rood, D., Nyaoke, A. C., Geary, S. J. & Silbart, L. K. (2007) *Chemokine and cytokine gene expression profiles in chickens inoculated with Mycoplasma gallisepticum strains R-low or GT5. Vaccine*, **25**, 8611-8621.
- Moon, J. C., McCoy, E. D., Mushinsky, H. R. & Karl, S. A. (2006) *Multiple Paternity and Breeding System in the Gopher Tortoise, Gopherus polyphemus. J Hered*, **97**, 150-157.
- Morris, W. F. & Doak, D. (2002) *Quantitative Conservation Biology*. Sinauer Associates, Inc., Sunderland.
- Mougeot, F., Redpath, S. M. & Piertney, S. B. (2006) *Elevated spring testosterone increases parasite intensity in male red grouse. Behavioral Ecology*, **17**, 117-125.
- Murtaugh, P. A. (2009) *Performance of several variable-selection methods applied to real ecological data. Ecology Letters*, **12**, 1061-1068.
- Mushinsky, H. R., Wilson, D. S. & McCoy, E. D. (1994) *Growth and sexual dimorphism of Gopherus polyphemus in Central Florida. Herpetologica*, **50**, 119-128.
- Nettles, V. F. (1992) *Wildlife diseases and population medicine. Journal of the American Veterinary Medical Association*, **200**, 648-652.
- Nolan, P. M., Hill, G. E. & Stoehr, A. M. (1998) *Sex, size, and plumage redness predict house finch survival in an epidemic. Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 961-965.

- Oftedal, O. T. & Allen, M. E. (1996) *Nutrition as a Major Facet of Reptile Conservation. Zoo Biology*, **15**, 491-497.
- Oftedal, O. T., Christopher, T. E. & Allen, M. E. (1996) URTD in hatchling tortoises. *Health Profiles, Reference Intervals, and Diseases of Desert Tortoises* (eds, pp. A44-A47. Soda Springs, CA.
- Ordorica, A. M., Pough, F. H., Cate, S. & Deutch, C. E. (2008) *Seasonal variations in microbial communities in the nasal passages of captive desert tortoises. Journal of the Arizona-Nevada Academy of Science*, **40**, 121-127.
- Origgi, F. C. (2007) Reptile immunology. *Infectious diseases and pathology of reptiles: color atlas and text*. (eds E. R. Jacobson), pp. 131-166. CRC Press.
- Origgi, F. C. & Jacobson, E. R. (2000) *Diseases of the respiratory tract of chelonians. Vet Clin North Am Exot Anim Pract*, **3**, 537-549, viii.
- Ostfeld, R. S., Meffe, G. K. & Pearl, M. C. (2002) Conservation Medicine: The Birth of Another Crisis Discipline. *Conservation Medicine: Ecological Health in Practice* (eds A. A. Aguirre, R. S. Ostfeld, G. M. Tabor, C. House & M. C. Pearl), pp. 17-25. Oxford University Press, Oxford.
- Ozgul, A., Oli, M. K., Bolker, B. M. & Perez-Heydrich, C. (2009) *Upper respiratory tract disease, force of infection, and effects on survival of gopher tortoises. Ecological Applications*, **19**, 786-798.
- Padgett, D. A. & Glaser, R. (2003) *How stress influences the immune response. Trends in Immunology*, **24**, 444-448.
- Parker, R. F., Davis, J. K., Cassell, G. H., White, H., Dziedzic, D., Blalock, D. K., Thorp, R. B. & Simecka, J. W. (1989) *Short-term exposure to nitrogen-dioxide enhances susceptibility to murine respiratory mycoplasmosis and decreases intrapulmonary killing of Mycoplasma pulmonis. American Review of Respiratory Disease*, **140**, 502-512.
- Pascarella, J. B. & Horvitz, C. C. (1998) *Hurricane Disturbance and the Population Dynamics of a Tropical Understory Shrub: Megamatrix Elasticity Analysis. Ecology*, **79**, 547-563.
- Pence, D. B. & Ueckermann, E. (2002) *Sarcoptic mange in wildlife. Revue Scientifique Et Technique De L Office International Des Epizooties*, **21**, 385-398.
- Perkins, S. E., Cagnacci, F., Stradiotto, A., Arnoldi, D. & Hudson, P. J. (2009) *Comparison of social networks derived from ecological data: implications for inferring infectious disease dynamics. Journal of Animal Ecology*, **78**, 1015-1022.

- Perkins, S. E., Cattadori, I. M., Tagliapietra, V., Rizzoli, A. P. & Hudson, P. J. (2003) *Empirical evidence for key hosts in persistence of a tick-borne disease. International Journal for Parasitology*, **33**, 909-917.
- Peterson, C. C. (1994) *Different rates and causes of high mortality in two populations of the threatened desert tortoise Gopherus agassizii. Biological Conservation*, **70**, 101-108.
- Pettan-Brewer, K. C. B., Drew, M. L., Ramsay, E., Mohr, F. C. & Lowenstine, L. J. (1996) *Herpesvirus particles associated with oral and respiratory lesions in a California desert tortoise (Gopherus agassizii). Journal of Wildlife Diseases*, **32**, 521-526.
- Pike, D. A. & Grosse, A. (2006) *Daily activity of immature gopher tortoises (Gopherus polyphemus) with notes on commensal species. Florida Scientist*, **69**, 92-98.
- Pike, D. A. & Seigel, R. A. (2006) *Variation in hatchling tortoise survivorship at three geographic localities. Herpetologica*, **62**, 125-131.
- Plowright, R. K., Sokolow, S. H., Gorman, M. E., Daszak, P. & Foley, J. E. (2008) *Causal inference in disease ecology: investigating ecological drivers of disease emergence. Frontiers in Ecology and the Environment*, **6**, 420-429.
- Poulin, R. (1996) *Sexual inequalities in helminth infections: A cost of being a male? American Naturalist*, **147**, 287-295.
- Rautiainen, E., Virtala, A. M., Wallgren, P. & Saloniemi, H. (2000) *Varying effects of infections with Mycoplasma hyopneumoniae on the weight gain recorded in three different multisource fattening pig herds. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health*, **47**, 461-469.
- Razin, S., Yogev, D. & Naot, Y. (1998) *Molecular biology and pathogenicity of mycoplasmas. Microbiology and Molecular Biology Reviews*, **62**, 1094-1156.
- R Development Core Team. (2007). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria
- Regula, G., Lichtensteiger, C. A., Mateus-Pinilla, N. E., Scherba, G., Miller, G. Y. & Weigel, R. M. (2000) *Comparison of serologic testing and slaughter evaluation for assessing the effects of subclinical infection on growth in pigs. Journal of the American Veterinary Medical Association*, **217**, 888-895.
- Rostal, D. C., Lance, V. A., Grumbles, J. S. & Schumacher, I. M. (1994) *Reproduction in the desert tortoise: Effects of upper respiratory tract disease. American Zoologist*, **34**, 22A.
- Ruby, D. E. & Niblick, H. A. (1994) *A Behavioral Inventory of the Desert Tortoise: Development of an Ethogram. Herpetological Monographs*, **8**, 88-102.

- Russell, C. A., Smith, D. L., Childs, J. E. & Real, L. A. (2005) *Predictive spatial dynamics and strategic planning for raccoon rabies emergence in Ohio. PLoS Biology*, **3**, 382-388.
- Saif, Y. M. (2003) *Diseases of poultry*. Iowa State Press, Ames, Iowa.
- Saito, M., Nakagawa, M., Suzuki, E., Kinoshita, K. & Imaizumi, K. (1981) *Synergistic Effect of Sendai Virus on Mycoplasma-Pulmonis Infection in Mice. Japanese Journal of Veterinary Science*, **43**, 43-50.
- Sandmeier, F. C., Tracy, C. R., duPre, S. & Hunter, K. (2009) *Upper respiratory tract disease (URTD) as a threat to desert tortoise populations: a reevaluation. Biological Conservation*, **142**, 1255-1268.
- Schumacher, I. M., Brown, M. B., Jacobson, E. R., Collins, B. R. & Klein, P. A. (1993) *Detection of antibodies to a pathogenic mycoplasma in desert tortoises (Gopherus agassizii) with Upper Respiratory Tract Disease. Journal of Clinical Microbiology*, **31**, 1454-1460.
- Schumacher, I. M., Hardenbrook, D. B., Brown, M. B., Jacobsen, E. R. & Klein, P. A. (1997) *Relationship between clinical signs of upper respiratory tract disease and antibodies to Mycoplasma agassizii in desert tortoises from Nevada. Journal of Wildlife Diseases*, **33**, 261-266.
- Schumacher, I. M., Rostal, D. C., Yates, R. A., Brown, D. R., Jacobson, E. R. & Klein, P. A. (1999) *Persistence of maternal antibodies against Mycoplasma agassizii in desert tortoise hatchlings. American Journal of Veterinary Research*, **60**, 826-831.
- Scott, M. E. (1988) *The impact of infection and disease on animal populations: implications for conservation biology. Conservation Biology*, **2**, 40-56.
- Seigel, R. A., Smith, R. B. & Seigel, N. A. (2003) *Swine flu or 1918 pandemic? Upper respiratory tract disease and the sudden mortality of gopher tortoises (Gopherus polyphemus) on a protected habitat in Florida. Journal of Herpetology*, **37**, 137-144.
- Senar, J. C. & Conroy, M. J. (2004) *Multi-state analysis of the impacts of avian pox on a population of Serins (Serinus serinus): the importance of estimating recapture rates. Animal Biodiversity and Conservation*, **27**, 133-146.
- Silva, J. F., Raventos, J., Caswell, H. & Trevisan, M. C. (1991) *Population Responses to Fire in a Tropical Savanna Grass, Andropogon Semiberbis: A Matrix Model Approach. The Journal of Ecology*, **79**, 345-355.
- Simecka, J. W. (2003) *Contrasting roles of immunity in Mycoplasma respiratory disease. Recent research developments in infection & immunity. Vol. 1. Part I*, 203-219.

- Simecka, J. W. (2005) Immune Responses Following Mycoplasma Infection. *Mycoplasmas: Molecular Biology, Pathogenicity, and Strategies for Control* (eds A. Blanchard & G. Browning), pp. 603. Horizon Bioscience, Norfolk.
- Simecka, J. W., Davis, J. K., Davidson, M. K., Ross, S. E., Stadtlander, C. T. K.-H. & Cassel, G. H. (1992) Mycoplasma disease of animals. *Mycoplasmas : molecular biology and pathogenesis* (eds J. Maniloff, R. N. McElhaney, L. R. Finch & J. B. Baseman), pp. 391-415. American Society for Microbiology, Washington, D.C.
- Skorping, A. & Jensen, K. H. (2004) *Disease dynamics: all caused by males? Trends in Ecology & Evolution*, **19**, 219-220.
- Skuldt, L. H., Mathews, N. E. & Oyer, A. M. (2008) *White-tailed deer movements in a chronic wasting disease area in south-central Wisconsin. Journal of Wildlife Management*, **72**, 1156-1160.
- Smith, K. F., Acevedo-Whitehouse, K. & Pedersen, A. B. (2009) *The role of infectious diseases in biological conservation. Animal Conservation*, **12**, 1-12.
- Smith, K. F., Sax, D. F. & Lafferty, K. D. (2006a) *Evidence for the Role of Infectious Disease in Species Extinction and Endangerment. Conservation Biology*, **20**, 1349-1357.
- Smith, L. L. (1992) Nesting Ecology, Female Home Range and Activity Patterns, and Hatchling Survivorship in the Gopher Tortoise (*Gopherus Polyphemus*). University of Florida.
- Smith, L. L. (1995) *Nesting ecology, female home range and activity, and population size-class structure of the gopher tortoise, Gopherus polyphemus, on the Katharine Ordway Preserve, Putnam County, Florida. Bulletin of the Florida Museum of Natural History*, **38**, 97-126.
- Smith, L. L., Tuberville, T. D. & Seigel, R. A. (2006b) *Workshop on the ecology, status, and management of the gopher tortoise (Gopherus polyphemus), Joseph W. Jones Ecological Research Center, 16-17 January 2003: final results and recommendations. Chelonian Conservation and Biology*, **5**, 326-330.
- Smith, R. B., Seigel, R. A. & Smith, K. R. (1998) *Occurrence of upper respiratory tract disease in gopher tortoise populations in Florida and Mississippi. Journal of Herpetology*, **32**, 426-430.
- Sokolow, S. H., Foley, P., Foley, J. E., Hastings, A. & Richardson, L. L. (2009) *Disease dynamics in marine metapopulations: modelling infectious diseases on coral reefs. Journal of Applied Ecology*, **46**, 621-631.
- Swinton, Harwood, Grenfell & Gilligan (1998) *Persistence thresholds for phocine distemper virus infection in harbour seal Phoca vitulina metapopulations. Journal of Animal Ecology*, **67**, 54-68.

- Swinton, J., Woolhouse, M. E. J., Begon, M., Dobson, A., Ferroglio, E., Grenfell, B. T., Guberti, V., Hails, R. S., Heesterbeek, J. A. P., Lavazza, A., Roberts, M. G., White, P. J. & Wilson, K. (2002) Microparasite transmission and persistence. *The Ecology of Wildlife Diseases* (eds P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek & A. P. Dobson), pp. 83-101. Oxford University Press, New York.
- Sydenstricker, K. V., Dhondt, A. A., Hawley, D. M., Jennelle, C. S., Kollias, H. W. & Kollias, G. V. (2006) *Characterization of experimental Mycoplasma gallisepticum infection in captive house finch flocks. Avian Diseases*, **50**, 39-44.
- Sydenstricker, K. V., Dhondt, A. A., Ley, D. H. & Kollias, G. V. (2005) *Re-exposure of captive house finches that recovered from Mycoplasma gallisepticum infection. Journal of Wildlife Diseases*, **41**, 326-333.
- Tableman, M. & Kim, J. S. (2002) *Survival Analysis Using S*. Chapman & Hall/CRC, Boca Raton, FL.
- Thomas, D. & Blankenship, E. (2002) *Mycoplasma testing in the gopher tortoise, Gopherus polyphemus, along a pipeline transect in Mississippi. Herpetological Review*, **33**, 101-103.
- Tompkins, D. M., Dobson, A., Arneberg, P., Begon, M., Cattadori, I. M., Greenman, J., Heesterbeek, J. A. P., Hudson, P. J., Newborn, D., Pugliese, A., Rizzoli, A., Rosa, R., Rosso, F. & Wilson, K. (2002) Parasites and host population dynamics. *The Ecology of Wildlife Diseases* (eds P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek & A. P. Dobson), pp. 45-62. Oxford University Press, New York, NY.
- Tuljapurkar, S. & Haridas, C. V. (2006) *Temporal autocorrelation and stochastic population growth. Ecology Letters*, **9**, 324-334.
- Turner, F. B., Berry, K. H., Randall, D. C. & White, G. C. (1987) Population ecology of the desert tortoise at Goffs, California, 1983-1987. Report to Southern California Edison Company, Research and Development Series 87-RD-81.
- Venables, W. N. & Ripley, B. D. (2002) *Modern Applied Statistics with S, Fourth Edition*. Springer, New York, NY.
- Verbisck-Bucker, G., Gonzalez-Candela, M., Galian, J., Cubero-Pablo, M. J., Martin-Atance, P. & Leon-Vizcaino, L. (2008) *Epidemiology of Mycoplasma agalactiae infection in free-ranging Spanish ibex (Capra pyrenaica) in Andalusia, southern Spain. Journal of Wildlife Diseases*, **44**, 369-380.
- Weissinger, M. D., Theimer, T. C., Bergman, D. L. & Deliberto, T. J. (2009) *Nightly and seasonal movements, seasonal home range, and focal location photo-monitoring of urban striped skunks (Mephitis mephitis): implications for rabies transmission. Journal of Wildlife Diseases*, **45**, 388-397.

- Wendland, L. D. (2007) Epidemiology of mycoplasmal upper respiratory tract disease in tortoises Ph.D., University of Florida.
- 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. & Brown, M. B. (in press) *Social behavior drives the dynamics of respiratory disease in threatened tortoises. Ecology.*
- Wendland, L. D., Zacher, L. A., Klein, P. A., Brown, D. R., Demcovitz, D., Littell, R. & Brown, M. B. (2007) *Improved enzyme-linked immunosorbent assay to reveal Mycoplasma agassizii exposure: a valuable tool in the management of environmentally sensitive tortoise populations. Clinical and Vaccine Immunology, 14, 1190-1195.*
- Westhouse, R., Jacobson, E., Harris, R., Winter, K. & Homer, B. (1996a) *Respiratory and pharyngo-esophageal iridovirus infection in a gopher tortoise (Gopherus polyphemus). J Wildl Dis, 32, 682-686.*
- Westhouse, R. A., Jacobsen, E. R., Harris, R. K., Winter, K. R. & Homer, B. L. (1996b) *Respiratory and pharyngo-esophageal iridovirus infection in a gopher tortoise (Gopherus polyphemus). Journal of Wildlife Diseases, 32, 682-686.*
- Wilcox, C. & Elderd, B. (2003) *The effect of density-dependent catastrophes on population persistence time. Journal of Applied Ecology, 40, 859-871.*
- Wilson, D. S., Mushinsky, H. R. & McCoy, E. D. (1994) Home Range, Activity, and Use of Juvenile Gopher Tortoises in Central Florida. *Biology of North American Tortoises* (eds R. B. Bury & D. J. Germano). National Biological Survey, Fish and Wildlife Research.
- Wilson, D. S. & Tracy, C. R. (2003) *Estimating age of turtles from growth rings: A critical evaluation of the technique. Herpetologica, 59, 178-194.*
- Wobeser, G. A. (2006) *Essentials of Disease in Wild Animals*, 1st edn. Blackwell Publishing Professional, Ames, IA.
- Woolhouse, M. E. (1989) *On the interpretation of age-prevalence curves for schistosome infections of host snails. Parasitology, 99 Pt 1, 47-56.*
- Wright, J. S. (1982) The distribution and population biology of the gopher tortoise, *Gopherus polyphemus*, in South Carolina. Master of Science, Clemson University.
- Yancey, A. L., Watson, H. L., Cartner, S. C. & Simecka, J. W. (2001) *Gender is a major factor in determining the severity of mycoplasma respiratory disease in mice. Infection and Immunity, 69, 2865-2871.*

- Yaremych, S. A., Novak, R. J., Raim, A. J., Mankin, P. C. & Warner, R. E. (2004) *Home range and habitat use by American Crows in relation to transmission of West Nile Virus. Wilson Bulletin*, **116**, 232-239.
- Zellweger, K., Stark, K. D. C. & Rufenacht, J. (2004) *Enzootic Pneumonia (EP) in Swiss swine herds after area-wide eradication: Epidemiological analysis of outbreaks occurring between 1999 and 2003. Schweizer Archiv Fur Tierheilkunde*, **146**, 471-478.
- Zimmermann, L., Jambresic, S., Giacometti, M. & Frey, J. (2008) *Specificity of Mycoplasma conjunctivae strains for alpine chamois Rupicapra r. rupicapra. Wildlife Biology*, **14**, 118-124.
- Zipser, J. C. & Ashton, K. G. (2003) *Do gopher tortoises at Archbold Biological Station, a long-protected area in Florida, have upper respiratory tract disease? Florida Scientist*, **66**, 294-299.
- Zuk, M. & McKean, K. A. (1996) *Sex differences in parasite infections: Patterns and processes. International Journal for Parasitology*, **26**, 1009-1023.

## BIOGRAPHICAL SKETCH

Carolina Perez-Heydrich was born and raised in Miami, FL. She earned a bachelor's degree in biology from Davidson College in 2003. In 2004, she began her Ph.D. in Infectious Diseases and Pathology in the College of Veterinary Medicine at the University of Florida. She was the recipient of a minority supplement from the National Sciences Foundation and a Morris Animal Foundation Fellowship. After completion of her doctoral program, she will continue her training in environmental biostatistics at the University of North Carolina at Chapel Hill, where she will be a Fellow on a T32 training grant from the National Institutes of Health. Currently, she lives in Durham, North Carolina with her husband and two children.