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INITIAL ASSESSMENT AND EFFECTS OF SNAKE FUNGAL DISEASE ON POPULATIONS OF SNAKES IN KENTUCKY

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INITIAL ASSESSMENT AND EFFECTS OF SNAKE FUNGAL DISEASE ON POPULATIONS OF SNAKES IN KENTUCKY

Thesis

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Forest and Natural Resource Sciences in the College of Agricultural, Food and Environment at the University of Kentucky

By

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Lexington, Kentucky

2018

ABSTRACT OF THESIS

INITIAL ASSESSMENT AND EFFECTS OF SNAKE FUNGAL DISEASE ON POPULATIONS OF SNAKES IN KENTUCKY

Pathogenic fungi are increasingly associated with epidemics in wildlife populations and represent a significant threat to global biodiversity. Snake fungal disease is an emerging disease caused by the fungus, *Ophidiomyces ophiodiicola,* and appears to be widespread in the eastern United States. Yet an evaluation of field diagnostics, and an understanding of the population-level consequences of the disease, are lacking. First, I evaluated the use of clinical signs to predict the presence of *O. ophiodiicola* across season and snake habitat affiliation (aquatic or terrestrial) and I compared two sampling methods to see if collection method impacts PCR result. Overall, snakes with clinical signs had a higher probability of testing positive regardless of season or habitat association. However, terrestrial snakes had a lower overall probability of testing positive for *O. ophiodiicola* compared to aquatic snakes. I found no significant difference between sampling methods. Second, I used Passive Integrated Transponder (PIT) telemetry, and multistate capture-mark-recapture modelling to determine if SFD affects the short-term survival, movement, and behavior of wild snakes. I found no difference in short-term survival for snakes with SFD. Snakes with SFD spend more time surface-active and have lower permanent emigration and temporary immigration rates than snakes without SFD.

KEYWORDS: snake, populations, snake fungal disease, survivorship, clinical sign, capture-mark-recapture

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May 29, 2018
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CHAPTER 1: INTRODUCTION

Pathogenic fungi are increasingly associated with epidemics in animal populations and represent a significant threat to global biodiversity (Fisher et al. 2012). Prominent examples include the emergence of the fungi *Batrachyochytrium dendrobatidis,* and *B. salamandrivorans* which both lead to chytridiomycosis in amphibians (Berger et al. 1998; Martel et al. 2013), and *Pseudogymnoascus destructans*, the fungus responsible for white-nose syndrome in bat populations (Blehert et al. 2008). Due to the reproductive potential of most pathogenic fungi, host populations can decline at a rate that quickly leads to extirpation or extinction (Fisher et al. 2012).

Snake fungal disease (SFD), caused by the fungus *Ophidiomyces ophiodiicola,* is an emerging infectious disease affecting both wild and captive snakes (Rajeev et al. 2009; Lorch et al. 2016). The fungus causes crusts on scales, thickening of the skin, cloudiness of the eyes, separation of the outermost layer of skin from the underlying layer (i.e., *stratum corneum*) and facial swelling (Lorch et al. 2015). Individual mortality due to infection has occurred (Allender et al. 2011; Lind et al. 2018), yet many aspects of SFD remain poorly understood. Among the most important factors requiring research include disease diagnostics, host susceptibility, and the population-level effects of SFD. Understanding these factors is important to develop essential management and conservation strategies for this disease.

Snake fungal disease was first documented in Kentucky in a single wild caught Queensnake (*Regina septemvittata*) in 2014 and has since been confirmed in several other snake species within the Commonwealth (Price et al. 2015; Lorch et al. 2016). In my first chapter, I examined disease diagnostics and host susceptibility in Kentucky. Specifically,

I evaluated the use of clinical signs to predict the presence of *O. ophiodiicola* across seasons and habitat affiliation. I also compared two sampling methods, scale clip collection and swabbing, for *O. ophiodiicola* to see if collection method impacts the results of polymerase chain reaction (PCR). My second chapter focuses on the impacts of SFD on wild populations of *R. septemvittata* and *Nerodia sipedon* in central Kentucky. By utilizing Passive Integrated Transponder (PIT) telemetry and a multistate capture-mark-recapture model, I assess how SFD affects the short-term survival, movement and behavior of these two species. My first chapter is in review with EcoHealth, and slight formatting differences between chapters reflect journal submission requirements.

CHAPTER 2: INITIAL ASSESSMENT

Introduction

Pathogenic fungi are increasingly associated with epidemics in animal populations and represent a significant threat to global biodiversity (Fisher et al. 2012). Prominent examples include the emergence of *Batrachyochytrium dendrobatidis* and *B. salamandrivorans*, which causes chytridiomycosis in amphibians (Berger et al. 1998; Martel et al. 2013), and *Pseudogymnoascus destructans*, the fungus responsible for white-nose syndrome in some bat populations (Blehert et al. 2008). As of 2012, fungi have been implicated in at least 54 species-level extirpations and are a major cause (e.g., 65%) of pathogen-driven host loss (Fisher et al. 2012). Since fungal pathogens have caused widespread declines of many host populations, intensive monitoring of the distribution, host susceptibility, and development of field diagnostics for newly emerging fungal pathogens have become essential first steps for management and conservation actions.

Snake fungal disease (SFD), caused by the fungus *Ophidoimyces ophiodiicola*, is an emerging disease of wild and captive snakes (Sigler et al. 2013; Lorch et al. 2015; Lorch et al. 2016). Clinical signs of SFD include skin ulcers, subcutaneous nodules, increased molt frequency, localized thickening of the skin, and facial swelling (Lorch et al. 2015). Behavioral changes, such as an increase in basking, have also been noted in infected individuals (Clark et al. 2011; Lorch et al. 2015). Secondary effects of *O. ophiodiicola* infection may include starvation, poor body condition, and bacterial infection, that may result in mortality (Allender et al. 2011; Lorch et al. 2016; McCoy et al. 2017). Despite its recent description, research suggests that *O. ophiodiicola* is a fungus native to North America, yet is now recently emerging for unknown reasons (Lorch et al. 2016).

Recent work suggests that *O. ophiodiicola* displays traits of a well-adapted pathogen that has a broad host range and can likely persist in the environment (Allender et al. 2015; Lorch et al. 2016; Burbrink et al. 2017). Yet, seasonal variation in clinical sign severity indicate that environmental conditions can influence infection status (McCoy et al. 2017). While *O. ophiodiicola* has been documented in 23 U.S. states, one Canadian Province in eastern North America, and recently in Europe (Lorch et al. 2016; Franklions et al. 2017), information remains limited on effective field diagnostic methods, particularly the relationship between clinical signs (i.e., skin lesions) and infection by *O. ophiodiicola*.

Clinical signs of SFD are nonspecific and used to describe "symptoms" commonly observed with infection by *O. ophiodiicola*. Swabs and tissue samples (e.g., scale clips) are used to test for the presence of *O. ophiodiicola* by polymerase chain reaction (PCR) (Allender et al. 2015; Bohuski et al. 2015); however, the relationship between PCR-based detection of the fungus and disease state has not been fully elucidated. Furthermore, some snakes harboring *O. ophiodiicola* do not have clinical signs of SFD (Paré et al. 2003; Bohuski et al. 2015), but the extent to which wild snakes may have subclinical infections or act as carriers of *O. ophiodiicola* has not been investigated. Assessing the percentage of "asymptomatic" snakes with *O. ophiodiicola* would be helpful in further defining the geographic distribution, host range and disease dynamics of *O. ophiodiicola*.

My primary objective was to test the relationship between field observations of SFD and the presence of *O. ophiodiicola.* Since previous studies have alluded to seasonal trends of *O. ophiodiicola* infection (reviewed by Lorch et al. 2016; McCoy et al. 2017), I examined the relationship between both clinical signs and season (spring, summer and fall) on fungal presence. In addition, since moist environments are thought to be important for

fungal growth (Lorch et al. 2016), I considered habitat affiliation (aquatic or terrestrial) of snake species sampled to determine if snakes with a certain habitat affiliation are more likely to be exposed or infected with *O. ophiodiicola*. Second, I examined the effectiveness two sampling methods for *O. ophiodiicola*: scale clips and swabbing.

Methods - Study Sites

Snakes were captured using a variety of field methods at six sampling locations within the Inner Bluegrass, Eastern Kentucky Coalfields, Knobs, and Jackson Purchase physiographic regions of Kentucky (USA) between March 2015 and May 2016 (Figure 2.1). At sample locations in the Knobs and Eastern Kentucky, most snakes were captured under artificial cover (i.e., wood coverboards and roofing tin). Within the Inner Bluegrass, snakes were captured opportunistically and later recaptured using passive integrated transponder (PIT) telemetry (Oldham et al. 2016). In the Jackson Purchase, snakes were captured via nighttime road surveys.

Methods - Field Sampling and Laboratory Assessment

Upon each capture, snakes were identified to species; dates of capture and locality information were also recorded. I recorded the presence/absence of visible dermal lesions on the head or body of the animal. Specifically, I defined visible dermal lesions to include regional or local edema, crusts, ulcers, dysecdysis and other forms of damage to the dermis (i.e., Lorch et al. 2015; Guthrie et al. 2016). I considered these dermal lesions as indicative of *O. ophiodiicola* infection. After recording clinical signs, I used up to two methods to collect samples for PCR assay for *O. ophiodiicola*. If lesions were present, one lesion was

swabbed with a sterile polyester tipped swab saturated with pure water (Fisher Scientific, BP2484-100) and/or a scale clip was collected by removing a small section of one scale. The swab was brushed over the lesion five times, then placed into a plastic vial, and stored in a -40°C freezer (J. Lorch pers. comm.). If no lesions were present, a snake was swabbed on the dorsal side of its midline. Scale clips were taken using scissors and forceps; sampling equipment was treated with 10% bleach to sterilize and remove nucleic acid between snakes. Scale clips were either taken from lesioned areas of skin (if lesions were present) or a grossly normal ventral scale on the transverse midline (if lesions were absent). After sampling, all snakes were released at their capture location.

Real-time PCR was used to determine presence of *O. ophidiocola* according to the protocols in Bohuski et al. (2015) for the internal transcribed spacer region (ITS) assay. *Ophiodiomyces ophiodiicola* was considered present on a snake if the threshold was ≤ 40 cycles (Bohuski et al. 2015). This threshold does not confirm the presence of SFD; it only confirms the presence of *O. ophiodiicola.* Samples were considered negative for *O. ophiodiicola* if amplification did not occur within 40 cycles. All research was compliant with University of Kentucky IACUC protocol (2013-1073). Permits were obtained from the Kentucky Department of Fish and Wildlife Resources (SC1511017, SC1611043, SC1611136).

Statistical Analyses

I compared scale clips and swab samples using 173 snakes for which a scale clip and a swab sample were both taken at the same instance of capture. I used the package "MASS" in R v.3.2.1 to run a McNemar's test (Venables and Ripley, 2002; R Core Team,

2016) to compare the effectiveness of scale clips against swab samples at detecting presence of *O. ophiodiicola*. Because some snakes had multiple instances of paired samples (e.g., swab and scale clip collected at the same time), for this analysis I used the first instance of a paired sample with one positive sampling method for *O. ophiodiicola.* If an individual did not have any instances where one sampling method was positive within a paired sample, I used the first set of paired samples collected to determine disease status.

To evaluate the probability of a positive PCR result given the presence or absence of clinical signs, season and species habitat affiliation, I used the package "lme4" to fit a generalized linear mixed model in R v.3.2.1 (Bates et al. 2015; R Core Team, 2016). I used the package "AICcmodavg" to generate predicted infection probabilities using an inverse-logit transformation (Mazerolle, 2016). For this analysis, I included all available PCR results from both sampling methods, even if a snake had multiple PCR results over time. I used PCR result (0=negative, 1=positive) as the response variable with season, presence/absence of clinical signs and habitat affiliation as predictor variables. I treated individual ID as a random effect to account for non-independence of multiple measurements of the same snakes. Season was divided into three groups: spring (March-May), summer (June-August), and fall (September-November). In addition, I divided snakes into two groups based on habitat affiliation (aquatic and terrestrial) to examine how this affiliation was related to the probability of *O. ophiodiicola* presence. Specifically, I placed *Regina septemvittata* and *Nerodia sipedon* in the aquatic category (n=188), with all other snakes in the terrestrial category (n=83) (Table 2.1). I generated seven competing models to predict PCR result: 1) habitat affiliation, 2) clinical signs, 3) season, 4) clinical signs and habitat affiliation, 5) season and habitat affiliation, 6) season and clinical signs,

and 7) season, clinical signs and habitat affiliation (Table 2.2). I compared models using the Akaike Information Criterion (AIC; Burnham and Anderson, 2002).

Results

I collected 639 swab or scale clip samples from 271 individual snakes, represented by fifteen species. Fifteen snake species were represented in this study (Table 2.1). Out of the 271 snakes sampled, 140 (51.66%) had clinical signs. *Ophidiomyces ophiodiicola* was detected in at least one sample from each species, except for *Opheodrys aestivus* which was represented by just two individuals. I collected 196 samples from 100 individuals in spring, 273 samples from 147 individuals in summer, and 170 samples from 71 individuals in fall. I also detected *O. ophiodiicola* in nearly every combination of season, PCR result, and clinical sign status (Table 2.3). From the 271 individuals, aquatic snakes (i.e., *R. septemvittata* and *N. sipedon*) comprised 188 samples, with 125 samples testing positive for *O. ophiodiicola* (66.50%). Terrestrial snakes comprised 83 samples, with 42 samples (50.60%) testing positive. Most clinical signs were considered mild (i.e., see Gutherie et al. 2016; Lorch et al. 2016), although some individuals had moderate to severe infections as denoted by skin ulcers, large patches of thickened skin, and infection of the eyes (Figure 2.2). When comparing scale clip and swab sampling, I found no significant difference between the two sampling methods for detecting presence of *O. ophiodiicola* (McNemar's chi-squared = 1.59, df = 1, p-value = 0.21) (Figure 2.3).

The presence of clinical signs, snake habitat affiliation and season were all important predictors of PCR results (AIC weight = 0.95; Table 2). Specifically, aquatic snakes with clinical signs had an 81.4% (spring; n= 196), 75.5% (summer; n = 273) and

24.1% (fall; n = 170) probability of having a positive PCR result in each season, whereas terrestrial snakes with clinical signs had 65.8%, 57.5%, and 12.2% chances of a positive PCR result in spring, summer, and fall, respectively (Figure 2.4). A post-hoc Tukey test showed that spring and fall were significantly different ($p<0.001$), and summer and fall were significantly different ($p<0.001$). There was no significant difference between spring and summer samples.

I found that some wild snakes may have subclinical infections or act as "asymptomatic" carriers of *O. ophiodiicola*. For example, aquatic snakes without clinical signs had a 42.2%, 33.9% and 5.0% chance of having a positive PCR result in spring, summer, and fall respectively, whereas terrestrial snakes lacking clinical signs had the following chances of PCR-based *O. ophiodiicola* detection across seasons: 24.3%, 18.41% and 2.28%. For all snakes, the probability of *O. ophiodiicola* being present was lowest in the fall samples.

Discussion

Snake fungal disease is widely distributed in North America, and *O. ophiodiicola* has a broad host range (Burbrink et al. 2017), with infections documented in 30 species across six snake families (reviewed by Lorch et al. 2016; Burbrink et al. 2017). My research is consistent with these findings, albeit at a smaller spatial scale, as I detected *O. ophiodiicola* at each sampling location and in 14 of 15 (93.3%) species examined. I added one wild-caught snake species, *Pantherophis spiloides,* to the known host range of *O. ophiodiicola.*

I found no significant difference between sampling methods (i.e., swabs versus tissue samples) for detection of *O. ophiodiicola* by real-time PCR. Based on my results, I suggest sampling snakes via swab because it is less invasive and can be done rapidly in the field compared to scale clipping. Swabbing may also decrease the risk of disease transmission between individuals because swabs are less likely to compromise the surface of the skin and allow a point of entry for *O. ophiodiicola* (Lorch et al. 2015). Furthermore, swabbing is more efficient in the field because tools (i.e., scissors and forceps) do not need to be disinfected and decontaminated between individual animals. More work is needed to determine whether certain sample methods might be better for a particular type of lesion or stage of infection.

I found that snakes with lesions had a higher probability of a positive PCR result for *O. ophiodiicola* than snakes without lesions. However, I found that some "asymptomatic" snakes tested positive for *O. ophiodiicola*. This is consistent with previous findings by Bohuski et al. (2015) that 12.5% of snakes that lacked clinical signs of SFD tested positive for *O. ophiodiicola* by real-time PCR. This indicates that while clinical signs are a conspicuous predictor of the presence of *O. ophiodiicola,* instances where the fungus is present, but the snake is without lesions, do occur. Snakes without clinical signs could be in the early stages of infection with *O. ophiodiicola* or they could be carriers of the fungus (Lorch et al. 2016). Certain clinical signs, such as crusts, may be more likely to have *O. ophiodiicola* on the surface and thus more likely to yield a positive PCR result compared to other clinical signs indicative of a fungal infection deeper in the skin.

I found that snakes with aquatic habitat affiliations had a higher probability of testing positive for *O. ophiodiicola* than snakes with terrestrial habitat affiliations. Thus,

my results indicate that species affiliated within aquatic habitats vary in either their susceptibility or the distribution of *O. ophiodiicola* may vary between terrestrial and aquatic habitats. Lorch et al. (2016) suggested that moist conditions could promote growth of *O. ophiodiicola* and its persistence in the environment, as seen in other fungal pathogens (Kriger and Hero, 2007). Based upon the detection of *O. ophiodiicola* on individuals without clinical signs of infection, aquatic snakes may have higher exposure rates compared to the fungal pathogen than terrestrial snakes in my study area. Conversely, the aquatic snake species examined could be more susceptible to *O. ophiodiicola.* The snake species, *R. septemvittata,* which comprised most of my aquatic snakes, has some of the thinnest skin of all snake species which could make it more vulnerable to abrasions which would provide an entry point for *O. ophiodiicola* infection (Stokes and Dunson, 1982). Most previous work on SFD has focused on terrestrial snake species (Allender et al. 2013; McCoy et al. 2017), but my findings demonstrate that aquatic snake species should be more closely studied to better understand how host natural history may affect disease dynamics. For example, comparing aquatic and terrestrial environments could provide insights into how environmental loads of the pathogen vary between habitats (i.e., host exposure) and the ability of infected snakes to locate suitable microclimates for fighting infection. Closely examining species-level differences in natural histories of various host species could also provide insights into which snake species are most vulnerable to developing severe infections caused by *O. ophiodiicola*.

I found that the probability of disease was lowest in fall, compared to spring and summer. The higher probability of positive PCR results in spring closely tracks previous reports of SFD being more frequent in snakes after spring emergence (Lorch et al. 2016).

This suggests that infections by *O. ophiodiicola* may initiate in winter or spring during a time when host immune function is suppressed (Nelson and Demas, 2012) and snakes are exposed to potentially high loads of the fungus in moist underground hibernacula. Snakes that emerge from hibernation infected may subsequently expose unaffected animals by direct contact or by shedding large amounts of the pathogen into the environment. Snakes that are PCR-positive for *O. ophiodiicola* in the absence of clinical signs, may indicate exposure to the fungus without the establishment of an active infection. Snake fungal disease is often a chronic condition (Lorch et al. 2015) which may explain persistence of clinical signs and detection of *O. ophiodiicola* into summer. However, the percentage of snakes with clinical signs of SFD is reduced in fall compared to spring and summer, consistent with recovery or removal of many infected animals within the population or because the wild snakes may have already been in inaccessible hibernacula at the time when infections would be expected to reoccur. More work is needed to determine the roles of seasonal changes in host immune physiology, rainfall, and air temperature in driving disease dynamics, as these factors are correlated with season.

Habitat and species-specific differences could also result in different observed seasonal patterns. For example, aquatic snake species that occupy more shaded habitats could experience cooler summer temperatures, which could cause a delayed response to infection, with infections lingering longer into summer. In Florida (USA), a mean fungal score, which included the presence and severity of observed clinical signs, negatively correlated with increasing temperature (McCoy et al. 2017). This is contrary to my results, which demonstrate high probabilities of testing positive in spring and summer. This discrepancy could exist because Florida temperatures allow this population of snakes to be

active year-round, and temperatures may exceed the upper growth limit for *O. ophiodiicola* (>35°C; Allender et al. 2015), and never become too cold to prevent growth (7°C; Allender et al. 2015), unlike in Kentucky.

Studying the fungal load of specific lesions, and the predictive ability of certain lesions would be beneficial in understanding how the infection progresses. In snakes that have clinical signs but test negative for *O. ophiodiicola*, other etiologies (e.g., other fungi, bacteria, traumatic injuries, etc.) could be responsible for the presence of lesions, which further confounds diagnosing snakes with SFD using clinical signs alone (see Lorch et al. 2016 for a description of other fungi). While confirming the presence of *O. ophiodiicola* via PCR is vital for a definitive diagnosis of SFD, I found that clinical signs appear to be a relatively accurate predictor of *O. ophiodiicola* presence in spring and summer. On the other hand, I found that clinical signs were not particularly effective at diagnosing snakes in fall because the overall the probability of a snakes testing positive for *O. ophiodiicola* was lower. I only tested for additive effects of sign, habitat, and season and that, as a result, I do not know whether the effectiveness of clinical sign as a disease indicator depends on season.

Overall, my results provide insight into the range of host species that can become infected by, or carry, *O. ophiodiicola*, differences in detection probabilities of *O. ophiodiicola* for snakes with certain habitat affiliations, and seasonal variation in the presence of clinical signs of SFD and the presence of *O. ophiodiicola*. Results may vary in other regions, but in my study area, I recommend surveying snake populations for *O. ophiodiicola* in the spring and summer as that corresponds to when clinical signs are most conspicuous and predictive of positive PCR results. Although I make an effort to address

the lack of multi-species assessments for infection by *O. ophiodiicola,* the effects this fungus has on populations of snakes are unknown. My study provides information that will be vital to understanding infection patterns and developing effective management strategies for populations of snakes affected by SFD.

Table 2.1. Morbidity Table. Morbidity table showing species, habitat affiliation, number of individual samples, presence or absence of clinical signs and real-time PCR results (positive or negative) for snake species tested for *O. ophiodiicola* in Kentucky, USA (2016).

Aquatic Species	**Number Sampled**	**Clinical Signs Present**	**Clinical Signs Present and Positive**	**Clinical Signs Present and Negative**	**Clinical Signs Absent**	**Clinical Signs Absent and Positive**	**Clinical Signs Absent and Negative**
Nerodia sipedon	72	17	17	0	55	21	34
Regina septemvittata	116	73	61	12	43	26	17
Total	188	90	78	12	98	47	51
Terrestrial Species							
Agkistrodon contortrix	18	9	7	2	9	0	9
Carphophis amoenus	6	4	4	0	2	0	2
Coluber constrictor	13	8	5	3	5	1	4
Crotalus horridus	3	3	2	1	0	0	0
Diadophis punctatus	9	4	3	1	5	0	5
Lampropeltis getula	9	7	7	0	2	1	1
L. triangulum	5	5	4	1	0	0	0
Nerodia erythrogaster	2	1	1	0	1	0	1
Opheodrys aestivus	2	1	0	1	1	0	1
Pantherophis spiloides	2	1	1	0	1	0	1
Storeria occipitomaculata	3	1	1	0	2	0	2

Table 2.1 (continued)

Terrestrial Species	Number Sampled	Clinical Signs Present	Clinical Signs Present and Positive	Clinical Signs Present and Negative	Clinical Signs Absent	Clinical Signs Absent and Positive	Clinical Signs Absent and Negative
Thamnophis sirtalis	8	5	4	1	3	0	3
Virginia valeriae	3	1	0	1	2	1	1
Total	83	50	39	11	33	3	30
Overall Total	**271**	**140**	**117**	**23**	**131**	**50**	**81**

Table 2.2 Model Output from GLMM Analysis. Model outputs from GLMM analysis examining the ability of clinical signs to predict disease status in snakes with certain habitat affiliations over three seasons. The table shows the model, AIC_c value, model weight, number of parameters, and coefficient estimates and standard errors for explanatory variables. Asterisks indicate significance ($p<0.001$).

Model	AIC_c	*w*	k	Intercept	Habitat Affiliation (HA) - Terrestrial	Season - Fall	Season - Spring	Clinical Signs (CS) - Present
Season + CS + HA	706.23	0.95	6	-0.664 ± 0.2263	-0.8224 ± 0.3051	-2.2706 ± 0.3424 ***	0.3521 ± 0.2715	1.7890 ± 0.2763 ***
Season + CS	712.11	0.05	5	-0.8274 ± 0.2185 ***	---	-2.1869 ± 0.3376 ***	0.2820 ± 0.2655	1.7072 ± 0.2667 ***
Season + HA	756.00	0.00	5	0.2243 ± 0.1893	-0.6611 ± 0.3006	-2.3247 ± 0.3408 ***	0.7263 ± 0.2750	---
Season	759.05	0.00	4	0.0571 ± 0.1730	---	-2.2700 ± 0.3383 ***	0.6596 ± 0.2703	---
Clinical + HA	772.01	0.00	4	-1.2374 ± 0.2174 ***	-0.6168 ± 0.3236	---	---	2.1229 ± 0.2995 ***
Clinical	773.95	0.00	3	-1.3429 ± 0.2094 ***	---	---	---	2.0237 ± 0.2841 ***
Habitat Affiliation	840.62	0.00	3	-0.0983 ± 0.1615	-0.3705 ± 0.3158	---	---	---

Table 2.3 Number of Samples Collected By Season, Habitat Affiliation, PCR Result, and Clinical Sign Status. The number of samples collected for each season, habitat affiliation, real-time PCR result and clinical sign status combination.

Season	Habitat Affiliation	PCR Result	Clinical Signs	Number of Samples Collected
Spring	Aquatic	Negative	Absent	20
Spring	Aquatic	Negative	Present	27
Spring	Aquatic	Positive	Absent	15
Spring	Aquatic	Positive	Present	70
Spring	Terrestrial	Negative	Absent	13
Spring	Terrestrial	Negative	Present	14
Spring	Terrestrial	Positive	Absent	1
Spring	Terrestrial	Positive	Present	36
Summer	Aquatic	Negative	Absent	49
Summer	Aquatic	Negative	Present	43
Summer	Aquatic	Positive	Absent	46
Summer	Aquatic	Positive	Present	70
Summer	Terrestrial	Negative	Absent	24
Summer	Terrestrial	Negative	Present	15
Summer	Terrestrial	Positive	Absent	2
Summer	Terrestrial	Positive	Present	24
Fall	Aquatic	Negative	Absent	83
Fall	Aquatic	Negative	Present	44
Fall	Aquatic	Positive	Absent	1
Fall	Aquatic	Positive	Present	22
Fall	Terrestrial	Negative	Absent	13
Fall	Terrestrial	Negative	Present	4
Fall	Terrestrial	Positive	Absent	0
Fall	Terrestrial	Positive	Present	3

Figure 2.1 Sampling Locations. Red dots indicate sampling locations across multiple physiographic regions in Kentucky. At least one positive individual was found at each sampling location.

Figure 2.2 Clinical Signs of Snake Fungal Disease. Mild clincal signs of snake fungal disease on A) Northern Copperhead (*Agkistrodon contortrix*) captured in Breathitt Co., Kentucky, B) Moderate clinical signs on Queensnake (*Regina septemvittata*) captured in Jessamine Co., Kentucky and C) severe clinical signs on Southern Black Racer (*Coluber constrictor*) captured in Madison Co., Kentucky.

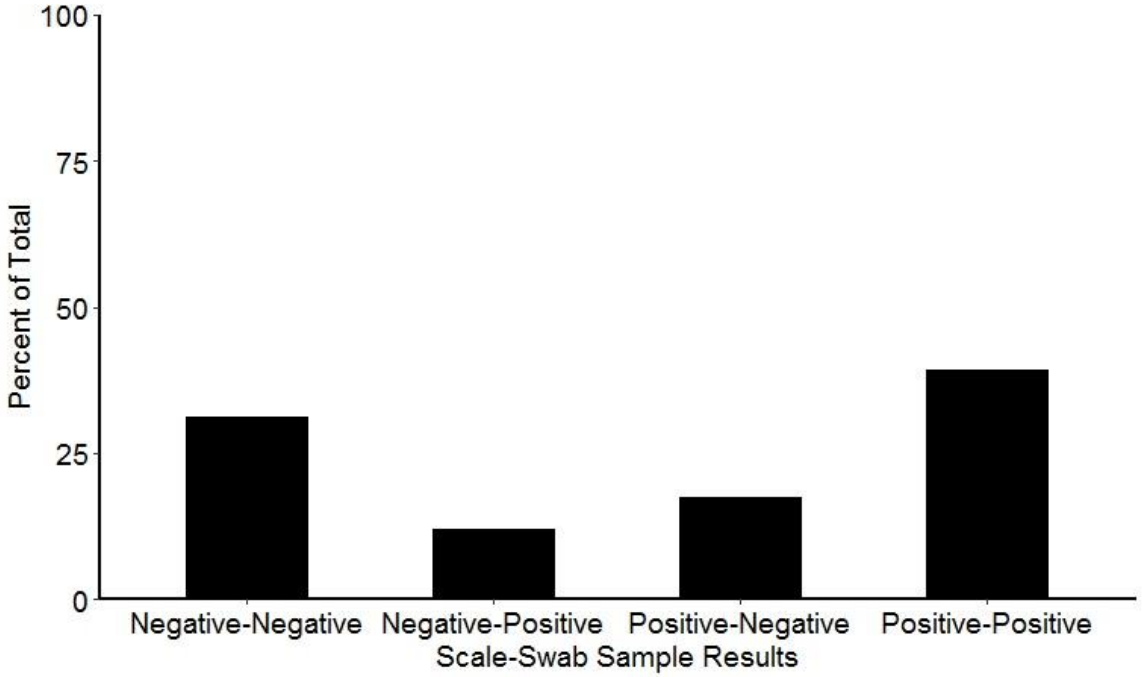

Figure 2.3 PCR Results for Scale and Swab Samples. Percentages of the total number of paired swab and scale clips in each combination: scale clip and swab negative, scale clip negative and swab positive, scale clip positive and swab negative, and scale clip and swab positive.

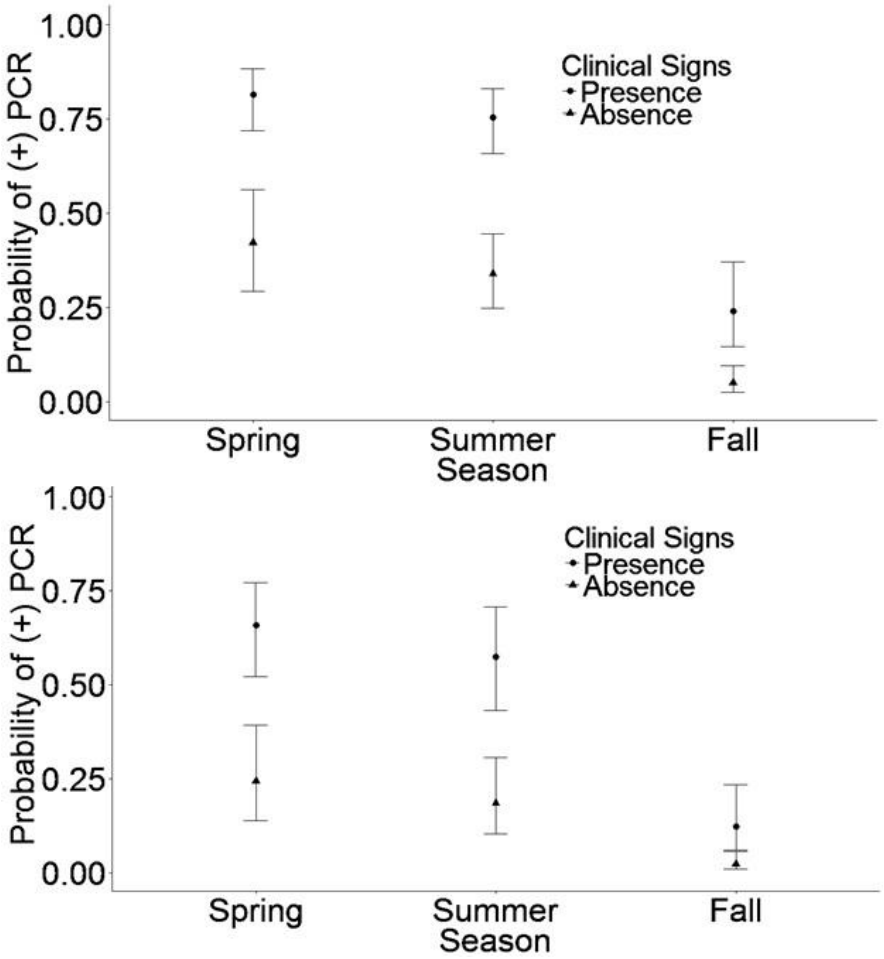

Figure 2.4 Disease Probabilities by Clinical Sign Presence/Absence, Season and Habitat Affiliation. Probability of a positive real-time PCR detection for *Ophidiomyces ophiodiicola* in aquatic snakes (top) and terrestrial snakes (bottom) with SFD clinical sign status.

CHAPTER 3: POPULATION IMPACTS

Introduction

Recently, pathogenic fungi are emerging as a significant threat to global biodiversity (Fisher et al. 2012). Studies of these emerging diseases often focus on describing spatial or temporal patterns of disease prevalence and infection rates (Cooch et al. 2012). However, the rate of disease transmission and the effectiveness of particular disease management strategies depend on an understanding of the behavior of the host species, disease-associated mortality rates, seasonal variation of pathogen spread and spatiotemporal patterns in disease prevalence across landscapes (Grassly and Fraser 2008, Cross et al. 2009, McCallum 2012). Assessing these complex disease dynamics in free-ranging wildlife populations is challenging because demographic data are typically collected from incomplete samples of individuals (Cooch et al. 2012), individual infection status may be incorrectly identified (McClintock et al. 2010, Miller et al. 2012), and susceptible species may emigrate from study sites, resulting in biased estimates of mortality rates (Faustino et al. 2004). Furthermore, whereas most quantitative research on disease dynamics has focused on animals that are easily observed or trapped (See Faustino et al. 2004; Lachish et al. 2007), many high-profile diseases infect species that are elusive or potentially difficult to sample (e.g., amphibians and chytridiomycosis, Berger et al. 1998).

Snake fungal disease (SFD) is an emerging threat to wild snake populations (Rajeev et al. 2009; Lorch et al. 2016). Snake fungal disease was first hypothesized as a causative factor resulting in declines in snake populations in 2006 (Clark et al. 2011), and afflicted snakes have since been documented throughout eastern North America and

recently in Europe (Lorch et al. 2016; Franklinos et al. 2017). The disease is caused by the fungus *Ophidiomyces ophiodiicola* and is characterized by severe skin infections (Lorch et al. 2015; Lorch et al. 2016; Hileman et al. 2017). In laboratory settings, severe infections alter host behavior, and lead to morbidity and mortality (Lorch et al. 2016). To date, 23 North American snake species have been reported to have the disease, and a recent analysis suggests that most snake species, regardless of ecological traits and phylogeny, are susceptible to SFD (Burbrink et al. 2017). The number of individuals exhibiting clinical signs varies both spatially and temporally, with infection rate often associated with hibernation and spring emergence in temperate climates and cooler temperatures where snakes do not hibernate (Lorch et al. 2016; Lind et al. 2018; McCoy et al. 2017). Due to its recent emergence, this disease has been described as among the most significant yet under-studied risks to global biodiversity (Sutherland et al. 2014). To date, the majority of research on SFD has focused on identifying the causative agent, documenting the geographic distribution and host range of SFD and identifying spatial and temporal dynamics of infection (Allender et al. 2015; Lorch et al. 2016).

Understanding the individual and population-level impacts of SFD in field settings has been challenging due to the secretive nature of snakes and the subsequent difficulty associated with collecting health monitoring data. Most snake species have notoriously low detection rates, preventing the collection of adequate samples to make inferences regrading population status or demographic rates (Steen et al. 2012). Thus, only a few studies have examined population-level effects of SFD. Tetzlaf et al. (2017) and Lind et al. (2018) found that Viperid snakes (i.e., *Sistrurus catenatus* and *S. miliarius*, respectively) with clinical signs of SFD exhibit altered behaviors, including

increased basking and reduced movements, which may influence survival. However, traditional CMR models are unable to estimate true survival when individuals permanently emigrate from the study population (Lebreton et al. 1992) and disease-associated mortality cannot be directly estimated since permanent emigration and disease-associated mortality are confounded. Thus, novel field techniques to monitor large numbers of individuals and appropriate analyses are needed to assess the population-level impacts of SFD on wild snake populations. Recent efforts by Connette and Semlitsch (2015) describe a method that uses detections of individuals marked with Passive Integrated Transponder tags (i.e., PIT tags; Connette and Semlitsch 2012; Oldham et al. 2016) to populate a multistate capture-mark-recapture model (Lebreton et al. 1992), which overcomes some of the problems encountered by traditional CMR methods (i.e. Cormack Jolly Seber) including distinguishing between two sources of loss within a population, mortality and permanent emigration, and therefore more accurately estimating true survival within a population.

Herein, I utilize a monitoring technique (i.e., PIT telemetry; Connette and Semlitsch 2012; Oldham et al. 2016) and multistate capture-mark-recapture models to examine the consequences of SFD on wild populations of Queensnakes (*Regina septemvittata*) and Northern Watersnakes (*Nerodia sipedon*) in central Kentucky streams. Specifically, I address the following question: does SFD affect the short-term survival, movement, and behavior of wild snakes? This study will be the first to yield estimates for key demographic (survival, emigration) and behavioral parameters (e.g., movement, surface activity) of snakes in wild populations with and without SFD.

Methods - Study Sites

My study was conducted at six streams from Madison, Fayette, Jessamine and Woodford Counties, in the inner Bluegrass Region of Kentucky (USA) (Table 3.1). This region is characterized by Karst topography, gently rolling hills, and mixed land-use, dominated by pasture, forest, and urban/suburban land cover. I sampled streams that ranged from first to fourth order; each stream was part of the Kentucky River basin and had ample rock cover with bedrock substrate. Surveyed stream reaches ranged from 293 to 1005 meters (Table 3.1). Stream water chemistry at my study sites was consistent with other streams in the region (i.e., conductivity values between 300-500 umohs/L) (Table 3.2). Average canopy cover was similar at all sites (between 78.6% and 95.0%), except Otter Creek, which had a more open canopy (Table 3.1).

Methods - Field Sampling

I conducted area-constrained searches of natural cover objects within the creek channel and banks at all field sites for *R. septemvittata* and *N. sipedon* between April and early June 2016 to capture and mark snakes with PIT tags prior to beginning PIT telemetry surveys. Snakes were transported back to the lab in individual snake bags or containers where they were photographed, weighed, measured (snout-vent length and total length), sexed and visually assessed for clinical signs of SFD (presence/absence). Clinical signs were considered "present" if dermal lesions were observed. If clinical signs were present, a sterile polyester tipped swab was first dipped in sterile water (Fisher Scientific, BP2484-100) and then brushed across the lesion five times. If clinical were absent, snakes were swabbed five times on the dorsal midline, over a small section of

scales. Swabs were placed into plastic vials and stored in a -40°C freezer. Snakes were subcutaneously marked with passive integrated transponder (PIT) tags, using the Biomark MK10 implanter with 134.2 kHz, 12.5 mm PIT tag (Figure 3.1), along the posterior third of the venter above the cloaca (Oldham et al. 2016). The injection site was sanitized with iodine or a 10% hydrogen peroxide solution. After processing, snakes were kept in individual enclosures before being returned to their original location within 48 hours of capture. To reduce cross contamination between snakes, gloves were worn, and all equipment was sanitized with a 10% bleach solution.

I conducted sixteen PIT telemetry surveys at each study site between early June and October of 2016 to detect marked snakes and collect encounter data for capture-mark-recapture analyses. Passive Integrated Transponder telemetry surveys consisted of one person holding the Biomark HPR Plus portable PIT tag reader and the Biomark BP Portable Antenna Plus and sweeping the antenna over creek banks, and any exposed rocks (Oldham et al. 2016). The antenna is capable of detecting PIT tags up to 43.2 cm away for the type of tags used. When a PIT tag was detected, I attempted locating the snake to visually confirm its presence. If a snake was not located, it was recorded as a non-visual detection. Recaptured snakes were photographed, weighed, measured, clinical signs recorded, and the snake was swabbed for *O. ophiodiicola.* Subsequent measuring occurred in the field only if the snake had not been captured within the past 30 days. Otherwise, the snake was confirmed alive and immediately released. On some occasions snakes were seen, but I were unable to recapture them. If unmarked snakes were opportunistically discovered, they were implanted with a PIT tag and processed (see

above). Surveys occurred every 7 to 10 days. I recorded time, air temperature, wind speed, day of last rain, and cloud cover at the beginning and end of each survey.

Methods - Laboratory Assessment/Determining Disease Status

I used two methods, in conjunction, to determine disease status for each snake. First, I reviewed photos of snakes for the presence or absence of specific clinical signs. These clinical signs were separated into two categories, indicative of SFD and non-indicative of SFD. Clinical signs that were indicative of SFD included: regional edema, local (scale) edema, crust with *stratum corneum*, crust without *stratum corneum*, nodule, and ulcer. Clinical signs that were not indicative of SFD included: healed, discoloration, non-SFD lesion (i.e., skin damage that did not appear infected, like a wound, puncture or scrape; see Appendix for definitions of clinical signs). Second, I used real-time PCR to determine the presence *O. ophidiocola* according to protocols in Bohuski et al. (2015). If a snake had less than 116 copies of fungal DNA, and no clinical signs indicative of SFD, I defined the snake as SFD negative (J. Lorch pers. comm.). If a snake had greater than 116 copies of fungal DNA, and at least one clinical sign that was indicative of SFD, I considered the snake to be SFD positive. Finally, snakes with less than 116 copies of fungal DNA, and at least two clinical signs indicative of SFD were also considered positive for SFD.

All research was compliant with University of Kentucky IACUC protocol (2013-1073). Permits were obtained from the Kentucky Department of Fish and Wildlife Resources (SC1511017, SC1611043, SC1611136).

Statistical Methods

I used a multistate capture-recapture model to estimate the probability that individuals transition from "alive", "dead", "temporarily emigrated" or "permanently emigrated" states by utilizing visual resighting and PIT tag detections (Connette and Semlitsch, 2015; Figure 3.3). Each individual was recorded as either not detected (0), detected with the PIT tag antenna only (1), or visually detected (2). During a survey, a snake can be: alive and present in the survey area, alive and dead within the survey area, alive and absent from the survey area, dead and absent from the survey area, or permanently emigrated. Snakes can transition between states until they reach an "absorbing state" (i.e. dead or permanently emigrated from the study area) (Connette and Semlitsch 2015). The populations of snakes at these study sites were open to mortality, permanent emigration and temporary emigration between surveys. Permanent emigration is when a snake leaves the study area (outside of the survey reach, or out of range of the PIT-tag reader) and is not detected again. Temporary emigration occurs when a snake leaves the study reach or is out of range of the PIT-tag reader but returns and is detected again. The model assumes individuals can be visually encountered when they are alive and present within the study area and that PIT-tag detections are possible for those individuals, as well as those dead within the study area. Survey data were summarized in an encounter matrix, where an individual was (1) detected with the PIT tag reader only, (2) visually resighted, and (3) not detected during the survey (Connette and Semlitsch 2015). I used a state-space formulation (Kéry and Schaub 2012), where the true state of an individual at time $t + 1$ is conditional on the state of the individual at the previous survey.

To examine the effect of SFD on monthly survival, permanent emigration, visual detection, temporary emigration, and temporary immigration, I incorporated the following covariates into my model: species (*R. spetemvittata, N. sipedon*) and disease status (SFD, or no SFD). Disease status was treated as constant, and if a snake had SFD at one occasion during the sampling season, I assumed it had SFD for the duration of the project. I considered cloud cover and temperature as covariates for visual detection because I believe these factors influence basking behavior of snakes within the site (Sun et al. 2001). In other words, on warmer and less cloudier days snakes are more likely to be surface active than on cooler, cloudier days. Day of year was used as a covariate for temporary emigration because snakes may have seasonal movement patterns and may prefer to occupy a specific section of a stream at a specific time of year (Mushinsky et al. 1980).

I used the program R version 3.4.0 (R Development Core Team 2017), and the package jagsUI 1.4.4 (Kellner 2016) to access JAGS 4.2.0 (Plummer 2003). I assigned uninformative uniform priors (0,1) to the parameters for survival, permanent emigration, and PIT-tag detection. I assigned uniform priors (-5,5) to the parameters for visual detection, temporary emigration, and temporary immigration. I assigned uniform priors (-10, 10) to the covariates for visual detection (cloud cover, temperature), and temporary emigration (day of year). This model ran three parallel Markov chains comprised of 800,000 MCMC iterations, a thinning rate of 50, and an initial burn-in of 400,000 which yielded 48,000 samples from the joint posterior. Convergence was and evaluated using the Gelman-Rubin statistic ($\hat{r}$, Gelman et al. 2004) for which convergence occurs when $\hat{r} < 1.01$. This model had successful convergence of all parameters.

Results

During 2016, I marked and processed 525 individuals across my six study sites, with most individuals being marked in April-early June. I marked 232 *R. septemvittata* and 293 *N. sipedon.* I considered 98/232 (42.24%) of *R. septemvittata* and 56/293 (19.11%) of *N. sipedon* with SFD based on clinical signs and results of PCR test. Through my 16 PIT telemetry surveys at each site, I recorded 1330 PIT-tag detections. I classified 902 of these detections as confirmed alive if I capture the snake in hand, or visually identified it (Table 3.3). My recapture rates, if only visual detections are included (i.e. snake confirmed alive), for *R. septemvittata* was 45.7% (102/232) and for *N. sipedon*, was 35.5% (104/293).

Within-season monthly survival was high (0.99, 95% CRI: 0.96-1.00) for both snake species and I found no difference between diseased and non-diseased snakes (Table 3.5, Figure 3.3). However, I found that disease status affected behavior and movement (Table 3.5, Figure 3.3). Specifically, permanent emigration was lower in *R. septemvittata* with SFD (0.07, 95% CRI: 0.01-0.14) compared to non-diseased *R. septemvittata* (0.23, 95% CRI: 0.16-0.30). Furthermore, in *R. septemvittata* with SFD, temporary immigration was lower (0.54, 95% CRI: 0.52-0.57) than the estimate for *R. septemvittata* without SFD (0.58, 95% CRI: 0.55-0.62). I found that *N. sipedon* with SFD, had reduced temporary immigration rates (0.55, 95% CRI: 0.52-0.59) compared to *N. sipedon* without SFD (0.58, 95% CRI: 0.55-0.61). Temporary emigration probabilities for *R. septemvittata* and *N. sipedon* did not differ between diseased and non-diseased snakes (Table 3.5, Figure

3.3). However, the probability of a snake temporarily emigrating increased as day of year increased (Figure 3.4).

I found that the estimated probability of detecting an individual marked snake within range of the PIT tag reader, regardless of species or disease status was 0.43 (CRI: 0.33-0.55) and was positively associated with increasing temperature and negatively associated with increasing cloud cover for all snakes (Figure 3.5, 3.6). Furthermore, I found that disease state affected detection probabilities (Figure 3.5). The mean probability for visual detection was higher for diseased snakes (*R. septemvittata*: 0.61, 95% CRI: 0.54-0.68; *N. sipedon*: 0.52, 95% CRI: 0.40-0.64) than non-diseased snakes (*R. septemvittata*: 0.42, 95% CRI: 0.35-0.49; *N. sipedon*: 0.44, 95% CRI: 0.39-0.50), indicating greater degree of surface activity.

Discussion

Using a multi-state capture-mark-recapture model and PIT telemetry, I examined the population-level impacts of SFD by addressing the question: does SFD affect the short-term survival, movement, and behavior of wild snakes? I found no evidence that SFD impacted short-term survival in either of the species examined. Yet, I found an increased probability of visual detection for SFD positive snakes, indicating the SFD may alter behavior. Additionally, lower rates of temporary and permanent emigration and temporary immigration indicate the SFD may also impact movements.

I found high monthly survival over four and a half months of field sampling with monthly survival estimates approaching 0.99 for both species. My results differ from other studies that allude to decreased short-term survival of snakes afflicted with SFD

(Allender et al. 2011, Tetzlaff et al. 2017). Individual mortality has been documented and appears more commonly documented than population declines (Tetzlaff et al. 2017, Allender et al. 2011, Lorch et al. 2015**).** Snakes infected with *O. ophiodiicola* often have severe infections of the head, mouth and eyes that may directly limit the procurement of prey, that may result in death if the infection cannot be cleared, and normal feeding behaviors resume (Lorch et al. 2016). Indeed, in both field and lab settings infected snakes have shown either emaciation, or refusal to eat when presented with food (Lorch et al. 2015, Lorch et al. 2016, McCoy, Lind and Farrell 2017). Additionally, McCoy, Lind and Farrell (2017) documented a negative relationship between the severity of clinical signs and body condition index. Although the snakes that I captured and assessed had clinical signs on the head, mouth and eyes, the severity and extent of these clinical signs was less severe than those documented in previous studies (Allender et al. 2011, McCoy, Lind and Farrell 2017). Therefore, the inability to procure food, likely did not impact the snakes in this study in the short-term.

A possible explanation for the high rates of visual detection in snakes with SFD may be that diseased snakes increase basking to overcome infection (Burns et al. 1996). A previous laboratory study noted that *Patherophis guttatus* infected with *O. ophiodiicola* spent more time in conspicuous areas of their enclosures, despite having access to a shelter and being kept in stable environmental conditions (Lorch et al. 2015). In field settings, increased basking may result in "risky" behaviors including emerging early from hibernation to bask and subsequently dying from exposure. Furthermore, increased basking makes snakes more vulnerable to predators, as snakes are spending more time in conspicuous places (Lorch et al. 2016). If initial body temperatures are low

while a snake is engaging in "risky" basking behavior, its ability to escape predators could be impaired as there is a strong relationship between body temperature and flight distance (Layne and Ford 1984). However, in my study populations it appears that this increased surface activity does not influence short-term survival. This could be because of the proximity of available cover objects, that were abundant at all study sites. Additionally, consistently warm temperatures (Table 3.4) experienced during my study period (June-October) would allow snakes to maintain higher initial body temperatures, suggesting little impact to their ability to escape predators while basking.

More importantly, increased surface activity of infected individuals complicates estimating the proportion of diseased snakes within a population. Since diseased individuals are more surface-active, they may be over-represented in surface counts which could lead to an over-estimation of diseased individuals within a population. Surveys of snake populations should account for this behavioral change to yield appropriate estimates of disease rates in a population.

Furthermore, snakes afflicted with SFD showed reduced movement (permanent emigration, temporary immigration). These results are consistent with a study on 25 *Sistrurus catenatus* in Michigan that found individuals with *O. ophiodiicola* or with clinical signs of SFD moved less frequently than individuals deemed uninfected (Tetzlaff et al. 2017). Overall, reductions in movement could have consequences for behaviors such as foraging, mate finding, and dispersal. Snakes with SFD are often emaciated which could be the result of reduced foraging (Lorch et al. 2015; Lorch et al. 2016; Tetzlaff et al. 2017; McCoy, Lind and Farrell 2017). Additionally, decreased activity in response to infection has been documented in other reptiles, such as reduced activity in

Sceloporus occidentalis infected with malaria (Dunlap and Church 1996). Reduced movement in diseased snakes which would further overestimate surface counts of diseased individuals in a population, as diseased individuals are less likely to leave the study area.

Long-term consequences on survival and population persistence cannot be addressed by my data, which are limited to one active season (4.5 months). Despite documented population declines caused by *O. ophiodiicola* in timber rattlesnakes and the Lake Erie watersnake (Clark et al. 2011; Lorch et al. 2016), it is possible that these declines may not manifest until snakes emerge from overwintering or that these populations had specific demographic traits that make them especially vulnerable to a fungal outbreak (small population size, inbreeding depression, loss of habitat). Previous SFD infections can reoccur in individuals emerging from hibernation and physiological changes induced by hibernation may make snakes more vulnerable to infection (Lorch et al. 2016). The need to thermoregulate can drive snakes to emerge early from hibernation and succumb to low overnight temperatures (Lorch et al. 2016). Long-term studies are underway to examine changes in densities and long-term survival within these populations (J. Lorch pers. comm.).

By utilizing PIT tags to mark individuals and focusing my sampling efforts on two species of relatively common snakes in Kentucky (*R. septemvittata*, and *N. sipedon*) I was able to procure a large sample size and enhance detection probabilities. I had higher detection probabilities than a previous capture-mark-recapture study on *R. septemvittata*, which reported recapture rates of only 18.6% (13/70) of marked snakes (Branson and Baker 1974). My recapture rates for marked *R. septemvittata* was 45.7% (102/232), and

35.5% (104/293) of marked *N. sipedon*. In comparison, recapture rates for *Thamnophis atratus,* a snake often associated with streams, similar to *N. sipedon,* annual recapture rates ranged from 13 to 32% (Lind et al. 2005). By using PIT-tags and PIT telemetry I were able to recapture more individuals than if I were using traditional methods. Furthermore, traditional CMR methods can underestimate true survival because it is not possible to differentiate between emigration and mortality, my use of PIT telemetry allows us to use auxiliary data (when a snake is detected with the PIT tag reader, but not detected visually) to distinguish between these two sources of loss within a population. Without distinguishing between these two sources of loss, it cannot be determined if a disease is responsible for population declines, or if another factor exists within the population that is driving permanent emigration from the study site. Since snake populations are traditionally touted as challenging to study, I present methodologies that substantially improves data collection for these secretive species (Durso, Wilson and Winne 2011). With SFD considered a threat to snake populations, and a current lack of population-level studies, I feel these methods will be incredibly useful for exploring the effect SFD has on snake populations.

My results indicate that in free-ranging snakes, behavioral consequences due to SFD are occurring at the population-level. Research on SFD thus far has focused on documenting fungal characteristics, geographic range of *O. ophiodiicola* and individual consequences of infection. I suggested increased monitoring of snake populations over multiple years to understand seasonal patterns that could be driving infection dynamics (Lind et al. 2018; McCoy et al. 2017). The fungus that causes SFD has a broad host range and can persist in the environment, although environmental traits that facilitate

persistence are not known at this time (Lorch et al. 2016). Understanding the role environmental temperature, and rainfall play in disease dynamics would allow us to locate geographically vulnerable snake populations. Additionally, characterizing demographics (small population, isolated, increased conspecific interactions) that might make snakes more vulnerable to contracting or spreading SFD would allow the allocating of resources of protect these populations.

Table 3.1 Descriptive Characteristics of Study Site. County, stream order, geographic coordinates, survey length and canopy cover for study sites.

Study Site	County	Stream Order	Geographic Coordinates (Latitude, Longitude)	Survey Length (meters)	Canopy Cover*
Little Hickman Creek	Jessamine	1	37.774879, -84.566452	755	14.29 ± 9.68
Tates Creek	Madison	2	37.76375, -84.35667	510	13.67 ± 4.78
Otter Creek	Madison	2	37.84716, -84.24734	470	6.90 ± 7.31
Glenns Creek	Woodford	3	38.13435, -84.82336	293	11.79 ± 6.69
Elias and Hickman Creek	Fayette	1, 4	37.954559, -84.510384	1005	12.45 ± 8.80
Elkhorn Creek	Fayette	1	38.03981, -84.42525	399	14.06 ± 4.94

*Average number of points obscured on a densiometer.

Table 3.2 Water Chemistry. Average water chemistry with standard deviations for all field sites.

Site	Cond (umohs/L)	TOC (mg/L C)	PO4 (mg/L)	pH (H+)	Mn (mg/L)	Cl (mg/L)	SO4 (mg/L)	NO3-N (mg/L)	NH4-N (mg/L)	Ca (mg/L)
Elias and Hickman Creek	532.63 ± 131.37	12.08 ± 6.48	2.55 ± 4.08	6.85 ± 0.20	0.04 ± 0.04	1.07 ± 0.52	31.75 ± 30.16	0.44 ± 0.27	0.04 ± 0.06	54.01 ± 11.92
Elkhorn Creek	493.00 ± 154.47	10.83 ± 9.94	4.34 ± 3.71	6.69 ± 0.11	0.02 ± 0.02	1.04 ± 0.35	47.38 ± 76.77	0.23 ± 0.26	0.05 ± 0.08	50.64 ± 8.33
Glenns Creek	519.25 ± 101.15	14.12 ± 8.43	0.74 ± 0.54	7.5 ± 0.07	0.02 ± 0.02	1.07 ± 0.32	22.66 ± 24.42	1.81 ± 0.47	0.005 ± 0.01	57.26 ± 11.12
Otter Creek	316.75 ± 55.06	13.79 ± 7.29	5.36 ± 4.63	7.25 ± 0.10	0.07 ± 0.10	0.94 ± 0.35	101.82 ± 109.83	0.02 ± 0.02	0.03 ± 0.07	31.85 ± 9.94
Little Hickman Creek	340.25 ± 40.41	12.16 ± 7.41	6.92 ± 8.63	6.97 ± 0.10	0.03 ± 0.02	0.88 ± 0.31	75.33 ± 25.36	0.30 ± 0.27	0.02 ± 0.03	45.44 ± 5.84
Tates Creek	450.50 ± 51.32	10.95 ± 6.61	0.02 ± 0.04	7.20 ± 0.12	0.02 ± 0.02	1.15 ± 0.53	58.00 ± 21.04	0.16 ± 0.13	0.005 ± 0.01	50.57 ± 11.08

Table 3.2 (continued)

Site	Mg (mg/L)	K (mg/L)	Na (mg/L)	NO2-N (mg/L)
Elias and Hickman Creek	5.91 ± 2.71	2.58 ± 0.53	13.35 ± 3.89	0.08 ± 0.18
Elkhorn Creek	6.33 ± 2.83	3.13 ± 0.89	12.49 ± 1.65	0.11 ± 0.14
Glenns Creek	5.53 ± 2.68	5.1 ± 1.04	16.79 ± 1.02	0.08 ± 0.06
Otter Creek	10.65 ± 0.69	3.88 ± 0.32	4.12 ± 0.42	0.06 ± 0.04
Little Hickman Creek	4.92 ± 1.93	3.67 ± 0.45	3.68 ± 0.72	0.08 ± 0.06
Tates Creek	9.77 ± 0.80	3.9 ± 0.47	12.63 ± 1.69	0.10 ± 0.11

Table 3.3 Frequency of PIT-tag Detections by Type of Detection, Disease Status and Species. Number of snakes, by species, marked, detected, detected and visually confirmed alive, PIT-tag only detections, with SFD, and without SFD in central Kentucky, USA 2016.

Species	Number Marked	Number of Detections	Total Visual Detections	Total PIT-tag	With SFD	Without SFD
Regina septemvittata	232	645	438	207	98	127
Nerodia sipedon	293	685	464	221	56	226
Total	525	1330	902	428	154	353

Table 3.4 Air Temperature, Windspeed, Water Temperature, Day Since Last Rain and Cloud Cover for All Surveys. Averages and standard deviations for covariates (air temperature, windspeed, water temperature, days since last rain, and cloud cover) recorded at the start and end of PIT-tag telemetry surveys, when specified.

Site	Starting Air Temperature (°C)	Ending Air Temperature (°C)	Starting Windspeed (mph)	Ending Windspeed (mph)	Water Temperature (°C)	Days since Last Rain	Cloud Cover (eighths)
Elias and Hickman Creek	23.78 ± 1.54	26.14 ± 1.99	0.56 ± 0.90	0.94 ± 0.77	22.52 ± 2.44	2.24 ± 2.66	3 ± 2.97
Elkhorn Creek	24.66 ± 1.91	26.95 ± 2.83	0.69 ± 0.62	1.39 ± 1.13	21.55 ± 2.12	1.88 ± 1.80	1.76 ± 2.28
Glenns Creek	24.88 ± 2.63	27.21 ± 2.95	0.36 ± 0.48	0.55 ± 0.69	22.45 ± 1.88	4.19 ± 5.32	2.56 ± 2.18
Otter Creek	23.95 ± 2.38	27.62 ± 3.20	0.44 ± 0.46	1.08 ± 0.98	24.35 ± 2.95	1.94 ± 1.43	4 ± 3.12
Little Hickman Creek	23.25 ± 2.25	26.83 ± 2.56	0.72 ± 0.56	0.59 ± 0.66	21.13 ± 2.16	1.89 ± 1.64	3.00 ± 3.33
Tates Creek	23.84 ± 2.35	27.34 ± 3.22	0.70 ± 0.70	0.39 ± 0.58	22.03 ± 3.06	2.75 ± 2.21	2.94 ± 2.91

Table 3.5 Model Probability Rates. Model probability rates (posterior mean ± 1 SD) for monthly survival, permanent emigration, visual detection, temporary emigration and temporary immigration for both species, with and without SFD.

	Regina septemvittata		*Nerodia sipedon*	
Diseased	-	+	-	+
Monthly Survival	0.99 ± 0.01	0.99 ± 0.01	0.99 ± 0.01	0.99 ± 0.01
Permanent Emigration	0.23 ± 0.03	0.07 ± 0.03	0.25 ± 0.03	0.21 ± 0.06
Visual Detection	0.42 ± 0.04	0.61 ± 0.04	0.45 ± 0.03	0.52 ± 0.06
Temporary Emigration	0.44 ± 0.10	0.40 ± 0.08	0.51 ± 0.09	0.51 ± 0.09
Temporary Immigration	0.58 ± 0.02	0.54 ± 0.01	0.58 ± 0.01	0.55 ± 0.02

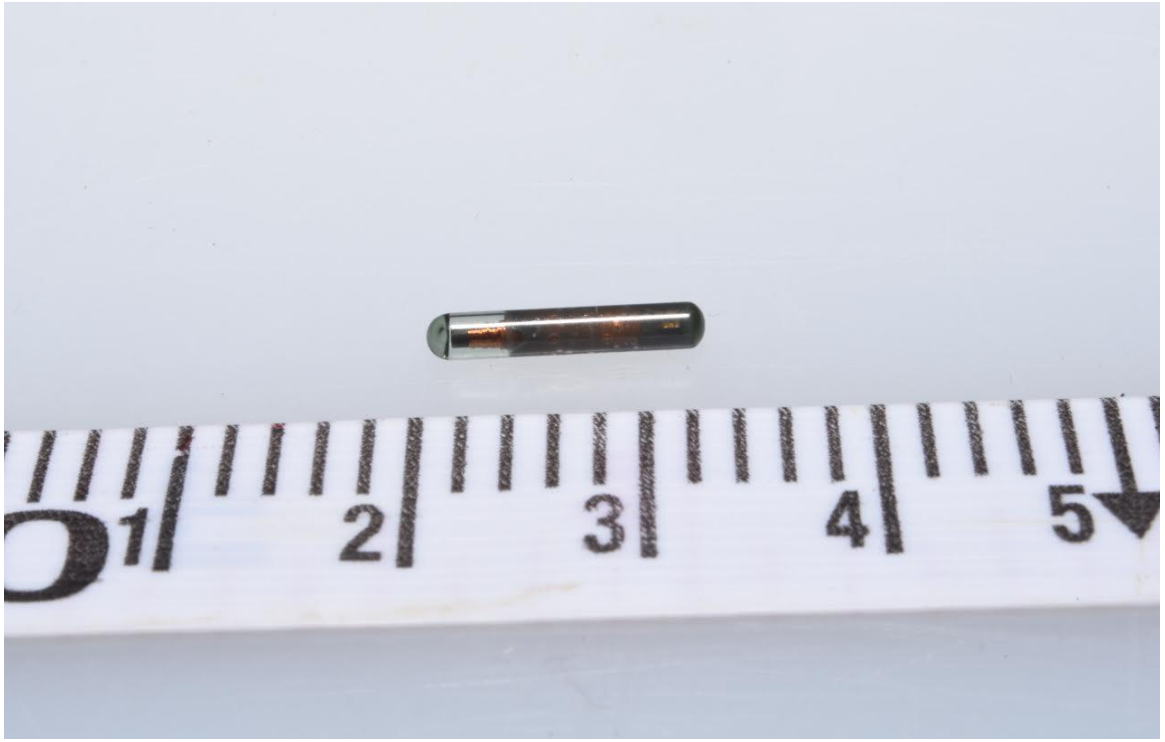

Figure 3.1 Passive Integrated Transponder (PIT) Tag. An 134.2 kHz 12.5 mm passive integrated transponder (PIT) tag used to subcutaneously mark snakes.

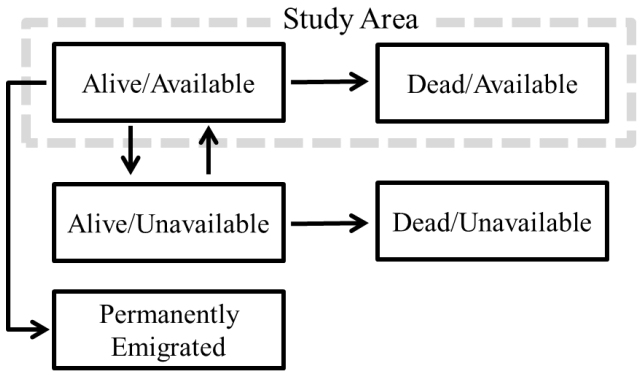

Figure 3.2 Conceptual Diagram of Multi-State Model. Conceptual diagram of model for estimating emigration, survival, and availability for PIT-tag detection

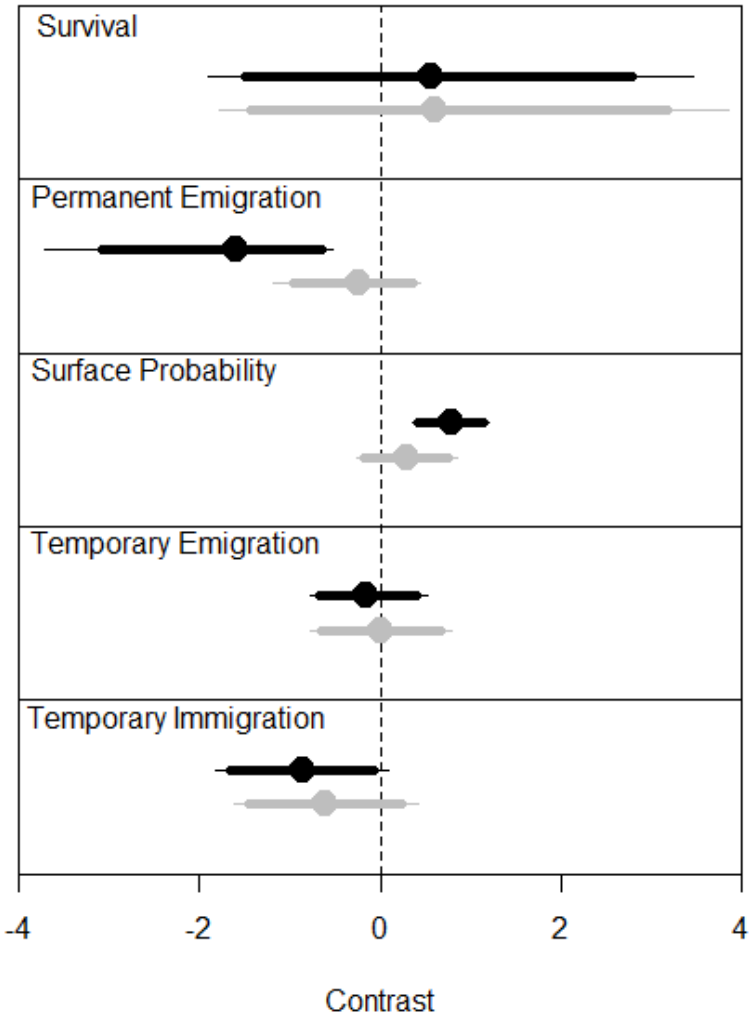

Figure 3.3 Estimated Difference in Parameters Estimates for Diseased and Non-Diseased Snakes. Estimated difference in parameter estimates for diseased and non-diseased snakes (logit-scale). The black bars represent *R. septemvittata* and the gray bars represent *N. sipedon*. Points indicate posterior means, thin bars indicate 90% credible intervals, and thick bars indicate 95% credible intervals.

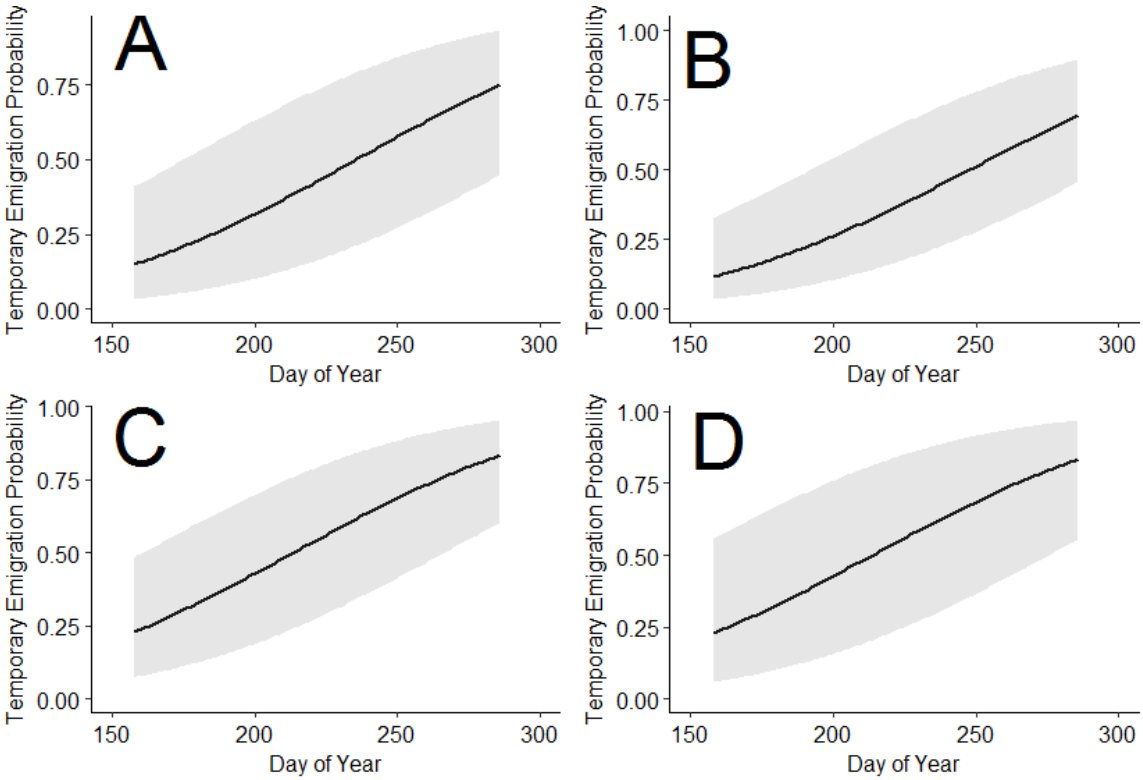

Figure 3.4 Predicted Relationship Between Temporary Emigration and Day of Year. The predicted relationship and 95% credible interval between temporary emigration probability and day of year for (A) *R. septemvittata* without SFD, (B) *R. septemvittata* with SFD, (C) *N. sipedon* without SFD, and (D) *N. sipedon* with SFD. For all snakes, temporary emigration probability increasing as day of year increases.

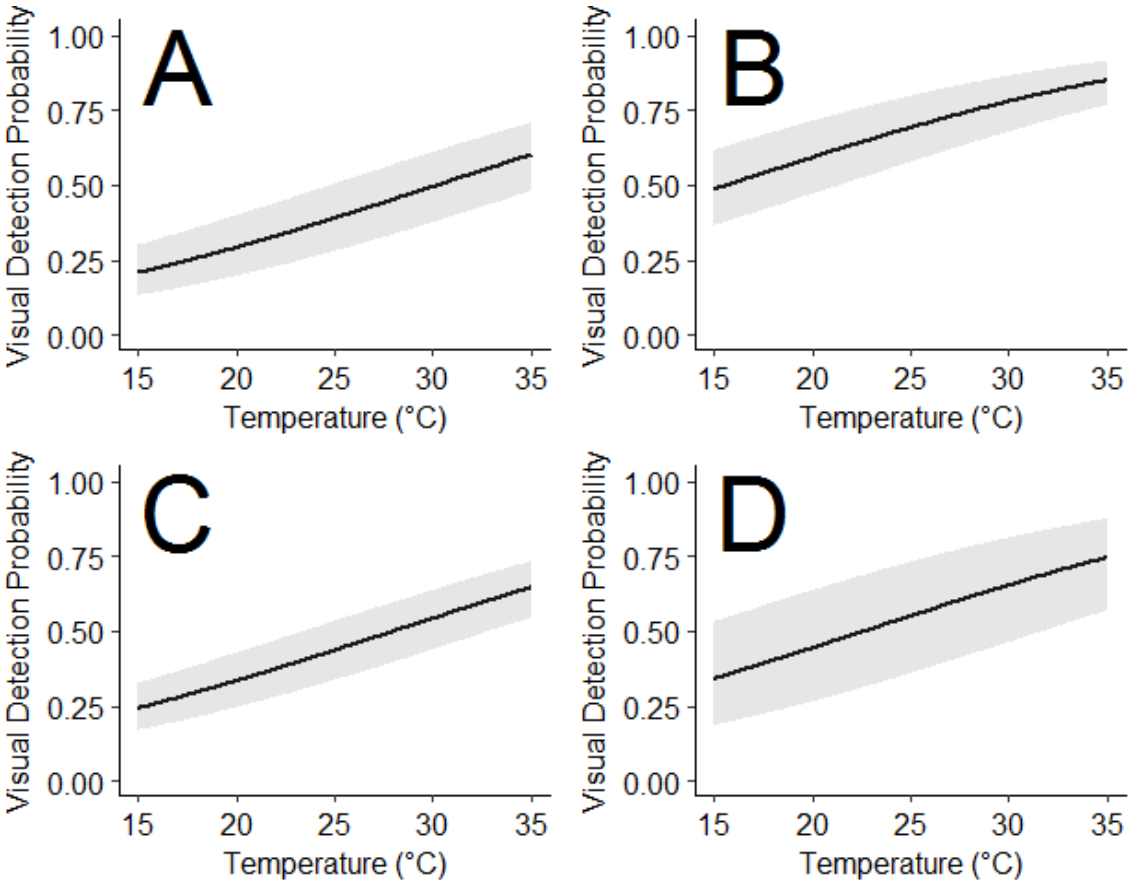

Figure 3.5 Predicted Relationship Between Visual Detection and Temperature. The predicted relationship and 95% credible interval between visual detection probability and temperature for (A) *R. septemvittata* without SFD, (B) *R. septemvittata* with SFD, (C) *N. sipedon* without SFD, and (D) *N. sipedon* with SFD. For all snakes, visual detection probability is positively correlated with increasing temperature.

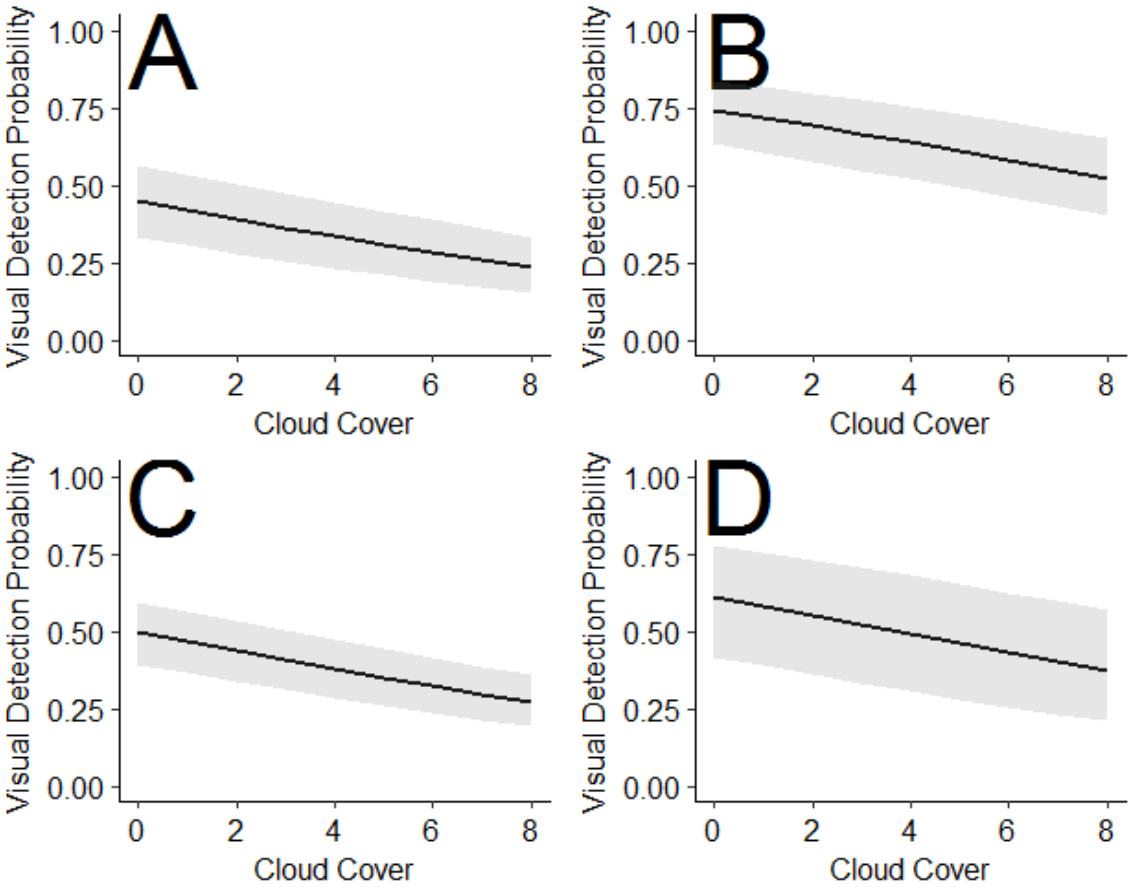

Figure 3.6 Predicted Relationship Between Visual Detection and Cloud Cover. The predicted relationship and 95% credible interval between visual detection probability and cloud cover for (A) *R. septemvittata* negative for SFD, (B) *R. septemvittata* positive for SFD, (C) *N. sipedon* negative for SFD, and (D) *N. sipedon* positive for SFD. For all snakes, visual detection probability is negatively associated with increasing cloud cover.

APPENDICES

Appendix A: Description of Specific Clinical Signs

In Chapter 3, photographs of captured snakes were reviewed to classify any clinical sign observed into specific categories. Certain clinical signs (regional edema, local [scale] edema, crust with *stratum corneum*, crust without *stratum corneum*, nodule and ulcer) were considered indicative of SFD. The following is a detailed description with examples of each clinical sign category used.

Regional edema involves generalized swelling of an entire area of the body and is among the first clinical signs to occur during initial infection by *O. ophiodiicola* (Figure A.1). Regional edema typically lasts briefly but can persist if infection is severe. Scale damage has likely not yet occurred during this stage of infection. *Ophidiomyces ophiodiicola* is under the outer layer of skin at this stage of infection. Local (scale) edema is occurs when immune cells move to the exact scales that are infected (Figure A.2). This clinical sign manifests as swelling and discoloration (whitening or yellowing) of infected scales. Scales can become inflated with fluid and resemble blisters. This can last for several days and can be present at the edge of advanced lesions. At this stage of infection, *O. ophiodiicola* is present under the skin. Crust with stratum corneum occurs when immune cells respond to the site of infection and release reactive oxygen compounds to kill the fungus (Figure A.3). However, this kills the snakes' own skin cells in the process. When these cells die, the skin becomes necrotic and turns yellowish-brown. While not technically crusts, they become thickened and hardened. When crusts first start to form the stratum corneum is still intact and covers the crust which may make the crust look smooth and

shiny. At this stage, *O. ophiodiicola* begins rapidly producing spores underneath the stratum corneum. Crust without *stratum corneum* occur when the *stratum corneum* detaches from the lesion, exposing the underlying crust before the snake can molt (Figure A.4). When the stratum corneum detaches, the crust appears dull with a roughened surface. This clinical sign can be common on ventral scales where the snakes' movement against the substrate facilitates removal of the stratum corneum. Without the stratum corneum, *O. ophiodiicola* and its spores are present in greater abundance on the surface of the skin. A snake at this stage of infection may molt and rid themselves of the infection, or restart the infection process. Ulcers occur when the epidermis is removed and the underlying layers of skin are exposed to show the presence of blood or pink skin (Figure A.5). Ulcers can occur when a crust detaches during a molt, or due to mechanical removal. Unless part of the crust is still attached to the skin, it can be very difficult to determine when if an ulcer is the result of SFD, or some other injury or infection. Ulcers can facilitate infection by providing an invasion site for *O. ophiodiicola.* Edema or crusts can be present surrounding the ulcer. Ulcers can take a long time to heal and may present as areas of the skin lacking scales. Healed scales occur when a snake with SFD, or some other form of skin damage molts, and the new scales or skin appear to be abnormal (Figure A.6). Several molt cycles are often needed for the skin to regain its typical appearance. Snakes that have had SFD but subsequently shed, will often have scales that appear small, wrinkled, or with irregular edges. It can take several days or weeks for a recurring *O. ophiodiicola* infection to begin showing clinical signs again after a molt. Snakes that had SFD and very recently shed may have residual *O. ophiodiicola* on the surface of their new skin, even though they may never re-develop the disease. A nodule occurs when immune cells form a granuloma around a

pathogen in living tissue (Figure A.7). In SFD granulomas will only form if *O. ophiodiicola* penetrates the dermis. Nodules may be covered by crusted skin, or they may be covered by healthy-looking skin. The granuloma will remain for a long time, even after the skin appears healed. Nodules are non-specific and can result from bacterial, fungal or parasitic infections. Sometimes crusts can become so thickened that they form a mass that resembles a nodule, but true nodules occur deeper in the skin. Snakes sometimes have abnormally-colored scales (discoloration) without signs of edema or crusting (Figure A.8). These snakes likely do not have skin infections, unless they have other types of lesions present. Non-SFD lesions include skin damaged that appeared to be mechanical (i.e., crushing wounds, punctures, scrapes) (Figure A.9). Dysecdysis covers any type of abnormal shedding, including skin flaking off in pieces, rather than molting off in one piece, or old skin sticking to the new skin after a molt (Figure A.10). While this is common with SFD, it can be caused by other issues as well.

Figure A.1. Regional edema caused by *O. ophiodiicola* near the head of captured *R. septemvittata* (left) and *N. sipedon* (right).

Figure A.2. Local (scale) edema on three locations (dorsal, ventral and head) on different *R. septemvittata*.

Figure A.3. Two *N. sipedon* (left and right) and one *R. septemvittata* (center) with instances of crusts with the *stratum corneum* intact.

Figure A.4. Crusts without the *stratum corneum* on two *R. septemvittata* (left, center) and one *N. sipedon* (right).

Figure A.5. Ulcers found on one *N. sipedon* (left) and two *R. septemvittata* (center, right). They are characterized by exposed pink skin.

Figure A.6. Healed scales are in one *N. sipedon* (left) and two *R. septemvittata* (center, right). These scales often appear to be irregularly shaped or abnormal.

Figure A.7. A *R. septemvittata* with multiple nodules along its body. Nodules may be covered by healthy-looking or by crusty skin.

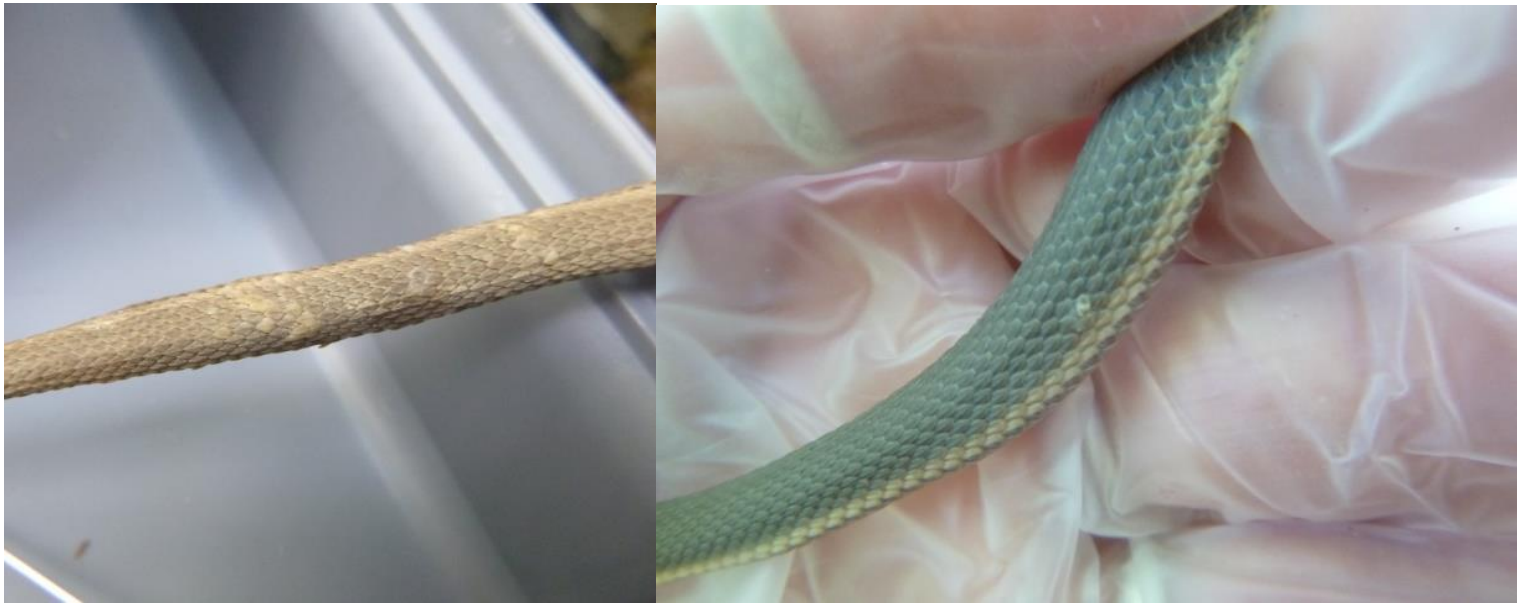

Figure A.8. Discoloration in two *R. septemvittata.* Discoloration is not always an indicator of *O. ophiodiicola.*

Figure A.9. Three *R. septemvittata* are depicted with non-SFD lesions, which includes any mechanical-looking skin damage.

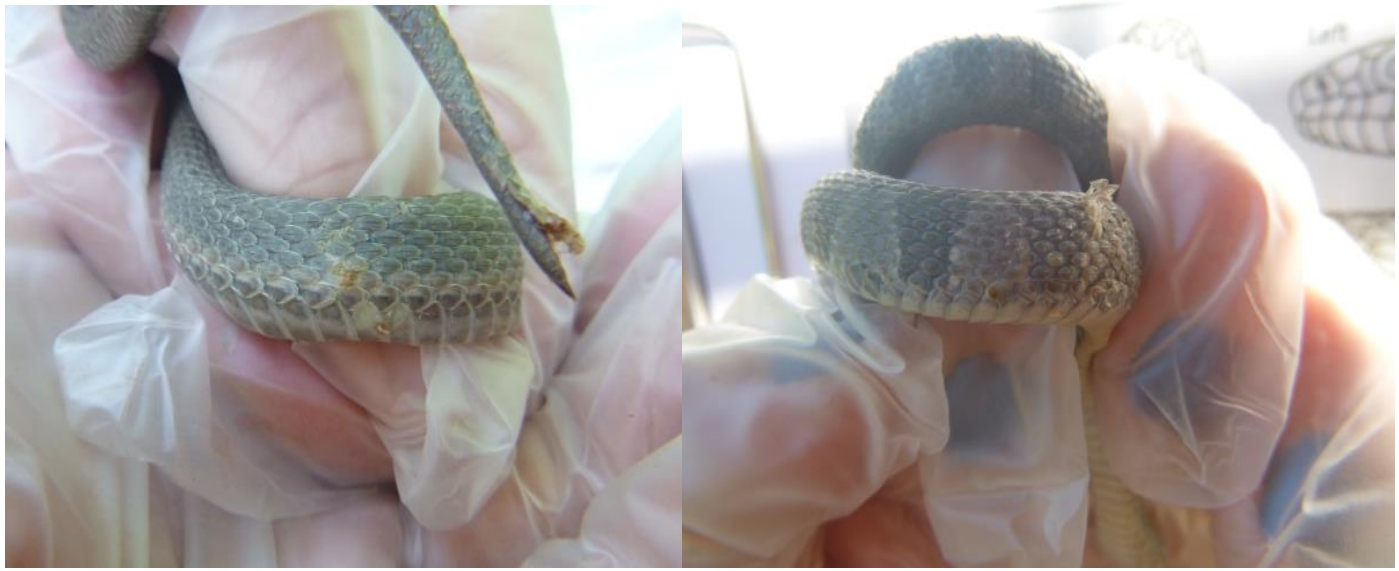

Figure A.10. Examples of dysecdysis in one *R. septemvittata* (left) and one *N. sipedon* (right).

Appendix B: R Code For Chapter 2

B.1. Swab and Scale Clip Comparison R Code

```
#######################Swab v. Scale Clip
Comparision#################################################
##########################December 14,
2016############################################################

#clear all variables
rm(list=ls())
graphics.off()

#set working directory
getwd()
setwd("C:\\")

#load data and rename for easier typing
sc<-read.csv(file.choose(), fill = NA, header = TRUE) #swabscaleminimal.csv
test<-read.csv(file.choose(), fill = NA, header = TRUE)
View(sc)
View(test)
#making a contingency table
#loading MASS package
library(MASS)

#creating a labelled table
table <- matrix(c(54,21,30,68),ncol=2,byrow=TRUE)
colnames(table) <- c("Swab Neg","Swab Pos")
rownames(table) <- c("Scale Neg","Scale Pos")
table <- as.table(table)
table

#chi squared test
chisq.test(table)

#mcnemar's test
mcnemar.test(table)

#making a barplot for the contingency table
library(ggplot2)
install.packages("ggplot2")

#reading in a different data set
graph<-read.csv(file.choose(), fill = NA, header = TRUE)
View(graph)

graph1<-ggplot(data=graph, aes(x=graph$Scale.Swab.Sample.Results, y=graph$Percent.of.Total)) +
  geom_bar(stat="identity", width = 0.5, fill="black") + xlab("Scale-Swab Sample Results") +
ylab("Percent of Total")+
  theme_bw() + theme(axis.text.x = element_text(colour="black",size=14),axis.text.y =
element_text(colour="black",size=14),
  axis.title.x = element_text(colour="black",size=14), axis.title.y = element_text(colour="black",size=14 ))
+
```

```
  theme(panel.border = element_blank(), panel.grid.major = element_blank(),
  panel.grid.minor = element_blank(),axis.line.x = element_line(color="black", size = 0.8),
  axis.line.y = element_line(color="black", size = 0.8)) + scale_y_continuous(expand = c(0,0)) +
  expand_limits(y=c(0,100))

graph1
```

B.2. Chapter 2 Model and Graph R Code

```
##Model Code For Chapter 2

#clear all variables
rm(list=ls())
graphics.off()

#set working directory
getwd()
setwd("C:\\")

#load data and rename for easier typing
ct<-read.csv(file.choose(), fill = NA, header = TRUE) #using all snakes cleaned.csv

View(ct)

#checking the structure of clinical and PCR
str(ct$clinical)
str(ct$pcr)

ct$clinical <- factor(ct$clinical)
ct$pcr <- factor(ct$pcr)
#viewing data
ct[1:5,]

#loading package
library(lme4)

#checking what type of variable submitter id
str(ct$Submitter.ID)
as.numeric(table(ct$Submitter.ID))

#running season models
mod4<-glmer(pcr ~ season + (1|Submitter.ID), family="binomial", data=ct)
summary(mod4)

mod4.1<-glmer(pcr ~ clinical  + (1|Submitter.ID), family="binomial", data=ct)
summary(mod4.1)

mod4.2<- glmer(pcr ~ group  + (1|Submitter.ID), family="binomial", data=ct)
summary(mod4.2)

mod4.3<-glmer(pcr ~ season  + clinical + (1|Submitter.ID), family="binomial", data=ct)
summary(mod4.3)

mod4.4<-glmer(pcr ~ season  + group + (1|Submitter.ID), family="binomial", data=ct)
summary(mod4.4)
```

```
mod4.5<-glmer(pcr ~ clinical  + group + (1|Submitter.ID), family="binomial", data=ct)
summary(mod4.5)

mod4.6 <- glmer(pcr ~ season + clinical + group  + (1|Submitter.ID), family="binomial", data=ct)
summary(mod4.6)

# Make AIC table with other models included
table_AIC <- AIC(mod4, mod4.1, mod4.2, mod4.3, mod4.4, mod4.5, mod4.6)
table_AIC$AICc <- table_AIC[,2] + (2*table_AIC[,1]*(table_AIC[,1]+1))/(nrow(ct)-table_AIC[,1]-1)
table_AIC$dAICc <- table_AIC$AICc - min(table_AIC$AICc)
table_AIC$modLik <- exp(-table_AIC$dAICc/2)
table_AIC$weights <- table_AIC$modLik/sum(table_AIC$modLik)

#### Make AIC Table
rownames(table_AIC) <-  c("Season", "Clinical Signs", "Group", "Season + Clinical Signs", "Season +
Group", "Clinical + Group", "Season + Clinical + Group")
colnames(table_AIC) <- c("K","AIC","AICc","dAICc","Likelihood","weight")
table_AIC <- table_AIC[order(table_AIC[,5]),]
round(table_AIC,digits=2)

##create a data set to make predictions
new2 <- expand.grid(clinical = c(0,1), season=c("spring", "summer", "fall"), group=c("aquatic",
"terrestrial"))
new2

##predictions on logit link scale
install.packages("AICcmodavg")
library(AICcmodavg)

pred <- predictSE(mod = mod4.6, newdata = new2, se.fit = TRUE,
                  type = "link")
plogis(pred$fit)

#####Plotting data#####
##basic plotting, trying to get three points on the graph
#reading in new csv to graph
graphtest2<-read.csv(file.choose(), fill = NA, header = TRUE) #graphtest.csv
graphtest2
summary(graphtest2)

#adjusting variable names
graphtest2
graphtest2 <- within(graphtest2, (Season <- factor(Season, c("Spring", "Summer", "Fall"))))
graphtest2
library(ggplot2)

#changing the legend order
levels(graphtest2$Status)
graphtest2$Status<-factor(graphtest2$Status, levels =rev(levels(graphtest2$Status)))
levels(graphtest2$Status)
# geom_line(aes(linetype=status), size=1) + scale_linetype_discrete(name="Clinical Signs") +
# A line graph
p<-ggplot(data=graphtest2, aes(x=Season, y=Value, group=Status, shape=Status, colour=Status)) +
    # Set linetype by status
 geom_point(size=2, fill="white") +        # Use larger points, fill with white
 expand_limits(y=c(0,1)) +                 # Set y range to include 0
```

```
  scale_colour_hue(name="Clinical Signs",      # Set legend title
                   l=30)  +                    # Use darker colors (lightness=30)
  scale_shape_manual(name="Clinical Signs",
                     values=c(22,21)) +        # Use points with a fill color

  xlab("Season") + ylab("Probability of (+) PCR") + # Set axis labels
  ggtitle("Probability of Disease with Clinical Sign Status by Season") +     # Set title
  theme_pubr() +
  theme(legend.position=c(.9, .7))
#viewing graph
print(p)

#error bars for the plot
#defining error bars
limits<-aes(ymax=plogis(pred$fit + 1.96*pred$se.fit), ymin =plogis(pred$fit - 1.96*pred$se.fit))
#error bars on the graph
p1<-p + geom_errorbar(limits, width=0.1, size=0.68, position=position_dodge(0.001))
print(p1)

#making larger axis tic mark sizes
black.13.text <- element_text(color = "black", size = 13)
p1 + theme(axis.text = black.13.text)
#making larger axis labels
red.bold.italic.text <- element_text(color = "black", size=16)
p1 + theme(axis.title = red.bold.italic.text)
#making a larger graph title
graph.title<-element_text(color = "black", size = 17)
p1 + theme(plot.title = graph.title)+ theme(axis.text = black.13.text)+
  theme(axis.title = red.bold.italic.text)

###reading in data#############################################################################
graphtest<-read.csv(file.choose(), fill = NA, header = TRUE)
graphtest
summary(graphtest)
graphtest <- within(graphtest, (time <- factor(time, c("Spring", "Summer", "Fall"))))
graphtest

# A line graph
p<-ggplot(data = graphtest, aes(x=time, y=value, color = status, shape= status, group = interaction(status,
association))) +
        geom_point(aes(x = time, y = value, shape = status), size = 3, fill = "white") +
        geom_line(aes(linetype=association), size=1) +
        expand_limits(y=c(0,1)) +   scale_colour_hue(name="Clinical Signs", l=30, c("Presence",
"Absence"))+
        xlab("Season") + ylab("Probability of (+) PCR") +
        ggtitle("Probability of Disease with Clinical Sign Status by Season") +
        theme_bw() +
        scale_linetype_discrete(name="Natural History") +
        theme_bw() + theme(panel.border = element_blank(), panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),axis.line.x = element_line(color="black", size = 0.5),
        axis.line.y = element_line(color="black", size = 0.5))+
        scale_shape_discrete(name="Clinical Signs", c("Presence", "Absence"))

print(p)
```

```
#error bars for the plot
#defining error bars
limits<-aes(ymax=plogis(pred$fit + 1.96*pred$se.fit), ymin =plogis(pred$fit - 1.96*pred$se.fit) )
#error bars on the graph
p1<-p + geom_errorbar(limits, width=0.2, size= 0.68, position=position_dodge(0.001))
print(p1)

#making larger axis tic mark sizes
black.13.text <- element_text(color = "black", size = 13)
p1 + theme(axis.text = black.13.text)
#making larger axis labels
red.bold.italic.text <- element_text(color = "black", size=16)
p1 + theme(axis.title = red.bold.italic.text)
#making a larger graph title
graph.title<-element_text(color = "black", size = 17)
p1 + theme(plot.title = graph.title)+ theme(axis.text = black.13.text)+
  theme(axis.title = red.bold.italic.text)


##create a data set to make predictions
new4 <- expand.grid(clinical = c(0), season=c("spring", "summer", "fall"), group=c("aquatic",
"terrestrial"))
new4

##predictions on logit link scale

pred1 <- predictSE(mod = mod4.6, newdata = new4, se.fit = TRUE,
                   type = "link")
plogis(pred1$fit)
limits2<-aes(ymax=plogis(pred1$fit + 1.96*pred1$se.fit), ymin =plogis(pred1$fit - 1.96*pred1$se.fit) )
#error bars on the graph
abs1<-abs + geom_errorbar(limits2, width=0.2, size= 0.68, position=position_dodge(0.001))
print(abs1)
####for aquatic
aquatic<-subset(graphtest2, Group =="Aquatic")
View(aquatic)

aq<-ggplot(data = aquatic, aes(x=Season, y=Value, color = Status, shape= Status, group = Status)) +
  geom_point(aes(x = Season, y = Value, shape = Status), size = 3, fill = "white") +
  expand_limits(y=c(0,1)) +   scale_colour_hue(name="Clinical Signs", l=30, c("Presence", "Absence"))+
  xlab("Season") + ylab("Probability of (+) PCR") +

  theme_pubr() +
  scale_linetype_discrete(name="Clinical Signs", c("Presence", "Absence")) +
  scale_shape_discrete(name="Clinical Signs", c("Presence", "Absence"))+theme(legend.position=c(0.7,
.8))

print(aq)
##create a data set to make predictions
new5 <- expand.grid(clinical = c(0,1), season=c("summer", "fall", "spring"), group=c("aquatic"))
new5

##predictions on logit link scale
pred2 <- predictSE(mod = mod4.6, newdata = new5, se.fit = TRUE,
                   type = "link")
plogis(pred2$fit)
```

```
limits3<-aes(ymax=plogis(pred2$fit + 1.96*pred2$se.fit), ymin =plogis(pred2$fit - 1.96*pred2$se.fit) )
#error bars on the graph
aq1<-aq + geom_errorbar(limits3, width=0.2, size= 0.68, position=position_dodge(0.001))
print(aq1)

#making larger axis tic mark sizes
black.13.text <- element_text(color = "black", size = 34)
aq1 + theme(axis.text = black.13.text)
#making larger axis labels
red.bold.italic.text <- element_text(color = "black", size=34)
aq1 + theme(axis.title = red.bold.italic.text)

#making a larger graph title
graph.title<-element_text(color = "black", size = 36)
aq1 + theme(plot.title = graph.title)+ theme(axis.text = black.13.text)+
  theme(axis.title = red.bold.italic.text) +
  theme(legend.text =element_text(color="black", size = 30)) +
  theme(legend.title = element_text(color="black", size = 30))
print(tr1)
#####terrestrial
terrestrial<-subset(graphtest2, Group == "Terrestrial")
View(terrestrial)

tr<-ggplot(data = terrestrial, aes(x=Season, y=Value, color = Status, shape= Status, group = Status)) +
  geom_point(aes(x = Season, y = Value, shape = Status), size = 3, fill = "white") +

  expand_limits(y=c(0,1)) +   scale_colour_hue(name="Clinical Signs", l=30, c("Presence", "Absence"))+
  xlab("Season") + ylab("Probability of (+) PCR") +

  theme_pubr()+

  scale_shape_discrete(name="Clinical Signs", c("Presence", "Absence"))+theme(legend.position=c(0.7,
.8))

print(tr)

##create a data set to make predictions
new6 <- expand.grid(clinical = c(0,1), season=c("summer", "fall", "spring"), group=c("terrestrial"))
new6

##predictions on logit link scale
pred3 <- predictSE(mod = mod4.6, newdata = new6, se.fit = TRUE,
                   type = "link")
plogis(pred3$fit)
limits4<-aes(ymax=plogis(pred3$fit + 1.96*pred3$se.fit), ymin =plogis(pred3$fit - 1.96*pred3$se.fit) )
#error bars on the graph
tr1<-tr + geom_errorbar(limits4, width=0.2, size= 0.68, position=position_dodge(0.001))
print(tr1)

#making larger axis tic mark sizes
black.13.text <- element_text(color = "black", size = 34)
tr1 + theme(axis.text = black.13.text)
#making larger axis labels
red.bold.italic.text <- element_text(color = "black", size=34)
tr1 + theme(axis.title = red.bold.italic.text)
```

```
#making a larger graph title
graph.title<-element_text(color = "black", size = 36)
tr1 + theme(plot.title = graph.title)+ theme(axis.text = black.13.text)+
  theme(axis.title = red.bold.italic.text) +
  theme(legend.text =element_text(color="black", size = 30)) +
  theme(legend.title = element_text(color="black", size = 30))
```

B.3. Tukey Test R Code

```
library(multcomp)

summary(glht(mod4.6, linfct = mcp(season = "Tukey")))
```

Appendix C: R Code For Chapter 3

C.1. R Code for Multi-State Model

```
##############################################################################################
##########
# Set input parameters
options(scipen = 20)  # Turn off scientific notation for PIT-tag codes
##############################################################################################
##########
getwd()
# Set working directory to analysis folder
setwd('C:/Users/User/Desktop/Current Analysis')

# Import data to create observation histories
ObsDat <- read.csv(file="ObservationData_v4.csv",strip.white = T)[,c(1:8,13)]    # Import individual
observation data
ObsDat$Date <- julian(as.Date(ObsDat$Date, format="%m/%d/%Y"),origin = as.Date("2016-01-01"))    #
Convert dates to julian
ObsDat$scan <- as.numeric(substring(ObsDat$scan,5)) + 1

# Delete extra scan0 records (using the last visual detection as the "capture/release occasion")
ObsDat$tmp <- paste0(ObsDat$id,"_",ObsDat$scan)   # Create unique id x scan combination
ObsDat <- ObsDat[order(ObsDat$id, ObsDat$scan, -ObsDat$detection, -ObsDat$Date),]   # Order records
ObsDat <- ObsDat[!duplicated(ObsDat$tmp),]   # delete all but the first scan0 record for an individual
ObsDat <- ObsDat[,-ncol(ObsDat)]     # Delete the unique id x scan column

# Specify number of scans/surveys and individuals
numberofsurveys <- 17
numberofsnakes <- length(unique(ObsDat$id))  # Calculate total number of individuals

# Set up empty encounter matrices
CH <- PCR <- Clinical <- matrix(NA,numberofsnakes,numberofsurveys)
colnames(CH) <- colnames(PCR) <- colnames(Clinical) <- 1:17
rownames(CH) <- rownames(PCR) <- rownames(Clinical) <- unique(ObsDat$id)
site <- species <- firstdate <- numeric()

iter=0
for (i in unique(ObsDat$id)){   # Loop through Individuals (PIT tag Numbers)
  iter=iter+1   # counter for individual #

  ScanNumber <- ObsDat$scan[which(ObsDat$id==i)]
  site[iter] <- ObsDat$site[which(ObsDat$id==i)[1]]
  species[iter] <- ObsDat$Species[which(ObsDat$id==i)[1]]
  firstdate[iter] <- min(ObsDat$Date[(which(ObsDat$id==i & ObsDat$detection==2))])

  # Create detection histories
  DetectionType <- ObsDat$detection[which(ObsDat$id==i)]   # Extract whether visually observed or not -
convert to numeric
  CH[iter,colnames(CH) %in% ScanNumber] <- DetectionType      # Write those values to correct scan
number columns

  PCRType <- ObsDat$pcr[which(ObsDat$id==i)]      # Extract PCR results
```

```
  PCR[iter,colnames(PCR) %in% ScanNumber] <- PCRType  # Write those values to correct scan number column

  ClinicalType <- ObsDat$clincalsign[which(ObsDat$id==i)]      # Extract clinical sign results
  Clinical[iter,colnames(Clinical) %in% ScanNumber] <- ClinicalType  # Write those values to correct scan number column
}

# Find snakes never visually seen in 2016 and remove (Most from Little Hickman?)
NeverSeen <- which(apply(CH,1,max,na.rm=T)<2)
CH <- CH[-NeverSeen,]
PCR <- PCR[-NeverSeen,]
Clinical <- Clinical[-NeverSeen,]
site <- site[-NeverSeen]
species <- species[-NeverSeen]
firstdate <- firstdate[-NeverSeen]

# Calculate occasion of first capture for each individual
f <- apply(CH,1,function(x){min(which(x==2))})

# Import individual-level summary data
SurvDat <- read.csv(file="SurveyData.csv",strip.white = T)[,c(1:10)]    # Import survey data
SurvDat$sampledate <- julian(as.Date(SurvDat$sampledate, format="%m/%d/%Y"),origin =
as.Date("2016-01-01"))    # Convert dates to julian
SurvDat$scan <- as.numeric(substring(SurvDat$scan,5)) + 1   # Add 1 because there's no scan0

# Create matrix of survey dates by individual
SampleDatesM <- matrix(NA,nrow=nrow(CH),ncol=ncol(CH))
colnames(SampleDatesM) <- 1:17
rownames(SampleDatesM) <- rownames(CH)

iter=0
for (i in rownames(SampleDatesM)){   # Loop through Individuals (PIT tag Numbers)
  iter=iter+1   # counter for individual #

  SampleDatesM[iter,2:17] <- SurvDat$sampledate[which(as.numeric(SurvDat$site)==site[iter])] # Write dates surveyed

  # Any survey dates before/equal to the first observation become NA
  SampleDatesM[iter,(1:f[iter])] <- NA
  SampleDatesM[iter,f[iter]] <- firstdate[iter]
}

# Final data manipulations
Intervals <- t(diff(t(SampleDatesM)))  # Calculate intervals between surveys (including first capture)
Surv <- dim(CH)[2]   # Determine number of surveys
Ind <- dim(CH)[1]    # Determine number of individuals
CH[which(is.na(CH))] <- 3    # Make non-detections == 3

# Below uses regional weather stations to compile site/survey covariates - currently not implemented
#FrankWeather <- read.table(file="Frankfortweather.csv",sep=",",header=T, strip.white=T)  # Must be ordered by date
#FrankWeather$JDate <- julian(as.Date(FrankWeather$EDT,format="%m/%d/%Y"),origin =
as.Date("2016-01-01"))
#LexWeather <- read.table(file="Lexweather.csv",sep=",",header=T, strip.white=T)  # Must be ordered by date
```

```
#LexWeather$JDate <- julian(as.Date(LexWeather$EDT,format="%m/%d/%Y"),origin = as.Date("2016-
01-01"))

#Cloud <- CloudF <- CloudL <- SampleDatesM
#for (i in 1:length(Cloud)){
#  CloudF[i] <-
ifelse(is.na(SampleDatesM[i]),NA,FrankWeather$CloudCover[which(FrankWeather$JDate==SampleDates
M[i])])
#  Cloud[i] <-
ifelse(is.na(SampleDatesM[i]),NA,LexWeather$CloudCover[which(LexWeather$JDate==SampleDatesM[i
])])
#}
#Cloud[which(site==3),] <- CloudF[which(site==3),]  # Replace Glenn cloud cover with FrankWeather

SurvDat <- read.csv(file="SurveyData.csv",strip.white = T)    # Import survey-specific data
SurvDat$Date <- julian(as.Date(SurvDat$sampledate, format="%m/%d/%Y"),origin = as.Date("2016-01-
01"))    # Convert dates to julian
SurvDat <- SurvDat[order(SurvDat$site, SurvDat$Date),]   # Order records

Cloud <- matrix(SurvDat$cloud.cover,nrow=6,byrow=T)
Temp <- matrix(SurvDat$start.air,nrow=6,byrow=T)
SiteType <- c(1,1,1,1,1,1)   # 1 = Forest, 2 = Suburban, 3 = Agriculture

# Delete extra objects
rm(list= ls()[!(ls() %in%
c('f','Intervals','Cloud','Temp','SiteType','SampleDatesM','CH','PCR','Clinical','SurvDat','basedir','Surv',
                    'Ind','species','site','firstdate'))])

##############################################################################################
###########
# Multi-state mark-recapture analysis
##############################################################################################
###########

# DOY effect on TE (PIT requested)
# Cloud cover on visual (also PIT)
# Average temperature or average high temperature could be a covariate for temporary emmigration
#Site type (Otter, Glenns = agricultural; Tates, Little Hickman = forested, Elkhorn, Elias/HIckman =
suburban) could also be used as a covariate for survivorship.

#Frankweather=Glenns

basedir <- "C:/Users/User/Desktop/Current Analysis"   # Where is the project folder?

source(file = paste0(basedir,"/","DataProcessing_v3.R"))

# For Model 1 we are just treating each snake as "diseased" if it ever tested positive (PCR=positive)
SFD <- apply(PCR,1,max,na.rm=T)    # Calculate 0/1 for negative/positive
SFD[which(!is.finite(SFD))] <- NA  # Use NA to denote individuals with no test result

# Convert species to numeric
species <- as.numeric(species)   # Regsep=1, Nersip=2

##############################################################################################
###########
# Specify Model
```

```
sink("PIT.txt")
cat("
   model {

   ###################################
   # Sub-model for disease dynamics #
   ###################################

   # Priors and constraints
   for (sp in 1:2){
   Dprob[sp] ~ dunif(0,1)
   }

   # Likelihood
   for (i in 1:n.ind){
   SFD[i] ~ dbern(Dprob[Species[i]])
   }

   ###################################
   # Sub-model for survival dynamics #
   ###################################

   # Priors and constraints
   for (ds in 1:2){  # loop over disease states (1=not diseased, 2=diseased)
   for (sp in 1:2){  # loop over species (1=Regsep, 2=Nersip)
   pV.a[ds,sp] ~ dunif(-5,5)          # Prior for conditional encounter probability
   psiOI.a[ds,sp] ~ dunif(0,1)        # Prior for Out-In Temporary Emigration probability
   psiIO.a[ds,sp] ~ dunif(-5,5)       # Prior for In-Out Temporary Emigration probability
   mo.s[ds,sp] ~ dunif(0,1)         # Prior for survival
   mo.f[ds,sp] ~ dunif(0,1)         # Prior for site fidelity
   }
   }

   psiIO.b ~ dunif(-10,10)        # Prior for In-Out Temporary Emigration probability
   pV.b1 ~ dunif(-10,10)
   pV.b2 ~ dunif(-10,10)

   pT ~ dunif(0,1)               # Prior for PIT-tag detection probability

   for (i in 1:n.ind){
   for (t in first[i]:(n.surv-1)){
   psiOI[i,t] <- psiOI.a[(SFD[i]+1),Species[i]]
   logit(psiIO[i,t]) <- psiIO.a[(SFD[i]+1),Species[i]] + psiIO.b*DOY[i,t]
   logit(pV[i,t]) <- pV.a[(SFD[i]+1),Species[i]] + pV.b1*Cloud[site[i],t] + pV.b2*Temp[site[i],t]
   s[i,t] <- pow(mo.s[(SFD[i]+1),Species[i]], Intervals[i,t]/30)
   f[i,t] <- pow(mo.f[(SFD[i]+1),Species[i]], Intervals[i,t]/30)
   }
   }

   for (i in 1:n.ind){
   for (t in first[i]:(n.surv-1)){   # Loop through INTERVALS

   # Define transition matrix (probability of individual states at time t, conditional on states at t-1)
   ps[1,i,t,1] <- f[i,t]*s[i,t]*(1-psiIO[i,t])
   ps[1,i,t,2] <- f[i,t]*s[i,t]*psiIO[i,t]
   ps[1,i,t,3] <- (1-f[i,t])*s[i,t]
```

```
ps[1,i,t,4] <- (1-s[i,t])
ps[1,i,t,5] <- 0
ps[2,i,t,1] <- f[i,t]*s[i,t]*psiOI[i,t]
ps[2,i,t,2] <- f[i,t]*s[i,t]*(1-psiOI[i,t])
ps[2,i,t,3] <- (1-f[i,t])*s[i,t]
ps[2,i,t,4] <- 0
ps[2,i,t,5] <- (1-s[i,t])
ps[3,i,t,1] <- 0
ps[3,i,t,2] <- 0
ps[3,i,t,3] <- 1
ps[3,i,t,4] <- 0
ps[3,i,t,5] <- 0
ps[4,i,t,1] <- 0
ps[4,i,t,2] <- 0
ps[4,i,t,3] <- 0
ps[4,i,t,4] <- 1
ps[4,i,t,5] <- 0
ps[5,i,t,1] <- 0
ps[5,i,t,2] <- 0
ps[5,i,t,3] <- 0
ps[5,i,t,4] <- 0
ps[5,i,t,5] <- 1

# Define observation matrix
po[1,i,t,1] <- (1-pV[i,t])*pT
po[1,i,t,2] <- pV[i,t]*pT
po[1,i,t,3] <- 1-pT
po[2,i,t,1] <- 0
po[2,i,t,2] <- 0
po[2,i,t,3] <- 1
po[3,i,t,1] <- 0
po[3,i,t,2] <- 0
po[3,i,t,3] <- 1
po[4,i,t,1] <- pT
po[4,i,t,2] <- 0
po[4,i,t,3] <- 1-pT
po[5,i,t,1] <- 0
po[5,i,t,2] <- 0
po[5,i,t,3] <- 1
} #t
} #i

# Likelihood
for (i in 1:n.ind){
# Define latent state at first capture
z[i,first[i]] <- 1

for (t in (first[i]+1):n.surv){
# State process - current state (z[i,t]) given previous state (z[i,t-1])
z[i,t] ~ dcat(ps[z[i,t-1],i,t-1,])

# Observation process - current observation (y[i,t]) given current state (z[i,t])
y[i,t] ~ dcat(po[z[i,t],i,t-1,])
} #t
} #i
}
```

```
   ",fill = TRUE)
sink()

# Create known latent states z (only known state is when PIT & Visual detetion occurs)
Known.Z <- ifelse(CH==2,1,NA)
for (i in 1:Ind){
  Known.Z[i,f[i]] <- NA
}

# Bundle data
bugs.data <- list(y = CH, n.surv=Surv,
                  Temp=(Temp-mean(Temp))/sd(Temp),
                  Cloud=(Cloud-mean(Cloud))/sd(Cloud),
                  DOY=(SampleDatesM[,-1]-mean(SampleDatesM[,-1],na.rm=T))/sd(SampleDatesM[,-
1],na.rm=T),
                  n.ind=Ind, z = Known.Z, SFD=SFD,
                  first=f, site=site, SiteType=SiteType,
                  Intervals=Intervals, Species=species)

# Function to create initial values for unknown z
ms.init.z <- function(ch){
  ch <- ifelse(is.na(Known.Z),1,NA)
  return(ch)
}

ms.init.z <- function(ch, f){
  for (i in 1:dim(ch)[1]){ch[i,1:f[i]] <- NA}
  for (i in 1:dim(ch)[1]){ch[i,(f[i]+1):Surv] <- 1}
  for (i in 1:dim(ch)[1]){ch[i,which(Known.Z[i,]==1)] <- NA}
  return(ch)
}

# Initial values
inits <- function(){list(z=ms.init.z(CH,f),
                         pV.a = matrix(runif(4, -1, 1),nrow=2),
                         pV.b1 = runif(1, -1, 1),
                         pV.b2 = runif(1, -1, 1),
                         psiIO.b = runif(1, -1, 1))}

# Parameters monitored
parameters <- c("mo.f", "mo.s", "pT", "pV.a","pV.b1","pV.b2","psiIO.a","psiIO.b","psiOI.a","Dprob")

# MCMC settings  (let's aim for ~3,000 posterior samples)
ni <- 800000
nt <- 50
nb <- 400000
nc <- 3

library("jagsUI")
# Call WinBUGS from R (Approximate run time = 4 hr.)
system.time(out <- jags(bugs.data, inits, parameters, "PIT.txt", parallel = T, #codaOnly = c('po'),
                        n.chains = nc, n.thin = nt, n.iter = ni, n.burnin = nb))
print(out, digits = 3)

update(out,n.iter=800000,n.burnin=400000,n.thin=50)
```

C.2. R Code to Create Figure 3.3

```
library(jagsUI)


# Load workspace
load("model_run_021318.RData")
print(out,3)

dSurvivalRegsep <- qlogis(out$sims.list$mo.s[,2,1])-qlogis(out$sims.list$mo.s[,1,1])
dSurvivalNersip <- qlogis(out$sims.list$mo.s[,2,2])-qlogis(out$sims.list$mo.s[,1,2])
dPermEmigRegsep <- qlogis(1-out$sims.list$mo.f[,2,1])-qlogis(1-out$sims.list$mo.f[,1,1])
dPermEmigNersip <- qlogis(1-out$sims.list$mo.f[,2,2])-qlogis(1-out$sims.list$mo.f[,1,2])
dVisibleRegsep <- out$sims.list$pV.a[,2,1]-out$sims.list$pV.a[,1,1]
dVisibleNersip <- out$sims.list$pV.a[,2,2]-out$sims.list$pV.a[,1,2]
dTempEmiRegsep <- out$sims.list$psiIO.a[,2,1]-out$sims.list$psiIO.a[,1,1]
dTempEmiNersip <- out$sims.list$psiIO.a[,2,2]-out$sims.list$psiIO.a[,1,2]
dTempImmRegsep <- qlogis(out$sims.list$psiOI.a[,2,1])-qlogis(out$sims.list$psiOI.a[,1,1])
dTempImmNersip <- qlogis(out$sims.list$psiOI.a[,2,2])-qlogis(out$sims.list$psiOI.a[,1,2])

# Survival
plot(mean(dSurvivalRegsep),5.1,ylim=c(0.5,5.5),xlim=c(-
4,4),pch=19,cex=2,xaxs='i',yaxs='i',yaxt='n',ylab="",xlab="Contrast")
abline(v=0, lty=2)
points(mean(dSurvivalNersip),4.9,ylim=c(0,6),xlim=c(-4,4),pch=19,cex=2,col="gray")
segments(x0=quantile(dSurvivalRegsep,0.025),x1=quantile(dSurvivalRegsep,0.975),y0=5.1,y1=5.1)
segments(x0=quantile(dSurvivalRegsep,0.05),x1=quantile(dSurvivalRegsep,0.95),y0=5.1,y1=5.1,lwd=5)
segments(x0=quantile(dSurvivalNersip,0.025),x1=quantile(dSurvivalNersip,0.975),y0=4.9,y1=4.9,col="gra
y")
segments(x0=quantile(dSurvivalNersip,0.05),x1=quantile(dSurvivalNersip,0.95),y0=4.9,y1=4.9,lwd=5,col
="gray")

# Permanent Emigration
points(mean(dPermEmigRegsep),4.1,ylim=c(0,6),xlim=c(-4,4),pch=19,cex=2)
points(mean(dPermEmigNersip),3.9,ylim=c(0,6),xlim=c(-4,4),pch=19,cex=2,col="gray")
segments(x0=quantile(dPermEmigRegsep,0.025),x1=quantile(dPermEmigRegsep,0.975),y0=4.1,y1=4.1)
segments(x0=quantile(dPermEmigRegsep,0.05),x1=quantile(dPermEmigRegsep,0.95),y0=4.1,y1=4.1,lwd=
5)
segments(x0=quantile(dPermEmigNersip,0.025),x1=quantile(dPermEmigNersip,0.975),y0=3.9,y1=3.9,col
="gray")
segments(x0=quantile(dPermEmigNersip,0.05),x1=quantile(dPermEmigNersip,0.95),y0=3.9,y1=3.9,lwd=5
,col="gray")

# Visual Detection
points(mean(dVisibleRegsep),3.1,ylim=c(0,6),xlim=c(-4,4),pch=19,cex=2)
points(mean(dVisibleNersip),2.9,ylim=c(0,6),xlim=c(-4,4),pch=19,cex=2,col="gray")
segments(x0=quantile(dVisibleRegsep,0.025),x1=quantile(dVisibleRegsep,0.975),y0=3.1,y1=3.1)
segments(x0=quantile(dVisibleRegsep,0.05),x1=quantile(dVisibleRegsep,0.95),y0=3.1,y1=3.1,lwd=5)
segments(x0=quantile(dVisibleNersip,0.025),x1=quantile(dVisibleNersip,0.975),y0=2.9,y1=2.9,col="gray"
)
segments(x0=quantile(dVisibleNersip,0.05),x1=quantile(dVisibleNersip,0.95),y0=2.9,y1=2.9,lwd=5,col="
gray")

# Temporary Emigration
points(mean(dTempEmiRegsep),2.1,ylim=c(0,6),xlim=c(-4,4),pch=19,cex=2)
points(mean(dTempEmiNersip),1.9,ylim=c(0,6),xlim=c(-4,4),pch=19,cex=2,col="gray")
segments(x0=quantile(dTempEmiRegsep,0.025),x1=quantile(dTempEmiRegsep,0.975),y0=2.1,y1=2.1)
```

```
segments(x0=quantile(dTempEmiRegsep,0.05),x1=quantile(dTempEmiRegsep,0.95),y0=2.1,y1=2.1,lwd=5
)
segments(x0=quantile(dTempEmiNersip,0.025),x1=quantile(dTempEmiNersip,0.975),y0=1.9,y1=1.9,col="
gray")
segments(x0=quantile(dTempEmiNersip,0.05),x1=quantile(dTempEmiNersip,0.95),y0=1.9,y1=1.9,lwd=5,c
ol="gray")

# Temporary Immigration
points(mean(dTempImmRegsep),1.1,ylim=c(0,6),xlim=c(-4,4),pch=19,cex=2)
points(mean(dTempImmNersip),0.9,ylim=c(0,6),xlim=c(-4,4),pch=19,cex=2,col="gray")
segments(x0=quantile(dTempImmRegsep,0.025),x1=quantile(dTempImmRegsep,0.975),y0=1.1,y1=1.1)
segments(x0=quantile(dTempImmRegsep,0.05),x1=quantile(dTempImmRegsep,0.95),y0=1.1,y1=1.1,lwd=
5)
segments(x0=quantile(dTempImmNersip,0.025),x1=quantile(dTempImmNersip,0.975),y0=0.9,y1=0.9,col=
"gray")
segments(x0=quantile(dTempImmNersip,0.05),x1=quantile(dTempImmNersip,0.95),y0=0.9,y1=0.9,lwd=5,
col="gray")

# Extra lines
abline(h=4.5); text('Survival',x=-3.25,y=5.4)
abline(h=3.5); text('Permanent Emigration',x=-2.25,y=4.4)
abline(h=2.5); text('Surface Probability',x=-2.5,y=3.4)
abline(h=1.5); text('Temporary Emigration',x=-2.25,y=2.4)
text('Temporary Immigration',x=-2.25,y=1.4)
```

C.3. R Code to Create Figures 3.4 – 3.6

```
#Step 1: Load workspace from model run 400iterations
#clear variables
rm(list=ls())
print(out)
library(grid)
library(gridExtra)
library(ggplot2)
library(ggpubr)

#creating datasets
newTEMPdata <- seq(from=15,to=35,by=0.1)
newDOYdata <-seq(from=1,to=365, by=1)
newCLOUDdata<-seq(from=0, to=8, by=1)

#creating dataframes out of tempdata and probabilities
dftempdata <- data.frame(newTEMPdata)
dfDOYdata <- data.frame(newDOYdata)
dfclouddata <- data.frame(newCLOUDdata)

#positive queen snake, detection with temperature
logitposrs <- out$mean$pV.a[2,1] + out$mean$pV.b2*(newTEMPdata-mean(Temp))/sd(Temp)  #
Disease=0, species=1
ymax1=pnorm(0.146 + out$mean$pV.b2*(newTEMPdata-mean(Temp))/sd(Temp))
ymin1=pnorm(0.784 + out$mean$pV.b2*(newTEMPdata-mean(Temp))/sd(Temp))
probsposrs <- pnorm(logitposrs)
dfposrs <- data.frame(probsposrs)
View(dfposrs)
View(dftempdata)
```

```
df1<-cbind(dfposrs, dftempdata)

#ggplot2 line graph code
plot1<-ggplot(data=df1, aes(x=newTEMPdata, y=probsposrs, group=1)) + geom_line(size=1) +
  expand_limits(y=0) + ylim(0.0, 1.00)+annotate("text", x = 17, y = 0.9, label = "B", size=15)+
  xlab(expression("Temperature (°C)")) + ylab("Visual Detection Probability") +
  theme_pubr()+ geom_ribbon(aes(x=newTEMPdata, ymin=ymin1, ymax=ymax1), alpha=0.2,
fill="#808080")
plot1

#negative queen snake, visual detection and temperature
logitnegrs <- out$mean$pV.a[1,1] + out$mean$pV.b2*(newTEMPdata-mean(Temp))/sd(Temp)  #
Disease=0, species=1
ymax2=pnorm(-0.037 + out$mean$pV.b2*(newTEMPdata-mean(Temp))/sd(Temp))
ymin2=pnorm(-0.632 + out$mean$pV.b2*(newTEMPdata-mean(Temp))/sd(Temp))
probsnegrs <- pnorm(logitnegrs)
dfnegrs <- data.frame(probsnegrs)
View(dfnegrs)
df2<-cbind(dfnegrs, dftempdata)

plot2<-ggplot(data=df2, aes(x=newTEMPdata, y=probsnegrs, group=1)) + geom_line(size=1) +
   expand_limits(y=0) + ylim(0.0,1.00) +
  annotate("text", x = 17, y = 0.9, label = "A", size=15) +
  xlab(expression("Temperature (°C)")) + ylab("Visual Detection Probability") +
  theme_pubr()+geom_ribbon(aes(x=newTEMPdata, ymin=ymin2, ymax=ymax2), alpha=0.2,
fill="#808080")
plot2

#negative, nerodia, visual detection and temperature
logitnegns <- out$mean$pV.a[1,2] + out$mean$pV.b2*(newTEMPdata-mean(Temp))/sd(Temp)  #
Disease=0, species=1
ymax3=pnorm(0.036 + out$mean$pV.b2*(newTEMPdata-mean(Temp))/sd(Temp))
ymin3=pnorm(-0.469 + out$mean$pV.b2*(newTEMPdata-mean(Temp))/sd(Temp))
probsnegns <- pnorm(logitnegns)
dfnegns <- data.frame(probsnegns)
View(dfnegns)
df3<-cbind(dfnegns, dftempdata)

plot3<-ggplot(data=df3, aes(x=newTEMPdata, y=probsnegns, group=1)) + geom_line(size=1) +
  expand_limits(y=0) + ylim(0.0,1.00)+annotate("text", x = 17, y = 0.9, label = "C", size=15) +
  xlab(expression("Temperature (°C)")) + ylab("Visual Detection Probability") +
  theme_pubr()+geom_ribbon(aes(x=newTEMPdata, ymin=ymin3, ymax=ymax3), alpha=0.2,
fill="#808080")
plot3

#positive nerodia, temperature and visual detection
logitposns <- out$mean$pV.a[2,2] + out$mean$pV.b2*(newTEMPdata-mean(Temp))/sd(Temp)  #
Disease=0, species=1
ymax4=pnorm(0.567 + out$mean$pV.b2*(newTEMPdata-mean(Temp))/sd(Temp))
ymin4=pnorm(-0.408 + out$mean$pV.b2*(newTEMPdata-mean(Temp))/sd(Temp))
probsposns <- pnorm(logitposns)
dfposns <- data.frame(probsposns)
View(dfposns)
df4<-cbind(dfposns, dftempdata)

plot4<-ggplot(data=df4, aes(x=newTEMPdata, y=probsposns, group=1)) + geom_line(size=1) +
```

```
  expand_limits(y=0) +ylim(0.0, 1.00)+annotate("text", x = 17, y = 0.9, label = "D", size=15)+
  xlab(expression("Temperature (°C)")) + ylab("Visual Detection Probability") +
  theme_pubr()+geom_ribbon(aes(x=newTEMPdata, ymin=ymin4, ymax=ymax4), alpha=0.2,
fill="#808080")
plot4

#see multiplot function script at the end of this file
multiplot(plot2, plot3, plot1, plot4, cols=2)

####Psio in to out
##c

logitpsionegreg <- out$mean$psiIO.a[1,1] + out$mean$psiIO.b*(newDOYdata-
mean(SurvDat$Date))/sd(SurvDat$Date)  # Disease=0, species=1
ymax5=pnorm(0.567 + out$mean$psiIO.b*(newDOYdata-mean(SurvDat$Date))/sd(SurvDat$Date))
ymin5=pnorm(-1.040 + out$mean$psiIO.b*(newDOYdata-mean(SurvDat$Date))/sd(SurvDat$Date))
probpsionegreg <- pnorm(logitpsionegreg)
dfpsionegreg <- data.frame(probpsionegreg)
df5<-cbind(dfpsionegreg, dfDOYdata)
View(df5)

plot5<-ggplot(data=df5, aes(x=newDOYdata, y=probpsionegreg, group=1)) + geom_line(size=1) +
  expand_limits(y=0) + annotate("text", x = 50, y = 0.9, label = "A", size=15)+
  xlab("Day of Year") + ylab("Temporary Emigration Probability") +
  theme_pubr()+geom_ribbon(aes(x=newDOYdata, ymin=ymin5, ymax=ymax5), alpha=0.2,
fill="#808080")
plot5

#positive queen snakes, day of year
logitpsioposreg <- out$mean$psiIO.a[2,1] + out$mean$psiIO.b*(newDOYdata-
mean(SurvDat$Date))/sd(SurvDat$Date)  # Disease=0, species=1
ymax6=pnorm(0.343 + out$mean$psiIO.b*(newDOYdata-mean(SurvDat$Date))/sd(SurvDat$Date))
ymin6=pnorm(-1.021 + out$mean$psiIO.b*(newDOYdata-mean(SurvDat$Date))/sd(SurvDat$Date))
probpsioposreg <- pnorm(logitpsioposreg)
dfpsioposreg <- data.frame(probpsioposreg)
df6<-cbind(dfpsioposreg, dfDOYdata)
View(df6)

plot6<-ggplot(data=df6, aes(x=newDOYdata, y=probpsioposreg, group=1)) + geom_line(size=1) +
  expand_limits(y=0) +annotate("text", x = 50, y = 0.9, label = "B", size=15)+
  xlab("Day of Year") + ylab("Temporary Emigration Probability") + ylim(0, 1.00)+
  theme_pubr()+geom_ribbon(aes(x=newDOYdata, ymin=ymin6, ymax=ymax6), alpha=0.2,
fill="#808080")
plot6

#negative nerodia, day of year
logitpsionegner <- out$mean$psiIO.a[1,2] + out$mean$psiIO.b*(newDOYdata-
mean(SurvDat$Date))/sd(SurvDat$Date)  # Disease=0, species=1
ymax7=pnorm(0.758 + out$mean$psiIO.b*(newDOYdata-mean(SurvDat$Date))/sd(SurvDat$Date))
ymin7=pnorm(-0.649 + out$mean$psiIO.b*(newDOYdata-mean(SurvDat$Date))/sd(SurvDat$Date))
probpsionegner <- pnorm(logitpsionegner)
dfpsionegner <- data.frame(probpsionegner)
df7<-cbind(dfpsionegner, dfDOYdata)
View(df7)

plot7<-ggplot(data=df7, aes(x=newDOYdata, y=probpsionegner, group=1)) + geom_line(size=1) +
```

```
  expand_limits(y=0) +annotate("text", x = 50, y = 0.9, label = "C", size=15)+
  xlab("Day of Year") + ylab("Temporary Emigration Probability") +
  theme_pubr()+geom_ribbon(aes(x=newDOYdata, ymin=ymin7, ymax=ymax7), alpha=0.2,
fill="#808080")
plot7

#positive nerodia, day of year
logitpsioposner <- out$mean$psiIO.a[2,2] + out$mean$psiIO.b*(newDOYdata-
mean(SurvDat$Date))/sd(SurvDat$Date)  # Disease=0, species=1
ymax8=pnorm(0.939 + out$mean$psiIO.b*(newDOYdata-mean(SurvDat$Date))/sd(SurvDat$Date))
ymin8=pnorm(-0.772 + out$mean$psiIO.b*(newDOYdata-mean(SurvDat$Date))/sd(SurvDat$Date))
probpsioposner <- pnorm(logitpsioposner)
dfpsioposner <- data.frame(probpsioposner)
df8<-cbind(dfpsioposner, dfDOYdata)
View(df8)

plot8<-ggplot(data=df8, aes(x=newDOYdata, y=probpsioposner, group=1)) + geom_line(size=1) +
  expand_limits(y=0) +annotate("text", x = 50, y = 0.9, label = "D", size=15)+
  xlab("Day of Year") + ylab("Temporary Emigration Probability") +
  theme_pubr()+geom_ribbon(aes(x=newDOYdata, ymin=ymin8, ymax=ymax8), alpha=0.2,
fill="#808080")
plot8

#see multiplot function script at the end of this file
multiplot(plot5, plot7, plot6, plot8, cols=2)

#cloud cover attempt
#negative, queen snake, cloud
logitVnegreg <- out$mean$pV.a[1,1] + out$mean$pV.b1*(newCLOUDdata-mean(Cloud))/sd(Cloud)  #
Disease=0, species=1
ymax9=pnorm(-0.037 + out$mean$pV.b1*(newCLOUDdata-mean(Cloud))/sd(Cloud))
ymin9=pnorm(-0.632 + out$mean$pV.b1*(newCLOUDdata-mean(Cloud))/sd(Cloud))
probsVnegreg <- pnorm(logitVnegreg)
dfVnegreg <- data.frame(probsVnegreg)
df9<-cbind(dfVnegreg, dfclouddata)
View(df9)

plot9<-ggplot(data=df9, aes(x=newCLOUDdata, y=probsVnegreg, group=1)) + geom_line(size=1) +
  expand_limits(y=0) +ylim(0.0,1.00)+annotate("text", x = 0.5, y = 0.94, label = "A", size=15)+
  xlab("Cloud Cover") + ylab("Visual Detection Probability") +
  theme_pubr()+geom_ribbon(aes(x=newCLOUDdata, ymin=ymin9, ymax=ymax9), alpha=0.2,
fill="#808080")
plot9

#positive, queen snake, cloud
logitVposreg <- out$mean$pV.a[2,1] + out$mean$pV.b1*(newCLOUDdata-mean(Cloud))/sd(Cloud)  #
Disease=0, species=1
ymax10=pnorm(0.784 + out$mean$pV.b1*(newCLOUDdata-mean(Cloud))/sd(Cloud))
ymin10=pnorm(0.146 + out$mean$pV.b1*(newCLOUDdata-mean(Cloud))/sd(Cloud))
probsVposreg <- pnorm(logitVposreg)
dfVposreg <- data.frame(probsVposreg)
df10<-cbind(dfVposreg, dfclouddata)
View(df10)

plot10<-ggplot(data=df10, aes(x=newCLOUDdata, y=probsVposreg, group=1)) + geom_line(size=1) +
  expand_limits(y=0) +ylim(0.0,1.00)+annotate("text", x = 0.5, y = 0.94, label = "B", size=15)+
```

```
  xlab("Cloud Cover") + ylab("Visual Detection Probability") +
  theme_pubr()+geom_ribbon(aes(x=newCLOUDdata, ymin=ymin10, ymax=ymax10), alpha=0.2,
fill="#808080")
plot10

#negative, nerodia, cloud
logitVnegner<- out$mean$pV.a[1,2] + out$mean$pV.b1*(newCLOUDdata-mean(Cloud))/sd(Cloud)  #
Disease=0, species=1
ymax11=pnorm(0.036 + out$mean$pV.b1*(newCLOUDdata-mean(Cloud))/sd(Cloud))
ymin11=pnorm(-0.469 + out$mean$pV.b1*(newCLOUDdata-mean(Cloud))/sd(Cloud))
probsVnegner <- pnorm(logitVnegner)
dfVnegner <- data.frame(probsVnegner)
df11<-cbind(dfVnegner, dfclouddata)
View(df11)

plot11<-ggplot(data=df11, aes(x=newCLOUDdata, y=probsVnegner, group=1)) + geom_line(size=1) +
  expand_limits(y=0) +ylim(0.0,1.00)+annotate("text", x = 0.5, y = 0.94, label = "C", size=15)+
  xlab("Cloud Cover") + ylab("Visual Detection Probability") +
  theme_pubr()+geom_ribbon(aes(x=newCLOUDdata, ymin=ymin11, ymax=ymax11), alpha=0.2,
fill="#808080")
plot11

#postiive, nerodia, cloud
logitVposner <- out$mean$pV.a[2,2] + out$mean$pV.b1*(newCLOUDdata-mean(Cloud))/sd(Cloud)  #
Disease=0, species=1
ymax12=pnorm(0.567 + out$mean$pV.b1*(newCLOUDdata-mean(Cloud))/sd(Cloud))
ymin12=pnorm(-0.408 + out$mean$pV.b1*(newCLOUDdata-mean(Cloud))/sd(Cloud))
probsVposner <- pnorm(logitVposner)
dfVposner <- data.frame(probsVposner)
df12<-cbind(dfVposner, dfclouddata)
View(df12)

plot12<-ggplot(data=df12, aes(x=newCLOUDdata, y=probsVposner, group=1)) + geom_line(size=1) +
  expand_limits(y=0) +ylim(0.0,1.00)+ annotate("text", x = 0.5, y = 0.94, label = "D", size=15)+
  xlab("Cloud Cover") + ylab("Visual Detection Probability") +
  theme_pubr()+geom_ribbon(aes(x=newCLOUDdata, ymin=ymin12, ymax=ymax12), alpha=0.2,
fill="#808080")
plot12

multiplot(plot9, plot11, plot10, plot12, cols=2)

#attempting to graph two dataframes on one ggplot2 graph
a.temp <- newTEMPdata
a.value   <- probsnegrs
a.cat <- c("regina, negative")
dtframe.a <- data.frame(a.temp, a.value, a.cat)

a.temp <- newTEMPdata
a.value   <- probsposrs
a.cat <- c("regina, positive")
dtframe.b <- data.frame(a.temp, a.value, a.cat)

#combining the two dataframes above
df <- rbind(dtframe.a,dtframe.b)

#creating other two data frames for nerodia disease status
```

```
a.temp <- newTEMPdata
a.value   <- probsnegns
a.cat <- c("nerodia, negative")
dtframe.c <- data.frame(a.temp, a.value, a.cat)

a.temp <- newTEMPdata
a.value   <- probsposns
a.cat <- c("nerodia, positive")
dtframe.d <- data.frame(a.temp, a.value, a.cat)

#combining all of the dataframes into one
df1<-rbind(df,dtframe.c, dtframe.d)
View(df1)

#changing column headings
names(df1)[1] <- "Temp"
names(df1)[2] <- "Prob"
names(df1)[3] <- "Cat"
#

tempplot<-ggplot(data = df1, aes(x=Temp, y=Prob, color = Cat)) +
  geom_line(aes(color=Cat), size=1) +
  expand_limits(y=c(0,1)) +   scale_colour_hue(name="Species and Disease Status", l=30, c("Queensnake,
Negative","Queensnake, Positive",
                                                        "Northern Watersnake, Negative", "Northern
Watersnake, Positive"))+
  xlab("Temperature") + ylab("Probability of Visual Detection") +
  ggtitle("Probability of Visual Detection Depending on Temperature") +
  theme_bw() + theme(panel.border = element_blank(), panel.grid.major = element_blank(),
                panel.grid.minor = element_blank(),axis.line.x = element_line(color="black", size = 0.5),
                axis.line.y = element_line(color="black", size = 0.5)) +
  scale_colour_manual(values=c("red","green","blue","purple"))
print(tempplot)

ggplot(data=df1, aes(x=Temp, y=Prob, group = Cat, colour = Cat)) +
  geom_line()+ xlab("Temperature") + ylab("Probability of Visual Detection") +
  ggtitle("Probability of Visual Detection Depending on Temperature") + theme_bw()+
  theme(panel.border = element_blank(), panel.grid.major = element_blank(),
panel.grid.minor = element_blank(),axis.line.x = element_line(color="black", size = 0.5),
axis.line.y = element_line(color="black", size = 0.5))

#

#multiplot function code
#####################################################################
#3 will go all the way across the bottom.
#
multiplot <- function(..., plotlist=NULL, file, cols=1, layout=NULL) {
  require(grid)

  # Make a list from the ... arguments and plotlist
  plots <- c(list(...), plotlist)

  numPlots = length(plots)

  # If layout is NULL, then use 'cols' to determine layout
```

```
  if (is.null(layout)) {
    # Make the panel
    # ncol: Number of columns of plots
    # nrow: Number of rows needed, calculated from # of cols
    layout <- matrix(seq(1, cols * ceiling(numPlots/cols)),
                     ncol = cols, nrow = ceiling(numPlots/cols))
  }

  if (numPlots==1) {
    print(plots[[1]])

  } else {
    # Set up the page
    grid.newpage()
    pushViewport(viewport(layout = grid.layout(nrow(layout), ncol(layout))))

    # Make each plot, in the correct location
    for (i in 1:numPlots) {
      # Get the i,j matrix positions of the regions that contain this subplot
      matchidx <- as.data.frame(which(layout == i, arr.ind = TRUE))

      print(plots[[i]], vp = viewport(layout.pos.row = matchidx$row,
                                      layout.pos.col = matchidx$col))
    }
  }
}
```

REFERENCES

Allender MC, Baker S, Wylie D, Loper D, Dreslik MJ, Phillips CA, Maddox C, and Driskell EA (2015) Development of snake fungal disease after experimental challenge with *Ophidiomyces ophiodiicola* in cottonmouths (*Agkistrodon piscivorous*). Plos One 10:1–13.

Allender MC, Deslik M, Wylie S, Phillips C, Wylie DB, Maddox C, Delaney MA and Kinsel M (2011) *Chrysosporium sp*. infection in Eastern Massasauga rattlesnakes. Emerging Infectious Diseases 17:2383–2384.

Allender MC, Raudabaugh DB, Gleason FH and Miller AN (2015) The natural history, ecology, and epidemiology of *Ophidiomyces ophiodiicola* and its potential impact on free-ranging snake populations. Fungal Ecology 17:187–196.

Bates D, Maechler M, Bolker B and Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G and Parkes H (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences 95:9031–9036.

Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-zier BM, Buckles EL, Coleman JTH, Darling SR, Gargas A, Niver R, Okoniewski JC, Rudd RJ and Ward B (2008) Bat white-nose syndrome: An emerging fungal pathogen? Science 323:227.

Bohuski E, Lorch JM, Griffin KM and Blehert DS (2015) TaqMan real-time polymerase chain reaction for detection of *Ophidiomyces ophiodiicola*, the fungus associated with snake fungal disease. BMC Veterinary Research 11:1-10.

Branson BA and Baker EC (1974) An ecological study of the queen snake, *Regina septemvittata* (Say) in Kentucky. Tulane Stud. Zool. Bot. 18:153–171.

Burbrink FT, Lorch JM and Lips KR (2017) Host susceptibility to snake fungal disease is highly dispersed across phylogenetic and functional trait space. Science Advances 3:e170137.

Burnham KP and Anderson DR (2002) Model selection and inference—a practical information-theoretic approach, New York, New York: Springer-Verlag.

Burns G, Ramos A and Muchlinski A (1996) Fever response in North American snakes. Journal of Herpetology 30:133–139.

Clark RW, Marchand MN, Clifford BJ, Stechert R and Stephens S (2011) Decline of an isolated timber rattlesnake (*Crotalus horridus*) population: interactions between climate change, disease, and loss of genetic diversity. Biological Conservation 144:886–891.

Connette GM and Semlitsch RD (2015) A multistate mark-recapture approach to estimating survival of PIT-tagged salamanders following timber harvest. Journal of Applied Ecology 52:1316-1324.

Cooch EG, Conn PB, Ellner SP, Dobson AP and Pollock KH (2012) Disease dynamics in wild populations: modeling and estimation: a review. Journal of Ornithology 152:485-509.

Cross PC, Drewe J, Patrek V, Pearce G, Samuel MD and Delahay RJ (2009) Wildlife population structure and parasite transmission: implications for disease management. Pages 9-29 *in* R. J. Delahay, G. C. Smith, and M. R. Hutchings, editors. Management of Disease in Wild Mammals. Springer Japan.

Dai A, Trenberth KE and Karl TR (1999) Effects of clouds, soil moisture, precipitation, and water vapor on diurnal temperature range. Journal of Climate 12: 2451-2473.

Dunlap KD and Church DR (1996) Interleukin-1β reduces daily activity level in male lizards, *Sceloporus occidentalis*. Brain, Behavior, and Immunity 10:68–73.

Durso AM, Willson JD and Winne CT (2011) Needles in haystacks: Estimating detection probability and occupancy of rare and cryptic snakes. Biological Conservation 144:1508–1515.

Faustino CR, Jennelle CS, Connolly V, Davis AK, Swarthout EC, Dhondt AA and Cooch EG (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.

Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL and Gurr SJ (2012) Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186-194.

Franklinos LH, Lorch JM, Bohuski E, Fernandez JR, Wright ON, Fitzpatrick L, Petrovan S, Durrant C, Linton C, Baláž V and Cunningham AA (2017) Emerging fungal pathogen *Ophidiomyces ophiodiicola* in wild European snakes. Scientific Reports 7:3844-3850.

Gelman A, Carlin, JB, Stern, HS and Rubin, DB (2004) Bayesian Data Analysis. CRC/Chapman and Hall, Boca Raton, FL.

Gibbons JW and Dorcas ME (2004) North American Watersnakes: A Natural History. Norman University of Oklahoma Press.

Grassly NC and Fraser C (2008) Mathematical models of infectious disease transmission. Nat Rev Micro 6:477-487.

Guthrie AL, Knowles S, Ballmann AE and Lorch JM (2016) Detection of snake fungal disease due to *Ophidiomyces ophiodiicola* in Virginia, USA. Journal of Wildlife Diseases 52:57–69.

Hileman E, Allender M, Bradke D, Faust L, Moore J, Ravesi M and Tetzlaff S (2017) Estimation of *Ophidiomyces* prevalence to evaluate snake fungal disease risk. Journal of Wildlife Management 82(1):173–181.

Kellner KF (2015) jagsUI: A Wrapper Around rjags to Streamline JAGS Analyses. R package version 1.3.1. 2015.

Kéry M and Schaub, M (2012) Bayesian Population Analysis Using WinBUGS: A Hierarchical Perspective. Academic Press, San Diego, California.

Kriger KM and Hero JM (2007) Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. Journal of Zoology 271:352–359.

Lachish S, Jones M and 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.

Layne JR and N Ford (1984) Flight distance of the queen snake, *Regina septemvittata*. Journal of Herpetology 18(4):496-8.

Lebreton JD, Burnham KP, Clobert J and David R (1992) Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. Ecological Monographs 62:67–118.

Lind AJ, Welsh Jr. HH and Tallmon DA (2005) Garter snake population dynamics from a 16-year study: considerations for ecological monitoring. Ecological Applications 15(1) 294-303.

Lind C, Moore IT, Akçay Ç, Vernasco BJ, Lorch JM and Farrell TM (2018) Patterns of Circulating Corticosterone in a Population of Rattlesnakes Afflicted with Snake Fungal Disease: Stress Hormones as a Potential Mediator of Seasonal Cycles in Disease Severity and Outcomes. Physiological and Biochemical Zoology 2018 91:2, 765-775.

Lorch JM, Knowles S, Lankton JS, Michell K, Edwards JL, Kapfer JM, Staffen RA, Wild ER, Schmidt KZ, Ballmann AE, Blodgett D, Farrell TM, Glorioso BM, Last LA, Price SJ, Schuler KL, Smith CE, Wellehan JFX Jr. and Blehert DS (2016) Snake fungal disease: an emerging threat to wild snakes. Philosophical Transactions of the Royal Society B: Biological Sciences 371.

Lorch JM, Lankton J, Werner K, Falendysz EA, McCurley K and Blehert DS (2015) Experimental infection of snakes with *Ophidiomyces ophiodiicola* causes pathological changes that typify snake fungal disease. mBio 6:1-9.

Martel A, Spitzen-van der Sluijs A, Blooi M, Bert W, Ducatelle R, Fisher MC, Woeltjes A, Bosman W, Chiers K, Bossuyt F and Pasmans F (2013) *Batrachochytrium salamandrivorans sp. nov.* causes chytridiomicosis in amphibians. Proceedings of the National Academy of Sciences of the United States of America, 110(38): 15325–15329.

Mazerolle JM (2016) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.0-4. http://CRAN.R-project.org/package=AICcmodavg .

McCallum H (2012) Disease and the dynamics of extinction. Philosophical Transactions fo the Royal Soceity B: Biological Sciences 367:2828-2839.

McClintock BT, Nichols JD, Bailey LL, MacKenzie DI, Kendall WL and Franklin AB (2010) Seeking a second opinion: uncertainty in disease ecology. Ecology Letters 13:659-674.

McCoy CM, Lind CM and Farrell TM (2017) Environmental and physiological correlates of the severity of clinical signs of snake fungal disease in a population of pigmy rattlesnakes, *Sistrurus miliarius*. Conservation Physiology 5:1-10.

Miller DAW, Talley BL, Lips KR and Campbell Grant EH (2012) Estimating patterns and drivers of infection prevalence and intensity when detection is imperfect and sampling error occurs. Methods in Ecology and Evolution 3:850-859.

Mushinsky HR, Hebrard JJ and Walley MG (1980) The role of temperature on the behavioral and ecological associations of sympatric water snakes. Copeia 4: 744-754.

Nelson RJ and Demas GE (1996) Seasonal changes in immunce function. The Quarterly Review of Biology 71:511–548.

Oldham C, Fleckenstein JL, Boys W and Price SJ (2016) Enhancing ecological investigations of snakes with Passive Integrated Transponder (PIT) telemetry. Herpetological Review 47: 385-388.

Paré JA, Sigler L, Rypien KL and Gibas CC (2003) Survey for the *Chrysosporium* anamorph of *Nanizziopsis vriesii* on the skin of healthy captive squamate reptiles and notes on their cutaneous fungal mycobiota. Journal of Herpetological Medical Surgery, 13:10-15.

Plummer M (2003) JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. http://citeseer.ist.psu.edu/plummer03jags.html

R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria

Rajeev S, Sutton DA, Wickes BL, Miller DL, Giri D, Van Meter M, Thompson EH, Rinaldi MG, Romanelli AM, Cano JF and Guarro J (2009) Isolation and characterization of a new fungal species, *Chrysosporium ophiodiicola*, from a mycotic granuloma of a black rat snake (*Elaphe obsoleta obsoleta*). Journal of Clinical Microbiology 47:1264–1268.

Sigler L, Hambleton S, Pare JA (2013) Molecular characterization of reptile pathogens currently known as members of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* complex and relationship with some human-associated isolates. Journal of Clinical Microbiology 51:3338–3357.

Steen, DA, Mcclure CJW, Brock JC, Rudolph DC, Pierce JB, Lee JR, Humphries WJ, Gregory BB, Sutton WB, Smith LL, Baxley DL, Stevenson DJ and Guyer C (2012) Landscape-level influences of terrestrial snake occupancy within the southeastern United States. Ecological Applications 22:1084–1097.

Strickler GS (1959) Use of the densiometer to estimate density of forest canopy on permanent sample plots. USDA. For. Ser. Res. Note PNW-180.

Sun L, Shine R, Debi Z and Zhengren T (2001) Biotic and abiotic influences on activity patterns of insular pit-vipers (*Gloydius shedaoensis*, Viperidae) from north-eastern China. Biological Conservation 97 (2001) 387-398.

Sutherland, WJ, Aveling R, Brooks TM, Clout M, Dicks LV, Fellman L, Fleishman E, Gibbons DW, Keim B, Lickorish F, Monk KA, Mortimer D, Peck LS, Pretty J, Rockström J, Rodríguez JP, Smith RK, Spalding MD, Tonneijck FH and Watkinson AR (2014) A horizon scan of global conservation issues for 2014. Trends in Ecology and Evolution 29:15–22.

Tetzlaf SJ, Ravesi MJ, Allender MC, Carter ET, DeGregorio BA, Josimovich JM and Kingsbury BA (2017) Snake fungal disease affects behavior of free-ranging massasauga rattlesnakes (*Sistrurus catenatus*) Herptological Conservation and Biology 12(3):624-634.

Venables WN, Ripley BD (2002) Modern Applied Statistics with S. Fourth Edition, New York: Springer.

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Graduate Teaching Assistant, University of Kentucky
Bahamas Pulsed Subsidies Project Manager, University of California, Davis
Biological Aid, United States Forest Service
Herpetological Field Technician, Natural History Museum of Los Angeles County
Seasonal Avian Field Ecologist, Smithsonian Migratory Bird Center
Stream Salamander Field Assistant, University of Montana

Scholastic and professional honors:

Awards

Forest and Natural Resource Sciences Graduate Student Award for Excellence
Wildlife, Fish and Conservation Biology Departmental Citation for Excellence

Grants

Chicago Herpetological Society Grant
Kentucky Academy of Sciences
The Wildlife Society-Kentucky Chapter
Association of Southeastern Biologists, Graduate Student Travel Award
University of Kentucky, Department of Forestry, Graduate Student Travel Award
Herpetologists' League, E.E. Cummings Grant – Honorable Mention
American Society of Ichthyologists and Herpetologists, Gaige Award
University of Kentucky, Eller and Billings Student Research Award
Kentucky Society of Natural History, Student Research Grant
University of Kentucky, Department of Forestry, Graduate Student Travel Award
Northern California Herpetological Society Research Grant
Sigma Xi Grants-in-Aid of Research Program
University of California, Davis President's Undergraduate Fellowship

Professional publications:

Wetzel, W.C., R.M. Screen, I. Li, J.M. McKenzie, K.A. Phillips, M. Cruz, A. Greene, E. Lee, N. Singh, C. Tran, and L.H. Yang. 2016. Ecosystem engineering by a gall-forming wasp indirectly suppresses diversity and density of herbivores on oak trees. Ecology.

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