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EFFECTS OF SNAKE FUNGAL DISEASE ON THE SURVIVAL AND GROWTH OF THE QUEENSNAKE (*REGINA SEPTEMVITTATA*)

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EFFECTS OF SNAKE FUNGAL DISEASE ON THE SURVIVAL AND GROWTH OF THE QUEENSNAKE (*REGINA SEPTEMVITTATA*)

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 Agriculture, Food and Environment
at the University of Kentucky

By

Andrew Jerome Ibach

Lexington, Kentucky

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2022

ABSTRACT OF THESIS

EFFECTS OF SNAKE FUNGAL DISEASE ON THE SURVIVAL AND GROWTH OF THE QUEENSNAKE (*REGINA SEPTEMVITTATA*)

Having only emerged as a threat to snakes in 2006, relatively little is known of snake fungal disease's (SFD) impacts on demographic processes, particularly survival and growth. I used data from an extensive capture-mark-recapture study to examine survival and growth in central Kentucky Queensnake (*Regina septemivittata*) populations. I found that diseased snakes and healthy snakes possessed similar monthly survival estimates (SFD positive 0.9687, 95% CI 0.8444 to 0.9944; SFD negative 0.8735, 95% CI 0.7518 to 0.9402) and that disease state transition probability from SFD negative to SFD positive, and SFD positive to SFD negative were also similar (N-P 0.4807, 95% CI 0.3395 to 0.6251; P-N 0.3461 95% CI 0.2523 to 0.4536). Additionally, I found that diseased snakes exhibited heightened growth rates (1.12 mm/month females, 0.91 mm/month males) compared to healthy snakes (0.73 mm/month females, 0.59 mm/month males).

KEYWORDS: Snake Fungal Disease, SFD, Growth, Survival, Queensnake

Andrew Jerome Ibach

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INTRODUCTION

Fungal pathogens are an increasingly widespread problem for wildlife (Fisher et al. 2012). Examples of emerging fungal pathogens impacting wildlife include *Pseudogymnoascus destructans* (Pd), which leads to white nose syndrome (WNS) in bats (Frick et al. 2010), and *Batrachochytrium dendrobatidis* (Bd) that causes chytridiomycosis in amphibians (Berger et al. 1998). White nose syndrome has resulted in the population decline of over 75% within infected North American bat populations from 2006-2008 (Blehert et al. 2009). *Batrachochytrium dendrobatidis* causes epidermal infections of the keratinized tissues of amphibians that leads to chytridiomycosis and mortality (Berger et al. 1998). *Batrachochytrium dendrobatidis* infection has been documented in hundreds of amphibian species and is responsible for the largest, disease-associated loss of biodiversity ever recorded (Fisher et al. 2009, Fisher et al. 2012).

Snake fungal disease (SFD or Ophidiomycosis) is a recently emerging disease characterized by severe skin infections in snakes (Allender et al. 2015, Lorch et al. 2016). The disease is caused by the fungus *Ophidiomyces ophidiicola* (*Oo*) (Allender et al. 2015, Lorch et al. 2015, 2016). Snake fungal disease was first recognized as a serious threat to wild snake populations in 2006 (Clark et al. 2011). To date, most research on SFD has focused on identifying the causative agent, the geographic scope and host range, and the spatial and temporal dynamics of infection (Lorch et al. 2016). *Ophidiomyces ophidiicola* is known to reproduce asexually via anthroconidia and aleurioconidia; however, sexual reproduction has yet to be observed (Rajeev et al. 2009, Sigler et al. 2013, Allender et al. 2015). *Ophidiomyces ophidiicola* can grow successfully on a variety of carbon and nitrogen sources from dead material, persist in low matric pressure soil (-5MPa), various

pH levels (pH 5-11), and high sulfur concentrations (Allender et al. 2015). One study found that *Oo* is most likely to be found in soil from snake hibernacula but soil with an active microbial community exhibited no growth of *Oo* (Campbell et al. 2021).

Snake fungal disease has been documented throughout North America and Eurasia (Lorch et al. 2016, Franklinos et al. 2017, Haynes et al. 2021, Sun et al. 2021). Over 30 snake species from 5 different families are known to be afflicted with SFD (Lorch et al. 2016) suggesting a broad host range. Burbrink et al. (2017) found no significant phylogenetic or ecological traits to predict host susceptibility to *Oo*. Susceptibility to SFD may also increase with rising global temperatures since *Oo* seems to grow best at a temperature of 25° C, with growth inhibition occurring at 7° C and growth reduction occurring below 14° C and above 35° C (Allender et al. 2015). Environmental conditions, such as air temperature and seasonality, correlate with the severity of clinical signs and SFD prevalence (McCoy et al. 2017, McKenzie et al. 2018). Infection with *Oo* not only causes the formation of skin lesions but also leads to increased basking, rate of shedding, and anorexia in wild snakes (Lorch et al. 2015, Clark et al. 2011).

While SFD cases have been confirmed in North American snakes as far back as 1945 (Lorch et al. 2021), a lack of historical disease data has made it challenging to determine whether *Oo* is a recent arrival to North American or native and emerging in situ. Recently, Ladner et al. (2022) examined the genomes of 82 *Oo* strains to determine the pathogen's history in eastern North America. *Ophidiomyces* strains from eastern North America formed a clade distinct from European strains, and molecular dating indicated that these clades diverged too recently (~2,000 years ago) for transcontinental

dispersal of *Oo* to have occurred via natural snake movements across Beringia. Ladner et al. (2022) suggest that *Oo* has been introduced multiple times to North America, and molecular dating indicates that several of these introductions occurred within the last few hundred years. Thus, it appears that *Oo* has been introduced to North American snake populations, with the most likely mode of introduction being through the pet trade. Indeed, globalization of trade and environmental alteration have been cited as two of the most likely influential factors to the increase in emergence of fungal pathogens (Fisher et al. 2012).

Despite the recent research on SFD, the effects of SFD on wild snake populations remains uncertain. Given the various impacts on behavior and physiological processes it is logical that SFD infection may lead to population declines in wild snake populations. Indeed, some previous studies have documented mortality on afflicted, wild snakes (Allender et al. 2011; Franklinos et al. 2017; Rajeev et al. 2009), yet other researchers have found that SFD related mortality rates may be overstated (Davy et al. 2021; McKenzie et al. 2021). One study observed heightened surface activity and decreased emigration rates in SFD afflicted snakes; however, found no impact of SFD on survival in a single field season (McKenzie et al. 2021). The collection of adequate samples to make inferences regarding population status or demographic rates is likely causing this uncertainty. Thus, research on population-level impacts is needed to fully understand the impacts of SFD on wild snake populations.

Passive Integrated Transponder (PIT) tag telemetry is a prime example of a method that can improve capture and recapture rates of snakes and, thus, provide researchers with adequate samples to investigation population-level impacts of SFD

(Connette and Semlitsch 2012; Oldham 2016). Using this method of injecting captured individuals with a unique transponder and then scanning a site regularly with a transceiver has been shown to increase detection and recapture of individuals when compared with visual survey methods (Oldham 2016). McKenzie et al. (2021) marked and processed 526 individual snakes across six study sites in central Kentucky (USA) over one field season and estimated the probability of PIT tag detection for all snakes within range of the PIT tag reader as 0.41 (95% credible interval [CRI] = 0.33–0.52). Previous Queensnake studies have found lower recapture rates such as in Branson and Baker (1974) where they recorded recapture rate of 18.6% during visual surveys. Oldham (2016) documented a significant difference in Queensnake recapture rates between visual surveys (24.24%) and PIT telelmetry surveys (66.66%); this research further illustrates the utility of this method to study population-level consequences of SFD.

My research will build on the work of McKenzie et al. (2021) to further evaluate the impacts of SFD on snake populations by examining the effects of SFD on long-term survival and growth in Queensnakes (*Regina septemvittata*). My research objective is to model individual survival and growth of the Queensnake as related to SFD. My research question is: do snakes afflicted with Snake Fungal Disease (SFD) exhibit altered growth rates and survivorship? I hypothesized that snakes afflicted with SFD would exhibit decreased survivorship rates and reduced growth rates due to the anorexia observed in many SFD cases (Lorch et al. 2015). In order to evaluate my hypothesis, I will use multistate capture-mark-recapture (CMR) models to compare how survivorship dependent on disease state can change at each capture occasion as well as the probability of transition from one disease state or group to another (Buchanan and Skalski 2010).

These capabilities allow us to compare SFD afflicted and non-afflicted individuals and take into account that disease state is not constant. Second, I will use CMR data and hierarchical growth models to evaluate the effects of SFD on growth rates. The combination of modern growth modeling methods and new mark recapture techniques allow for more effective research of snakes that have previously circumvented investigators attempts to better understand concepts such as growth. In this way, the recaptures and multiple measurements required to model growth of individuals can be enhanced thereby increasing the likelihood of effectively modeling growth and the robustness of the model itself.

METHODS

Study Sites

This study was conducted within the Inner Bluegrass Region of Kentucky, USA. This region of Kentucky is known for its karst topography, rolling hills with pastures and forest, and generally mixed land use ranging from agricultural to urban areas. Surveys were conducted from 2013-2019 at 6 streams (Elkhorn, Glenns, Little Hickman, Hickman-Elias, Otter, and Tate) throughout 4 counties (Fayette, Jessamine, Madison, and Woodford) that are part of the Kentucky River basin. I continued surveying the same stream reaches at these sites that were initially designated in McKenzie et al. (2021) and Leuenberger et al. (2019). All of these stream reaches range in length from 293m to 1005m, primarily possess limestone bedrock substrate, and contain sufficient rocky cover, which provide refuges for snakes.

Study Species

The species of interest in this study was the Queensnake (*Regina septemvittata*). The Queensnake is an aquatic natricine species, generally found in shallow streams with plenty of rock cover (Conant 1960). The diet of *R. septemvittata* consists of recently molted crayfish making them a dietary specialist (Branson and Baker 1974). The ovoviviparous *R. septemvittata* has been reported to give birth to between 8 and 17 offspring in one litter and female fecundity increases with snout to vent length (SVL) (Branson and Baker 1974). Male Queensnakes grow to a size of ~642 mm SVL and may reach sexual maturity as soon as their second year, while females grow to ~695 mm SVL and reach sexual maturity at ~344 mm SVL (Branson and Baker 1974). I determined size at birth of the Queensnake by capturing gravid females, holding them in separate enclosures in the lab until parturition and then measuring the neonates on their first day of life. This process provided me with measures from 22 day one neonates.

Field Sampling

The data used in the study was collected over the course of 418 visual and PIT telemetry surveys at the same six streams as in McKenzie (2018) and Leuenberger et al. (2019). Surveys were conducted between the months of April and October by a variety of collectors from 2013-2019. During each survey, I scanned the entire stream reach with a Biomark HPR Plus portable PIT tag reader and Biomark BP Portable Antenna Plus. This tag reader and antenna combination enhanced my ability to recapture individuals and also provided me with precise geographic coordinates of where each snake was captured. Suitable cover objects were also visually searched for unmarked *R. septemvittata*. When

located, I placed snakes individually in snake bags to await processing. I determined snout to vent length (SVL), total length (TTL), mass, and sex for each snake, in addition to taking photographs of each individual. Newly captured individuals were implanted with a PIT tag with a unique identification number to aid in future recapture and identification. I visually inspected each snake for clinical signs of snake fungal disease. Clinical signs recorded include nodules, ulcers, regional edema, local scale edema, crust with *stratum corneum,* and crust without *stratum corneum* (McKenzie et al. 2021). When these clinical signs were identified, they were photographed and noted on a diagram of each snake. Additionally, I swabbed each snake for *Oo* using a sterile polyester swab dipped in sterile water. If the snake expressed any clinical signs indicative of SFD, I swabbed the clinical sign in question with a swab 5 times. I swabbed snakes with no clinical signs 5 times on the dorsum at mid-body and photographed this region. I placed used swabs inside labeled, sterile, micro-centrifuge tubes and froze them in a -40°C freezer before sending them to the USGS National Wildlife Health Laboratory for qPCR analysis to determine fungal load.

Determining Disease Status

I used a two-fold approach to determine disease status. This method combined visual analysis of clinical signs determined by the presence or absence of various clinical signs indicative of SFD (local (scale) edema, regional edema, crust with *stratum corneum*, crust without *stratum corneum*, ulcer, and nodule) from photos, and the number of fungal DNA copies determined during qPCR analysis (McKenzie et al. 2021). qPCR analysis was performed at the USGS National Wildlife Health Laboratory by Dr. Jeffrey

Lorch. Previous work done by McKenzie et al. (2021) and Bohuski et al. (2015) developed the protocols to quantify fungal load of *O.o.* and determined that 41.5 copies of fungal DNA was the minimum number needed for detection in the PCR assay. In order to designate a snake as SFD positive it had to possess 41.5 or more copies of fungal DNA and at least 1 clinical sign indicative of SFD, or less than 41.5 copies of fungal DNA and 2 or more clinical signs indicative of SFD. To be classified as SFD negative a snake had to have no clinical signs regardless of the number of fungal DNA copies. See McKenzie et al. (2021) for more details on sampling procedures. All research was compliant with University of Kentucky IACUC protocol (2013-1073). I obtained permits from the Kentucky Department of Fish and Wildlife Resources (SC1511017, SC1611043, SC1611136).

Survival Analysis

I selected a multistate mark recapture model, run in Program Mark (as described in Program Mark: A Gentle Introduction by E. G. Cooch and G. C. White; available at http://www.phidot.org/software/mark/docs/book/), to further analyze the data collected and assess impacts of disease state, time, and sex on survival and recapture rates of *R. septemvittata*. This model builds onto the 'typical' mark recapture model:

$$\{\varphi_{t*x}\ p_{t*x}\}$$

where φ_{t*s} symbolizes survival from (φ) dependent on time (t) and a given variable (x) such as sex, and p_{t*s} symbolizes recapture probability (p) dependent on time (t) and a given variable (x) such as sex. Estimates of survival probability describe the likelihood of an individual that is alive at time *i* will still be alive at time *i+1,* while estimates of

recapture probability describe the likelihood that an individual captured at time *i* will be recaptured at time *i+1*. The selected multistate model builds on this by allowing for a state or group to be assigned at each capture, and by adding a parameter to estimate transition probability from one state or group to another to the model.

$$\{S^{g}_{t*x}\, p^{g}_{t*x}\, \psi^{g}_{t*x}\}$$

In our case S^{g}_{t*x} represents survival (*S*) dependent on disease state (*g*), time (*t*), and sex (*x*), p^{g}_{t*x} represents recapture probability (*p*) dependent on disease state (*g*), time (*t*), and sex (*x*), and ψ^{g}_{t*x} represents probability to transition from SFD positive to SFD negative (ψ) dependent on disease state (*g*), time (*t*), and sex (*x*). This allowed us to compare survivorship and recapture probability of SFD positive and negative individuals as well as estimate probability of transitioning from one disease state to another. One potential downfall of this model is that it does not take into account emigration, which may impact survival and transition probability estimates. Additionally, this model assumes that survival from one capture occasion to another is not dependent on the state at the second capture. This means that the model assumes that all state transition happens directly after survival from one point in time to another.

Disease state, sex, and time were selected as parameters of interest for a variety of reasons. Disease state was primarily selected to answer the central question of whether SFD may impact survival since high survival probabilities have been reported within one field season at these sites in the past (McKenzie et al. 2021). I was also interested in assessing whether recapture rates varied between disease states due to observations of increased surface activity of SFD afflicted snakes (McKenzie et al. 2021). Sex was added to prospective models to assess whether males and females exhibited any differences in

survival, recapture, or disease state transition due to differences in body size and activity of males and females. Time, on a monthly scale, was added to prospective models to assess whether survival, recapture, and disease state transition varied month to month since seasonal trends have been previously reported within one field season at these sites (McKenzie et al. 2021).

The encounter matrix for this model was created with 18 capture occasions, each representing a month of surveying from May of 2016-October of 2018 at our Little Hickman Creek, Elkhorn Creek, and Glenns Creek sites (Table 5). These sites and years were selected for this model since survey effort and disease state determination were consistent. Captures were marked with either an "N" (SFD Negative) or "P" (SFD Positive), depending on their disease state within that month, and a "0" if the individual was not captured that month. In the event that an individual was determined both SFD positive and negative within a single month, its monthly disease status was recorded as positive. Using this multistate recapture model, with monthly disease state, allowed us to assess recapture and survival probability estimates of SFD positive and negative states while also taking into account the fact that disease state was not a constant. This model also allowed us to assess probability of a shift from SFD positive to negative, and SFD negative to positive.

I used a step down approach (Lebreton et al. 1992, Muncy et al. 2014) to first investigate the impact of disease state, sex, and time on recapture probability before doing the same for survival probability, and finally disease state transition probability. The best model was selected using AIC_c values. I evaluated goodness of fit on a fully time dependent model using the Median c Test in Program Mark (as described in

Program Mark: A Gentle Introduction by E. G. Cooch and G. C. White; available at http://www.phidot.org/software/mark/docs/book/), and AIC_c values were adjusted to $QAIC_c$ values when $\hat{c} > 1$.

Growth Analysis

I estimated growth of *R. septemvittata* by fitting a von Bertalanffy growth model (Fabens 1965) in a hierarchical Bayesian framework modified from Eaton and Link (2011) to incorporate variation among individuals as a function of covariates (SFD status, sex, site), and distinguish natural variation (among and within individuals) from measurement error. Measurement error commonly occurs with snakes because they have long, muscular vertebral columns, and when stretched out, measurements can vary depending on how relaxed a snake is (Madsen and Shine 2001, Luiselli 2005).

The Von Bertalanffy growth model describes population growth rates over time, or more specifically, the mean size of individuals in a population at a particular age (Fabens 1965) with the following equation:

$$S(A) = S_\infty (1 - be^{-kA})$$

Where *'A'* represents age (in days), '$S(A)$' is the mean population size at age *A,* *'*$S(\infty)$*'* is the asymptotic size, '*b'* is a constant relating birth size to asymptotic size, and '*-k'* is a growth rate coefficient.

Not all individuals will have the same asymptotic size, and individual growth patterns will not necessarily align well with the pattern described by *S(A)*. Thus, individual growth can be modeled as a Gamma process (a random process with independent increments from the gamma distribution), with a mean given by the Von

Bertalanffy model above, and I refer readers to Eaton and Link (2011) for details on the properties of the Gamma distribution as it relates to growth models. I model size (length) of an individual at age A as:

$$s_i(A) = \eta_i(S(A))$$

Where 'si' refers to the true (unknown) size of individual *i* and 'η_i' is a feature of the gamma distribution for individual *i* (Eaton and Link 2011). If I define p = λ, where p and λ are shape and scale parameters, respectively, of the gamma distribution, then the individual sizes at age A are given by:

$$_{si}(A) \sim \Gamma(\lambda S(A), \lambda)$$

As with sizes (S(A)), population-level growth increments also follow a Gamma process. The growth increments at each time step for each individual is given by:

$$I_{i,t} = \eta_i(S(A_{i,t})) - \eta_i(S(A_{i,t-1})) \sim \Gamma(\lambda[S(A_{i,t}) - S(A_{i,t-1})], \lambda)$$

For simplicity, $S(A_{i,t}) - S(A_{i,t-1})$ can be defined as $I^*_{i,t}$ (see Eaton and Link 2011) thus:

$$I_{i,t} \sim \Gamma(\lambda I * i, t, \lambda)$$

$$I_{i,t} \sim \Gamma(\lambda I^* i, t, \lambda)$$

Individual Captures can be defined as:

$$S(A_{i,t}) = S(A_{i,t-1}) + (S_\infty - S(A_{i,t-1}))(1 - e^{-k\Delta t})$$
$$I^*_{i,t} = S(A_{i,t}) - S(A_{i,t-1})$$

To account for measurement error, I model body length measurement h_{ij} for individual *i* at capture occasion *j* in our primary data set as:

$$h_{ij} = H_i(A_{ij}) + \varepsilon R_{ij}$$

where R is defined as a recapture and εR_{ij} are independent, normally distributed measurement errors, with a mean of zero and common variance σ^2_R (i.e., σ^2_R is the measurement error variance). I parameterized our model for asymptotic size as:

$$S(\infty) = \alpha_0 + \alpha_1 * Sex_i$$

I incorporated the effect of SFD on growth rate using a binary indicator for disease status (0 = SFD negative, 1 = SFD positive). Snakes that tested positive for SFD were classified as SFD-positive for all subsequent captures within that calendar year. I parameterized growth rates as:

$$log(k) = \beta_0 + \beta_1 Sex_i + \beta_2 SFD_{i,t}$$

I assigned uniform priors on [100mm,600mm] to $S(A_i1)$, the expected size range of an animal at the unknown age of first capture for animal *i*, and diffuse mean zero normal priors (SD=1000) to the logarithms of a and k; I denote this prior by $N(0, 1000^2)$. The measurement error variance σ^2_R was assigned a diffuse inverse gamma distribution (scale = shape = 0.001), denoted IG(0.001, 0.001). I implemented the model in a Bayesian framework using the software NIMBLE (de Valpine et al. 2017) as called from R version 4.0.2 (R Core Team 2020) using the package 'nimble' (de Valpine et al. 2020). I sampled from the posterior distribution using 3 independent chains of 120,000 iterations each after a burn-in period of 20,000 iterations, and thinned chains by a factor of 50 to base inference on 6,000 samples from the posterior distribution. To assess MCMC convergence I visually inspected trace plots for each monitored parameter, and I calculated the Gelman-Rubin statistic (Gelman and Rubin 1992) using the R package "coda" (Plummer et al 2006) where values below 1.1 indicate convergence; I observed no evidence for lack of convergence (all $\hat{R} < 1.1$ and history plots appeared well-mixed with no trends). Unless indicated otherwise, posterior distributions are summarized as median (0.025 quantile – 0.975 quantile).

RESULTS

Survival Analysis

I created capture histories from the 214 *R. septemvittata* individuals captured between May of 2016 and October of 2018. These consisted of 447 individual captures, and individuals captured during this time represented an approximately 1:1 sex ratio. The goodness of fit testing found $\hat{c}$ = 1.4350381 and thus $QAIC_c$ values were used in place of AIC_c values. Our top model ($\{S_{\cdot}^{g}\ p_t\ \psi_{\cdot}^{g}\}$) indicated state specific survival, time specific recapture probability, and state specific transition (Model weight 0.76918; Table 1). The survival probability mean estimates generated by the selected multistate recapture model (Table 2) indicate no significant difference in survival between SFD negative *R. septemvittata* (0.8735, 95% CI 0.7518 to 0.9402; Fig. 1) and SFD positive individuals (0.9687, 95% CI 0.8444 to 0.9944; Fig. 1), given the fact that the 95% confidence intervals overlap. Disease state transition probability estimates indicate no significant difference in probability of *R. septemvittata* transitioning from SFD negative to positive (0.4807, 95% CI 0.3395 to 0.6251; Fig. 2) than from SFD positive to negative (0.3461 95% CI 0.2523 to 0.4536; Fig. 2), with 95% confidence intervals overlapping. Monthly recapture probability estimates showed a cyclical pattern with generally higher values in the spring and summer decreasing through fall. This pattern was only broken with an increased recapture probability in September of 2016 (Fig. 3). With this said, recapture probability did not significantly differ between all months with often overlapping 95% confidence intervals.

Growth Analysis

I collected 625 *R. septemvittata* growth increments from 672 individuals, between the summer of 2013 and the fall of 2019. These consisted of 1295 individual captures, with the number of captures per individual ranging from one to eleven (Table 4). The individuals captured exhibited an approximately 1:1 sex ratio. Using the 625 *R. septemvittata* growth increments I was able to create the growth curves below (Fig. 5).

Growth rates and asymptotic size differed by disease state and sex respectively (Fig. 4). Female *R. septemvittata* reach higher asymptotic sizes (~500mm SVL) than males (~460mm SVL; Fig. 5). Females grew to the minimum size of sexual maturity (344mm SVL) previously reported (Branson and Baker 1974) within their first year of life. Uninfected female (0.73 mm/month) and male *R. septemvitatta* (0.59 mm/month; Fig. 5) grew faster than infected female (1.12 mm/month) than male *R. septemvitatta* (0.91 mm/month; Fig. 5). The standard deviation of the measurement error term (epsilon) was estimated as to be 18.63 mm (95% CI 16.60 to 20.98).

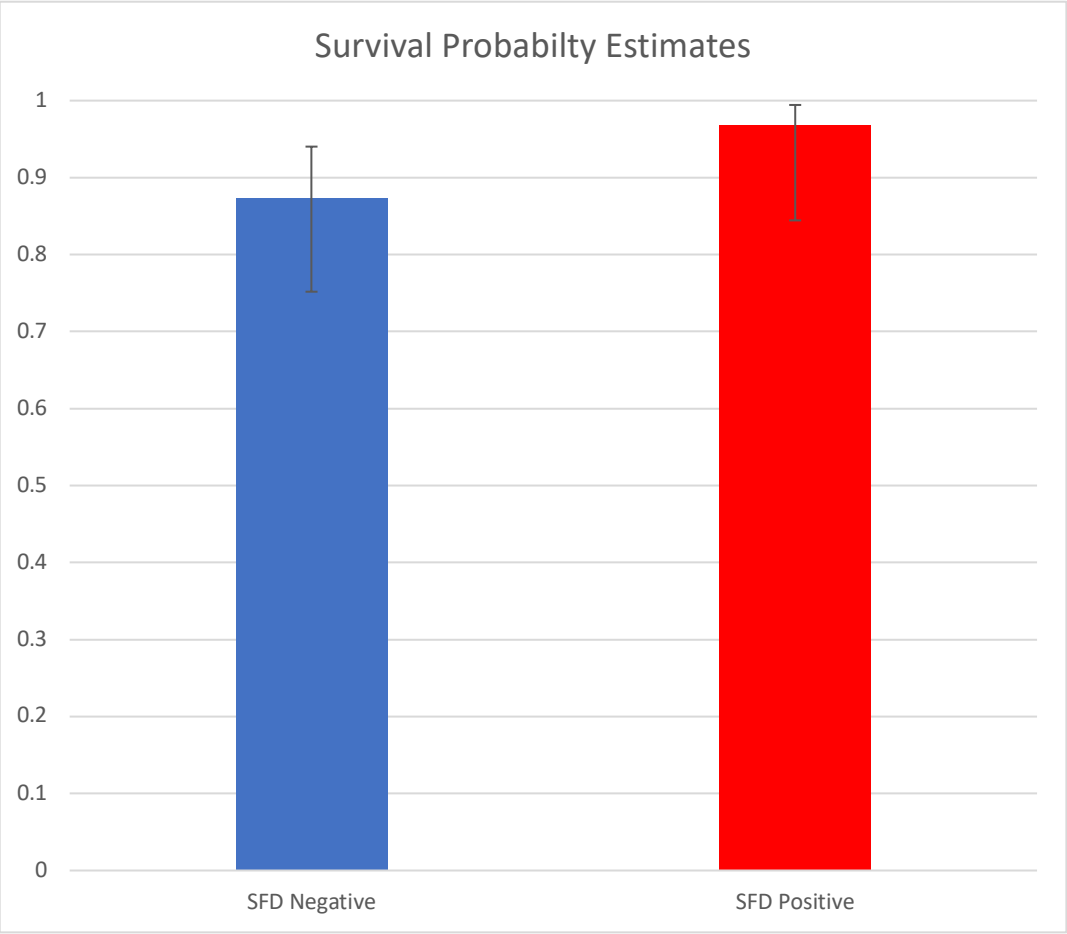

Figure 1: Monthly Survival probability estimates for SFD positive and negative *R. septemvittata*. Error bars represent 95% confidence intervals.

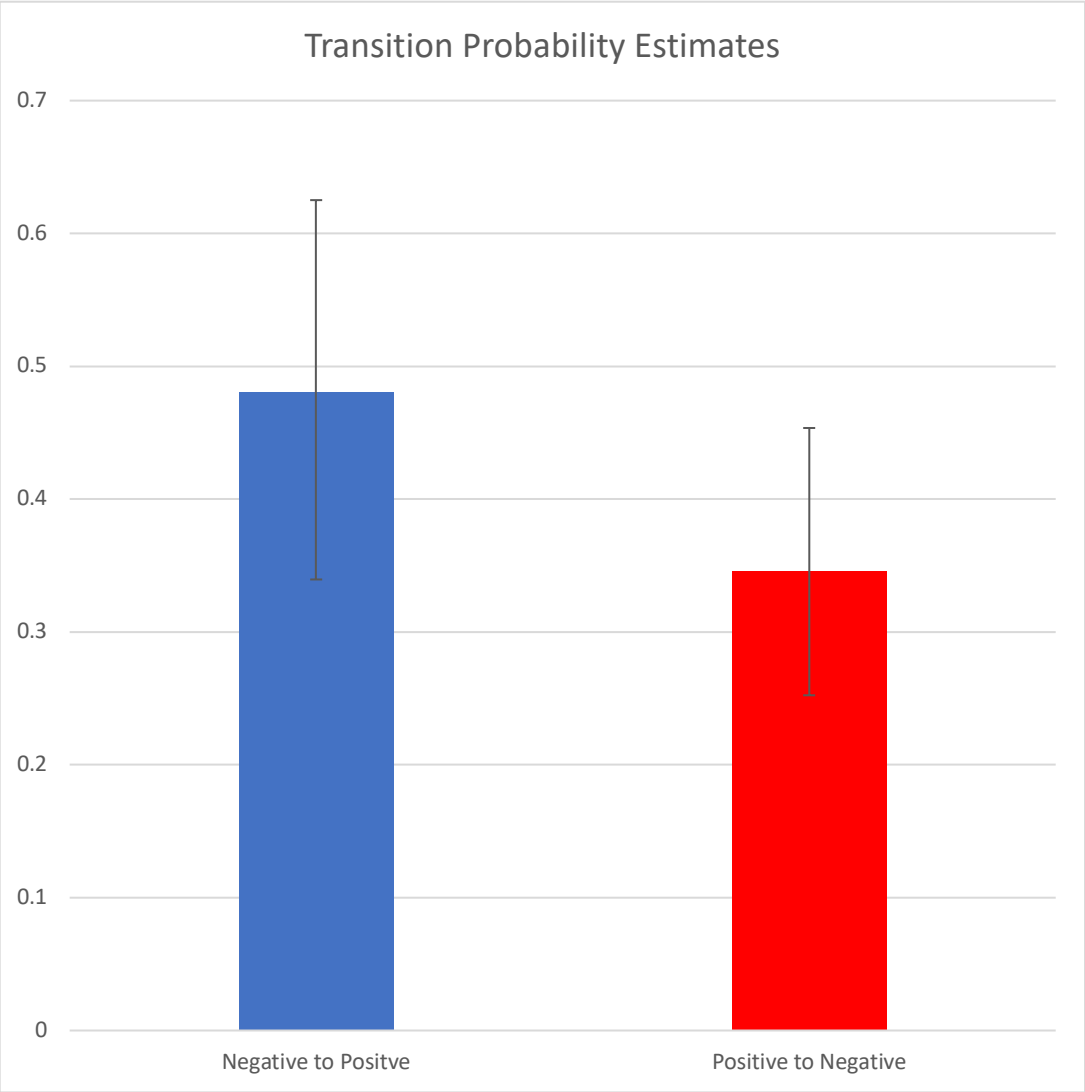

Figure 2. Disease state transition probability of *R. septemvittata* transitioning from SFD positive to negative, and SFD negative to positive. Error bars represent 95% confidence intervals.

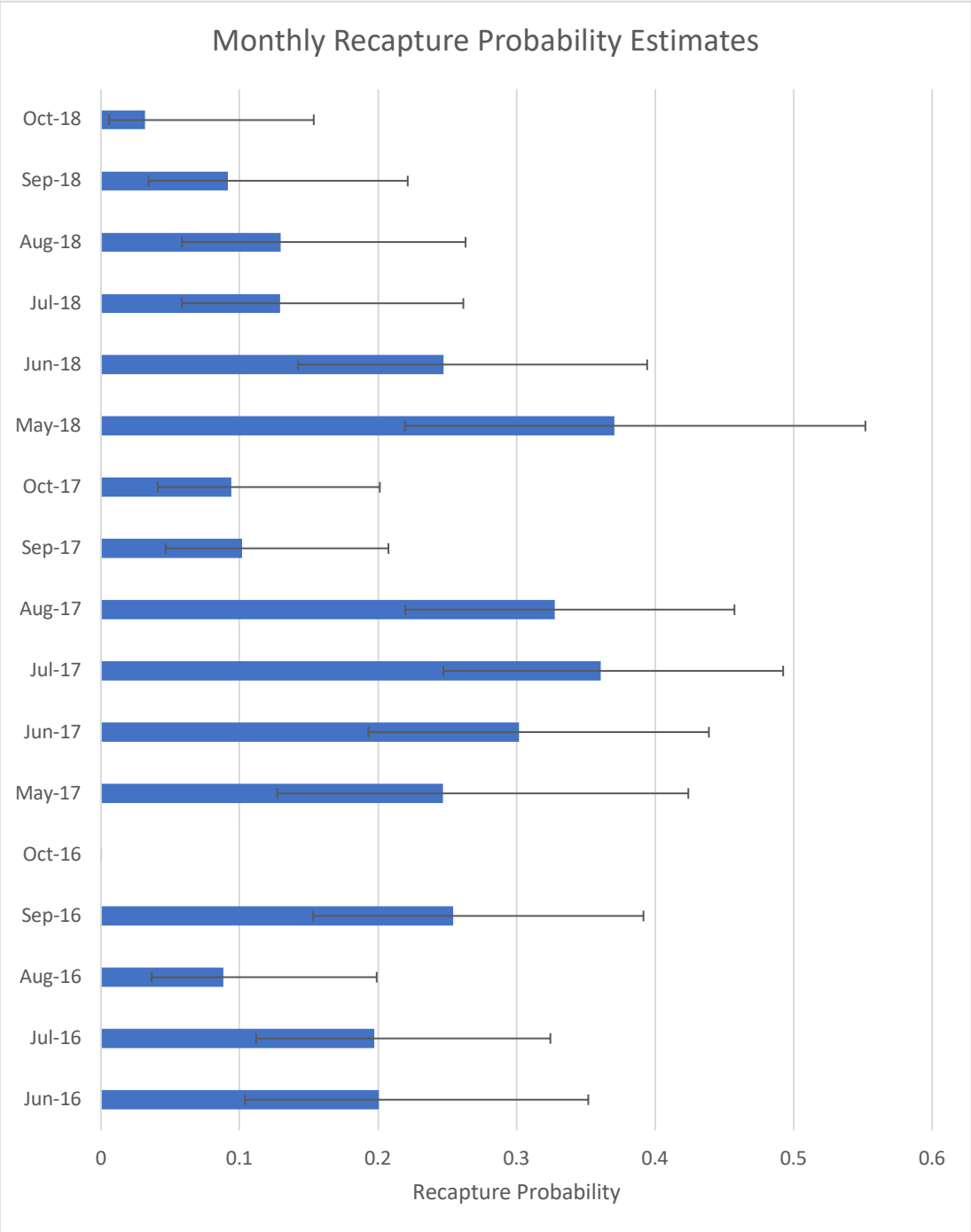

Figure 3: Monthly recapture probability estimates of SFD negative and positive *R. septemvittata.* Error bars represent 95% confidence intervals.

Table 1: Multistate CMR model set analyzing the impact of disease state, time, and sex on survival probability, recapture probability, and disease state transition probability. S = survival, p = recapture probability, Psi = transition probability, state = disease state, time = monthly intervals, sex = male and female. $QAIC_c$ values were calculated from AIC_c values with $\hat{c}$ = 1.4350381 value calculated from goodness of fit testing

Model	QAICc	Delta QAICc	AICc Weights	Number of Parameters	QDeviance
{S(state)p(time)Psi(state)}	1188.7659	0	0.76918	21	764.3276
{S(.)p(time)Psi(.)}	1191.1818	2.4159	0.22984	19	771.1378
{S(time)p(time)Psi(state)}	1202.6585	13.8926	0.00074	35	746.2629
{S(time)p(time)Psi(.)}	1205.3934	16.6275	0.00019	34	751.3525
{S(state)p(state*time)Psi(state)}	1208.9538	20.1879	0.00003	38	745.4249
{S(time)p(.)Psi(.)}	1209.3859	20.62	0.00003	19	789.3419
{S(time)p(state*time)Psi(state)}	1224.9262	36.1603	0	52	726.6722
{S(time)p(time)Psi(time)}	1230.9975	42.2316	0	50	737.8546
{S(state)p(.)Psi(.)}	1233.2373	44.4714	0	4	844.8827
{S(.)p(state)Psi(.)}	1233.3495	44.5836	0	4	844.9948
{S(state)p(state)Psi(state)}	1233.4297	44.6638	0	6	840.9747
{S(.)p(.)Psi(state)}	1233.4725	44.7066	0	4	845.1178
{S(sex)p(.)Psi(.)}	1238.472	49.7061	0	4	850.1173
{S(.)p(.)Psi(sex)}	1238.4744	49.7085	0	4	850.1197
{S(.)p(sex)Psi(.)}	1238.5344	49.7685	0	4	850.1797
{S(sex)p(sex)Psi(sex)}	1242.3803	53.6144	0	6	849.9252
{S(state*sex)p(state*sex)Psi(state*sex)}	1243.8782	55.1123	0	12	838.8951
{S(.)p(.)Psi(time)}	1256.7331	67.9672	0	19	836.689
{S(state)p(state*time)Psi(state*time)}	1268.7573	79.9914	0	69	724.8698
{S(state*time)p(state*time)Psi(state*time)}	1323.9353	135.1694	0	96	698.4596

Table 2: Output of parameter estimates, standard error, and 95% confidence intervals for the top model. The top model had disease state dependent survival (1-2), time (monthly) dependent recapture probability (3-19), and disease state dependent disease state transition (20-21).

Standard Error and Confidence Intervals Corrected for c-hat = 1.4350381				
Real Function Parameters of {S(state)p(time)Psi(state)}				
			95% Confidence Interval	
Parameter	**Estimate**	**Standard Error**	**Lower**	**Upper**
1:S N:SFD Negative	0.8734836	0.0464494	0.7518107	0.940248
2:S P:SFD Positive	0.9686667	0.0269431	0.8444005	0.994354
3:p	0.2005754	0.0631177	0.1039366	0.3517915
4:p	0.1974873	0.0540618	0.1119818	0.3244292
5:p	0.0885262	0.0386797	0.0365682	0.1990558
6:p	0.2544293	0.0614162	0.1532023	0.3916096
7:p	0	0	0	0
8:p	0.2468019	0.0767628	0.1272921	0.4240014
9:p	0.3019659	0.0636723	0.1930952	0.4388365
10:p	0.360797	0.0637387	0.2471995	0.4924484
11:p	0.3275511	0.0615898	0.2197129	0.4572983
12:p	0.1017748	0.0390709	0.0467373	0.2075154
13:p	0.0941123	0.0385482	0.041066	0.2012965
14:p	0.3704617	0.0878463	0.2195133	0.5518211
15:p	0.2473512	0.0649192	0.1423063	0.3942902
16:p	0.1291329	0.0499708	0.0584377	0.261591
17:p	0.1296181	0.0503598	0.0584557	0.2631952
18:p	0.0914244	0.044037	0.0343749	0.2214422
19:p	0.0318458	0.026868	0.0059257	0.1536238
20:Psi N to P	0.4807376	0.074927	0.3395302	0.6250908
21:Psi P to N	0.3460998	0.0519495	0.2523466	0.4535546

Table 3: Output of beta estimates, standard error, and 95% confidence intervals for the top model. The top model had disease state dependent survival (1-2), time (monthly) dependent recapture probability (3-19), and disease state dependent disease state transition (20-21).

Standard Error and Confidence Intervals Corrected for c-hat = 1.4350381				
SIN Link Function Parameters of {S(state)p(time)Psi(state)}				
			95% Confidence Interval	
Parameter	**Beta**	**Standard Error**	**Lower**	**Upper**
1:S N:SFD Negative	0.8434888	0.1397265	0.5696248	1.1173528
2:S P:SFD Positive	1.2148969	0.1546532	0.9117766	1.5180172
3:p	-0.6420634	0.1576242	-0.9510068	-0.33312
4:p	-0.6497978	0.1357983	-0.9159624	-0.3836332
5:p	-0.9665801	0.1361684	-1.2334702	-0.69969
6:p	-0.5133995	0.1410118	-0.7897826	-0.2370164
7:p	-1.5707964	0.1943667	-1.9517551	-1.1898377
8:p	-0.5310004	0.1780419	-0.8799624	-0.1820384
9:p	-0.4072309	0.1386862	-0.6790559	-0.1354059
10:p	-0.2821341	0.1327246	-0.5422744	-0.0219938
11:p	-0.3521299	0.1312319	-0.6093444	-0.0949154
12:p	-0.9214023	0.1292231	-1.1746795	-0.6681251
13:p	-0.9471859	0.1320214	-1.2059479	-0.6884239
14:p	-0.2620661	0.1819033	-0.6185965	0.0944643
15:p	-0.5297269	0.1504596	-0.8246278	-0.234826
16:p	-0.8356525	0.1490124	-1.1277168	-0.5435882
17:p	-0.8342066	0.1499325	-1.1280744	-0.5403388
18:p	-0.9564515	0.1527939	-1.2559276	-0.6569754
19:p	-1.2119664	0.1530161	-1.511878	-0.9120548
20:Psi N to P	-0.0385344	0.1499652	-0.3324663	0.2553975
21:Psi P to N	-0.3128803	0.1092005	-0.5269133	-0.0988473

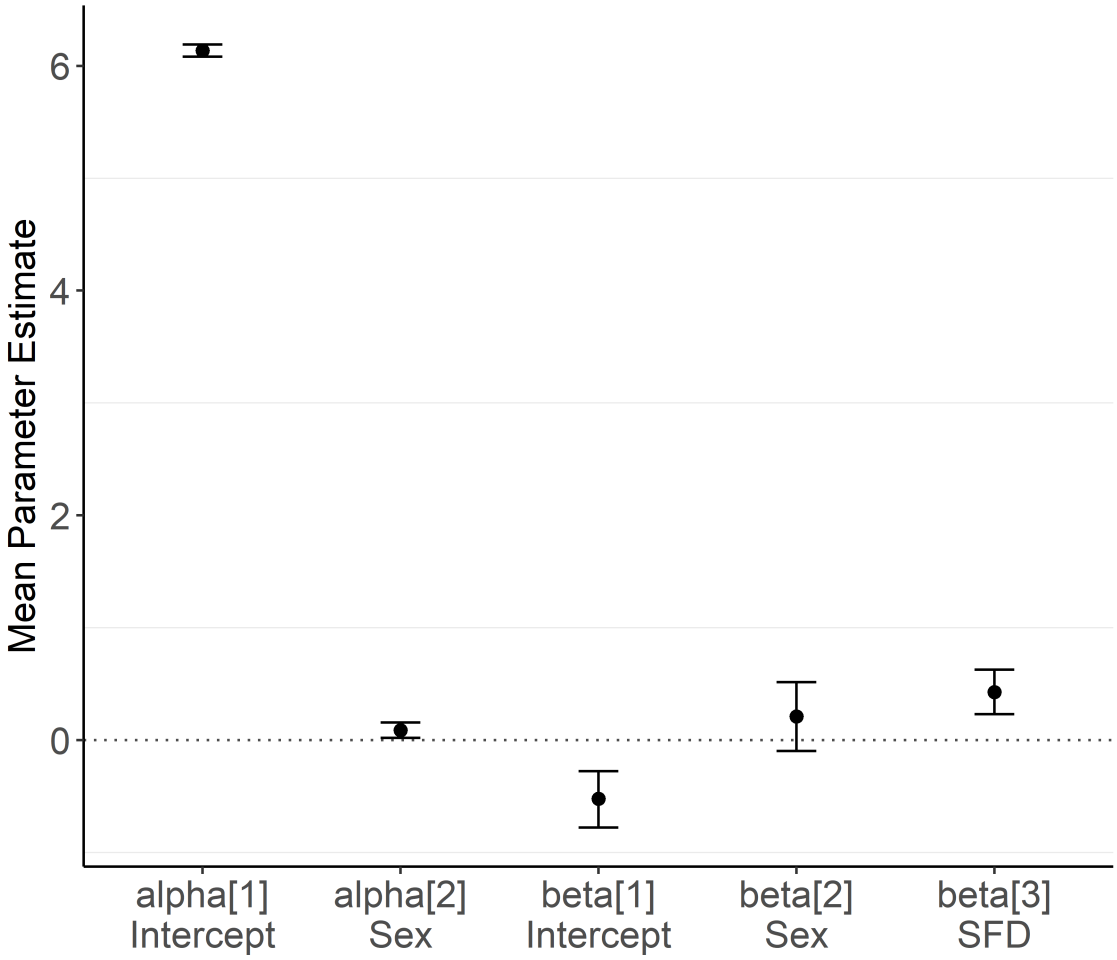

Figure 4: Mean parameter estimates for variables included in *R. septemvitatta* growth model. Error bars reflect 95% Bayesian credible intervals. Alpha and beta parameters represent individual and time-varying parameters, respectively. Credible intervals that do not overlap zero represent strong positive or negative relationships.

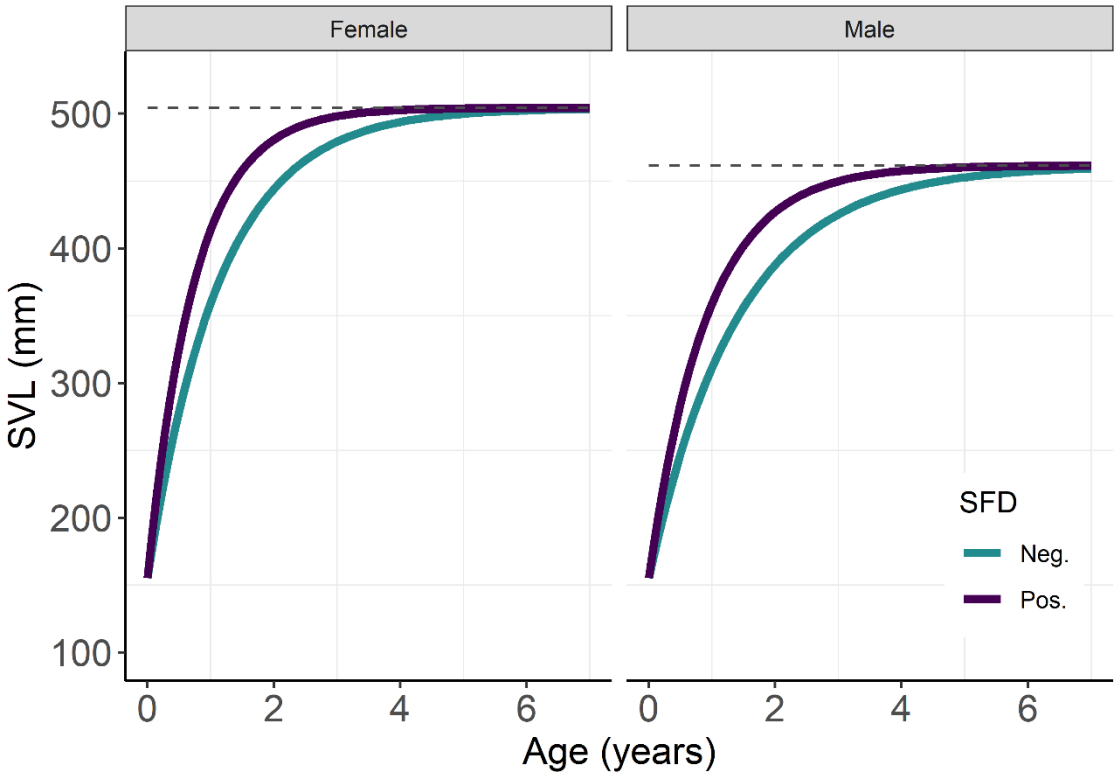

Figure 5: Population mean growth curves for both uninfected (Neg.) and infected (Pos.) conditions for female and male *R. septemvitatta.* Bold lines represent posterior means and dashed lines indicate estimated asymptotic size.

DISCUSSION

Emerging fungal pathogens are an increasingly common problem for wildlife worldwide (Fisher et al. 2012). Two of the most notable, white nose and chytrid fungus, have decimated bat and amphibian populations respectively with the latter responsible for the single largest disease related loss of biodiversity in recorded history (Frick et al. 2010, Fisher et al. 2012). Snake fungal disease is becoming similarly notable, with the decline of a Timber Rattlesnake population in Eastern North America attributed to it (Clark et al. 2011). However, sub-lethal impacts such as changes in behavior and growth may be just as notable and important to our understanding of how these pathogens impact their respective hosts.

I hypothesized that SFD afflicted snakes would exhibit lower survival probability estimates than unafflicted individuals. Survival probability estimates for SFD afflicted and unafflicted individuals did not significantly differ and were relatively high at over 0.85 (Table 2 and Fig. 1), rejecting this hypothesis. This result is in line with recent work done by Davy et al. (2021) which found little evidence to suggest that survival probability is reduced by SFD. The fact the survival probability estimates showed a statistically insignificant but slightly higher survival probability for SFD afflicted snakes may seem unintuitive; however, SFD afflicted snakes in this system have been reported to be more surface active than unafflicted individuals, making it likely for us to encounter and recapture them (McKenzie et al. 2021). Increased number of recaptures of more surface active SFD afflicted snakes in the spring may aid in slightly increasing survival estimates for afflicted individuals. The fact that September and October generally exhibited the lowest recapture probability estimates supports this assertion since

seasonality of SFD affliction has been documented with probability of affliction waning in the fall (McKenzie et al. 2018; Fig. 3). Disease state transition probability estimates suggest that snakes in this system are regularly going between SFD negative and positive states. With this said, seasonality in probability of affliction may explain statistically insignificant higher probability of transition from SFD negative to positive as well. Research has shown SFD affliction probability is highest in spring making it more likely that individuals would stay in or transition to SFD positive during the spring and early summer months that exhibited the highest recapture probability estimates (McKenzie et al. 2018; Fig. 2 and Fig. 3). In essence, the results of the multistate mark recapture model suggest that snakes regularly transition between disease states and disease state does not impact survival.

I hypothesized that SFD afflicted snakes would exhibit lower growth rates than unafflicted individuals. I found that both male and female *R. septemvittata* categorized as SFD positive exhibited heightened growth rates, in comparison to individuals categorized as SFD negative. It is important to note that while the survival analysis took into account monthly changes in disease state, the growth model assumed an individual deemed SFD positive stayed in this state for the rest of the year. This may make comparing results between these models difficult. With this in mind, the heightened growth rates exhibited by SFD positive snakes may be explained by the heightened metabolism documented within SFD afflicted snakes (Agugliaro et al. 2020). SFD afflicted Pygmy Rattlesnakes (*Sistrurus miliarius*) were found to have 30%-45% higher resting metabolic rates and 30%-40% higher evaporative water loss rates than unafflicted individuals (Agugliaro et al. 2020). While these changes in metabolism and water loss rate may negatively impact

terrestrial or food limited species, *R. septemvittata* at my central Kentucky sites may not share this problem. Since *R. septemvittata* is a primarily aquatic snake, increases in evaporative water loss rates are likely less impactful. Additionally, crayfish (the primary prey of *R. septemvittata*) seem to be abundant at all my sites, which may mitigate likelihood of emaciation due to increased metabolism requiring more food. In this way, with an abundance of prey, increased metabolism of *R. septemvittata* is likely responsible for the increased growth exhibited by individuals infected with *O. ophidiicola*.

Increased metabolism may be the result of a change in behavior that SFD afflicted snakes make to fight fungal infection. Snakes have been documented to induce fever like conditions by basking to raise their body temperature (Clark et al. 2011, Lorch et al. 2015, Lorch et al. 2016). When this is combined with the fact that prior work within my central Kentucky sites found snakes afflicted with SFD to be more surface active than unafflicted individuals (McKenzie et al. 2021), it seems likely that SFD afflicted individuals are taking this behavioral step to aid in fighting their fungal infection and in the process raise their metabolic rate. This increased surface activity may in turn explain the higher recapture probability estimates observed in the spring and when SFD infections have been reported at their highest rate (McKenzie et al. 2018). In this way, heightened growth rates seem to be a sub-lethal result of SFD in my populations of aquatic snakes. Conversely, in populations of terrestrial snakes, that may be more limited by food and water availability, this cascade of cause and effect would likely lead to starvation before increased growth rate. This may explain the apparent differences in reported SFD related mortality between my study system (McKenzie et al. 2021) and

those of other investigations such as Clark et al. (2011), where the decline of Timber Rattlesnake population was attributed to SFD.

The combination of heightened growth rates of SFD afflicted snakes and similar survival probabilities between SFD afflicted and unafflicted snakes paints a different picture than previous studies of impacts of SFD. While one prior study has reported a potential SFD related population decline and many studies have documented SFD related mortality, most of these studies deal with seemingly smaller populations and samples sizes than this study. For instance, the 50% population decline reported in Clark et al. (2011) was observed in a population where 40 Timber Rattlesnakes were captured between 2006 and 2009. In comparison, between 2016 and 2018 we captured 216 *R. septemvittata* individuals at three sites in Kentucky and estimated monthly survival probability above 0.85. The difference in sample sizes may account for some of this variation. Davy et al. (2021) suggests that small sample sizes and bias towards testing and observation of individuals displaying "gross lesions" may account for higher reported mortality than may actually exist. We swabbed and recorded clinical signs of every snake captured to determine SFD affliction status, thereby reducing the likelihood of sampling bias towards individuals with obvious clinical signs. These differences in scope of study may further explain why my conclusions on SFD mortality differ from those of other investigations and illustrate the importance of investigating sublethal impacts of SFD.

Sublethal impacts of SFD, such as increased growth rate, may alter our understanding of the life history of *R. septemvittata*. Female *R. septemvittata,* both afflicted and unafflicted with SFD, exhibited growth rates fast enough to put them at the

minimum reported size of sexual maturity (344mm SVL) within their first year of life (Branson and Baker 1974). Gravid snakes at my sites typically give birth between early August and early September, which means that a hypothetical individual born in August of 2018 could reach reproductive maturity by August of 2019 and give birth to its first litter during the summer of 2020. Previous reports put the age and size of sexual maturity within the second or third year of life (Branson and Baker 1974). This decrease in age at reproductive maturity also changes how we look at generational time within these populations and may have serious implications for our understanding of how *R. septemvittata* may be impacted by SFD at the population level given that SFD afflicted snakes may reach both the size of reproductive maturity, and their asymptotic size faster than snakes unafflicted with SFD. Future research into aspects such as body condition and fecundity of afflicted snakes is needed to assess whether this decrease in time to sexual maturity allows SFD afflicted snakes to successfully reproduce earlier in life.

This study also reconfirmed the presence of sexual size dimorphism within *R. septemvittata*. Sexual size dimorphism is well documented in various snake species (Shine 1994). *R. septemvittata* is no exception (Branson and Baker 1974.), and therefore it is unsurprising that *R. septemvittata* females within this study were found to have higher asymptotic sizes than males. It is advantageous for females to attain larger body sizes in order to carry more offspring (Branson and Baker 1974, and Weatherhead et al. 1995), while males may invest more energy in traveling and sperm production than in growth (Weatherhead et al. 1995). This difference in means to optimize reproductive fitness likely explains why female *R. septemvittata* seemed to grow to their asymptotic

size faster than *R. septemvittata* males. With female fecundity largely determined by size, fast growth is advantageous.

This study has provided novel information that will aid in future investigations. We successfully estimated growth of *R. septemvittata* using a model that distinguished natural variation from measurement error and have gained valuable insight into both the life history of *R. septemvittata* and various sublethal impacts of SFD on this species. Life history information such as male and female growth rates and age at sexual maturity are vital to filling knowledge gaps in our understanding of this species and will greatly enhance any management efforts pursued in the future. Additionally, while many studies have catalogued the mortality caused by SFD, here we show that snake species that have not experienced excessive SFD driven mortality and subsequent population decline may still be experiencing drastic sub-lethal impacts such as increased growth rates. These sub-lethal impacts highlight the conclusion that while *Oo* may be an opportunistic pathogen that infects a wide range of hosts, the ecology, physiology, and behavior of any given host may elicit drastically different impacts. To this end, the further study of basic biological metrics such as growth as well as their interactions with snake fungal disease is not only valuable but vital.

APPENDICES

Table 4: Summary of *Regina septemvittata* data collected between 2013-2019.

Regina septemvittata									
Site	**Site ID**	**Years Sampled**	**Sex**	**Individuals**	**Captures**	**Growth Increments**	**Mean Increment length (Days)**	**Median increment Length (Days)**	**Increment Length Range (Days)**
All		2013-2019	Female	332	643	312	150	58	2-1194
All		2013-2019	Male	340	652	313	168	67	2-1257
Little Hickman	1	2013-2019	Female	119	249	132	152	52	2-1107
Little Hickman	1	2013-2019	Male	134	268	135	164	62	2-694
Emmett	2	2013	Female	7	14	7	46	26	7-118
Emmett	2	2013	Male	7	9	4	256	128	6-763
Elias	3	2013, 2015-2017, 2019	Female	41	48	7	89	49	18-288
Elias	3	2013, 2015-2017, 2019	Male	35	45	11	546	633	54-1257
Elkhorn	4	2016-2019	Female	60	160	101	166	68	5-1184
Elkhorn	4	2016-2019	Male	62	165	103	146	84	7-715
Glenns	5	2016-2019	Female	36	68	32	144	63	7-432
Glenns	5	2016-2019	Male	32	56	24	153	85	7-492
Hickman	6	2013-2017, 2019	Female	17	27	10	122	38	3-716
Hickman	6	2013-2017, 2019	Male	19	28	9	366	257	10-1057
Otter	7	2016, 2019	Female	16	23	7	62	62	14-111
Otter	7	2016, 2019	Male	17	24	7	47	50	13-72
Tate	8	2016, 2019	Female	35	52	17	174	74	5-1097
Tate	8	2016, 2019	Male	35	55	20	52	33	16-139

Table 5: Example encounter history with the month and year corresponding to occasion.

Capture Occasion	**Month and Year**
N0P0PPN00P00PNN000	May 2016
N0P0PPN00P00PNN000	June 2016
N0P0PPN00P00PNN000	July 2016
N0P0PPN00P00PNN000	August 2016
N0P0PPN00P00PNN000	September 2016
N0P0PPN00P00PNN000	April 2017
N0P0PPN00P00PNN000	May 2017
N0P0PPN00P00PNN000	June 2017
N0P0PPN00P00PNN000	July 2017
N0P0PPN00P00PNN000	August 2017
N0P0PPN00P00PNN000	September 2017
N0P0PPN00P00PNN000	October 2017
N0P0PPN00P00PNN000	May 2018
N0P0PPN00P00PNN000	June 2018
N0P0PPN00P00PNN000	July 2018
N0P0PPN00P00PNN000	August 2018
N0P0PPN00P00PNN000	September 2018
N0P0PPN00P00PNN000	October 2018

Figure 6: Examples of clinical signs of SFD on *R. septemvittata*, exhibited on the ventral side, dorsal side, and snout.

Figure 7: Biomark HPR Plus portable PIT tag reader and Biomark BP Portable Antenna Plus used to conduct PIT telemetry surveys.

Figure 8: Hypodermic needle used to implant a PIT tag in captured *R. septemvittata.*

Figure 9: Left, sterile swab used to collect samples used for qPCR analysis to determine fungal load. Right, micro centrifuge tube used to contain collected swab samples until qPCR.

Figure 10: A snake being processed and examined for clinical signs of SFD.

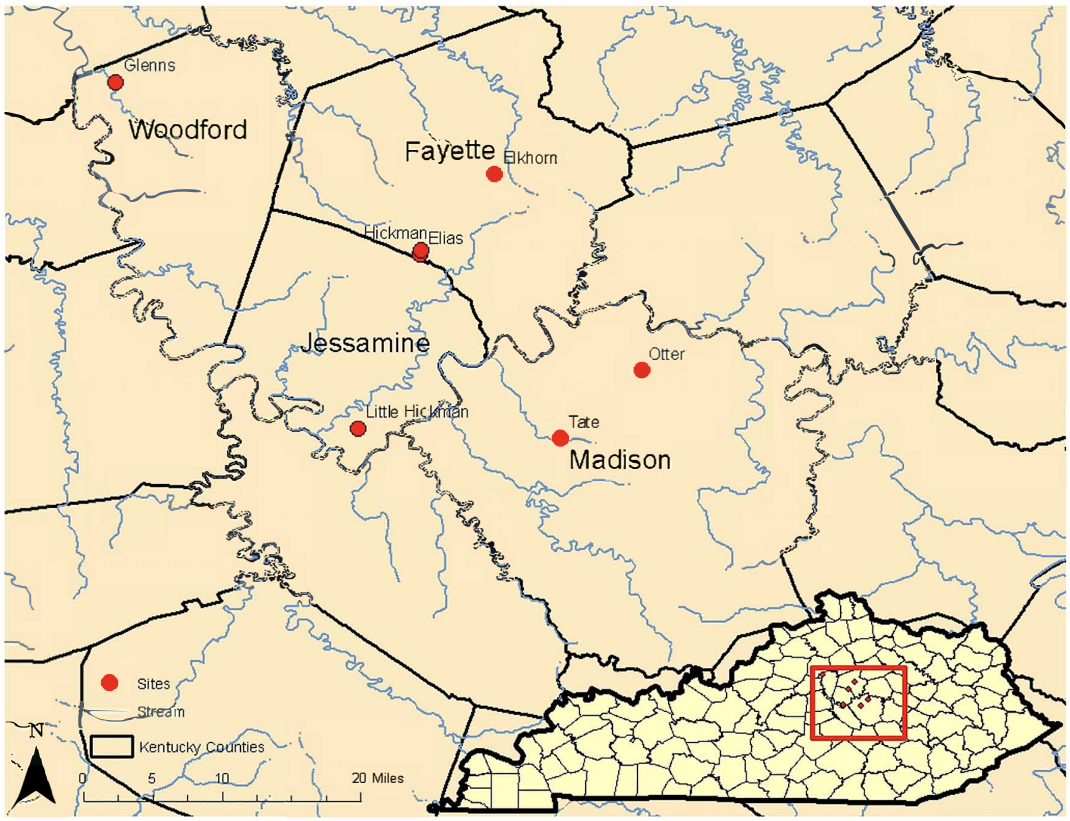

Figure 11: Map of central Kentucky showing the four counties where snakes were captured during this study. Red dots indicate our study sites: Glenns in Woodford county, Little Hickman in Jessamine County, Elkhorn and Hickman-Elias in Fayette county, and Tate and Otter in Madison county.

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EDUCATION

- University of Wisconsin-Whitewater
  Bachelor of Science in Biology- Graduation date: May 2017
  (Cumulative GPA: 3.782) Magna Cum Laude
  Emphasis in Ecology, Evolution, and Behavior
  Minor in Environmental Studies

POSITIONS HELD

- Research Technician ("Where have all the mussels gone?" study): University of Kentucky (February 2021-Present)

- Research Technician (Hellbender eDNA study): University of Kentucky (September 2020-November 2020)

- Research Technician (Freshwater Mussel Growth): University of Kentucky (July 2020-September 2020)

- Graduate Research Assistant: University of Kentucky (July 2018-June 2020)

- Financial Specialist, Senior: Wisconsin Department of Natural Resources (November 2017-present)

- Teaching Assistant (Field Techniques): University of Wisconsin-Whitewater (Fall 2016)

- Teaching Assistant (Yellowstone Travel Study): University of Wisconsin-Whitewater (Summer 2016)

- Research Assistant: University of Wisconsin- Whitewater (Summer 2016)

- Science Academy Student Mentor: University of Wisconsin-Whitewater (June 2016)

- Grandchildren's University: University of Wisconsin-Whitewater (June 2016)

- Summer Undergraduate Research Fellowship: University of Wisconsin-Whitewater (Summer 2015)

- Summer Assistant: University of Wisconsin-Whitewater (Summer 2015)

- Habitat Restoration Intern: Madison Audubon Society (Summer 2015)

HONORS

- University of Kentucky, Department of Forestry and Natural Resources Student Travel grant recipient (spring 2019 and 2020)

- Summer Undergraduate Research Fellowship from University of Wisconsin-Whitewater, "Use of Museum Specimens to Investigate Morphological Changes in Wisconsin Reptiles and Amphibians Over Time" (2015)

- University of Wisconsin-Whitewater Dean's List every semester of Undergraduate degree (fall 2013-spring 2017)

- Graduated magna cum laude from UW-Whitewater. (May 2017)

PUBLICATIONS

Kastle, M, J. Kapfer, A. R. Kuhns, W. Graser, G. Glowacki, **A. Ibach**, L. Mitchem, J. Mozuch, N. Rudolph, K. Rutzen, and R. King. 2021. Blanding's turtle hatchling survival and movements following natural vs. artificial incubation. Journal of Herpetology 55:167-173.

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