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Effects of snake fungal disease on short-term survival, behavior, and movement in free-ranging snakes

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Abstract. Pathogenic fungi are increasingly associated with epidemics in wildlife populations. Snake fungal disease (SFD, also referred to as Ophidiomycosis) is an emerging threat to snakes, taxa that are elusive and difficult to sample. Thus, assessments of the effects of SFD on populations have rarely occurred. We used a field technique to enhance detection, Passive Integrated Transponder (PIT) telemetry, and a multi-state capture–mark–recapture model to assess SFD effects on short-term (within-season) survival, movement, and surface activity of two wild snake species, *Regina septemvittata* (Queensnake) and *Nerodia sipedon* (Common Watersnake). We were unable to detect an effect of disease state on short-term survival for either species. However, we estimated Bayesian posterior probabilities of >0.99 that *R. septemvittata* with SFD spent more time surface-active and were less likely to permanently emigrate from the study area. We also estimated probabilities of 0.98 and 0.87 that temporary immigration and temporary emigration rates, respectively, were lower in diseased *R. septemvittata*. We found evidence of elevated surface activity and lower temporary immigration rates in diseased *N. sipedon*, with estimated probabilities of 0.89, and found considerably less support for differences in permanent or temporary emigration rates. This study is the first to yield estimates for key demographic and behavioral parameters (survival, emigration, surface activity) of snakes in wild populations afflicted with SFD. Given the increase in surface activity of diseased snakes, future surveys of snake populations could benefit from exploring longer-term demographic consequences of SFD and recognize that disease prevalence in surface-active animals may exceed that of the population as a whole.

Key words: capture–mark–recapture model; disease; fungi; Ophidiomyces ophidiicola*; reptile.*

INTRODUCTION

Pathogenic fungi are increasingly associated with emerging epidemics in wildlife and represent a substantial 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 understanding the behavior of 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 factors 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 infected individuals may emigrate from study sites, resulting in biased estimates of mortality rates (Faustino et al. 2004). Furthermore, whereas quantitative research on disease dynamics has often focused on animals that are easily observed or trapped (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).

Snake fungal disease (SFD, also known as ophidiomycosis) is an emerging threat to wild snake populations (Lorch et al. 2016, Burbrink et al. 2017). 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 (Lorch et al. 2016). Snake fungal disease was first hypothesized as a causative factor resulting

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in declines in snake populations in 2006 (Clark et al. 2011), and afflicted snakes have since been documented throughout North America and in Europe (Lorch et al. 2016, Franklinos et al. 2017). The disease is caused by the fungus *Ophidiomyces ophidiicola* and is characterized by severe skin infections (Allender et al. 2015, Lorch et al. 2015, 2016, Hileman et al. 2017). In laboratory settings, infections alter host behavior and lead to morbidity (Allender et al. 2015, Lorch et al. 2015). A recent analysis indicates that most snake species, regardless of ecological traits and phylogeny, are susceptible to SFD (Burbrink et al. 2017). However, the number of individuals exhibiting clinical signs varies both spatially and temporally, with higher infection rates often associated with hibernation and spring emergence in temperate climates and with cooler temperatures where snakes do not hibernate (Lorch et al. 2016, McCoy et al. 2017, Lind et al. 2018, McKenzie et al. 2019). Due to its recent emergence and uncertain effects, SFD has been described as one of the most important yet understudied risks to global biodiversity (Sutherland et al. 2014).

Understanding the individual and population-level effects of SFD in field settings has been challenging due to the secretive nature of snakes and the subsequent difficulty associated with collecting health data. Most snake species have notoriously low encounter rates, preventing the collection of adequate samples to make inferences regarding population status or demographic rates (Dorcas and Willson 2009, Durso et al. 2011, Steen et al. 2012). Furthermore, population analyses based on traditional capture–mark–recapture (CMR) models are unable to estimate true survival when individuals permanently emigrate from the study population because mortality and emigration are completely confounded (Lebreton et al. 1992). As a result, estimated effects of disease on mortality rates will be unreliable in cases where disease also affects movement behavior and related rates of permanent emigration. Thus, field techniques and analyses appropriate for monitoring large numbers of individuals are needed to assess the population-level effects of SFD on wild snake populations.

Recently, Connette and Semlitsch (2015) described a method that estimates true survival based on both live resightings and non-visual detections of individuals using Passive Integrated Transponder technology (PIT tags; Connette and Semlitsch 2015, Oldham et al. 2016). This method is based on the ability to remotely detect PIT tags of dead individuals that remained in the study area, which provides the additional information needed to distinguish mortality and emigration (Connette and Semlitsch 2015). This approach is similar to previous studies that used auxiliary data, such as spatial locations of individuals to distinguish between mortality and emigration in CMR studies (e.g., Barker 1997, Gilroy et al. 2012, Schaub and Royle 2014).

In this study, we performed CMR surveys in combination with non-visual monitoring of snake locations using PIT telemetry (Oldham et al. 2016). We then used multistate CMR models (Connette and Semlitsch 2015) to examine the consequences of SFD for wild populations of *Regina septemvittata* (Queensnake) and *Nerodia sipedon* (Common Watersnake) in central Kentucky, USA streams. Specifically, we addressed the following question: does SFD affect the short-term survival, movement, and behavior of wild snakes? This study is the first to yield estimates for key demographic and behavioral parameters (survival, emigration, surface activity) of a large number of snakes with and without SFD in wild populations.

Materials and Methods

Study sites

We conducted our study at six streams in Madison, Fayette, Jessamine, and Woodford Counties, in the inner Bluegrass Region of Kentucky, USA (Leuenberger et al. 2019). This region is characterized by karst topography, gently rolling hills, and mixed land-use including pasture, forest, and urban/suburban land cover. All sampled streams were part of the Kentucky River basin and ranged from first to fourth order. Streams in this region often have bedrock substrate and typically have many cover objects, primarily slabs of limestone often used by snakes as a refuge. Stream water chemistry at our study sites was consistent with other streams in the region (conductivity values between 300–500 μS/L). Riparian vegetation consisted of trees, shrubs and, in some cases, manicured lawns. Surveyed stream reaches ranged from 293 to 1,005 m in total length.

Field sampling

We collected *R. septemvittata* and *N. sipedon* during area-constrained searches of natural cover objects within the stream channel and along the banks between April and early June 2016. 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 signs 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. After swabbing, snakes were subcutaneously marked with passive integrated transponder (PIT) tags, using the Biomark MK10 implanter with 134.2 kHz, 12.5 mm PIT tag (Biomark, Boise, ID, USA), inserted 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 capture location within 48 h of capture. To reduce cross contamination between snakes, gloves were worn during capture and changed between processing of individuals, and all equipment was sanitized with a 10% bleach solution between samples.

To collect encounter data for capture–mark–recapture analyses, we conducted 16 PIT telemetry surveys at each study stream between early June and October of 2016. Surveys typically occurred every 7–10 d. PIT telemetry surveys consisted of one researcher 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 Biomark BP Portable Antenna has a maximum reading distance ranging from 30.5 cm to 43.2 cm (for 134.2 kHz Biomark 12.5 mm PIT tags), depending on tag orientation and electromagnetic interference (Ousterhout and Semlitsch 2014). However, reading distance is similar through any non-ferrous substrate and snakes could be detected through rock, under water, and within crayfish burrows (Oldham et al. 2016). When a PIT tag was detected, we attempted to locate the snake to visually confirm that it was alive. If a snake was not observed visually, it was recorded as a non-visual detection (i.e., PIT tag only). Recaptured snakes were photographed, weighed, measured, inspected for clinical signs, and swabbed for *O. ophidiicola*. However, in cases where recaptured snakes had been captured within the past 30 d, they were simply confirmed alive and immediately released. On a few occasions, snakes were seen and confirmed alive, but we were unable to recapture them. If unmarked snakes were opportunistically discovered during PIT telemetry surveys, they were implanted with a PIT tag, processed, and released using the methods described above. At the beginning and end of each survey, we recorded the time, air temperature (°C), wind speed (mph), day of last rain, and cloud cover (oktas; i.e., 0%, 12.5%, 25%, etc.).

Laboratory assessment and determining disease status

We used a two-step approach to determine disease status for each snake. First, we reviewed photos and field data 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. We considered clinical signs that were indicative of SFD to include regional edema, local (scale) edema, crust with *stratum corneum*, crust without *stratum corneum*, nodule, and ulcer (See Appendix S1, Fig. S1-S10 for definitions and images of clinical signs). Second, we used real-time polymerase chain reaction (PCR) to identify the presence of *O. ophidiicola* according to protocols in Bohuski et al. (2015). If a snake lacked clinical signs, we defined it as SFD negative, regardless of the number of copies of fungal DNA. If a snake had greater than 41.5 copies of fungal DNA (the “limit of detection for” the PCR assay based on preliminary data, the quantity of DNA at which 95% of samples containing that amount of DNA show evidence of amplification below the 40 cycle threshold of the assay), and at least one clinical sign that was indicative of SFD, we considered the snake to have SFD. Finally, snakes with less than 41.5 copies of fungal DNA, and at least two clinical signs indicative of SFD were also considered to have 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

We used a Bayesian state-space formulation of a multistate CMR model to obtain information about snake survival, surface activity, and emigration behavior (both permanent and temporary) based on a combination of visual resightings and PIT tag detections (Connette and Semlitsch 2015). For this purpose, we strictly define the study area as the three-dimensional area extending the surveyed length and width of the stream channel, and to a belowground depth equal to the range of the portable PIT tag reader. The model then assumes that PIT tag detections are possible for both live and dead individuals that are present in the study area and that, following PIT tag detection, live individuals may also be visually re-sighted on the ground surface. Visual detection always follows (i.e., is conditional on) a successful PIT tag detection. Thus, the probability of visual detection is considered synonymous with “surface activity” because only a snake under cover or below the ground surface could be detected by the PIT tag reader and fail to be visually re-sighted. Under certain assumptions (see Model assumptions), the ability to detect PIT tags of individuals that died within the three-dimensional limits of the study area provides the additional information necessary for distinctly estimating mortality and emigration (Connette and Semlitsch 2015). Because both permanent and temporary emigration require either vertical (i.e., belowground) or horizontal movement from and to the study area, we later refer to these processes collectively as “movement.”

Covariates.—To examine the effects of SFD on snake populations, our analysis incorporates a binary covariate for disease status (with or without SFD). Due to the short duration of our study and the inability to repeatedly test most individuals for disease, we treated an individual’s disease status as a constant. Because the majority of SFD diagnoses were performed early in the active season when observed disease prevalence is highest (McKenzie et al. 2019), we believe most individuals with disease were likely identified. Thus, if an individual snake was classified as having SFD, it was considered to have SFD for the duration of the study. If individuals were to regularly transition between diseased and non-diseased states, in contrast to this assumption, it could reduce our ability to detect disease-related changes in demographic and behavioral parameters. We also incorporated a binary covariate for species in order to generate species-specific estimates for key model parameters. We considered cloud cover and temperature at the beginning of each survey as covariates for visual detection probability because we expect these factors to influence basking behavior of snakes (Sun et al. 2001). In other words, we believe snakes are more likely to be surface active on warmer, clear days than on cooler, cloudier days (Dai et al. 1999). These covariates were not highly correlated (Pearson's $r < 0.11$). Finally, day of year was included as a covariate for temporary emigration because snakes may move deeper underground or into the banks as the season progresses (Durso et al. 2011). Cloud cover, temperature, and day of year were all standardized prior to analysis by subtracting the mean and dividing by the standard deviation of the observed values.

Model specification.—Our observation data (live resightings and PIT tag detections) were summarized as an encounter history matrix with values $y_{i,t}$, where $y_{i,t} = 1$ if individual i was detected with only the PIT tag reader at survey t, $y_{i,t} = 2$ if the individual was detected and visually confirmed alive, and $y_{i,t} = 3$ if the individual was not detected during the survey. We then assume that individuals may transition among five possible states representing whether or not individuals are alive, permanently emigrated, and available for detection with a PIT tag reader (Fig. 1). This "true" state of individual i at survey t is represented by a latent state variable $z_{i,t}$, where $z_{i,t} = 1$ if the individual is "alive and available" for PIT tag detection, $z_{i,t} = 2$ if the individual is "alive and unavailable" for PIT tag detection, $z_{i,t} = 3$ if the individual has "permanently emigrated" from the study area, $z_{i,t} = 4$ if the individual is "dead and available," and $z_{i,t} = 5$ if the individual is "dead and unavailable." Availability or unavailability for PIT tag detection is treated as the outcome of a Markovian temporary emigration process (e.g., Kendall et al. 1997, Schaub et al. 2004), where the availability of an individual at survey t depends on that individual's availability at time $t - 1$. Multi-state CMR models with this form of temporary emigration have been shown to be structurally identifiable except under certain parameterizations (Schaub et al. 2004). In our study, temporary emigration can be interpreted specifically as the probability that an individual has moved outside the study area or too deep belowground to be detected with a PIT tag reader (Connette and Semlitsch 2015).

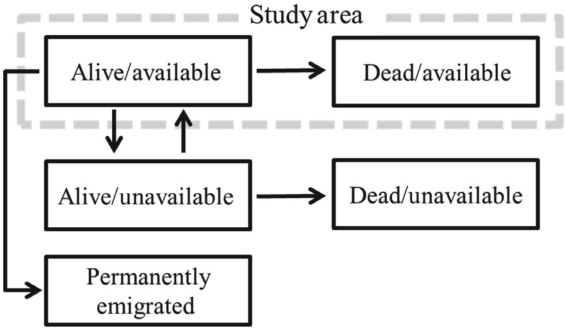

FIG. 1. Conceptual diagram of the multi-state model for estimating emigration, survival, and availability for PIT tag detection. Arrows indicate possible transitions among states.

A state process model describes transitions through time in the true state of each individual. Because new individuals were captured and marked throughout the study, the model conditions on the time of first capture for each individual, f_i. The initial state of each individual is then fixed as $z_{i,f_i} = 1$, because all individuals were observed to be alive and available at the occasion of first capture and marking. For all subsequent surveys, the state of individual i at survey $t + 1$ is described as a categorical random variable that is conditional on the state of the individual at the previous survey, t

$$z_{i,t+1}|z_{i,t} \sim \text{categorical}\left(\mathbf{\Omega}_{z_{i,t},i,t}\right).$$

The vector of transition probabilities, $\mathbf{\Omega}_{z,i,t}$, specifies the probabilities that individual i moves from state z to each of the five possible states between surveys t and $t + 1$. These state-specific transition probabilities are defined by the following matrix:

$$\begin{array}{l|ccccc} & \multicolumn{5}{c}{\text{State at survey } t+1} \\ \text{State at } t & \text{Alive/Available} & \text{Alive/Unavailable} & \text{Emigrated} & \text{Dead/Available} & \text{Dead/Unavailable} \\ \hline \text{Alive/Available} & S_{i,t}F_{i,t}(1-E_{i,t}) & S_{i,t}F_{i,t}E_{i,t} & S_{i,t}(1-F_{i,t}) & 1-S_{i,t} & 0 \\ \text{Alive/Unavailable} & S_{i,t}F_{i,t}I_{i,t} & S_{i,t}F_{i,t}(1-I_{i,t}) & S_{i,t}(1-F_{i,t}) & 0 & 1-S_{i,t} \\ \text{Emigrated} & 0 & 0 & 1 & 0 & 0 \\ \text{Dead/Available} & 0 & 0 & 0 & 1 & 0 \\ \text{Dead/Unavailable} & 0 & 0 & 0 & 0 & 1 \end{array}$$

where S represents the survival probability, F the probability of site fidelity (i.e., 1 – permanent emigration), E the temporary emigration probability, and I the temporary immigration probability. Individuals are assumed to transition among states until they reach an absorbing state (i.e., dead or permanently emigrated), from which no further transitions are possible. We assume the existence of separate "dead" states for animals on and off the study area because the former individuals are potentially detectable with the PIT tag reader and thus have different observation probabilities.

Snake populations at our study sites were assumed to be open to mortality, permanent emigration, and temporary emigration/immigration between all surveys. Survival from survey t to survey $t + 1$ was modeled as a function of both disease presence and species

$$S_{i,t} = (\text{mo}.S_{\text{SFD}_i,\text{SP}_i})^{\frac{\Delta_{\text{site}_i,t}}{30}}$$

where $\text{mo}.S_{\text{SFD}_i,\text{SP}_i}$ represents the monthly (30-d) survival rate specific to the disease state, SFD, and species, SP, of individual i. Although surveys were typically conducted at regular 7–10 d intervals, monthly survival rates were scaled according to number of days, $\Delta_{\text{site}_i,t}$, between survey t and survey $t + 1$ at the site containing individual i. Probabilities of site fidelity and temporary immigration were similarly dependent on disease presence and species, with the probability of site fidelity also depending on the length of the time interval between surveys at a site.

$$F_{i,t} = (\text{mo}.F_{\text{SFD}_i,\text{SP}_i})^{\Delta_{\text{site}_i,t}/30}$$

$$I_{i,t} = \alpha_{\text{SFD}_i,\text{SP}_i}$$

Probability of temporary emigration, $E_{i,t}$, was assumed to be a function of disease status, species and day of year on the logit scale

$$\text{logit}(E_{i,t}) = \beta_{0,\text{SFD}_i,\text{SP}_i} + \beta_1 \times \text{Day}_{i,t} + \beta_2 \times (\text{Day}_{i,t})^2.$$

The dependence of $E_{i,t}$ on day of year allowed for the expected decrease in snake availability for detection from spring to early fall. We also tested for a nonlinear (quadratic) relation between day of year and temporary emigration, but found little support for inclusion of the quadratic term.

An observation process model relates the encounter history data with the true, but imperfectly observed, state of an individual through time. This model represents the observation data, $y_{i,t}$, as a categorical random variable with outcome probabilities conditional on the true state of the individual, $z_{i,t}$, at survey t

$$y_{i,t}|z_{i,t} \sim \text{categorical}(\mathbf{\Theta}_{z_{i,t},i,t}).$$

The vector of outcome probabilities, $\mathbf{\Theta}_{z,i,t}$, specifies the probability of each possible observation type (i.e., PIT tag detection only, visual and PIT tag detection, non-detection) for individual i at survey t, conditional on the current state of the individual, z. These state-dependent outcome probabilities are defined by the following matrix:

$$\begin{array}{l|ccc}
 & \multicolumn{3}{c}{\text{Observation/outcome at survey } t} \\
\text{State at } t & \text{PIT-detection only} & \text{Visual \& PIT-detection} & \text{Not detected} \\
\hline
\text{Alive/Available} & \delta(1-\nu_{i,t}) & \delta\nu_{i,t} & 1-\delta \\
\text{Alive/Unavailable} & 0 & 0 & 1 \\
\text{Emigrated} & 0 & 0 & 1 \\
\text{Dead/Available} & \delta & 0 & 1-\delta \\
\text{Dead/Unavailable} & 0 & 0 & 1
\end{array}$$

where δ represents the probability an individual available for detection with the PIT tag reader is successfully detected and $\nu_{i,t}$ represents the probability that individual i is visually observed at time t, given that it is alive and detected with the PIT tag reader. Thus, an individual that is unavailable (i.e., temporarily emigrated) or permanently emigrated will be recorded as observation type $y_{i,t} = 3$, not detected, with a probability of 1.

Probability of visual observation, $\nu_{i,t}$, was modeled as a function of disease status, species, and site-survey-specific covariates for percent cloud cover and air temperature on the logit scale

$$\text{logit}(\nu_{i,t}) = \gamma_{0.\text{SFD}_i,\text{SP}_i} + \gamma_1 \times \text{Cloud}_{\text{site}_i,t} + \gamma_2 \times \text{Temp}_{\text{site}_i,t}.$$

We used Markov chain Monte Carlo (MCMC) simulation to fit the model using JAGS 4.20 (Plummer 2003). This software was accessed through program R 3.3.1 (R Development Core Team 2016) using the package jagsUI 1.4.4 (Kellner 2015). We assigned uninformative uniform priors (0,1) to the parameters representing monthly survival probability, monthly permanent emigration probability, temporary immigration probability and PIT tag detection probability. We assigned uniform priors (0,1) to the probability-scale intercept parameters for temporary emigration and visual detection probability. We assigned uniform priors (−10, 10) to the coefficients representing the effects of cloud cover and air temperature on visual detection, as well to the coefficient representing the effects of day of year on temporary emigration. We ran three arbitrarily long Markov chains composed of 800,000 MCMC iterations each, discarded the first 400,000 iterations as burn-in, and then sampled 1 in every 50 remaining iterations to yield a total of 24,000 samples representing the joint posterior distribution. Convergence was evaluated using the Gelman-Rubin statistic (R, Gelman et al. 2004). Values of R <1.01 were considered indicative of model convergence and we achieved successful convergence of all parameters. All model code was adapted from that provided in Appendix S1 of Connette and Semlitsch (2015).

Model assumptions

Our model depends on the standard assumptions of most multi-state CMR models (see Williams et al.

2002), except that explicit modeling of temporary emigration relaxes the assumption that all emigration must be permanent. Most multi-state CMR models with "unobservable" states, such as for temporary emigrants, typically require the additional assumption that survival is equal among observable and unobservable states (Kendall and Nichols 2002, Schaub et al. 2004). Similarly, our study separates permanent emigration and survival probabilities while assuming that both parameters are equal between individuals available and unavailable for detection (i.e., temporarily emigrated). Like many other discrete-time multi-state CMR models, we also assume that all individuals transition at the same time in the capture interval (e.g., Joe and Pollock 2002, Cooch 2008). Specifically, we treat all mortality as occurring prior to emigration, both permanent and temporary, but expect this to have little influence on survival or transition parameter estimation given that survival rates are modeled as constant over time and across states. Finally, a key assumption implicit in our use of PIT tag detections to distinguish between mortality and permanent emigration is that PIT tags of dead individuals do not leave the study area. This assumption might be violated if predators or downstream drift remove individuals from the study area, which would positively bias both permanent emigration and survival estimates.

RESULTS

We marked and processed 526 individuals (232 *R. septemvittata* and 294 N. *sipedon*) across our six study sites (Table 1), with most individuals being marked from April through early June. Disease status could not be determined for 34 snakes due to missing photographs of clinical signs (13 *R. septemvittata* and 21 N. *sipedon*). Of the remaining snakes, we considered 83/219 (37.9%) of *R. septemvittata* and 56/273 (20.5%) of *N. sipedon* to be SFD-positive based on clinical signs and results of PCR testing. We did not have any snakes with zero copies of fungal DNA and two or more clinical signs. Across 16 PIT telemetry surveys at each site, we recorded 1,331 PIT tag detections and were able to confirm that the individual was alive based on hand capture or visual identification for 903 of these detections. Overall, we resighted less than one-half of all tagged individuals through the combination of visual detections and hand captures (i.e., snake confirmed alive); 45.7% (106/232) were resighted for *R. septemvittata* and 34.7% (102/294) were resighted for *N. sipedon*.

We estimated the probability of PIT tag detection for all snakes within range of the PIT tag reader (i.e., not emigrated) as 0.41 (95% credible interval [CRI] = 0.33–0.52). We observed a clear increase in the probability of visual detection for diseased *R. septemvittata* of 46.68% (CRI = 19.21–79.46%) and estimated a 0.89 posterior probability that visual detection also increased in diseased *N. sipedon*, with an estimated increase of 19.75% (CRI = −10.55–53.41%) (Table 2). We also observed a negative association between visual detection probability and cloud cover ($\gamma_1 = -0.21$, CRI = −0.37 to −0.046) and estimated a 0.94 posterior probability that visual detection was positively associated with temperature ($\gamma_2 = 0.12$, CRI = −0.03–0.28; Figs. 2, 3).

Monthly survival estimates were similar during the active season for both non-diseased and diseased individuals of both snake species (Table 2), and the estimated change in monthly survival for diseased snakes was 0.11% (CRI = −2.61–2.76%) for *R. septemvittata* and 0.05% (CRI = −3.94–2.86%) for *N. sipedon* (Fig. 4). Estimated probability of permanent emigration (Fig. 4) was reduced in diseased *R. septemvittata*, with an observed change of −68.03% (CRI = −97.57 to −25.76%), while no significant change was detected in diseased *N. sipedon* (−12.27%, CRI = −58.44–39.29%). The product of these estimated rates of survival and permanent emigration yields traditional "apparent survival" estimates of 0.92 (CRI = 0.84–0.98) and 0.78 (CRI = 0.72–0.84) in diseased and non-diseased *R. septemvittata*, respectively. Apparent survival estimates for *N. sipedon* were 0.77 (CRI = 0.66–0.88) in diseased snakes and 0.74 (CRI = 0.69–0.79) in non-diseased snakes.

There was considerable uncertainty associated with rates of temporary emigration and immigration, likely due to limited statistical power to detect transitions to and from the unobservable temporarily emigrated state. However, we did identify a reduced rate of temporary immigration in *R. septemvittata* with SFD compared to non-diseased snakes (−48.87%, CRI = −77.25– −0.85%) despite finding no significant change in temporary

TABLE 1. Number of individuals marked for each snake species, disease status, and frequency of detections by type in central Kentucky, USA streams.

Species	Number marked	With SFD	Without SFD	Number of PIT detections	Total visual detections
Regina septemvittata	232	83	136	645	207
Nerodia sipedon	294	56	217	686	221
Total	526	139	353	1,331	428

Notes: We used presence or absence of specific clinical signs and real-time PCR to identify the presence of *Ophidiomyces ophidiicola* to determine disease status. We were unable to determine disease status for 13 *Regina septemvittata* and 21 *Nerodia sipedon;* thus, snakes with and without snake fungal disease (SFD) does not sum to the total number of snakes marked. Snakes were detected via Passive Integrated Transponder (PIT) telemetry.

TABLE 2. Model parameter estimates for non-diseased (SFD−) and diseased snakes (SFD+), estimated percent change due to disease, and Bayesian posterior probabilities that the given population parameter for diseased individuals exceeds that of non-diseased individuals (Prob>).

Parameter	SFD−	SFD +	Change (%)	Prob>
Regina septemvittata				
Monthly survival	0.99 [0.97–1.00]	0.99 [0.96–1.00]	0.11 [−2.61–2.76]	0.54
Permanent emigration	0.21 [0.15–0.27]	0.07 [0.01–0.14]	−68.03 [−97.57–−25.76]	0.00
Visual detection	0.43 [0.36–0.50]	0.63 [0.55–0.70]	46.68 [19.21–79.46]	1.00
Temporary emigration	0.47 [0.30–0.63]	0.39 [0.24–0.55]	−17.21 [−44.07–17.03]	0.13
Temporary immigration	0.31 [0.19–0.48]	0.15 [0.08–0.27]	−48.87 [−77.25–−0.85]	0.02
Nerodia sipedon				
Monthly survival	0.99 [0.97–1.00]	0.99 [0.95–1.00]	0.05 [−3.94–2.86]	0.58
Permanent emigration	0.25 [0.20–0.30]	0.22 [0.11–0.33]	−12.27 [−58.44–39.29]	0.30
Visual detection	0.45 [0.39–0.51]	0.54 [0.41–0.65]	19.75 [−10.55–53.41]	0.89
Temporary emigration	0.52 [0.35–0.67]	0.55 [0.35–0.74]	8.24 [−28.35–54.28]	0.64
Temporary immigration	0.33 [0.22–0.47]	0.22 [0.10–0.40]	−32.25 [−70.88 –31.95]	0.11

Notes: Parameter estimates for visual detection probability and temporary emigration are mean values of sampling covariates (i.e., those values represent the intercepts only). Values in brackets are 95% credible intervals.

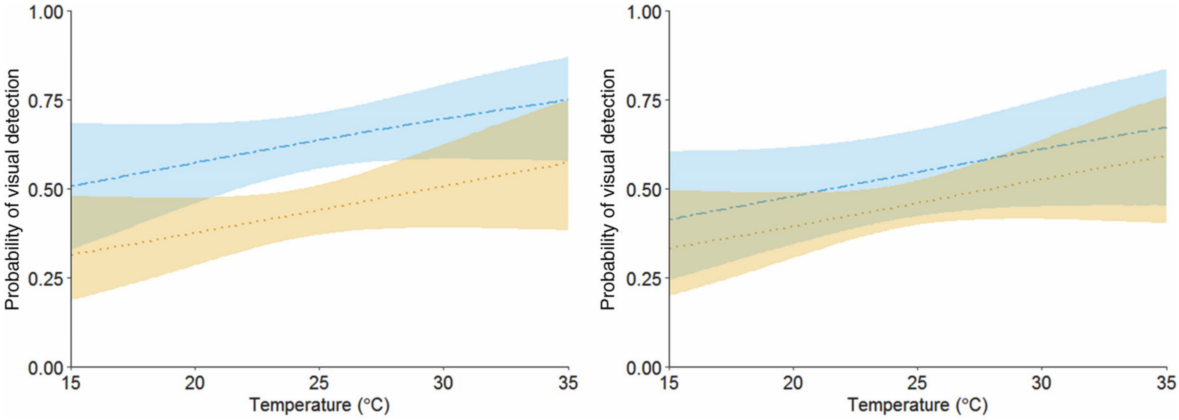

FIG. 2. The predicted relationship and 95% credible interval between visual detection probability and temperature for *R. septemvittata* (left) and *N. sipedon* (right). The dashed blue line indicates snakes with SFD and the dotted yellow line indicates snakes without SFD. For both species, visual detection probability is positively correlated with increasing temperature.

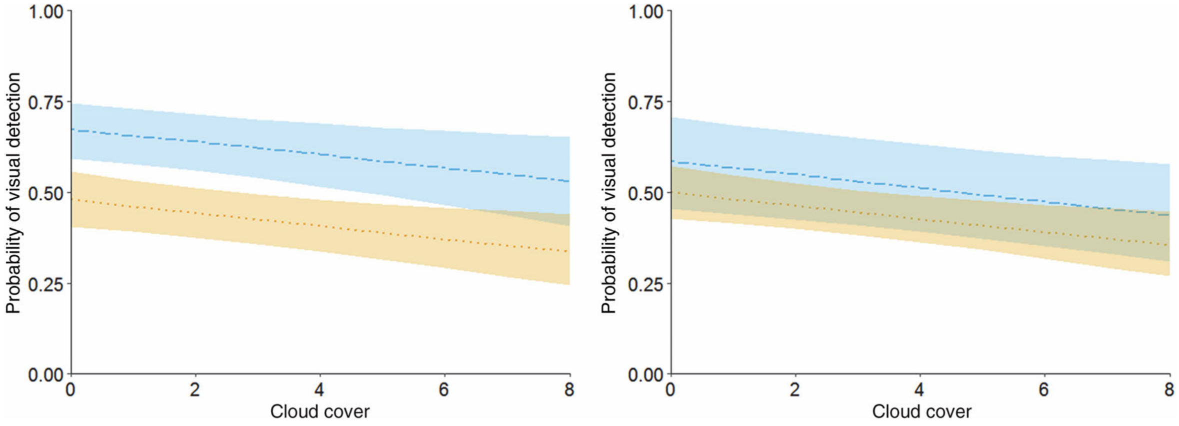

FIG. 3. The predicted relationship and 95% credible interval between visual detection probability and cloud cover for *Regina septemvittata* (left) and *Nerodia sipedon* (right). Cloud cover was measured in oktas with 0 being clear sky and 8 (100%) being fully cloudy sky. The dashed blue line indicates snakes with snake fungal disease (SFD) and the dotted yellow line indicates snakes without SFD. For both species, visual detection probability is negatively associated with increasing cloud cover.

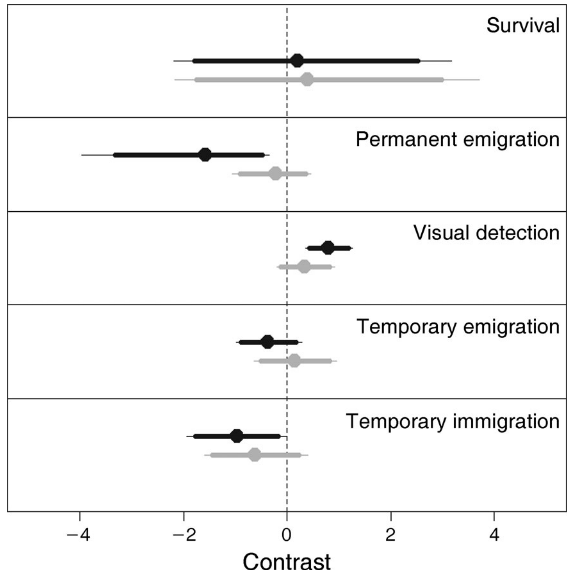

FIG. 4. Estimated difference in parameter estimates between diseased and non-diseased snakes. For comparison, contrasts are shown for parameter estimates on the logisticscale. Values greater than zero indicate that parameter estimates were higher for diseased individuals. The black bars represent *R. septemvittata* and the gray bars represent *N. sipedon*. Points indicate posterior means, thin bars indicate 95% credible intervals, and thick bars indicate 90% credible intervals.

emigration rates (−17.21%, CRI = −44.07–17.03%). We found no significant change in either temporary immigration (−32.25%, CRI = −70.88–31.95%) or temporary emigration (8.24%, CRI = −28.35–54.28%) rates in *N. sipedon* with SFD. We found an overall positive relationship between day of year and temporary emigration probability despite the lack of support for a quadratic term (Fig. 5), with an estimated probability of 1.00 that temporary emigration rates were higher on our last survey date than on our first survey date.

DISCUSSION

Snake fungal disease is considered a serious threat to snake populations, yet there is a lack of population-level studies. We found a relatively high percentage of the snakes we evaluated (37.9% of *R. septemvittata* and 20.5% of *N. sipedon*) with SFD in our sampled populations. Yet, we were unable to identify an effect of SFD on survival in either of the species examined; monthly survival estimates were universally high across our single field season (~4.5 months), regardless of species or disease state. Despite tracking over 500 individual snakes of two species, it is possible that the duration of our study was insufficient to detect differences in survival. In contrast, Tetzlaff et al. (2017) used radio telemetry to track *Sistrurus catenatus* (Eastern Massasauga) for up to 1,439 d in a Michigan, USA population, and ultimately found two of eight infected snakes died due to infection while a third was euthanized after being brought into the lab for treatment. Thus, multi-year surveys that span the dormant season may be needed to estimate effects of SFD on snake survival. Furthermore, the fungus *O. ophidiicola* has likely been present in North America for a long time with certain environmental factors (moisture, temperature, etc.) influencing disease severity and outcome (Lorch et al. 2016). Therefore, findings from our study may not necessarily translate to other snake species or populations that have specific genetic, demographic, or life history traits that may make them especially vulnerable to SFD outbreaks (i.e., small population size, inbreeding depression, sensitivity to habitat loss).

Although we found uncertainty associated with estimates of temporary emigration and immigration, we did detect that *R. septemvittata* afflicted with SFD showed reduced emigration and immigration rates compared to snakes that did not have SFD. Specifically, lower rates of permanent emigration and temporary immigration indicate that SFD may also affect individual movement

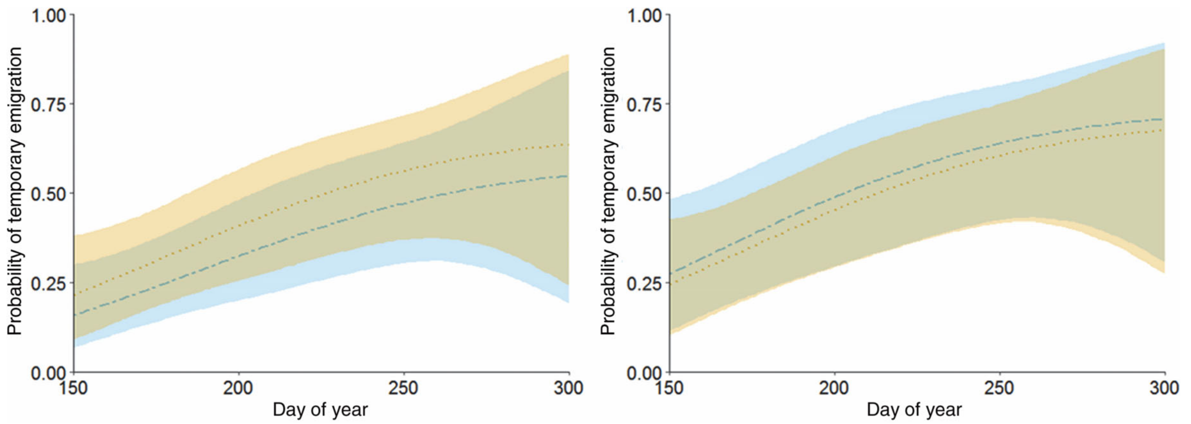

FIG. 5. The predicted relationship and 95% credible interval between temporary emigration probability and day of year for *R. septemvittata* (left) and *N. sipedon* (right). The dashed blue line indicates snakes with SFD and the dotted yellow line indicates snakes without SFD. For all snakes, temporary emigration probability increases as day of year increases.

behavior. These results are consistent with a study on *S. 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). Additionally, decreased activity in response to infection has been documented in other reptiles, such as reduced activity in *Sceloporus occidentalis* (Western Fence Lizard) infected with malaria (Dunlap and Church 1996). Conversely, it could be possible that infected snakes move less frequently because the microhabitats they select for fighting infection are spatially restricted. Reductions in movement could lead to reductions in foraging, locating mates, and dispersal that ultimately affect population health.

We observed that SFD resulted in behavioral changes for both snake species examined. Probability of visual detection, which we consider an indicator of surface activity levels, was clearly elevated in diseased *R. septemvittata* and there was a high probability that diseased *N. sipedon* show a similar response. A possible explanation for the high rates of visual detection in snakes with SFD may be that diseased snakes increase basking to overcome infection (Kluger et al. 1975, Burns et al. 1996). A previous laboratory study noted that *Pantherophis guttatus* (Red Cornsnake) infected with *O. ophidiicola* 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). However, Tetzlaff et al. (2017) found that free-ranging *S. catenatus* with *O. ophidiicola* or with clinical signs of SFD were less visible than their non-infected counterparts. This suggests that it is possible that snake species could respond differently to infection. 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 our study populations it appears that this increased surface activity did not correspond with reduced short-term survival. We speculate this is likely due to the consistently warm temperatures experienced during our study period (June–October) that allowed snakes to maintain higher initial body temperatures, suggesting little effect to their ability to escape predators while basking.

Long-term consequences on survival and population persistence cannot be addressed from our data, which are limited to one active season. Despite these limitations, we provide a quantitative framework and field-based approach to assess the effects of SFD and other diseases on cryptic animals that are challenging to study. Specifically, traditional CMR methods can confound mortality and emigration parameters. Our modeling approach used PIT tag detections to distinguish between mortality and permanent emigration and assumed that PIT tags of dead individuals do not leave the study area. This assumption might be violated if predators capture and remove diseased individuals at a greater frequency than non-diseased individuals from the study area; this would result in positively biasing both permanent emigration and survival estimates for diseased snakes. However, we found that that apparent survival (mortality × emigration) did not differ between disease states for *N. sipedon* and was lower in *R. septemvitatta* without SFD due to a larger number of individuals that permanently leave the study area. By accounting for differences in movement between diseased and non-diseased snakes, we provide vital rate estimation with less bias than traditional CMR analyses. Failure to account for higher permanent emigration rates in non-diseased snakes could result in under-estimation of survival for healthy individuals. Our field-based approach, particularly the use of PIT telemetry, achieved comparatively high re-sighting rates and documented increased surface activity in diseased snakes. The documentation of increased surface activity in diseased individuals has important implications for quantifying disease prevalence within snake populations. Because diseased individuals are more likely to be visually observed on the ground surface, they may be over-represented in surface counts, which would lead to an over-estimation of disease prevalence within a population. Future surveys of snake populations could be improved by accounting for this potential behavioral change to yield appropriate estimates of disease rates in snake populations.

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Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.2251/full

Data Availability

Snake capture–mark–recapture data, survey covariates, and JAGS code are available from Dryad Digital Repository (Price et al. 2020): https://doi.org/10.5061/dryad.np5hqbzqt.