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Original Contribution

Field Diagnostics and Seasonality of *Ophidiomyces ophiodiicola* in Wild Snake Populations

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Abstract: Snake fungal disease (SFD) is an emerging disease caused by the fungal pathogen, *Ophidiomyces ophiodiicola*. Clinical signs of SFD include dermal lesions, including regional and local edema, crusts, and ulcers. Snake fungal disease is widespread in the Eastern United States, yet there are limited data on how clinical signs of SFD compare with laboratory diagnostics. We compared two sampling methods for *O. ophiodiicola*, scale clip collection and swabbing, to evaluate whether collection method impacted the results of polymerase chain reaction (PCR). In addition, we evaluated the use of clinical signs to predict the presence of *O. ophiodiicola* across seasons, snake habitat affiliation (aquatic or terrestrial) and study sites. We found no significant difference in PCR results between sampling methods. Clinical signs were a strong predictor of *O. ophiodiicola* presence in spring and summer seasons. Snakes occupying terrestrial environments had a lower overall probability of testing positive for *O. ophiodiicola* compared to snakes occupying aquatic environments. Although our study indicates that both clinical signs of SFD and prevalence of *O. ophiodiicola* vary seasonally and based on habitat preferences of the host, our analysis suggests that clinical signs can serve as a reliable indicator of *O. ophiodiicola* presence, especially during spring and summer.

Keywords: Clinical signs, Fungal pathogens, PCR, Reptiles, Snake fungal disease

INTRODUCTION

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

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

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

Clinical signs of SFD are non-specific and used to describe "symptoms" commonly observed with infection by *O. ophiodiicola*. Swabs and tissue samples (e.g., scale clips) are used to test for the presence of *O. ophiodiicola* by polymerase chain reaction (PCR) (Allender et al. 2015; Bohuski et al. 2015); however, the relationship between PCR-based detection of the fungus and disease state has not been fully elucidated. For example, the presence of non-specific clinical signs alone (without confirmation of the presence of *O. ophiodiicola*) has been used to classify snakes as SFD positive (McCoy et al. 2017). Furthermore, some snakes harboring *O. ophiodiicola* do not have clinical signs of SFD (Paré et al. 2003; Bohuski et al. 2015), but the extent to which wild snakes may have subclinical infections or act as carriers of *O. ophiodiicola* has not been investigated. Assessing the percentage of "asymptomatic" snakes with *O. ophiodiicola* would be helpful in further defining the geographic distribution, host range, and disease dynamics of *O. ophiodiicola*.

Our primary objective was to test the relationship between field observations of SFD and the presence of *O. ophiodiicola*. As previous studies have alluded to seasonal trends of *O. ophiodiicola* infection (reviewed by Lorch et al. 2016; McCoy et al. 2017), we examined the relationship between both clinical signs and season (spring, summer, and fall) on fungal presence. In addition, since moist environments are thought to be important for fungal growth (Lorch et al. 2016), we considered habitat affiliation (aquatic or terrestrial) of snake species sampled to determine whether snakes with a certain habitat affiliation are more likely to be exposed or infected with *O. ophiodiicola*. Second, we examined the effectiveness of two sampling methods for *O. ophiodiicola*: scale clips and swabbing.

METHODS

Study Sites

Snakes were captured using a variety of field methods at six sampling locations within the Inner Bluegrass, Eastern Kentucky Coalfields, Knobs, and Jackson Purchase physiographic regions of Kentucky (USA) between March 2015 and May 2016 (Fig. 1). At sample locations in the Knobs and Eastern Kentucky, most snakes were captured under artificial cover (i.e., wood cover boards and roofing tin). Within the Inner Bluegrass, snakes were captured opportunistically and later recaptured using passive integrated transponder (PIT) telemetry (Oldham et al. 2016). In the Jackson Purchase, snakes were captured via nighttime road surveys.

Field Sampling and Laboratory Assessment

Snakes were identified to species, and we recorded locality information and date upon each capture. We recorded the presence/absence of visible dermal lesions on the head or body of the animal. Specifically, we defined visible dermal lesions to include regional or local edema, crusts, ulcers, dysecdysis, and other forms of damage to the dermis (i.e., Lorch et al. 2015; Guthrie et al. 2016). We considered these dermal lesions as indicative of *O. ophiodiicola* infection. After recording clinical signs, we used up to two methods to collect samples for PCR assay for *O. ophiodiicola*. If lesions were present, one lesion was swabbed with a sterile polyester-tipped swab saturated with pure water (Fisher Scientific, BP2484-100) and/or a scale clip was collected by removing a small section of one scale with a lesion. The swab was brushed over the lesion five times, then placed into a plastic vial, and stored in a − 40°C freezer. If no
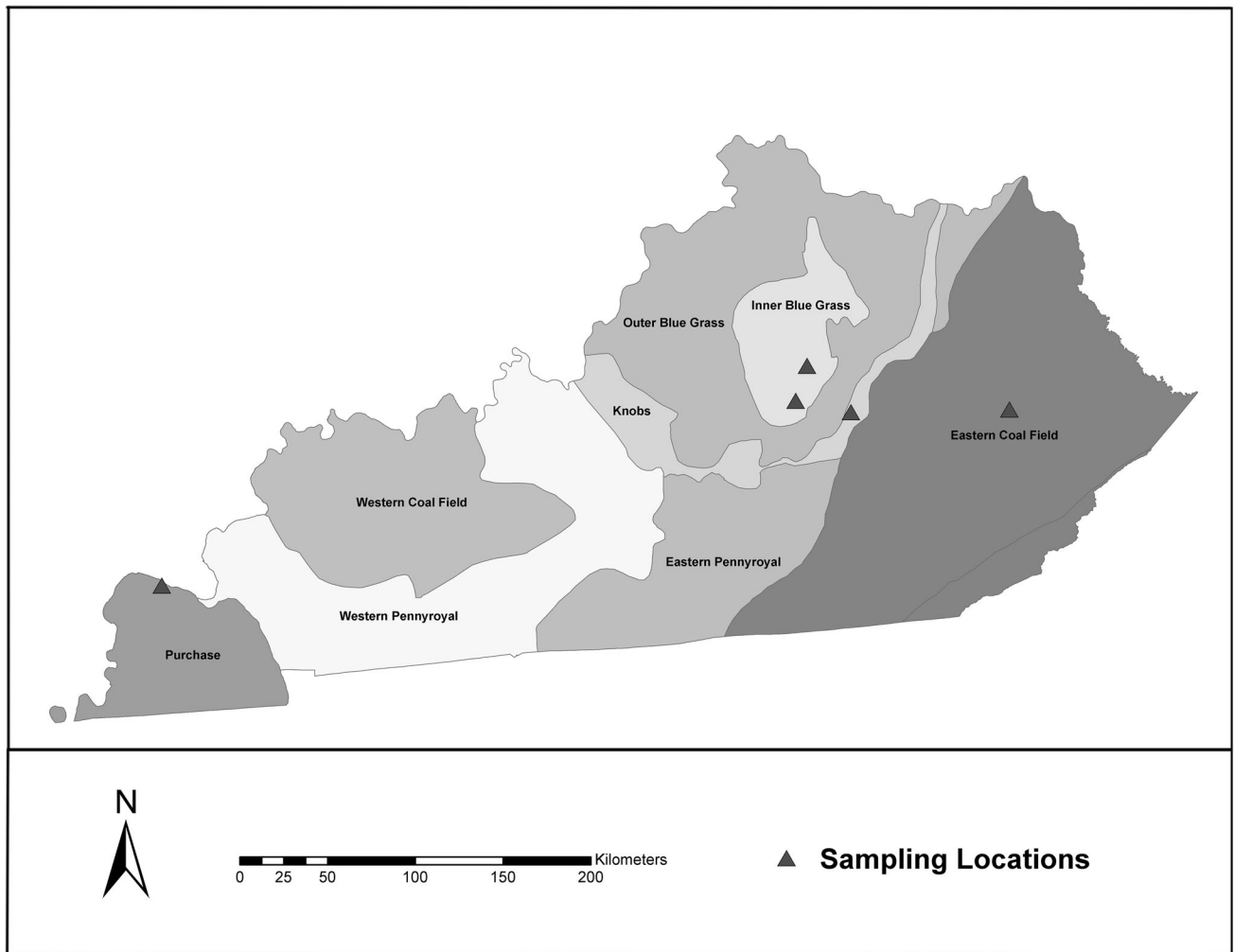
Figure 1. Sampling locations in Kentucky. Dark gray triangles indicate locations where snakes that tested positive for *O. ophiodiicola* via real-time PCR. At least one positive individual was found at each location.

lesions were present, a snake was swabbed on the dorsal side of its midline and/or a scale clip was taken from the same location. Scale clips were taken using scissors and forceps; sampling equipment was treated with 10% bleach to sterilize and remove nucleic acid between snakes. Scale clips were either taken from lesioned areas of skin (if lesions were present) or a grossly normal ventral scale on the transverse midline (if lesions were absent). After sampling, all snakes were released at their capture location.

Real-time PCR was used to determine the presence of *O. ophiodiicola* according to the protocols in Bohuski et al. (2015) for the internal transcribed spacer region (ITS) assay. A standard curve of tenfold dilutions ranging from 5 to 50,000 fg of genomic DNA from the type isolate of *O. ophiodiicola* was run on each plate, and samples were rerun if the efficiency (as calculated from the standard curve) was below 90% or above 110%. *Ophidiomyces ophiodiicola* was

considered present on a snake if the threshold was ≤ 40 cycles (Bohuski et al. 2015). This threshold does not confirm the presence of SFD; it only confirms the presence of *O. ophiodiicola*. Samples were considered negative for *O. ophiodiicola* if amplification did not occur within 40 cycles. All research was compliant with University of Kentucky IACUC protocol (2013-1073). Permits were obtained from the Kentucky Department of Fish and Wildlife Resources (SC1511017, SC1611043, SC1611136). The use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

Statistical Analyses

We compared scale clips and swab samples using 173 snakes for which a scale clip and a swab sample were both taken at the same instance of capture. We used the package

Table 1. Morbidity Table Showing Species, Habitat Affiliation, Number of Individual Samples, Presence or Absence of Clinical Signs, and Real-time PCR Results (Positive or Negative) for Snake Species Tested for *O. ophiodiicola*.

Snake species	Number sampled	Clinical signs present	Clinical signs present and positive	Clinical signs present and negative	Clinical signs absent	Clinical signs absent and positive	Clinical signs absent and negative
Aquatic species							
N. sipedon	72	17	17	0	55	21	34
Regina septemvittata	116	73	61	12	43	26	17
Total	188	90	78	12	98	47	51
Terrestrial species							
Agkistrodon contortrix	18	9	7	2	9	0	9
Carphophis amoenus	6	4	4	0	2	0	2
Coluber constrictor	13	8	5	3	5	1	4
Crotalus horridus	3	3	2	1	0	0	0
Diadophis punctatus	9	4	3	1	5	0	5
Lampropeltis getula	9	7	7	0	2	1	1
L. triangulum	5	5	4	1	0	0	0
Nerodia erythrogaster	2	1	1	0	1	0	1
Opheodrys aestivus	2	1	0	1	1	0	1
Pantherophis spiloides	2	1	1	0	1	0	1
Storeria occipitomaculata	3	1	1	0	2	0	2
Thamnophis sirtalis	8	5	4	1	3	0	3
Virginia valeriae	3	1	0	1	2	1	1
Total	83	50	39	11	33	3	30
Overall total	271	140	117	23	131	50	81

"MASS" in R version 3.2.1 to run a McNemar's test (Venables and Ripley 2002; R Core Team 2016) to compare the effectiveness of scale clips against swab samples at detecting the presence of *O. ophiodiicola*. Because some snakes had multiple instances of paired samples (e.g., swab and scale clip collected at the same time), for this analysis we used the first instance of a paired sample with one positive sampling method for *O. ophiodiicola*. If an individual did not have any instances where one sampling method was positive within a paired sample, we used the first set of paired samples collected to determine disease status.

To evaluate the probability of a positive PCR result given the presence or absence of clinical signs, season, and species habitat affiliation, we used the package "lme4" to fit a generalized linear mixed model in R version 3.2.1 (Bates et al. 2015; R Core Team 2016). We used the package "AICcmodavg" to generate predicted infection probabili-

ties using an inverse logit transformation (Mazerolle 2016). For this analysis, we included all available PCR results from both sampling methods, even if a snake had multiple PCR results over time. We used PCR result (0 = negative, 1 = positive) as the response variable with season and the presence/absence of clinical signs habitat affiliation as fixed effects. For the random effect, we nested individual ID within sampling site to account for non-independence of multiple measurements of the same snakes, and for potential site-level differences. Season was divided into three groups: spring (March–May), summer (June–August), and fall (September–November). In addition, we divided snakes into two groups based on habitat affiliation (aquatic and terrestrial) to examine how this affiliation was related to the probability of *O. ophiodiicola* presence. Specifically, we placed *Regina septemvittata* and *Nerodia sipedon* in the aquatic category (*n* = 188), with all other snakes in the terrestrial category (*n* = 83) (Table 1). We

Table 2. Model Outputs from GLMM Analysis Examining the Ability of Clinical Signs (CS) to Predict Disease Status in Snakes with Certain Habitat Affiliations (HA) over Three Seasons. Each model includes a random effect of individual ID nested within sampling site. The table shows the model, AIC$_c$ value, model weight, number of parameters, and coefficient estimates and standard errors for explanatory variables.

Model	AIC$_c$	w	k	Intercept	Season—fall	Habitat affiliation (HA)—terrestrial	Season—spring	Clinical signs (CS)—present
Season + CS + HA	706.76	0.74	7	− 0.6650 ± 0.2988	− 2.2786 ± 0.3371***	− 0.8512 ± 0.3956	0.3555 ± 0.2693	1.8170 ± 0.2722***
Season + CS	708.82	0.26	6	− 1.0317 ± 0.3235	− 2.2519 ± 0.3368***	–	0.3717 ± 0.2722	1.7846 ± 0.2703***
Season + HA	759.61	0.00	6	0.1844 ± 0.2479	− 2.2860 ± 0.3329***	− 0.6279 ± 0.3541	0.7401 ± 0.2717	–
Season	760.01	0.00	5	− 0.1346 ± 0.2896	− 2.2821 ± 0.3336***	–	0.7571 ± 0.2761	–
Clinical signs + HA	774.25	0.00	5	− 1.2270 ± 0.2645***	–	− 0.6092 ± 0.3815	–	2.1129 ± 0.2924***
Clinical signs (CS)	774.82	0.00	4	− 1.4229 ± 0.2667***	–	–	–	2.0692 ± 0.2886***
Habitat affiliation (HA)	843.49	0.00	4	− 0.1318 ± 0.1618	–	− 0.3210 ± 0.3126	–	–

Asterisks indicate significance ($p < 0.001$).

generated seven competing models to predict PCR result: (1) habitat affiliation, (2) clinical signs, (3) season, (4) clinical signs and habitat affiliation, (5) season and habitat affiliation, (6) season and clinical signs, and (7) season, clinical signs, and habitat affiliation (Table 2). We compared models using the Akaike information criterion (AIC; Burnham and Anderson 2002).

RESULTS

We collected 639 swab or scale clip samples from 271 individual snakes. Fifteen snake species were represented in this study (Table 1). Out of the 271 snakes sampled, 140 (51.66%) had clinical signs. *Ophidiomyces ophiodiicola* was detected in at least one sample from each species, except for *Opheodrys aestivus* which was represented by just two individuals. We collected 196 samples from 100 individuals in spring, 273 samples from 147 individuals in summer, and 170 samples from 71 individuals in fall (Table 3). From the 271 individuals, aquatic snakes (i.e., *R. septemvittata* and *N. sipedon*) comprised 188 samples, with 125 samples testing positive for *O. ophiodiicola* (66.50%). Terrestrial snakes comprised 83 samples, with 42 samples (50.60%) testing positive. Most clinical signs were considered mild (i.e., see Guthrie et al. 2016; Lorch et al. 2016), although some individuals had moderate to severe infections as denoted by skin ulcers, large patches of thickened skin, and infection of the eyes (Fig. 2). When comparing scale clip and swab sampling, we found no significant difference between the two sampling methods for detecting the presence of *O. ophiodiicola* (McNemar's Chi-squared = 1.59, $df = 1$, $p = 0.21$) (Fig. 3).

The presence of clinical signs, snake habitat affiliation, and season were all important predictors of PCR results (AIC weight = 0.74; Table 2). Specifically, aquatic snakes with clinical signs had an 81.9% (spring; $n = 196$), 76.0% (summer; $n = 273$), and 24.5% (fall; $n = 170$) probability of having a positive PCR result in each season, whereas terrestrial snakes with clinical signs had 65.8%, 57.5%, and 12.2% chances of a positive PCR result in spring, summer, and fall, respectively (Fig. 4). For the random effects in the top model, the variance explained by the sampling site (0.09 ± 0.30) was small compared to the variance explained by the nested random effect (sampling site within individual ID) (1.12 ± 1.05). The trend of less variation explained by sampling site compared to the variation explained by the nested random effect was consistent across

Table 3. Number of Samples for Each Combination of Season, Habitat Affiliation, and Real-time PCR Result for Snakes Tested for *O. ophiodiicola*, Presence or Absence of Clinical Sign.

Season	Habitat affiliation	PCR result	Clinical signs	Samples collected
Spring	Aquatic	Negative	Absent	20
Spring	Aquatic	Negative	Present	27
Spring	Aquatic	Positive	Absent	15
Spring	Aquatic	Positive	Present	70
Spring	Terrestrial	Negative	Absent	13
Spring	Terrestrial	Negative	Present	14
Spring	Terrestrial	Positive	Absent	1
Spring	Terrestrial	Positive	Present	36
Summer	Aquatic	Negative	Absent	49
Summer	Aquatic	Negative	Present	43
Summer	Aquatic	Positive	Absent	46
Summer	Aquatic	Positive	Present	70
Summer	Terrestrial	Negative	Absent	24
Summer	Terrestrial	Negative	Present	15
Summer	Terrestrial	Positive	Absent	2
Summer	Terrestrial	Positive	Present	24
Fall	Aquatic	Negative	Absent	83
Fall	Aquatic	Negative	Present	44
Fall	Aquatic	Positive	Absent	1
Fall	Aquatic	Positive	Present	22
Fall	Terrestrial	Negative	Absent	13
Fall	Terrestrial	Negative	Present	4
Fall	Terrestrial	Positive	Absent	0
Fall	Terrestrial	Positive	Present	3

Figure 2. Mild clinical signs of snake fungal disease on **a** northern copperhead (*Agkistrodon contortrix*) captured in Breathitt Co., Kentucky, **b** Moderate clinical signs on queen snake (*Regina septemvittata*) captured in Jessamine Co., Kentucky and **c** severe clinical signs on southern black racer (*Coluber constrictor*) captured in Madison Co., Kentucky.

all candidate models. A post hoc Tukey test showed that spring and fall were significantly different ($p < 0.001$), and summer and fall were significantly different ($p < 0.001$). There was no significant difference between spring and summer samples ($p = 0.3780$).

We found that some wild snakes may have subclinical infections or act as "asymptomatic" carriers of *O. ophiodiicola*. For example, aquatic snakes without clinical signs had a 42.3%, 34.0%, and 5.0% chance of having a positive PCR result in spring, summer, and fall, respec-
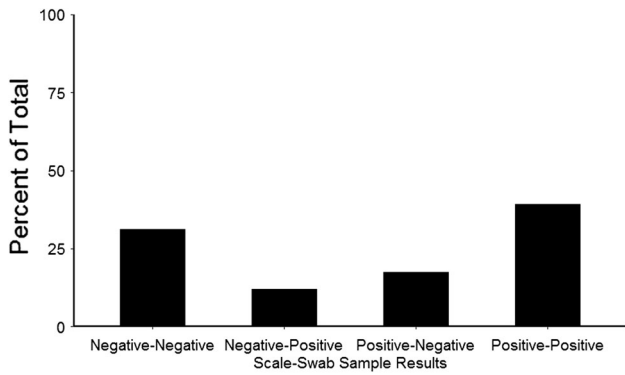
Figure 3. Percentages of the total number of paired swab and scale clips in each combination: scale clip and swab negative, scale clip negative and swab positive, scale clip positive and swab negative, and scale clip and swab positive.

tively, whereas terrestrial snakes lacking clinical signs had the following chances of PCR-based *O. ophiodiicola* detection in each season: 23.9%, 18.0%, and 2.20%. For all snakes, the probability of *O. ophiodiicola* being present was lowest in the fall samples.

DISCUSSION

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

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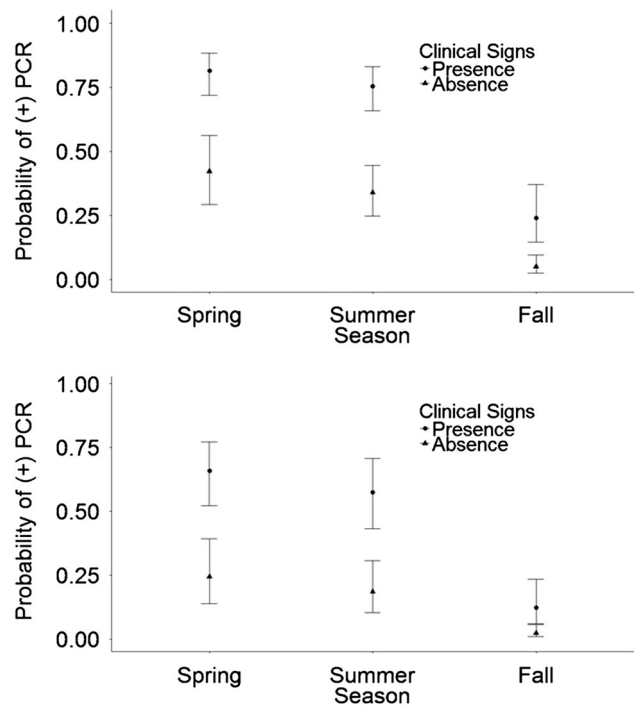

Figure 4. Probability of a positive real-time PCR detection for *Ophidiomyces ophiodiicola* in aquatic snakes (top) and terrestrial snakes (bottom) with SFD clinical sign status.

lesion or stage of infection. Certain clinical signs, such as crusts, may be more likely to have *O. ophiodiicola* on the surface and thus more likely to yield a positive PCR result compared to other clinical signs indicative of a fungal infection deeper in the skin.

We found that snakes with lesions had a higher probability of a positive PCR result for *O. ophiodiicola* than snakes without lesions. However, we found that some "asymptomatic" snakes tested positive for *O. ophiodiicola.* This is consistent with previous findings in which approximately 12% of snakes that lacked clinical signs of SFD tested positive for *O. ophiodiicola* by real-time PCR (Bohuski et al. 2015; Hileman et al. 2018). This indicates that while clinical signs are a conspicuous predictor of the presence of *O. ophiodiicola,* instances where the fungus is present, but the snake is without lesions, do occur. Snakes without clinical signs could be in the early stages of infection with *O. ophiodiicola*, or they could be carriers of the fungus (Lorch et al. 2016).

We found that snakes with aquatic habitat affiliations had a higher probability of testing positive for *O. ophiodiicola* than snakes with terrestrial habitat affiliations. Thus, our results indicate that species affiliated within aquatic habitats vary in either their susceptibility or the

distribution of *O. ophiodiicola* may vary between terrestrial and aquatic habitats. Lorch et al. (2016) suggested that moist conditions could promote growth of *O. ophiodiicola* and its persistence in the environment, as seen in other fungal pathogens (Kriger and Hero 2007). Based upon the detection of *O. ophiodiicola* on individuals without clinical signs of infection, aquatic snakes may have higher exposure rates to the fungal pathogen compared to terrestrial snakes in our study area. Conversely, the aquatic snake species examined could be more susceptible to *O. ophiodiicola*. The snake species, *R. septemvittata,* which comprised most of our aquatic snakes, has some of the thinnest skin of all snake species which could make it more vulnerable to abrasions that provide an entry point for *O. ophiodiicola* infection (Stokes and Dunson 1982). Most previous work on SFD has focused on terrestrial snake species (Allender et al. 2015; McCoy et al. 2017), but our findings demonstrate that aquatic snake species should be more closely studied to better understand how host natural history may affect disease dynamics. For example, comparing aquatic and terrestrial environments could provide insights into how environmental loads of the pathogen vary between habitats (i.e., host exposure) and the ability of infected snakes to locate suitable microclimates for fighting infection. Closely examining species-level differences in natural histories of various host species could also provide insights into which snake species are most vulnerable to developing severe infections caused by *O. ophiodiicola*.

We found that the probability of disease was lower in fall, compared to spring and summer. The higher probability of positive PCR results in spring closely tracks previous reports of SFD being more frequent in snakes after spring emergence (Lorch et al. 2016). This suggests that infections by *O. ophiodiicola* may initiate in winter or spring during a time when host immune function is suppressed (Nelson and Demas 1996) and snakes are exposed to potentially high loads of the fungus in moist underground hibernacula. Snakes that emerge from hibernation infected may subsequently expose unaffected animals by direct contact or by shedding large amounts of the pathogen into the environment. Snakes that are PCR positive for *O. ophiodiicola* in the absence of clinical signs may indicate exposure to the fungus without the establishment of an active infection. Snake fungal disease is often a chronic condition (Lorch et al. 2015) which may explain persistence of clinical signs and detection of *O. ophiodiicola* into summer. However, the number of snakes with clinical signs of SFD is lower in fall compared to spring and

summer (Table 3), consistent with recovery or removal of many infected animals within the population or because the wild snakes may have already been in inaccessible hibernacula at the time when infections would be expected to reoccur. More work is needed to determine the roles of seasonal changes in host immune physiology, rainfall, and air temperature in driving disease dynamics, as these factors are correlated with season.

Habitat and species-specific differences could also result in different observed seasonal patterns. For example, aquatic snake species that occupy more shaded habitats could experience cooler summer temperatures, which could cause a delayed response to infection, with infections lingering longer into summer. In Florida (USA), a mean fungal score, which included the presence and severity of observed clinical signs, negatively correlated with increasing temperature (McCoy et al. 2017). This is contrary to our results, which demonstrate high probabilities of testing positive in spring and summer. This discrepancy could exist because Florida temperatures allow this population of snakes to be active year-round, and temperatures may exceed the upper growth limit for *O. ophiodiicola* (> 35°C; Allender et al. 2015), and never become too cold to prevent growth (7°C; Allender et al. 2015), unlike in Kentucky.

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

Overall, our results provide insight into the range of host species that can become infected by, or carry, *O. ophiodiicola*, differences in detection probabilities of *O. ophiodiicola* for snakes with certain habitat affiliations, and seasonal variation in the presence of clinical signs of SFD and the presence of *O. ophiodiicola*. Results may vary in

other regions, but in our study area, we recommend surveying snake populations for *O. ophiodiicola* in the spring and summer as that corresponds to when clinical signs are most conspicuous and predictive of positive PCR results. Although we make an effort to address the lack of multispecies assessments for infection by *O. ophiodiicola,* the effects this fungus has on populations of snakes are unknown. Our study provides information that will be vital to understanding infection patterns and developing effective management strategies for populations of snakes affected by SFD.

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