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Article *in* Environmental Monitoring and Assessment · May 2023

DOI: 10.1007/s10661-023-11259-w

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RESEARCH

Prevalence of *Ophidiomyces ophidiicola* and epizootiology of snake fungal disease in free-ranging Northern Pine Snakes (*Pituophis melanoleucus melanoleucus*) in New Jersey

Joanna Burger · Michael Gochfeld · Robert Zappalorti · John Bunnell · Christian Jeitner · David Schneider · Kelly Ng · Emile DeVito · Jeffrey M. Lorch

Received: 3 January 2023 / Accepted: 18 April 2023

Abstract Snake fungal disease, caused by *Ophidiomyces ophidiicola*, is recognized as a potential concern for North American snakes. We tested skin swabs from Northern Pine Snakes (*Pituophis melanoleucus melanoleucus*) in the New Jersey pinelands for the presence of *O. ophidiicola* before emergence from hibernation. We used qPCR to test the collected swabs for the presence of *O. ophidiicola*, then determined pathogen prevalence as a function of sampling year, sampling location (skin lesion, healthy ventral skin, healthy head skin) sex, and age. There were no temporal trends in *O. ophidiicola* detection percentages on snakes, which varied from 58 to 83% in different years. *Ophidiomyces ophidiicola* detection on snakes was highest in swabs of skin lesions (71%) and lowest in head swabs (29%). Males had higher prevalence than females (82% versus 62%). The fungus was not detected in hatchling snakes (age 0) in the fall, but 75% of juveniles tested positive at the end of hibernation (age 1 year). We also screened hibernacula soil samples for the presence of *O. ophidiicola*. Where snakes hibernated, 69% of soil samples were positive for *O. ophidiicola*, and 85% of snakes lying on positive soil samples also tested positive for the pathogen. Although a high proportion of snakes (73%) tested positive for *O. ophidiicola* during

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Published online: 11 May 2023

our 4-year study, the snakes appeared healthy except for small skin lesions. We conclude that *O. ophidiicola* prevalence is high on hibernating Northern Pine Snakes and in the hibernacula soil, with a strong association between snakes and positive adjacent soil. This is the first demonstration that snakes likely become infected during hibernation.

Keywords Skin lesions · Snake disease · Disease prevalence · Sex differences · Hibernation sores · Transmission of SFD in soil

Introduction

Emerging infectious diseases represent a major threat to wildlife, as well as to humans. The emergence of fungal pathogens has already had devastating effects on global populations, for example, (1) white-nose syndrome in bats, caused by *Pseudogymnoascus destructans* (Blehert et al., 2009; Hoyt et al., 2018), and (2) chytridiomycosis in amphibians (caused by *Batrachochytrium dendrobatidis* and *B. salamandrivorans*) (Rachowicz et al., 2005; Skerratt et al., 2007Martel et al., 2013). There are concerns that fungal diseases may cause local extinction in snakes, in concert with habitat fragmentation and perhaps climate change (Allender et al., 2015a; Clark et al., 2011). Snake fungal disease (SFD, also called ophidiomycosis) is caused by *Ophidiomyces ophidiicola*, which can cause severe disease and mortality in some species (Allender et al., 2015a, 2016; Latney & Wellehan, 2020; Lorch et al., 2016). SFD has been identified in many snake species in the eastern USA. SFD often causes mild skin infections in snakes emerging from hibernation and is perhaps responsible for some or most lesions called "hibernation sores" (Lorch et al., 2016).

Laboratory experiments have confirmed that SFD is caused by *O. ophidiicola* and that snakes experimentally infected develop the abnormalities and altered behavior seen in some wild snake species from which the fungus has been isolated (Allender et al., 2015b; Lorch et al., 2015; McKenzie et al., 2020, 2021). Allender et al. (2015a) demonstrated that *O. ophidiicola* can utilize many carbon sources and is capable of growth (albeit slower than optimal growth) at low temperatures consistent with what might be encountered in snake hibernacula. Paré and Sigler (2016) subsequently hypothesized that hibernacula could serve as transmission sites for snakes and that mortality could be "substantial" in hibernacula where snakes congregate. Indeed, suspected transmission of the fungus between snakes through direct and indirect contact has been reported (Britton et al., 2019; Stengle et al., 2019). It has also been suggested that large, terrestrial snakes that overwinter communally are susceptible to SFD (Burbrink et al., 2017; Chandler et al., 2019; Lorch et al., 2016; Paré & Sigler, 2016). Continuous monitoring of populations of vulnerable snake species is essential to understand the prevalence in individuals and population effects of the disease and to aid in forming conservation goals and designing management strategies (Baker et al., 2019; McKenzie et al., 2019). Yet, monitoring snakes is difficult for many species because they are secretive, often fossorial, and may be rare.

The Northern Pine Snake (*Pituophis melanoleucus melanoleucus*, family Colubridae) is an ideal model for monitoring *O. ophidiicola* in wild snakes because it can be located reliably while hibernating. It is a large constrictor that can reach nearly 2 m in length (Burger & Zappalorti, 2011). In New Jersey, the Northern Pine Snake's population is disjunct from the core range by several hundred kilometers (Burger & Zappalorti, 2011, 2016; Golden et al., 2009). The Northern Pine Snake (hereafter referred to as Pine Snake) is the only North American snake known to always excavate its own nests and hibernation sites. Pine Snakes usually hibernate communally. They have high fidelity to these hibernation sites, and other species sometimes hibernate with them (Burger & Zappalorti, 2015). Often several Pine Snakes hibernate in the same den and share side chambers, sometimes in contact with one another (Burger et al., 1988). Communal denning provides favorable conditions for transmission of disease (Burbrink et al., 2017), and the snakes move in and out of the hibernation entrance tunnels for several weeks in the fall, encountering one another both above and below ground (Burger, 2019). Campbell et al. (2021) have previously shown that the soil of the hibernation dens in our study sites has a reservoir of *O. ophidiicola*, demonstrating the potential for environmental transmission as well.

In this study, we examined the prevalence of *O. ophidiicola* (based on DNA detections by qPCR) in free-ranging Northern Pine Snakes near the end of hibernation in the New Jersey Pinelands from 2018 to

2021. We sampled snakes at the end of the hibernation period, just before spring emergence. In addition, we sampled some hatchlings in the fall, as well as in hibernation. Our objectives were to determine how *O. ophidiicola* prevalence on snakes varied by year, age, and sex, whether hatchling snakes enter hibernation with *O. ophidiicola* or acquire it during the hibernation period, and whether soil directly beneath hibernating snakes contained *O. ophidiicola*. We thus test the hypotheses that (1) *O. ophidiicola* prevalence on snakes has no relationship to the soil on which they are hibernating; (2) infection rates have no differences by year, age class, or sex; (3) the snakes have no differences among sampling sites; and (4) *O. ophidiicola* prevalence in hatchlings has no differences in the fall and spring. Our overall goal was to understand the epizootiology of SFD in hibernating Northern Pine Snakes. We were concerned that the fungus might be causing substantive morbidity and mortality among Pine Snakes, particularly in their communal hibernation dens. Many snake species are in rapid decline because of many causes, including habitat loss, pollution, predation, over-exploitation and poaching, road mortality, and diseases, particularly emerging infections. Such threats may be amplified by habitat fragmentation, climate change, or other global changes (Gibbons et al., 2000; Lorch et al., 2016; Reading et al., 2010). The relative importance of different stressors likely varies among species and even in different populations within a species. Understanding how diverse stressors affect populations is key to conservation and management.

Methods and materials

The population of Pine Snakes sampled for this work have been studied since the 1980s as part of a long-term monitoring program (Burger & Zappalorti, 2015, 2016). In New Jersey, Pine Snakes emerge from hibernation in late March–April, mate in April–May, and nest in late June–early July. Hatchlings typically emerge in early September, remain in vegetative cover and forage over the autumn, and then must find a place to hibernate in the late fall, which is usually a site used by older conspecifics (Burger & Zappalorti, 2011; Burger et al., 2018). A snake that hatched in September (either in the laboratory or in the wild) is designated age 0 in our dataset if encountered in the fall of its hatching year. The same snake, encountered in the hibernacula when they are excavated in early March, is designated as 1-year old. The following March, this snake (ca 18 months old) is designated a 2-year old. We report results separately for the 1-year-old snakes and for snakes that are designated 2 years or older (age 2+).

We conducted this study at three "hibernacula complexes" that were excavated, modified, and subsequently reconstructed. These hibernacula complexes consisted of 3–5 dens each in Burlington and Ocean Counties, New Jersey. The exact locations are not divulged here to protect snakes from potential poaching (Burger & Zappalorti, 2016). Each active den had 1–15 Pine Snakes hibernating in it each year. This study was approved by the Rutgers University Animal Care and Use Committee (permit # E6-017), the New Jersey Department of Environmental Protection (Endangered and Nongame Species Program), the New Jersey Division of Parks and Forestry, and with permission from private landowners. The welfare of the snakes was given greatest consideration in the design of our studies and timing of our activities.

We initiated a pilot study of SFD at our study sites in 2018 based on 12 individual snakes and conducted a more detailed study from 2019 to 2021. We excavated snakes from the hibernacula once per year in late February to mid-March, depending upon weather conditions. At our study sites, Pine Snakes normally emerge from hibernation from mid-March to mid-April, and all excavations were performed prior to the animals emerging in spring. After sampling (see below) and removing all the snakes encountered, we reconstructed the hibernacula by using cement blocks to form a main chamber for each den, with a wooden board for a roof. After a hibernaculum was rebuilt, snakes were released at the entrance the same day (Burger & Zappalorti, 2011, 2015; Burger et al., 1988).

When snakes enter in the fall, they go into the main chamber and dig their own tunnels and then excavate side chambers in the hard-packed sand (Burger & Zappalorti, 2011). Figure 1 illustrates a typical hibernaculum that is rebuilt each year.

Field sampling

During excavation of the hibernacula, snakes were removed, and examined visually for the presence

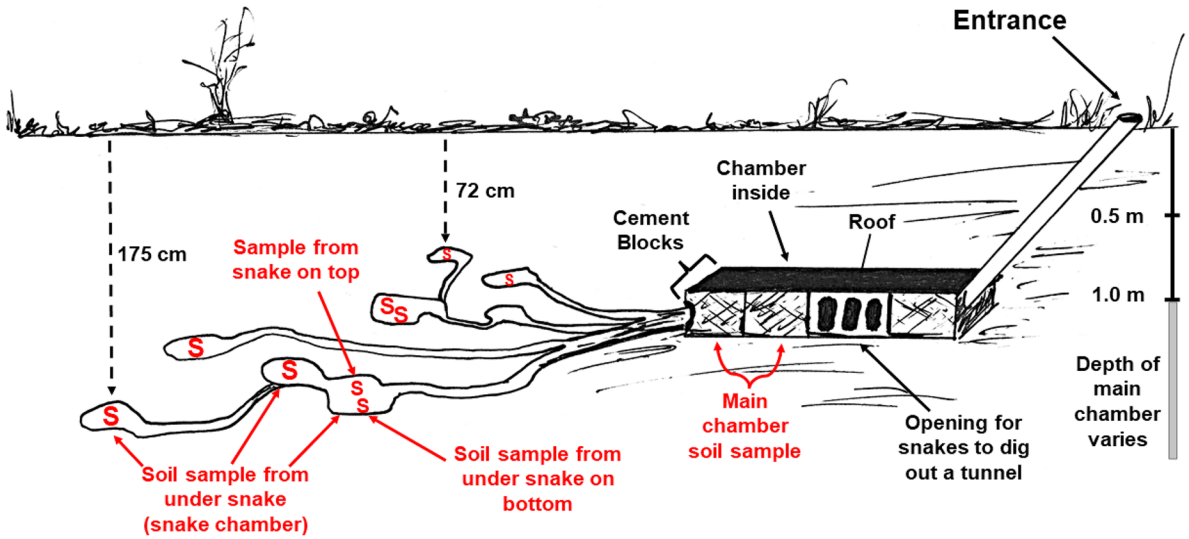

Fig. 1 Design of typical reconstructed hibernaculum showing locations of snakes and soil sample collection. All S represent a snake in a side chamber. All snakes were sampled, and in some years, soil was removed from beneath them

of gross skin lesions, head lesions, or other abnormalities. They were swabbed for detection of *O. ophidiicola*, measured (snout-vent length, total length), weighed, and injected with a passive integrated transponder (PIT) tag (AVID Identification Systems, Inc. Norco, California) if not previously marked. All persons handling the snakes wore disposable nitrile examination gloves (VWR International, Radnor, Pennsylvania) and changed gloves after each snake. When SFD was first identified in New Jersey, we initiated practices to prevent cross-contamination from one hibernaculum to another. All shovels, digging equipment, and our boots were washed with a 10% bleach solution when moving from one site to another.

Our protocol for sampling individual snakes included swabbing different parts of the body using a sterile polyester tipped swab (#MW113, MWE Medical Wire, Corsham, UK) premoistened with 20 µL of sterile deionized water. In 2018, single skin swab samples were collected from lesions and from the ventral surfaces of 12 Pine Snakes as a pilot project. In 2019–2021, we sampled the ventral surface of each snake by firmly swabbing downward from the neck to just anterior to the cloaca, using a single continuous pass and excluding any lesions present on the ventral surface. In 2019, 2020, and 2021, additional swabs were taken from the head (regardless of whether lesions were present) and from each individual lesion observed on the body. Lesions included discolored elevated scales, discolored ragged margins of ventral scales, abraded scales, and swollen or mounded scales (see Fig. 2). The sample of the head was collected by stroking the moistened swab over the top of the head and along the mandibles on both sites of the face. We targeted the head for sampling because SFD has previously been described as affecting the head in rattlesnakes (McBride et al., 2015). Swabs were then stored in screwcap tubes (Thermo Scientific, Waltham, Massachusetts), placed on ice in the field, and stored frozen at −30 °C in a freezer for later analysis.

In 2018, we collected samples from only 12 Pine Snakes. In 2019–2021, we sampled all Pine Snakes found in the study hibernacula ($n=72$). In all, we sampled 41 hatchlings (0-year old), 20 one-year olds, and 84 snakes that were ≥ 2 years old. We swabbed the ventral surface of hatchlings located in the fall (prior to entering hibernacula, $n=41$), and tested juveniles ($n=20$) from the end of hibernation. We also swabbed the ventral surface of nesting gravid females ($n=4$) and the surface of eggs ($n=18$, eggs from females that had recently laid). Nesting females in June, and hatchlings in the fall, were swabbed ventrally (none had lesions).

In 2019 and 2020, soil samples were collected from the bottom of the main hibernation chamber (that we constructed, $n=8$), and from beneath snakes that

Fig. 2 Examples of skin lesions or "hibernation sores" observed in Northern Pine Snakes at the study sites

excavated their own tunnels and chambers ($n=39$, see Fig. 1). Soil samples were collected after snake removal, stored in plastic bags, and later frozen at −30 °C).

Sample sizes are also provided on all graphs and figures.

Detection of *Ophidiomyces ophidiicola*

Nucleic acid was extracted from swab samples and screened for the presence of *Ophidiomyces ophidiicola* using a specific qPCR targeting the internal transcribed spacer region of the fungus as described previously (Bohuski et al., 2015). Soil samples were processed and screened using the qPCR as described in Campbell et al. (2021). We defined a sample as positive for *O. ophidiicola* if it had 15 or more copies of target DNA (as determined based on standard curves included on each PCR run) detected in a sample; this represents the limit of detection for the PCR assay. We considered a snake to be positive for *O. ophidiicola* if at least one of its swab samples was positive.

Statistical analysis

To determine if there was a significant relationship between the detection of *O. ophidiicola* on a given snake and in soil collected directly under that snake, we used the non-parametric test Fisher's exact (Siegel, 1956) which is a conservative test for small sample sizes (McDonald, 2022). We also used Fisher's exact test to determine differences among years, sexes, and sampling sites. To determine the relative risk of a snake being positive if it was on top of qPCR-positive soil, we divided the proportion of detections in snakes found in contact with positive soil by the proportion of detections if the soil under the snake was negative. A value of 1 equals no additional risk. A probability level of $p<0.05$ was considered significant. Graphics were generated with DeltaGraph 7 (RedRock Software, 2013).

Results

Relationship between detection of *O. ophidiicola* in hibernacula soils and in snakes in those hibernacula

The snakes move through the main chamber at the beginning of hibernation to get to soil where they dig side chambers and remain in those side chambers for the rest of hibernation. The *O. ophidiicola* positivity percentage for soil samples in the main hibernaculum chamber (4 of 8 were positive, 50%) was lower than in the side chambers where snakes hibernated (27 of 39 were positive, 69%).

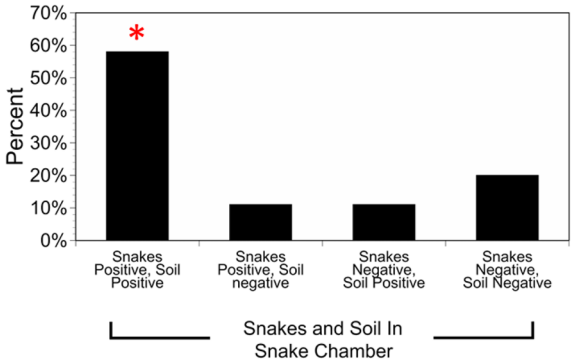

Fig. 3 Percentage of soil and snake paired samples testing positive for *Ophidiomyces ophidiicola* (based on qPCR) in hibernating chambers. Breakdown (by percent) of snakes testing positive for *O. ophidiicola* relative to the detection of *O. ophidiicola* in the soil beneath those snakes. An asterisk indicates a significant relationship between the detection of the pathogen on a snake and in the soil directly underneath that individual snake. The dataset only includes snakes that were ≥ 2 years old ($n = 39$)

In the side chambers where snakes hibernated, the distribution of snake and soil positivity for *O. ophidiicola* were related: 23 of 27 snakes on a positive soil sample tested positive for the fungus, whereas only 4 of 12 snakes collected from on top of negative soil samples were positive for *O. ophidiicola*; these differed from chance (Fisher's exact $p = 0.002$). Overall, 20% of the snakes were negative and on negative soil. Most snakes in chambers where the soil tested positive were also positive. However, some snakes located in chambers where the soil tested positive were themselves negative, and vice versa (Fig. 3). The relative risk of a snake being positive if it was lying in soil that tested positive for *O. ophidiicola* was 2.55 (95% confidence interval of 1.13 to 5.78). One-year-old snakes were not included in this analysis because they were often in soft sand without any obvious chamber; this made it difficult to collect soil that was in direct contact with the snakes.

Ophidiomyces ophidiicola prevalence in snakes based on collection year, sex, and location on snake

To test the hypothesis that prevalence of *O. ophidiicola* on snakes does not differ by year, we only included snakes ≥ 2 years of age because in some years there were few 1-year-old snakes. The overall prevalence of *O. ophidiicola* in Pine Snakes by sampling year was 58% (2018), 72% (2019), 83% (2020), and 70% (2021) (Table 1). Because 2018 was a pilot year with few snakes and a slightly different sampling regime, they were not included in the statistical analysis. There was no consistent temporal pattern in the overall positivity for ventral surface or head samples for 2019–2021. There was no statistical difference among or between years. We also tested for significance of the difference between the year with the lowest prevalence year (2018), and it did not differ significantly from the year with the highest prevalence (2020) (Fisher's exact $p = 0.125$).

It should be noted that in 2018, we analyzed only 12 snakes, and they were swabbed slightly differently than the other snakes. They were swabbed along the ventral, including ventral skin lesions as well. In 2019 to 2021, we followed a protocol of sampling the snake ventrally (avoiding the lesions), and then sampled each lesion separately. Thus, when examining

Table 1 Prevalence of *Ophidiomyces ophidiicola* as a function of sampling year and portion of the body targeted for sampling of Northern Pine Snakes ≥ 2 years of age sampled at the end of hibernation (from the New Jersey Pinelands)

Sampling site	2018[a]	2019	2020	2021	Overall % for 2 years and older[a]
Sample size	12	25	24	23	
Any sample positive (%)	7 (58%)	18 (72%)	20 (83%)	16 (70%)	73% ($n = 84$)
Ventral positive (%)	7 (58%)	6 (24%)	7 (29%)	9 (39%)	40% ($n = 72$)
Head positive (%)		4 (16%)	7 (29%)	4 (18%)	21% ($n = 72$)
Snakes with at least one positive skin lesion (%)		13(52%)	17(71%)	14 (61%)	61% ($n = 72$)

[a]In 2018, only skin lesions/ventral samples were collected. Ventral is perhaps higher in 2018 because skin lesions on the ventral surface were sampled with the ventral body swab. Note that the 20 hatchlings found in hibernation are not part of the above table

temporal differences, we did not use the 2018 snakes (e.g., sample size was 72 snakes for yearly analysis).

We examined the differences in pathogen prevalence between males and females for snakes ≥ 2 years of age that were sampled from 2018 to 2021 (Fig. 4). Males had significantly higher prevalence of *O. ophidiicola* (82%) than females (62%) (Fig. 4; Fisher's exact $p = 0.014$). Similarly, males had significantly higher prevalence of *O. ophidiicola* for ventral swabs (Fisher's exact $p = 0.005$), and males had significantly more gross (i.e., obvious) skin lesions (Fisher's exact $p = 0.03$) than did females.

Skin lesions were significantly more likely to test positive for *O. ophidiicola* (44 of 72 snakes) compared with ventral samples (22 of 72 snakes; Fisher's exact $p < 0.0001$) or head samples (15 of 72 snakes were positive; Fisher's exact $p < 0.0001$). Many skin lesion swabs did not test positive by qPCR (i.e., 21%). The variation in detection of *O. ophidiicola* from different sites on the snakes indicates that swabbing lesions is more reliable to determine whether the fungus is present on a snake. Almost all lesions detected were on the ventral surface (90%, 149/166), and not the head or dorsal surfaces.

Ophidiomyces ophidiicola prevalence in hatchling Northern Pine Snakes

The change in prevalence of *O. ophidiicola* in hatchling (first year) snakes prior to entering hibernation and near the end of the hibernation season is significant. In the fall, none of the 41 lab-raised and field hatchlings that were sampled were positive for the fungus. However, in March first year snakes (now considered 1-year olds, n = 20) had a prevalence of 75%. Prevalence percentages by the end of the first hibernation season were like that found in adult snakes ≥ 2 years old (73%; $n = 84$ for all adult snakes).

Differences in *Ophidiomyces ophidiicola* prevalence of 1-year olds versus older snakes

The differences between 1-year olds (hatchlings from the previous August or September) and snakes ≥ 2 years old are shown in Fig. 5. Although there were no discernible differences in the overall prevalence from different sampling sites on the body, a significantly higher percentage of snakes ≥ 2 years old had skin lesions compared to 1-year-old snakes (Fisher's exact $p = 0.048$). That is, snakes in their first year of life were less likely to be symptomatic than older snakes.

Female Pine Snakes and their eggs

We captured and swabbed four nesting females in early July (2019 and 2020). *Ophidiomyces ophidiicola* was not detected on these females at the time of egg-laying, although some had been positive months earlier in the hibernaculum. We also collected 18 swab samples from eggs in nests, all of which tested negative for the presence of *O. ophidiicola*.

Other snakes in Pine Snake hibernacula

Although we did not concentrate on other species, corn snakes (*Pantherophis guttatus*), timber rattlesnakes (*Crotalus horridus*), and North American Racers (*Coluber constrictor*) were found co-habiting hibernacula with Pine Snakes at our study sites and some of these species tested positive for *O. ophidiicola* without lesions.

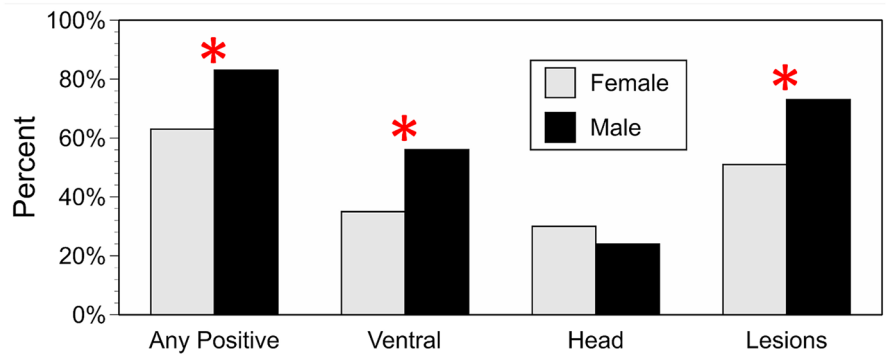

Fig. 4 Prevalence of *Ophidiomyces ophidiicola* for male and female Northern Pine Snakes from the New Jersey Pinelands ($n = 84$ for "any positive" categories, $n = 72$ for head, ventral, and skin lesions categories). *Significant difference between males and females for snakes ≥ 2 years of age

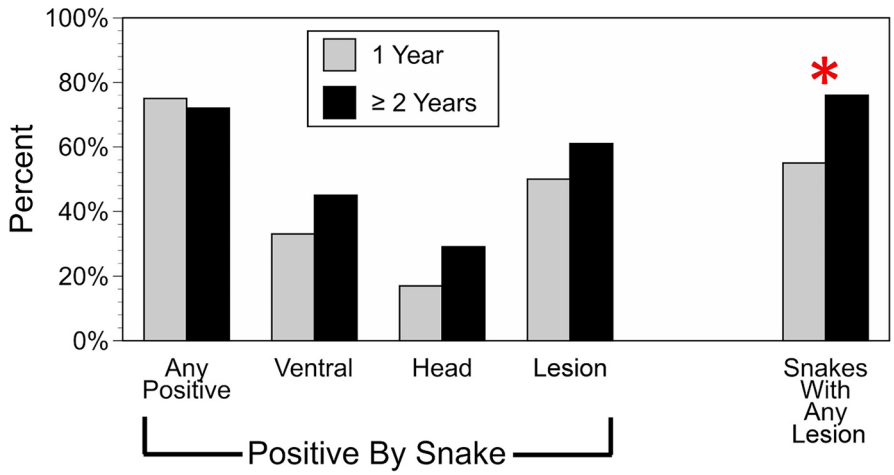

Fig. 5 Prevalence of *Ophidiomyces ophidiicola* in 1-year old Pine Snakes ($n=20$) compared to older (≥ 2 years old) snakes ($n=72$). *Significant difference. The last two bars indicate the percent of snakes that had any gross skin lesion ("hibernation sore")

Overall, our results indeed show a high prevalence of the fungus in Northern Pine Snakes from hibernacula that we studied in three areas within Burlington and Ocean Counties, NJ. Hatchlings did not test positive before entering hibernation but subsequently tested positive in spring prior to emergence from their first hibernation season. Prevalence in these first-year snakes was like that of older snakes. Despite the presence of skin lesions in hibernating snakes, we did not observe mortality or moribund animals. Snakes and lesions examined during this study looked similar to snakes we have been observing at these sites since the 1980s, and it is likely that these animals have been exposed to *O. ophidiicola* for quite some time (although officially testing for *O. ophidiicola* in this population did not occur until 2018).

Discussion

Prevalence

Classifying any Pine Snake with at least one qPCR-positive swab sample (head, ventral, skin lesion) as positive, the prevalence of *O. ophidiicola* in our study populations, varied from 58 to 83% between sites and years. We did not observe any moribund or dead snakes during our work, indicating that Pine Snakes may tolerate infections by *O. ophidiicola* without being as severely affected as species such as the Eastern Massasauga (*Sistrurus catenatus*, Allender et al., 2011). Such a high prevalence of *O. ophidiicola* has previously been reported in some wild snake populations. Specifically, McKenzie et al. (2019) reported a prevalence of 66% in aquatic snake species at their study sites. Others have previously reported that pathogen prevalence is higher in snake populations than clinical signs of SFD. For example, in the abovementioned example, 42.3% of aquatic snakes sampled in the spring without clinical signs of SFD were PCR-positive for the fungus (McKenzie et al., 2019); similarly, Haynes et al. (2020) reported that prevalence of lesions ranged up to 43% in several species in Georgia. It is unclear whether the snakes testing positive for *O. ophidiicola* without clinical signs in our study had subclinical infections. However, as noted above, several Pine Snakes in the present study that tested positive in one year were recaptured and appeared healthy and tested negative in following years. We note that at the end of sampling in 2020, 13 snakes that had tested positive in previous years were still alive and had returned to our hibernacula. Understanding the survival of infected snakes would benefit from further analysis and is planned as the subject of future studies.

Prevalence of *O. ophidiicola* on asymptomatic snakes is reportedly highest in spring (McKenzie et al., 2019) after emergence from hibernation. Although infection with *O. ophidiicola* has been postulated to occur during hibernation (Paré & Sigler, 2016), data has previously been lacking to support this, primarily because snakes are difficult to access and sample within hibernacula. The high prevalence of *O. ophidiicola* in asymptomatic Pine Snakes sampled in early spring and the presence of skin lesions in some of these animals provides the first direct evidence that

snakes are exposed to *O. ophidiicola* in winter and are infected during hibernation.

We found that *O. ophidiicola* was most commonly detected on swabs of skin lesions. The overall prevalence of *O. ophidiicola* among all lesions was 79%. Lesions occurred largely on the ventral scales (about 90%), and 40% of ventral swabs were positive. Ventral swabs avoided sores because the sores were swabbed separately. Swabs of the head skin of Pine Snakes also frequently tested positive for *O. ophidiicola* (21%), although fewer than 3% of the all sores were on the head. We sampled the head because others have found a high prevalence of lesions on the heads of snakes (McBride et al., 2015), and an infection on the head could greatly impact fitness by affecting feeding and predator detection. However, despite detection of *O. ophidiicola* on the head, lesions on the head were rare (< 5%). The reasons why Pine Snakes do not develop clinical signs of infection on the head is unclear. The head and rostral scale of Pine Snakes is adapted for digging, and we hypothesize that the thicker and tougher head scales may be more resistant to infection.

Although skin lesions frequently tested positive for *O. ophidiicola*, swabs collected from lesioned skin occasionally tested negative in this study (specifically, 21%). Whether lack of detections of *O. ophidiicola* from lesioned skin represent actual false negatives is difficult to discern. Other etiologies can result in clinical signs of dermatitis that are similar to those clinical signs caused by *O. ophidiicola* (Maas 3rd, 2013); thus, some of the skin lesions that tested negative for the fungus could have resulted from other causes. More likely, the technique of swabbing the surface of the skin overlooks fungus residing farther down in the epidermis. Swabbing multiple lesions on a single snake may therefore be necessary to detect *O. ophidiicola* on infected animals (Hileman et al., 2018).

Males and females may have different prevalence of infection due to sex differences in immune or hormonal function or in morphology, behavior, and ecology (Chandler et al., 2019; Dunlap & Schall, 1995; Lind et al., 2018; Long et al., 2019). Several studies, however, have reported no difference in prevalence by sex with respect to *O. ophidiicola* infection. For example, no sex, age-class, or body-size effects were noted for Eastern Indigo Snakes (*Drymarchon couperi*) (Chandler et al., 2019); similarly, there was no effect of sex, body length, or body mass for North American Racers, Gray Ratsnakes (*Pantherophis spiloides*), and Eastern Gartersnakes (*Thamnophis sirtalis sirtalis*) studied in Ohio (Long et al., 2019). Lind et al. (2018) reported that male Pygmy Rattlesnakes (*Sistrurus miliarius*) in Florida tended to have a higher prevalence of infection than females, although the differences were not significant. Ophidiomycosis may be related to seasonal patterns of reproduction, ecdysis, and thermoregulatory behavior. Our results showed that males had a significantly higher prevalence of *O. ophidiicola* than did females. Older male Pine Snakes may travel longer distances during the active season and have lower hibernation site fidelity (Burger, 2019; Burger & Zappalorti, 2015; Zappalorti et al., 2015), potentially increasing the chances that they will come into contact with the pathogen.

Transmission in Pine Snake hibernacula

Snake hibernacula are potential transmission sites for *O. ophidiicola* because of the congregation of large numbers of hosts for long periods of time. In this study, *O. ophidiicola* was found in hibernacula soil at all dens sampled. The pathogen was more frequently detected in soil samples collected directly beneath hibernating snakes in chambers (69%), compared to soil in the main chambers (50%). Specifically, soil under snakes had a 1.4 times greater risk of testing positive for the fungus than soil collected from parts of the hibernacula where there were no snakes (the main chamber). Thus, the fungus was less commonly detected in the main chamber where snakes pass through (but do not reside for long periods of time) compared to side chambers where the snakes spend ≥ 3 months.

The relationship between detection of *O. ophidiicola* on snakes and detection of the fungus in soil residing under snakes was significant. In our paired sampling study, qPCR results for snakes and their corresponding soil samples matched 80% of time. The discordant results for the remaining 20% of cases could be the result of snakes occasionally moving within the hibernaculum, snakes hibernating on top of other snakes (such that they were not in direct contact with the soil), fungus being established in the soil without establishing an infection on the snake, or limitations in the detection capabilities of the qPCR assay (especially in soil extracts where inhibitors may reduce the sensitivity of PCR). Several authors have suggested that hibernation

sites, including Gopher Tortoise (*Gopherus polyphemus*) burrows, are characterized by stable temperatures and high humidity, which is ideal for growth of *O. ophidiicola* (Campbell et al., 2021; Chandler et al., 2019; Paré & Sigler, 2016). We cannot definitively conclude whether hibernacula soil serves as a reservoir to infect Pine Snakes during the winter or whether the presence of *O. ophidiicola* in soil is simply due to shedding of the fungus from infected animals. A previous study conducted at some of our study sites demonstrated that *O. ophidiicola* is viable and has the capacity to grow in hibernacula soil (Campbell et al., 2021). In the same study, it was unclear whether the fungus could compete with the microbial community in the soil to replicate without a snake host; however, *O. ophidiicola* conidia were able to persist and likely remain infectious (Campbell et al., 2021). Furthermore, we observed that adult Pine Snakes do not usually have skin lesions prior to entering hibernation ($n=4$), and that *O. ophidiicola* is detected for the first time on juvenile Pine Snakes at the end of their first hibernation season. Pine Snakes in our study also had lesions most frequently on the ventral scales which are the part of the body in direct contact with the soil. These findings strongly indicate that snakes become exposed to *O. ophidiicola* within the hibernaculum (most likely initiated by pathogen reservoirs residing in the soil). Snakes that become infected because of that exposure then likely shed *O. ophidiicola* back into the soil where the reservoir is re-established.

Our findings have important management implications for snakes that communally hibernate because they indicate that intervention strategies targeting hibernacula, or the hibernation period, could prevent or limit infections in snakes. Furthermore, these data indicate that movement of snakes between hibernation sites could facilitate spread of *O. ophidiicola*, similar to what has been described for white-nose syndrome in bats (Hoyt et al., 2018). Pine Snakes have been observed moving between hibernation sites in proximity (sometimes within the same day) (Burger, 2019) or sites located up to 3 km away (Zappalorti et al., 2015).

Potential effects of *O. ophidiicola* infections on Northern Pine Snakes

We observed high prevalence of fungus detections in Pine Snakes over the 4-year study period, and we had noted clinical lesions now suggestive of SFD for nearly 40 years prior to initiating this study. Those anecdotal observations are consistent with studies demonstrating that *O. ophidiicola* has been present in wild snake populations in the USA for decades (Ladner et al., 2022; Lorch et al., 2021). Thus, it is likely that SFD is enzootic at our study sites.

Despite high prevalence of positive detections, we saw no evidence that SFD was having an immediate effect on the Pine Snake population. We did not observe severe manifestations of disease as has been reported by others (e.g., Allender et al., 2011; McBride et al., 2015). Paré and Sigler (2016) pointed out the potential for mortality due to SFD to occur in snake hibernacula, but we have not found any dead or moribund snakes in our study due to SFD. Many snakes with *O. ophidiicola* positive lesions appear to recover (Chandler et al., 2019; Lorch et al., 2015, 2016). Indeed, four female snakes that had skin lesions when sampled during hibernation, tested positive for *O. ophidiicola*, but were free of clinical signs when sampled 3 months later.

The reasons that *O. ophidiicola* infections appeared so mild in the populations we examined are unclear. Host susceptibility to SFD is likely highly variable taxonomically and geographically (Burbrink et al., 2017; Lorch, 2016). Thus, Northern Pine Snakes possibly exhibit lower susceptibility than some other snake species or perhaps populations in the New Jersey Pinelands experience environmental conditions that are inconducive to the development of severe disease. Studies exploring disease prevalence and severity of Pine Snake populations in other areas (e.g., Tennessee, Kentucky, North Carolina, and South Carolina) could lead to a better understanding of which factors influence disease outcome in this species. Variability in pathogenicity between strains of *O. ophidiicola* may also occur (Ladner et al., 2022). We did not characterize the strains or genetic lineages of *O. ophidiicola* that occur at our study sites. We should note, however, that pathogen strain characteristics or environmental factors seem to be the most plausible explanation for the predominantly mild infections we observed.

Conclusions

Northern Pine Snakes in the New Jersey Pinelands have a high prevalence of *Ophidiomyces ophidiicola* at the end of hibernation. In nearly 60% of the cases,

the snake and soil beneath it were both positive for the pathogen. Eggs and hatchlings all tested negative, but over the winter, about 75% of the hatchlings, then 1-year olds, became positive. Other species found in the hibernacula with them have also tested positive, but the prevalence and fate in these other species are unknown. Sampling methods matter for Pine Snakes. Gross lesions have a higher positivity percentage than ventral surface or head swabs in Pine Snakes. This indicates, given the variability in methods and techniques applied for different studies, that development of standard terminologies and methods would be beneficial for futures studies. Although the positivity rate in Pine Snakes, even if using only ventral swabs, is over 30%, we found no evidence of obvious health effects beyond the presence of skin lesions. We did not observe dead or dying snakes linked to severe skin disease at our study sites in the over 35 years of working at these locations. Additionally, snakes found in summer usually have no "hibernation sores," and those swabbed had no *O. ophidiicola* detected. The apparent high rate of SFD at the end of the Pine Snake hibernation period appears to have no discernible effect, and skin lesions seem to heal quickly after shedding in the spring. The lack of apparent health effects (and mortality) indicates Pine Snakes may be tolerant to *O. ophidiicola* infection and that SFD is enzootic in these populations. The lack of severe disease could also be due to less virulent strains of *O. ophidiicola* occurring in the New Jersey Pinelands.

Acknowledgements The authors thank the many people who have helped throughout these studies, particularly Taryn Pittfield and Dave Burkett for sample collection and Daniel Taylor and Megan Winzeler for assistance with sample processing in the laboratory. The authors thank the several individuals, NGOs, and state agencies who provided permission to access their lands, the NJ Division of Parks and Forests, NJ Division of Fish and Wildlife, NJ Natural Lands Trust, New Jersey Conservation Foundation, and the Pinelands Preservation Alliance. We thank the NJ Department of Environmental Protection and Division of Parks and Forests for permits. All snake studies were conducted with approval from the Rutgers University Institutional Animal Care and Use Committee (Protocol 86-017, renewed every 3 years).

Author contribution Joanna Burger: designed and executed the study, field collection, data analysis, writing, editing. Michael Gochfeld: conducted QA/QC, sample collection, data analysis, editing. Robert Zappalorti: sample collection, long-term study, editing. John Bunnell: study design, sample collection, editing. Christian Jeitner: aided in long-term study, sample collection, statistical analysis. David Schneider: sample collection. Kelly Ng: sample collection, statistical analysis, graphics. Emile DeVito: aided in long-term study, editing. Jeffrey Lorch: study design, conducted SFD analysis, editing.

Funding Specific funding for the SFD studies came from the Pinelands Commission, Pinelands Research Station, Rutgers University, and the Lorch laboratory. Funding for the long-term studies was largely from Rutgers University, the Tiko Fund, Herpetological Associates, and the NIEHS Center of Excellence (NIH-NIEHS P30ES005022).

Data availability The data are available from the senior author upon request.

Declarations

Disclaimer Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

Ethics approval and consent to participate Not applicable. All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of authors" as found in the Instructions for Authors and are aware that with minor exceptions, no changes can be made to authorship once the paper is submitted.

Conflict of interest The authors declare no competing interests.

Compliance with ethical standards This project was completed with appropriate protocols from Rutgers University, and within all ethical standard guidelines.

References

Allender, M. C., Dreslik, M., Wylie, S., Phillips, C., Wylie, D. B., Maddox, C., Delaney, M. A., & Kinsel, M. J. (2011). *Chrysosporium* sp. infection in Eastern Massasauga rattlesnakes. *Emerging Infectious Diseases, 17*, 2383–2384.

Allender, M. C., Raudabaugh, D. B., Gleason, F. H., & Miller, A. N. (2015a). The natural history, ecology, and epidemiology of *Ophidiomyces ophiodiicola* and its potential impact on free-ranging snake populations. *Fungal Ecology, 17*, 187–196.

Allender, M. C., Baker, S., Wylie, D., Loper, D., Dreslik, M. J., Phillips, C. A., Maddoz, C., & Driskell, E. A. (2015b). Development of snake fungal disease after experimental challenge with *Ophidiomyces ophiodiicola* in Cottonmouths (*Agkistrodon piscivorous*). *PloSOne, 10*, E014093.

Allender, M. C., Hileman, E. T., Moore, J., & Tetziaff, S. (2016). Detection of *Ophidiomyces ophiodiicola*, the causative agent of snake fungal disease, in the Eastern Massasauga (*Sistrurus catenatus*) in Michigan. *Journal of Wildlife Diseases, 52*, 694–698.

Baker, S. J., Haynes, E., Gramhofer, M., Standord, K., Bailey, S., Christman, M., Conley, K., Frasca, S., Jr., Ossiboff, R. J., Lobato, D., & Allender, M. C. (2019). Case definition

and diagnostic testing for snake fungal disease. *Herpetological Review, 50*, 279–285.

Blehert, D. S., Hicks, A. C., Behr, M., Meteyer, C. U., Berlowski-Zier, B. M., Buckles, E. L., Coleman, J. T. H., Darling, S. R., Gargas, A., Niver, R., Okoniewski, J. C., Rudd, R. J., & Stone, W. R. (2009). Bat white-nose syndrome: An emerging fungal pathogen? *Science, 323*, 227.

Bohuski, E., Lorch, J. M., Griffin, K. M., & Blehert, D. S. (2015). TaqMan real-time polymerase chain reaction for detection of *Ophidiomyces ophiodiicola*, the fungus associated with snake fungal disease. *Veterinary Research, 11*, 95–105.

Britton, M., Allender, M. C., Hsiao, S. -H., & Baker, S. J. (2019). Postnatal mortality in neonate rattlesnakes associated with *Ophidiomyces ophiodiicola*. *Journal of Zoo and Wildlife Medicine, 50*, 672–677.

Burbrink, F. T., Lorch, J. M., & Lipps, K. P. (2017). Host susceptibility to snake fungal disease is highly dispersed across phylogenetic and functional trait space. *Science Advances, 3*, e1701387.

Burger, J. (2019). Vulnerability of Northern Pine Snakes (*Pituophis melanoleucus*, Daudin, 1803) during fall den ingress in New Jersey, USA. *Amphibian and Reptile Conservation., 13*, 102–114.

Burger, J., & Zappalorti, R. T. (2011). The Northern Pine Snake (*Pituophis melanoleucus*) in New Jersey: Its life history, behavior and conservation. Nova Science Publisher, Inc. New York, New York.

Burger, J., & Zappalorti, R. T. (2015). Hibernation site philopatry in Northern Pine Snakes (*Pituophis melanoleucus*) in New Jersey. *Journal of Herpetology, 49*, 245–251.

Burger, J., & Zappalorti, R. T. (2016). Conservation and protections of threatened Pine Snakes (*Pituophis melanoleucus)* in the New Jersey Pinelands USA. *Herpetological Conservation and Biology., 11*, 304–314.

Burger, J., Zappalorti, R. T., Gochfeld, M., Boarman, W., Caffrey, M., Doig, V., Garber, S., Mikovsky, M., Safina, C., & Saliva, J. (1988). Hibernacujla and summer dens of Pine Snakes (*Pituophis m. melanoleucus*) in the New Jersey Pinelands. *Journal of Herpetology, 22*, 425–433.

Burger, J., Zappalorti, R. T., & Gochfeld, M. (2018). Hatchling survival to breeding age in Northern Pine Snakes (*Pituophis melanoleucus*) in the New Jersey Pinelands: Human effects on recruitment from 1986 to 2017. *PLoS ONE, 13*, e0195676.

Campbell, L. J., Burger, J., Zappalorti, R. T., Bunnell, J. F., Winseler, M. E., Taylor, D. R., & Lorch, J. M. (2021). Soil reservoir dynamics of *Ophidiomyces ophidiicola*, the causative agent of snake fungal disease. *Journal of Fungi, 4*, 461. https://doi.org/10.3390/jof7060461

Chandler, H. C., Allender, M. C., Stegenga, B. S., Haynes, E., Ospina, E., & Stevenson, D. J. (2019). Ophidomycosis prevalence in Georgia's Eastern indigo snake (*Drymarchon couperi*) populations. *PLOS One*. June 2019. https://doi.org/10.1371/journal.pone.021835

Clark, R. W., Marchand, M. N., Clifford, B. J., Stechert, R., & Stephens, S. (2011). Decline of an isolated timber rattlesnake (*Crotalus horridus*) population: Interactions between climate change, disease, and loss of genetic diversity. *Biological Conservation, 144*, 886–891.

Dunlap, K. D., & Schall, J. J. (1995). Hormonal alterations and reproductive inhibition in lizards, *Scheloporus occidentalis* infected with the malarial parasite, *Plasmodium mexicanum*. *Physiological Zoology, 68*, 608–621.

Gibbons, J. W., Scott, D. E., Ryan, R. J., Buhhmann, K. A., Tuberville, T. D., Metts, B. S., Greene, J. L., Mills, T., Leiden, Y., Poppy, S., & Winne, C. T. (2000). The global decline of reptiles: Déjà vu amphibians. *BioScience, 50*, 653–666.

Golden, D. M., Winkler, P., Woerner, P., Fowles, G., Pitts, W., & Jenkins, D. (2009). Status assessment of the Northern Pine Snake (*Pituophis m. melanoleucus*) in New Jersey: An evaluation of trends and threats. NJ Dept Environmental Protection, Div Fish and Wildlife, Endangered and Nongame Species Progr Trenton, NJ. 53 pp.

Haynes, E., Chandler, H. C., Stegenga, B. J., Adamovicz, L., Ospinal, E., Zerpa-catanhom, D., Stevenson, D. J., & Allender, M. C. (2020). Ophidiomycosis surveillance of snakes in Georgia, USA reveals new host species and taxonomic associations with disease. *Scientific Reports, 10*, 10870. https://doi.org/10.1038/s41598-020-67800-1

Hileman, E. R., Allender, M. C., Bradke, D. R., Faust, L. J., Moore, J. A., Ravesi, M. J., & Tetzlaff, S. J. (2018). Estimation of *Ophidiomyces* prevalence to evaluate snake fungal disease risk. *Journal of Wildlife Management, 82*, 173–181.

Hoyt, J. R., Langwig, K. E., White, J. P., Kaarakka, H. M., Redell, J. A., Kurta, A., DePue, J. E., Scullon, W. H., Parise, K. L., Foster, J. T., Frick, W. F., & Kilpatrick, A. M. (2018). Cryptic connections illuminating pathogen transmission within community networks. *Nature, 563*, 711–713.

Ladner, J. T., Palmer, J. M., Ettinger, C. L., Stajich, J. F., Glorioso, B. M., Lawson, B., Price, S. J., Stengle, A. G., Great, D. A., & Lorch, J. M. (2022). The population genetics of the causative agent of snake fungal disease indicate recent introductions to the USA. *PLoS Biology, 20*(6), e3001676. https://doi.org/10.1371/journal.pbio.3001676

Latney, L. T. V., & Wellehan, J. F. X. (2020). Selected emerging infectious diseases of squamata: An update. *Veterinary Clinics of North America Exotic Animal Practice, 23*, 353–371.

Lind, C. M., Lorch, J. M., Moore, I. T., Vernasco, B. J., & Farrell, T. M. (2018). Seasonal sex steroids indicate reproductive costs associated with snake fungal disease. *Journal of Zoology,* 0952–8369.

Long, R. B., Love, D., Seeley, K. E., Patel, S., Allender, M. C., Garner, M. M., & Ramer, J. (2019). Host factors and testing modality agreement associated with *Ophidiomyces* infection in a free-ranging snake population in southeast Ohio. *USA Journal of Zoology & Wildlife Medicine, 50*(2), 405–413. https://doi.org/10.1638/2018-0143

Lorch, J. M. (2016). Snake fungal disease: An emerging threat to wild snakes: U.S. Geological Survey data release. https://doi.org/10.5066/F7Z31WRB

Lorch, J. M., Knowles, S., Lankton, J. S., Mitchell, K., Edwards, J. L., Kapfer, J. S., Staffen, R. A., Wild, E. R., Schmidt, K. Z., Ballmann, E. A., Blodgett, D., Farrell, T. M., Glorioso, B. M., Last, L. A., Price, S. J., Schuler, K. L., Smith, C. E., Wellehan, J. F., Jr., & Blehert, D. S. (2016). Snake fungal disease: An emerging threat to wild snakes. *Philosophical Transactions Royal Society. B, 371*, 2015–2457.

Lorch, J. M., Lankton, J., Werner, K., Falendysz, E. A., McCurley, K. & Biehert, D. S. (2015). Experimental infection of snakes with *Ophidiomyces ophiodiicola*

causes pathological changes that typify snake fungal disease. *mBio, 6*, e01534–15,

Lorch, J. M., Price, S. J., Lankton, J. S., & Drayer, A. N. (2021). Confirmed cases of *Ophidiomyces* in museum specimens from as early as 1945, United States. *Emerging Infectious Diseases, 27*, 1986–1989.

Maas, A. K., 3rd. (2013). Vesicular, ulcerative, and necrotic dermatitis of reptiles. *The Veterinary Clinics of North America: Exotic Animal Practice, 16*(3), 737–755.

Martel, A., Spitzen-van der Sluijs, A., Blooi, M., Bert, W., Ducatelle, R., Fisher, M. C., Woeltjes, A., Bosman, W., Chiers, K., Bossuyt, F., & Pasmans, F. (2013). Batrachochytrium salamandrivorans sp. Nov. causes lethal chytridiomycosis in amphibians. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(38), 15325–15329. https://doi.org/10.1073/pnas.1307356110

McBride, M. P., Wojick, K. B., Georoff, T. A., Kimbro, J., Garner, M. M., Wang, X., ... & Wellehan, J. F. (2015). *Ophidiomyces ophiodiicola* dermatitis in eight free-ranging timber rattlesnakes (Crotalus horridus) from Massachusetts. *Journal of Zoo and Wildlife Medicine, 46*, 85–94.

McDonald, J. H. (2022). Fisher's Exact Test of Independence. Handbook of Biological Statistics—On Line. https://www.biostathandbook.com/fishers.html#:~:text=Fisher%27s%20exact%20test%20is%20more,test%20for%20larger%20sample%20sizes.

McKenzie, C. M., Oesterle, P. T., Stevens, B., Shirose, L., Mastromonaco, G. F., Lillie, B. N., Davy, C. M., Jardine, C. M., & Nemeth, N. M. (2020). Ophidiomycosis in Red Cornsnakes (*Pantherophis guttatus*): Potential roles of brumation and temperature on pathogenesis and transmission. *Veterinary Pathology, 57*, 825–837.

McKenzie, J. M., Price, S. J., Connette, G. M., Bonner, S. J., & Lorch, J. M. (2021). Effects of snake fungal disease on short-term survival, behavior, and movement of free-ranging snakes. *Ecological Applications, 31*, e02251.

McKenzie, J. M., Price, S. J., Fleckenstein, J. L., Drayer, A. N., Connette, G. M., Bohuski, E., & Lorch, J. M. (2019). Field diagnostics and seasonality of *Ophidiomyces ophiodiicola* in wild snake populations. *EcoHealth, 16*, 141–150.

Paré, J. A., & Sigler, L. (2016). An overview of reptile fungal pathogens in the genera *Nannizziopsis*, *Paranannizziopsis*, and *Ophidiomyces*. *Journal of Herpetological Medicine and Surgery., 26*, 46–53.

Rachowicz, L. J., Hero, J., Alford, R. A., Taylor, J. W., Morgan, J. A. T., Vredenburg, V. T., Collins, J. P., & Briggs, C. J. (2005). The novel and endemic pathogen hypotheses: Competing explanations for the origin of emerging infectious diseases of wildlife. *Conservation Biology, 19*, 1441–1448.

Reading, C. J., Luisell, L. M., Akan, G. C., Bonner, X., Amori, G., Ballouard, J. M., Filippi, E., Naulleau, G., Pearson, D., & Rugiero, L. (2010). Are snake populations in widespread decline? *Biology Letters, 6*, 777–780.

RedRock Software. (2013). DeltaGraph 7.

Siegel, S. L. (1956). Nonparametric Statistics. MGraw Hill, New York, NY.

Skerratt, I. F., Berger, L., Speare, R., Cashins, S., McDonald, K. R., Phillott, A. D., Hines, H. B., & Kenyon, N. (2007). Spread of Chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth, 4*, 125–134.

Stengle, A. G., Farrell, T. M., Freitas, K. S., Lind, C. M., Price, S. J., Butler, B. O., Tadevosyan, T., Isiodoro-Ayza, M., Taylor, D. R., Winzeler, M., & Lorch, J. M. (2019). Evidence of vertical transmission of the snake fungal pathogen *Ophidiomyces ophiodiicola*. *Journal of Wildlife Diseases, 55*, 961–964.

Zappalorti, R. T., Burger, J., & Peterson, F. (2015). Home range size and distance travelled from hibernacula in Northern Pine Snakes in the New Jersey Pinelands. *Herpetologica, 7*, 26–36.