

Apparent Ophidiomycosis Alters Eastern Copperhead (*Agkistrodon contortrix*) Behavior and Habitat Use

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ABSTRACT: Pathogens not only cause mortality but also impose nonlethal fitness consequences. Snakes experience trade-offs associated with behaviors that combat disease but divert time and energy away from other critical activities. The impacts of such behaviors on fitness remain poorly understood, raising concerns amid the emergence of novel herpetofaunal diseases. Ophidiomycosis, caused by the ascomycete fungus *Ophidiomyces ophidiicola*, impacts free-ranging snakes across North America and has been implicated in declines of several imperiled populations. Although previous ophidiomycosis research has primarily focused on disease-related mortality, few studies have evaluated nonlethal impacts on snake fitness. To address this knowledge gap, we investigated the effects of apparent ophidiomycosis on the behavior, habitat use, and movement of snakes in central New Jersey, USA, from 2020 to 2021. Our focal species was the eastern copperhead (*Agkistrodon contortrix*), a state species of special concern with limited representation in the ophidiomycosis literature. Although we did not observe mortality in our study population, we found that copperheads with apparent ophidiomycosis (8/31 individuals) displayed significantly different thermoregulatory behaviors than snakes without ophidiomycosis. Specifically, individuals with apparent ophidiomycosis favored areas with less canopy cover, less rock cover, and more coarse woody debris. Our findings suggest that snakes with apparent ophidiomycosis select habitats conducive to initiating behavior-mediated fever, potentially facilitating recovery.

Key words: *Agkistrodon contortrix*, habitat use, *Ophidiomyces ophidiicola*, sickness behavior.

INTRODUCTION

Fungal pathogens threaten wildlife globally, endangering biodiversity and ecosystem function (Eskew and Todd 2013). Over the last half-century, fungal pathogens have driven both population declines, as well as species-level extinctions across various taxa, including corals, amphibians, and bats (Berger et al. 1998; Kim and Harvell 2004; Frick et al. 2010). Moreover, such declines may have substantial indirect trophic effects, impacting broader ecosystem structure and function (Whiles et al. 2006). Thus, monitoring and mitigating wildlife diseases are increasingly central for conservation (Smith et al. 2009b).

Ophidiomycosis, caused by the ascomycete fungus *Ophidiomyces ophidiicola*, is a widespread disease affecting snakes in North America and Eurasia that typically manifests as dermatitis with

necrotic lesions, but symptoms vary among species (Allender et al. 2015b; Ladner et al. 2022). Ophidiomycosis is believed to spread via direct contact with infected individuals or contaminated soil, or vertically from mother to offspring, especially in live-bearing species (Lorch et al. 2016; Stengle et al. 2019; Campbell et al. 2021). Since 1990, ophidiomycosis has been detected in at least 62 species across multiple families (Di Nicola et al. 2022). Further, the broad phylogenetic dispersion and ecological characteristics of known hosts suggest that the disease potentially threatens an extremely wide range of species (Burbrink et al. 2017). Ophidiomycosis can impact populations directly by reducing reproductive success (Lind et al. 2018a) and causing mortality. The disease is thought to be partially responsible for the decline of several imperiled populations (Clark et al. 2011; Baker et al. 2018). Unfortunately, previous ophidiomycosis work

has been limited to *O. ophidiicola* challenge experiments in the laboratory or population-level, rather than individual-level, descriptions of disease prevalence in wild animals. As a consequence, and because snakes are notoriously enigmatic and difficult to sample (Steen 2010), we need more information about *O. ophidiicola* infection dynamics and ophidiomycosis impacts in wild populations to effectively inform conservation efforts (Allender et al. 2015b).

Field studies suggest that the effects of ophidiomycosis are context-dependent and influenced by various factors, such as season and host species (Lind et al. 2018b). Temporal trends in fungal load and disease severity have been reported in multiple studies (McKenzie et al. 2019; Dillon et al. 2022). Snake populations may exhibit the worst ophidiomycosis symptoms during spring, then recover as temperatures increase (Guthrie et al. 2016; McCoy et al. 2017). Conversely, some studies have reported peak *O. ophidiicola* prevalence during summer (Grisnik et al. 2018). The impacts of ophidiomycosis on behavior also vary widely across studies, species, and demographics. For example, ophidiomycosis has been associated with decreased movement in eastern massasaugas (*Sistrurus catenatus*; Tetzlaff et al. 2017) but increased movement in timber rattlesnakes (*Crotalus horridus*; Tutterow, 2020), possibly a consequence of habitat or species differences. Even within the same timber rattlesnake population, symptomatic females traveled and foraged less than expected, while the opposite was true for symptomatic males (Tutterow 2020). Although species-specific ophidiomycosis symptoms vary, many species appear to experience disease-mediated changes to typical behavior.

Importantly, even nonlethal ophidiomycosis cases may subtly contribute to population declines by altering fitness-related behaviors, such as movement, foraging, and thermoregulation. As ectotherms, snakes can induce behavioral fevers in response to infection (Burns et al. 1996). Snakes with ophidiomycosis have been observed to alter microhabitat use or increase the duration of thermoregulatory behaviors, such as basking, and using warm, sunny habitats

(McBride et al. 2015). Although basking conspicuously to raise body temperature may reduce pathogen growth, increase shedding frequency, and enhance immune function (Richards-Zawacki 2010), it also poses trade-offs, such as increased exposure to predators and adverse environmental conditions (Tetzlaff et al. 2017). Moreover, maintaining a higher temperature increases metabolic rate, diverting energy from growth and reproduction (Bonneaud et al. 2003), which can be especially taxing for species that eat infrequently and reduce their metabolic demands to tolerate food limitation (McCue 2007). Consequently, disease-related behavioral changes may impose substantial energetic costs, potentially impairing food- and mate-seeking; directly impacting individual fitness; and having cascading effects on population health.

Eastern copperheads (*Agkistrodon contortrix*) are medium-sized, venomous pit vipers (Viperidae: Crotalinae) that occupy a variety of terrestrial habitats across the eastern and central US (Ernst and Ernst 2003). Like other vipers, copperheads employ low-energy ambush hunting and have long generation times with low annual reproductive outputs (Ernst 1992). They also exhibit seasonal changes in habitat use and activity patterns (Smith et al. 2009a). At northern latitudes, copperheads typically overwinter communally in rocky slopes (Smith et al. 2009a), but forage in a variety of open and forested habitats during the active season (Reinert 1984). Basking sites, which typically are rare on the landscape, are usually open habitats with cryptic refugia and substantial solar exposure (Shoemaker and Gibbs 2010). Copperheads are known hosts of *O. ophidiicola* (Lorch et al. 2016), but information about the behavioral, physiological, and demographic consequences of ophidiomycosis on copperheads is lacking. Although copperheads are considered of Least Concern (IUCN 2023), some populations are imperiled (MassWildlife 2023; New Jersey Fish & Wildlife 2023) because of factors such as habitat loss and road mortality (Davenport 2011). In regions where copperheads are already at risk, ophidiomycosis might

exacerbate existing conservation stressors and further threaten vulnerable populations.

We aimed to clarify how ophidiomycosis influences behavior, habitat use, and movements of free-ranging copperheads to determine whether copperheads with apparent ophidiomycosis used significantly different microhabitats than individuals without apparent ophidiomycosis. We also tested whether copperheads with apparent ophidiomycosis would alter their movement behavior relative to their counterparts without apparent ophidiomycosis.

MATERIALS AND METHODS

Study site

We conducted our study in the Sourlands of New Jersey, USA (approximately 40°24'36.0"N, 74°48'36.0"W), a region characterized by a mosaic of habitats, including rocky diabase features and deciduous forest interspersed with wetlands and grasslands. The forest primarily consists of a mixed-oak community, with white ash (*Fraxinus americanus*) dominating successional areas (Collins and Anderson 1994). Within the forest, open habitats formed by tree falls and artificial clearings are important thermoregulatory habitats for copperheads. Large canopy gaps, which have been observed in other copperhead studies (Reinert 1984), are relatively uncommon in the Sourlands because the region lacks extensive rock features capable of creating such openings in the canopy.

Eastern copperhead tracking

For two years (2020–21), we radio-tracked copperheads from hibernaculum egress in late March until their return in October. We weighed newly captured snakes and determined their sex via cloacal probing (Reed and Tucker 2012). We considered a female to be gravid if we felt embryos during palpation (Farr and Gregory 1991) or if she exhibited an enlarged abdomen and prolonged sedentary behavior in the summer. Presumed gravidity was confirmed in late summer when we observed females or their neonates postparturition.

We tracked copperheads by implanting them with VHF radio transmitters (Újvári and Korsós 2000). Using a precision isoflurane vaporizer (Vapomatic 2, A.M. Bickford, Wales, New York, USA), we

induced anesthesia using 5% isoflurane gas in oxygen at 2 L/min. Once induced, we maintained anesthesia at 1.5% isoflurane (Bryant et al. 2010). We then implanted a radio transmitter (Model R1680, Advanced Telemetry Systems, Isanti, Minnesota, USA) in the rear third of the coelomic cavity (Reinert and Cundall 1982; Bryant et al. 2010). We ran antennas anteriorly using a 2-mm diameter stainless steel catheter and then closed incisions with absorbable sutures (4-0 violet braided polyglycolic acid, Oasis, Mettawa, Illinois, USA). We allowed snakes to recover at room temperature and released them within 48 h of implantation, after which, we tracked them using radio telemetry every 48–72 h until fall ingress to hibernacula. At each snake location, we recorded GPS coordinates and noted whether the snake had remained stationary or moved to a new location compared to the previous tracking. We performed all activities in accordance with Rutgers Institutional Animal Care and Use Committee protocol PROTO202100005.

Sampling for *O. ophidiicola*

Once monthly during the active season (March–October), we recaptured individual copperheads and swabbed them for *O. ophidiicola*. If we were unable to recapture a snake after multiple attempts, we skipped swabbing it for that month. Our swabbing procedure involved placing a snake in a container and guiding it head-first into a clear acrylic tube. Once inside the tube, one researcher secured the snake by hand, leaving the posterior three-quarters exposed. After documenting whether the snake had gross lesions indicative of ophidiomycosis as defined by Baker et al. (2019), another researcher used a sterile polyester-tipped applicator moistened with 80 μ L RNA-grade water to 1) swab the face and around the mouth, nostrils, and facial pits; 2) perform five longitudinal passes along the dorsum; and 3) perform five longitudinal passes along the ventrum, including the cloaca and tail. We placed applicators in 1.5-mL microcentrifuge tubes and stored all swabs at -80 C until laboratory analysis. To prevent *O. ophidiicola* transmission between snakes and contamination between swabbings, all researchers wore new nitrile gloves during snake capture and handling. Between snakes we also sterilized all equipment (snake hooks, acrylic tubes, and holding containers) by thoroughly scrubbing with wipes soaked in a 10% bleach solution.

Eastern copperhead habitat data collection

We used a 1-m² quadrat centered on the snake to collect data on seven habitat covariates that we deemed important microhabitat features for copperheads. We estimated the percentage of the quadrat occupied by rock, coarse woody debris (dead vegetation >1 cm in diameter), ground layer woody vegetation (<0.5 m in height, e.g., seedlings), shrub layer woody vegetation (0.5–3 m in height, e.g., mature shrubs, tree saplings, vines), graminoids (grasses, sedges, rushes), forbs (nongraminoids), and total ground layer vegetation (<0.5 m in height). We also measured percent canopy cover to evaluate the solar exposure of a snake location. To measure canopy cover, we took nonhemispherical zenithal photos using a smartphone positioned 1.22 m (4 ft) directly above the snake and analyzed the images using binary thresholding in ImageJ (Schneider et al. 2012).

Fungal load quantification and ophidiomycosis diagnosis

We extracted *O. ophidiicola* DNA from swabs using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA) following the protocol outlined by the manufacturer but with an extended incubation overnight at 56 C. We then performed qPCR following the methods of Bohuski et al. (2015) using 2X QuantiFast Probe PCR Master Mix (Qiagen) and Oo-rt-ITS primers and probes (IDT, Coralville, Iowa, USA). We used a total per well reaction volume of 25 µL consisting of: 12.5 µL Master Mix; 1 µL each of the forward and reverse primer; 0.5 µL probe; 5 µL RNase-free water; and 5 µL template DNA.

We ran a seven-point standard curve using a G-block control (IDT) with gDNA concentrations ranging from 5000 to 0.005 fg (34,569,460–34.56 copies of DNA) and the following cycling protocol: 95 C for 3 min, then 40 cycles of 95 C for 3 s, and 60 C for 30 s. We ran every swab in triplicate and considered a swab positive for *O. ophidiicola* if at least one replicate amplified, to improve detection of low-level infections (Kriger et al. 2006). We quantified the fungal load of a swab as the mean of the positive (nonzero) replicates. We diagnosed a snake with apparent ophidiomycosis if it exhibited appropriate clinical signs and tested positive for *O. ophidiicola* DNA through qPCR analysis on the same date (Baker et al. 2019).

Relationship between fungal load and presence of clinical signs

To assess the influence of fungal load on the likelihood of snakes displaying clinical signs (either present or absent), we ran a generalized linear model with a binomial response family and logit link function. Before applying the model, we added 1 to all fungal load values, then applied a log₁₀ transformation. This allowed a 1-unit increase in the transformed value to correspond to a 10-fold increase in fungal load. We used transformed fungal load and year as predictors. We included year in the model to account for fungal load variations between years. We performed all statistical analyses using R v3.3.0+ (R Core Team 2022), manipulating data using the *dplyr* package (Wickham et al. 2023) and creating plots using the *ggplot2* package (Wickham 2016).

Habitat use analysis

Because ophidiomycosis affects snakes for extended durations, sometimes lasting >1 mo (Allender et al. 2015a), we wanted to determine how snakes with and without apparent ophidiomycosis used habitats in the period surrounding a sampling date, referred to as a “kernel.” Using the *FactoMineR* package (Lê et al. 2008), we conducted a principal component analysis (PCA) to condense our initial eight habitat covariates into a smaller set of principal components (PCs) that explained most of the variance (Budaev 2010). We considered variables with coefficients $|\beta| > 0.4$ as important factors contributing to the PCs (Gorsuch 2014). We compared the mean habitat use of snakes with and without apparent ophidiomycosis along the top two PCs using linear mixed-effects models (LMM) from the *lme4* package (Bates et al. 2015). We used disease status (i.e., either with or without apparent ophidiomycosis) as the binary predictor and either PC1 or PC2 as continuous responses. We included two random effects, reproductive class and individual, to account for sex differences in habitat use, home range, and movement patterns (Christensen pers. comm.) and individual variation in habitat use, and to minimize biases from copperheads that we swabbed for *O. ophidiicola* more often.

To assess how varying time kernels influenced our ability to detect differences in habitat use between snakes with and without apparent ophidiomycosis, we incrementally increased the kernel from 3 d to 61 d. For each increment, we conducted a

separate PCA as described above to compare mean PC1 and PC2 scores between snakes with and without apparent ophidiomycosis. We quantified habitat dissimilarity by calculating the Euclidean distance between the means of snakes with and without apparent ophidiomycosis in PC space (PC1 as the x -axis and PC2 as the y -axis), with greater distances indicating less similarity in habitats used by the two groups. Based on our observation of attenuated habitat dissimilarity as we increased the kernel, we selected the 9-d kernel because it balanced sample size considerations while maximizing habitat dissimilarity. Further details regarding kernel optimization can be found in Supplementary Materials Fig S1.

Home range and movement analyses

We defined the home range of an individual as the area of the 100% minimum convex polygon (MCP; the smallest polygon with no interior angles exceeding 180° that encompasses the GPS coordinates of every snake location within a given year). We chose the MCP for its accuracy in representing herpetofaunal home ranges compared to traditional kernel estimators (Row and Blouin-Demers 2006a). Using the *adehabitatHR* package (Calenge and Fortmann-Roe 2023), we calculated separate home ranges for each year tracked, resulting in two distinct home ranges for snakes monitored in both 2020 and 2021. We categorized home ranges of snakes as either with or without apparent ophidiomycosis based on whether we diagnosed them with apparent ophidiomycosis at least once during that year (snakes can change their disease status, i.e., recover from or contract ophidiomycosis, within an active season; Lind et al. 2018b). This approach allowed us to evaluate whether having ophidiomycosis, even briefly, influenced movement behavior throughout the entire active season. To assess whether having apparent ophidiomycosis at any point in the active season affected home range, we fit an LMM using disease status as the binary predictor, home range as the continuous response, and reproductive class and individual as random effects.

We defined step length as the Euclidean distance between the GPS coordinates of consecutive trackings of an individual. Using the *trajr* package (McLean and Skowron Volponi 2018), we calculated the cumulative annual distance traveled by each copperhead, defined as the sum of step lengths during a single active season. To investigate whether having apparent ophidiomycosis at any point in the active season

influenced cumulative annual distance traveled, we fit another LMM using disease status as the binary predictor, cumulative distance as the continuous response, and reproductive class and individual as random effects.

Additionally, we calculated movement frequency, indicating the proportion of trackings where an individual was in a new location compared to the previous one. A higher movement frequency suggests more movement and less rest. To evaluate whether having apparent ophidiomycosis at any point in the active season affected movement frequency, we fit another LMM using disease status as the binary predictor, movement frequency as the continuous response, and reproductive class and individual as random effects.

RESULTS

During the 2-yr study period, we monitored 28 copperheads, with 16 individuals tracked in 2020, 24 in 2021, and 12 tracked in both years (Supplementary Material Table S1). We swabbed 24/28 tracked copperheads for *O. ophidiicola* and opportunistically swabbed seven more copperheads, resulting in 31 total individuals swabbed for *O. ophidiicola*: 18 in 2020, 19 in 2021, and six in both years. We collected 69 swabs total: 40 in 2020 and 29 in 2021. Throughout the active season, we tracked snakes 4–76 times ($\bar{x}=20$) and swabbed snakes for *O. ophidiicola* 2–4 times ($\bar{x}=2$).

Fungal load quantification and ophidiomycosis diagnosis

During the 2-yr study period, we observed no mortalities in any of our tracked snakes, indicating that none of our copperheads succumbed to infections. Based on qPCR analysis, we detected *O. ophidiicola* DNA on 15/31 copperheads. We diagnosed only eight of those individuals with apparent ophidiomycosis (Table 1). All snakes diagnosed with apparent ophidiomycosis in 2021 were gravid females. Gravid females also comprised half (5/10) of all diagnosed cases. We diagnosed snakes throughout the active season and observed no consistent temporality in *O. ophidiicola* loads (Supplementary Materials Fig. S2). We also detected instances where snakes tested positive for *O. ophidiicola*

TABLE 1. Eastern copperheads (*Agkistrodon contortrix*) diagnosed with apparent ophidiomycosis from the Sourlands of New Jersey, USA, between March and October in 2020–21.

Individual	Diagnosis date ^a	Reproductive class	Log ₁₀ (fungal load + 1) ^b
AGCO-29	5/2/20	Nongravid	3.67
AGCO-21	6/3/20	Male	3.07
AGCO-27	6/8/20	Male	3.13
AGCO-21	7/7/20	Male	3.30
AGCO-17	8/20/20	Nongravid	2.62
AGCO-18	5/18/21	Gravid	5.72
AGCO-46	5/18/21	Gravid	5.26
AGCO-17	7/25/21	Gravid	3.69
AGCO-48	7/25/21	Gravid	2.81
AGCO-57	9/10/21	Gravid	4.73

^aDate that we observed clinical signs on an individual and collected a dermal swab that tested positive for *Ophidiomyces ophidicola* DNA using quantitative PCR analysis.

^bLog₁₀-transformed load (copies of DNA/swab) of *O. ophidicola* fungus detected on the dermal swab taken on the diagnosis date.

DNA without showing clinical signs (*Ophidiomyces* present; $n=14$), and vice versa (possible ophidiomycosis; $n=9$; see Supplementary Materials Table S1).

Relationship between fungal load and presence of clinical signs

Our analysis revealed a significant positive correlation between fungal load and presence of ophidiomycosis clinical signs ($\beta=0.30\pm 0.15$

SE; Fig. 1), indicating that snakes with higher quantities of fungal DNA were more likely to exhibit clinical signs and therefore be diagnosed with apparent ophidiomycosis. The effect of year on the presence of clinical signs was not significant ($p=0.47$).

Habitat use analysis

Our analysis revealed significant differences in habitat use between snakes with and without apparent ophidiomycosis. Specifically, individuals with apparent ophidiomycosis had significantly lower PC2 scores ($\beta=-0.99\pm 0.28$ SE), indicating a preference for areas with less canopy cover, less rock cover, and more coarse woody debris (generally more structurally open habitats) compared to their counterparts without apparent ophidiomycosis (Fig. 2). However, disease status was not significantly correlated with PC1 score, which was positively associated with low ground and graminoid cover. Shrub layer woody vegetation, ground layer wood vegetation, and forbs were not important predictors of copperhead habitat use. Detailed PCA loadings are provided in Supplementary Materials Table S2.

Home range and movement analyses

We observed no significant differences in home range ($\beta=-0.07\pm 0.27$ SE), cumulative

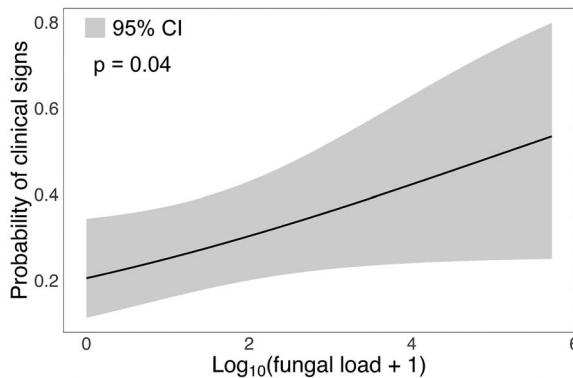


FIGURE 1. Probability of eastern copperheads (*Agkistrodon contortrix*) from the Sourlands of New Jersey, USA, sampled March–October in 2020–21, displaying clinical signs of ophidiomycosis (e.g., lesions, scars, etc.) based on the quantity of *Ophidiomyces ophidicola* fungal DNA detected on its dermal swab according to a generalized linear model. Before applying the model, we added 1 to all fungal load values then applied a log₁₀ transformation. We included year in the model as a predictor. The shaded area represents the 95% confidence interval.

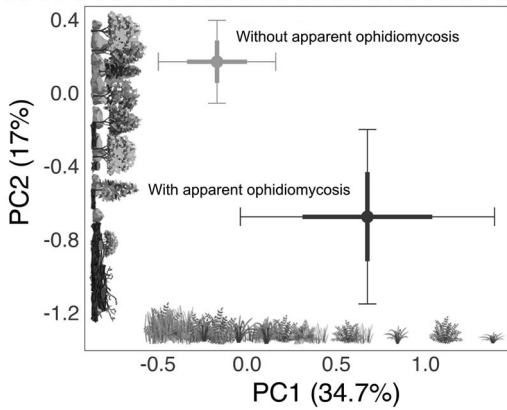


FIGURE 2. Mean principal component (PC) scores describing microhabitat features used by eastern copperheads (*Agkistrodon contortrix*) with and without apparent ophidiomycosis from the Sourlands of New Jersey, USA, between March and October in 2020–21. Mean PC2 score was significantly different between copperheads with and without apparent ophidiomycosis. Graphics along the two axes depict the gradients of habitat features associated with each PC. Thin and thick lines denote the 95% confidence intervals and standard errors for the means, respectively.

annual distance traveled ($\beta=0.69\pm0.45$ SE), or movement frequency ($\beta=0.02\pm0.05$ SE) between snakes with and without apparent ophidiomycosis (Fig. 3a). However, when

considering reproductive class, differences in all metrics became apparent (Fig. 3b). Males and nongravid females had larger home ranges and higher movement frequencies, and covered greater cumulative distances than did gravid females.

DISCUSSION

Copperheads tended to use different habitats based on their disease status: Individuals with apparent ophidiomycosis used areas with less canopy cover, less rock cover, and more coarse woody debris compared to conspecifics without apparent ophidiomycosis. Such behavior is consistent with snakes with ophidiomycosis seeking suitable basking conditions (Lorch et al. 2015), such as areas with increased solar exposure and aboveground physical structures such as rocks or woody debris (Whiles and Grubaugh 1996). Such areas have higher average temperatures and greater microclimate heterogeneity, enhancing their thermoregulatory quality (Row and Blouin-Demers 2006b). Copperheads with apparent ophidiomycosis were possibly attempting to elevate their body temperature, enhance their immune function, and

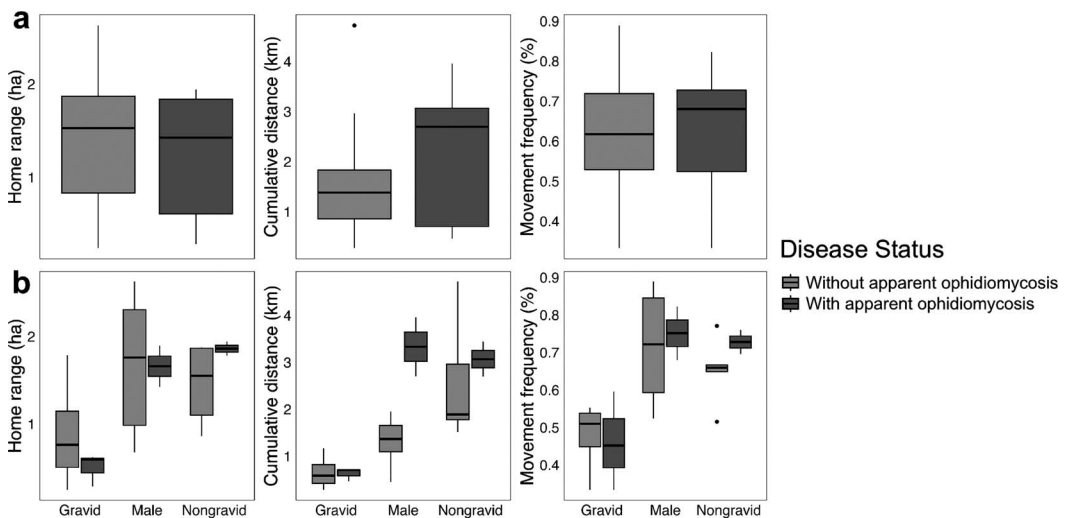


FIGURE 3. (a) Boxplots comparing home range, cumulative annual distance traveled and movement frequency between eastern copperheads (*Agkistrodon contortrix*) with and without apparent ophidiomycosis from the Sourlands of New Jersey, USA, between March and October in 2020–21. Copperheads with and without apparent ophidiomycosis did not differ significantly in their movement behaviors. (b) Differences in such movement parameters were better explained by reproductive class.

increase their shedding frequency to facilitate recovery (Lorch et al. 2015). Our findings align with studies showing that vipers with ophidiomycosis exhibit heightened thermoregulatory behaviors. For example, pygmy rattlesnakes (*Sistrurus miliarius*), timber rattlesnakes, and eastern massasaugas (*Sistrurus catenatus*) with ophidiomycosis are more likely to select sites with greater solar exposure, and to bask at the end of the active season when snakes without ophidiomycosis are already hibernating (Clark et al. 2011; Tetzlaff et al. 2017; Lind et al. 2023).

In contrast to prior research, we found no significant differences in home range, cumulative distance traveled, or movement frequency between copperheads with and without apparent ophidiomycosis. It is possible that suitable thermoregulation sites were already present within snake home ranges, eliminating the need for extensive travel to adequate convalescing habitats. Additionally, the severity of apparent ophidiomycosis might have been low enough to cause subtle or no changes to movement behavior. Other factors, such as body size, which often correlates positively with home range in snakes (Whitaker and Shine 2003), may also confound disease-related movement differences. For example, if ophidiomycosis was to increase home ranges (as seen in Tutterow 2020), smaller snakes with ophidiomycosis might have similar home ranges to larger snakes without ophidiomycosis. Therefore, it is important to consider the additive effects of disease and individual characteristics such as size, age, and body condition on movement behavior. In our study, movement variations were primarily explained by reproductive class, with gravid females being less active than nongravid females and males, consistent with earlier studies (Christensen pers. comm.). Some evidence suggests that transmitter implantation may affect snake behavior (Lentini 2008); however, this approach remains the best method for reliably locating free-ranging snakes over long time periods. Any transmitter-related behavioral changes within our study population would probably be consistent among individuals because all snakes within this study were implanted.

Although ophidiomycosis can acutely affect some North American vipers (Allender et al. 2011; Clark et al. 2011), we did not observe any disease-related mortality during this study. Nevertheless, over 8 yr of radio tracking copperheads, we observed two individuals with apparent ophidiomycosis presenting with extensive gross lesions (Christensen unpubl. data). Both snakes were brought into captivity and died before treatment. During our current study, we diagnosed 8/31 (26%) copperheads with apparent ophidiomycosis, which is consistent with disease prevalence estimates from other studies. Prior surveys have reported disease detection rates in copperheads ranging from 0 to 39%, but sample sizes and diagnosis criteria varied (Grisnik et al. 2018; McKenzie et al. 2019; Haynes et al. 2020; Lizarraga 2020; Snyder et al. 2020). *Ophidiomyces ophidiicola* has been detected on New Jersey copperheads since at least 2002 (Paré et al. 2016), suggesting that the fungus has been circulating for over two decades.

Our findings indicate that higher *O. ophidiicola* loads increase the likelihood of a snake exhibiting ophidiomycosis clinical signs, supporting previous findings that snakes with clinical signs are more prone to harboring fungal DNA (McKenzie et al. 2019). Although fungal load alone might serve as an ophidiomycosis diagnostic criterion, our study revealed instances where snakes tested positive for fungal DNA without showing clinical signs, and vice versa (Supplementary Materials Table S1). Moreover, current literature often advocates a combination of visual assessment and molecular methods or culturing to diagnose ophidiomycosis (Baker et al. 2019).

Although ophidiomycosis typically displays seasonality in many snake populations (Guthrie et al. 2016; McCoy et al. 2017), we observed no consistent temporality in *O. ophidiicola* loads on copperheads. Individual fungal load histories varied widely, with some snakes having peak loads early in the season, others in the middle, and still others later (Supplementary Materials Fig. S3). There might be several explanations for such variability. First,

transmission may occur sporadically as snakes move through the environment, utilize communal sites, and interact with one another (Allender et al. 2015b; Campbell et al. 2021). Copperheads are highly gregarious at hibernacula and gestation sites (Christensen et al. 2022), potentially increasing transmission risk. Second, our ability to detect ophidiomycosis might depend on snake condition and behavior, environmental conditions, or disease progression (Baker et al. 2019). Such factors might have produced enough variability in individual disease progressions that we observed no overall patterns in fungal load temporality.

While gravid females constituted a minority in our study population, they represented half of all diagnoses of apparent ophidiomycosis. Due to our limited sample size, we could not determine if certain reproductive classes were statistically more prone to infection. Nevertheless, it is noteworthy that in 2021 all snakes that we diagnosed with apparent ophidiomycosis were gravid females. Few studies have examined the interactions between ophidiomycosis and reproduction (but see Lind et al. 2019; Stengle et al. 2019), despite this connection having direct implications for population vital rates. Live-bearing snakes often experience reduced food intake and undernourishment during gestation, which can weaken their immune system and increase their infection susceptibility (Neuman-Lee et al. 2015). Moreover, copperheads often gestate contemporaneously and repeatedly at the same sites (Christensen et al. 2022), potentially heightening the risk of direct transmission. Consequently, the physiological and behavioral changes associated with reproduction, such as increased metabolism and reduced movement (Schultz et al. 2008), may impact ophidiomycosis susceptibility and spread.

Conversely, ophidiomycosis can impact reproduction. For instance, pygmy rattlesnakes with ophidiomycosis have depressed levels of sex hormones and forgo reproductive bouts (Lind et al. 2019). We observed copperheads with apparent ophidiomycosis mating and gestating, indicating that disease did not prevent reproduction. Although some or all females with

apparent ophidiomycosis successfully gave birth, we did not assess whether parental infection affected neonate viability. Vertical transmission of *O. ophidiicola* has been documented in several live-bearing snake species (Stengle et al. 2019) and is believed to be responsible for the mortality of entire eastern massasauga clutches (Britton et al. 2019). Considering that copperheads typically produce four to eight offspring biennially (Ernst 1992), vertical transmission could significantly contribute to ophidiomycosis spread within their populations. Failing to consider juveniles in studies may underestimate disease impacts on population dynamics, especially if juveniles are disproportionately affected by ophidiomycosis in terms of both severity and prevalence. Hence, it is vital to examine snakes across all age groups to understand the complex interplay between ophidiomycosis and reproduction.

Understanding the nonlethal fitness effects of disease, especially for imperiled species, is crucial for conservation efforts. Preserving habitats that support convalescence, which may differ from those used by healthy individuals, may be critical for safeguarding populations affected by ophidiomycosis. Additionally, examining disease-related behaviors may elucidate trade-offs between recovery and maintaining fitness, which might influence population dynamics. Further exploration is needed on other nonlethal fitness consequences to convalescing, such as depredation and exposure risks. Comprehensive insights into the impacts of sickness behavior on snake populations require long-term studies, expanding observations to encompass more species, and developing more quantitative methods for assessing the impacts of infection on individual fitness.

ACKNOWLEDGMENTS

This project was partially funded by the Association for Reptile and Amphibian Veterinarians. Thank you to Friends of Hopewell Valley Open Space for allowing us to swab copperheads in their existing radio-tracking study. We also acknowledge Vasilisa Tyurina for her assistance with laboratory work.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at <http://dx.doi.org/10.7589/JWD-D-24-00081>.

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Submitted for publication 16 May 2024.

Accepted 3 July 2024.