

SNAKE FUNGAL DISEASE AFFECTS BEHAVIOR OF FREE-RANGING MASSASAUGA RATTLESNAKES (*SISTRURUS CATENATUS*)

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Abstract.— Snake fungal disease (SFD) caused by *Ophidiomyces ophiodiicola* can lead to morbidity and mortality in snakes. However, we know little about the behavior of free-ranging individuals with the disease and the implications this may have for threatened taxa. We discovered Massasaugas (*Sistrurus catenatus*), a federally protected rattlesnake in eastern USA, with SFD in northern Michigan during a radio-telemetry study we conducted from 2013–2017. We were consequently provided with an opportunity to investigate differences in movement, visibility, thermoregulation, and overwintering site selection between eight infected and 17 uninfected snakes. Across the active season, infected snakes moved distances ≥ 10 m less frequently and were less visible than uninfected snakes. This suggests disease imposed an energetic cost of movement too great for debilitated snakes that was possibly outweighed by other behaviors, such as avoiding predators. Monthly body temperatures of infected snakes differed from uninfected snakes only near the end of the active season, supporting observations of infected snakes surface basking when uninfected snakes had retreated to overwintering refugia. Most infected individuals overwintered in a concentrated area, suggesting environmentally driven hotspots for the fungus could exist within the landscape. Our findings provide a baseline for future studies investigating more consequential behavior for infected snakes. Linking snake behavior with the distribution of the fungus and habitat features at localized scales will ultimately lead to increased epidemiological knowledge of SFD, which could aid management and conservation efforts for imperiled species such as Massasaugas.

Key Words.—clinical signs; fungal pathogen; infectious disease; *Ophidiomyces ophiodiicola*; reptile; wildlife diseases

INTRODUCTION

Fungal diseases have been identified as significant global threats to biodiversity and can have devastating effects on numerous taxa (Fisher et al. 2012). For example, white-nose syndrome caused by *Pseudogymnoascus destructans* (Warnecke et al. 2012) has decimated populations of North American bats (e.g., Frick et al. 2010), and chytridiomycosis caused by *Batrachochytrium dendrobatidis* has led to global declines of anurans (Skerratt et al. 2007; Lanoo et al. 2011). Snake fungal disease (SFD) is another mycosis of growing concern because it affects free-ranging and captive snakes and can lead to morbidity and mortality in otherwise healthy individuals (Allender et al. 2015c). Documented in over 30 species (Lorch et al. 2016; Ravesi et al. 2016), commonly reported clinical signs consistent

with SFD include localized swelling or thickening of the skin with crust or scabs, lesions, and dysecdysis (Allender et al. 2011, 2016; Tetzlaff et al. 2015; Fig. 1). Evidence suggests *Ophidiomyces ophiodiicola*, originally characterized as *Chrysosporium ophiodiicola* (Rajeev et al. 2009), is the primary causative agent synonymous with contemporary diagnosis of SFD (Allender et al. 2015a; Lorch et al. 2015). However, *O. ophiodiicola* may act in conjunction with other pathogens to cause disease (Allender et al. 2015c), which makes it difficult to universally define what constitutes SFD (Lorch et al. 2016). Much has been learned about the disease, but there is still lack of understanding as to how it affects free-ranging individuals.

Free-ranging snakes with SFD are often emaciated (Lorch et al. 2016). Thus, we might expect diseased individuals to limit movement because extensive



FIGURE 1. Progression of *Ophidiomyces ophiodiicola* infection in a male Massasauga (*Sistrurus catenatus*; ID #12) from Grayling, Michigan, USA, in 2013. The snake showed signs of dysecdysis and skin blistering on 1 August (indicated by red arrows in A), and a crusty lesion developed on its face three days later (indicated by red arrow in B). There was considerable fungal growth in the oral cavity by the time a biopsy of a mandibular lesion was taken by 21 August (C). The animal had considerable mandibular warping, fungal growth in the continued oral cavity, and severe dysecdysis (noted by retained shed skin and cloudy eyes) had developed by 24 November, just before the snake was euthanized (D). (A–C photographed by Sasha Tetzlaff and D by Matthew Allender).

locomotion can reduce immune function and is energetically costly (Altizer et al. 2011). Successfully foraging to maintain energy reserves for combating infection may conflict with movement for Massasaugas and other snakes that predominantly hunt by ambush (i.e., sit-and-wait foraging; Ernst and Ernst 2003). Furthermore, because ectotherms primarily regulate body temperature behaviorally, substantial movements are incompatible with comparatively stationary thermoregulation, which could also be critical for disease mitigation.

Snakes coping with infection have been found to elevate their body temperature, exhibiting a behavioral fever to reduce pathogen load under laboratory conditions (Burns et al. 1996). Like many ectotherms, Massasaugas adjust body temperature by altering the duration and amount of their body exposed to solar radiation (Harvey and Weatherhead 2010). The pathological effects of SFD might intensify these behaviors if snakes frequently need to bask to fend off infection. Lorch et al. (2015) observed that Corn Snakes (*Pantherphis guttatus*) experimentally infected

with *O. ophiodiicola* under laboratory conditions were more exposed than uninfected snakes, which spent more time in shelters. However, to what degree SFD affects visibility of free-ranging snakes is unknown. Temperate vipers with SFD continue to bask when presumably uninfected conspecifics have retreated to overwintering locations. For example, infected Timber Rattlesnakes (*Crotalus horridus*) have been observed basking outside communal winter dens (Clark et al. 2011; McBride et al. 2015), whereas this and other temperate species typically remain in overwintering sites once entered until warmer temperatures suitable for surface activity arise in spring.

It has been suggested that communal overwintering in conjunction with potentially concentrated distribution (i.e., hotspots) of *O. ophiodiicola* in the landscape may have consequences for disease susceptibility and/or transmission (Allender et al. 2016; Lorch et al. 2016). Thus, documenting where infected snakes overwinter is of particular importance to further our understanding of the ecology of SFD. Massasaugas have been observed communally overwintering (Smith 2009), but

the distribution of overwintering locations of infected individuals remains unknown.

Our goal was to examine if the behavior of infected snakes differed from uninfected individuals by using radio-telemetry data opportunistically collected on Massasaugas from 2013–2017. Because this species is listed as threatened under the Endangered Species Act of the United States (U.S. Fish and Wildlife Service 2016), our study was intended to inform management and conservation efforts for this imperiled rattlesnake. Relative to uninfected snakes, we predicted infected Massasaugas would move less frequently, be visible (i.e., exposed) more often, and maintain higher body temperatures across the active season. Additionally, we describe overwintering site selection of infected and uninfected snakes and the collective potential population-level implications of our findings.

MATERIALS AND METHODS

Study site.—Our study took place from May 2013 to May 2017 in the northern Lower Peninsula of Michigan, USA, at Camp Grayling Joint Maneuver Training Center (44.6615°N, 84.7148°W), the largest National Guard training facility in the United States. The site is unique compared to most other Massasauga study areas because it is heavily forested with sandy substrates. The snakes we studied occupied an approximately 800-ha portion of Camp Grayling less disturbed by military operations than other parts of the base. Several habitat types are distributed throughout the heterogeneous landscape including coniferous and hardwood forests comprised of spruce (*Picea* spp.), cedar (*Thuja* spp.), pine (*Pinus* spp.), maple (*Acer* spp.), oak (*Quercus* spp.), and aspen (*Populus* spp.) stands; scrub-shrub wetlands comprised of willow (*Salix* spp.) and Speckled Alder (*Alnus incana*); and barrens dominated by lichen and blueberry (*Vaccinium* spp.). Six clear-cuts about 6 ha each were created in the central and northern portion of the study area in winter of 2006 (DeGregorio 2008), and an unintended fire burned about 130 ha of coniferous forest and barrens in the eastern and southern portions of the site in 2010 (Ravesi 2016).

Radio-telemetry.—We captured adult Massasaugas during May to August from 2013–2016. We surgically implanted a 5 g or 9 g temperature sensitive radio-transmitter (model SB-2T or SI-2T, respectively; Holohil Systems, Ltd., Carp, Ontario, Canada) into the body cavity of each snake following methods adapted from Reinert and Cundall (1982). Transmitters did not exceed 6% of the mass of an individual. We recorded the mass (g), snout-to-vent length (cm), and sex of each snake at the time of transmitter implantation. We attempted to maintain sterile handling protocols by disinfecting all

capture gear and surfaces snakes contacted with bleach or household cleaners demonstrated to inactivate *O. ophiodiicola* (Rzadkowska et al. 2016). We sterilized all surgical instruments in an autoclave. We soaked radio-transmitters in zephiran chloride for at least one hour and rinsed each one with saline before implantation. We typically held implanted snakes for 3–5 d for post-operative recovery and then released each at its respective capture location. We located individuals every 48–72 h during daylight hours (0800–2000) using a handheld receiver (R-1000, Communications Specialists, Inc., Orange, California, USA) and 3-element mini Yagi antenna. We recorded the position of each snake when located in Universal Transverse Mercator units (UTM, North American Datum of 1983) with a handheld GPS (Garmin eTrex® 30, Garmin International, Inc., Olathe, Kansas, USA; 3 m accuracy) and varied the time of day we located an individual for subsequent tracking events. We noted the fate of each snake throughout the study until its conclusion in May 2017.

Disease classification and pathogen detection.—We categorized snakes as infected if they tested positive for *O. ophiodiicola* using established sampling and diagnostic techniques (Allender et al. 2015b) or, in the absence of pathogen detection, had clinical signs consistent with SFD previously reported for the species (see Allender et al. 2011, 2016; Tetzlaff et al. 2015). We considered all other individuals as uninfected. We predominantly sampled snakes by swabbing lesions with cotton-tipped or nylon flocked applicators. We did not begin a formal sampling regime for *O. ophiodiicola* until 2014 because snakes exhibiting clinical signs of SFD were not detected (and thus not tested) until the latter portion of the 2013 active season. The first two cases at our site were confirmed from biopsies of lesions (Tetzlaff et al. 2015), and since (2014–2016), we have only swabbed snakes for testing, including any snakes radio-tracked during 2013 that survived the 2013–2014 winter (nine of 12 snakes). In general, we collected samples either at the time of capture in the field or during transmitter implantation, which occurred within 48 h of capture. Because we sampled snakes once per year from 2014–2016, we sampled some snakes tracked in multiple years up to three times to test for presence or absence of the pathogen in a given year. We tested samples for *O. ophiodiicola* using qPCR according to Allender et al. (2015b).

Movement frequency.—Using a similar radio-tracking regime as ours, DeGregorio et al. (2011) documented adult male and non-pregnant Massasaugas at Camp Grayling consistently made average daily movements > 10 m across most of the active season, and we assumed the snakes we studied moved at similar

rates. We thus calculated movement frequency for each snake as the number of times it had moved ≥ 10 m from its previous location divided by the number of radio-locations. We compared movement frequencies between snake groups (infected or uninfected) using a generalized linear mixed model assuming a binomially-distributed error and fit by maximum likelihood (Laplace approximation) using the lme4 package (Bates et al. 2015) in R 3.1 (R core team 2014). We used snake group and time between successive locations as fixed effects and subject (snake identity) and numbered calendar week as random intercepts.

Exposure.—We estimated the visibility of each snake during radio-tracking events using categorical exposure levels. We noted exposure of snakes as full (the entire snake was visible), partial (at least some of the snake was exposed), or none (the snake was not visible). We compared proportions of each exposure category between snake groups using general linear models in R.

Thermoregulation.—We used temperature sensitive transmitters to obtain body temperature of radio-tracked snakes. We measured time to 10 pulses twice each time a snake was located to obtain two estimates of pulse rate, averaged them, and transformed to body temperature from calibration curves supplied by the transmitter manufacturer ($r^2 \geq 0.99$ for all equations). Massasaugas have a preferred body temperature range of 30.0–33.6° C (Harvey and Weatherhead 2011), but the thermal environment at the latitude of our study site is broadly challenging for Massasaugas because environmental temperatures are often too low for regulating body temperature at preferred levels (Tetzlaff 2015). Therefore, we limited our analysis to body temperatures collected from all snakes from 1100–1800, as this is the general daily time frame that best allows snakes to maintain body temperatures closest to or within their preferred range at the site. We analyzed body temperatures for each month of tracking (May - September) with a linear mixed model fit by maximum likelihood using the nlme package (Pinheiro et al. 2016) in R. We used the interaction of snake group by month as a fixed effect and subject as a random intercept. For all relevant analyses, we confirmed residuals approximately conformed to a normal distribution by examining Q-Q plots and tested equality of variances using Levene's tests, and we detected no significant violations. Means are reported with standard error unless noted otherwise.

Overwintering site selection.—We tracked snakes to their overwintering locations if they survived the duration of the active season. We recorded GPS points

of these sites in UTM units and the overwintering habitat type (as described above). We mapped overwintering locations of infected and uninfected individuals on aerial imagery of the study site using ArcMap 10.1 (Esri, Redlands, California, USA).

RESULTS

We radio-tracked 25 individuals from May 2013 to May 2017 resulting in 1,303 radio-tracking events. We tracked 12 males (four infected and eight uninfected) and 13 females (four infected and nine uninfected). The duration for which we tracked an individual ranged between 25 and 1,439 d, depending on factors such as cause of death, transmitter failure or removal, or disease status and severity. Nine of the 17 (53%) uninfected snakes died: five perished over winter, one died from predation, two died from vehicular strikes, and one died due to an unknown cause. Six of the eight (75%) infected snakes died. We removed three snakes from the field for treatment given the severity of their clinical signs of disease; we euthanized one individual, whereas the other two died in captivity. We found two others depredated, and the intact carcass of another was found in the field (Monica Matthews, pers. comm.) but cause of death was not confirmed (Table 1).

Seven infected snakes tested positive for *O. ophiodiicola* using qPCR, and one with clinical signs of SFD tested negative (ID #27; Fig. 2). No snakes that we sampled multiple times initially tested positive and then negative later (or vice versa). The severity of clinical signs varied between individuals; some had numerous lesions, others had only one lesion, and one individual (ID #29) that tested positive had no clinical signs (Table 2).

Movement frequency.—We calculated movement frequency based on 1,303 telemetry events for infected ($n = 400$) and uninfected ($n = 903$) snakes. Infected snakes moved less frequently ($\bar{x} = 0.612 \pm 0.100$ SE) than uninfected ones ($\bar{x} = 0.779 \pm 0.085$; $z = 2.353$, $P = 0.019$). Time between successive telemetry locations was not a predictor of movement frequency ($z = 1.125$, $P = 0.261$).

Exposure.—We compared exposure between snake groups based on 1,145 telemetry events for infected ($n = 298$) and uninfected ($n = 847$) snakes. The visibility of snakes differed between groups for each exposure category. Uninfected snakes were more often fully exposed than infected snakes ($\beta = 0.265 \pm 0.074$, $P = 0.001$). We found the opposite pattern for partial exposure, where infected snakes were more often partially exposed than uninfected snakes ($\beta = 0.148 \pm$

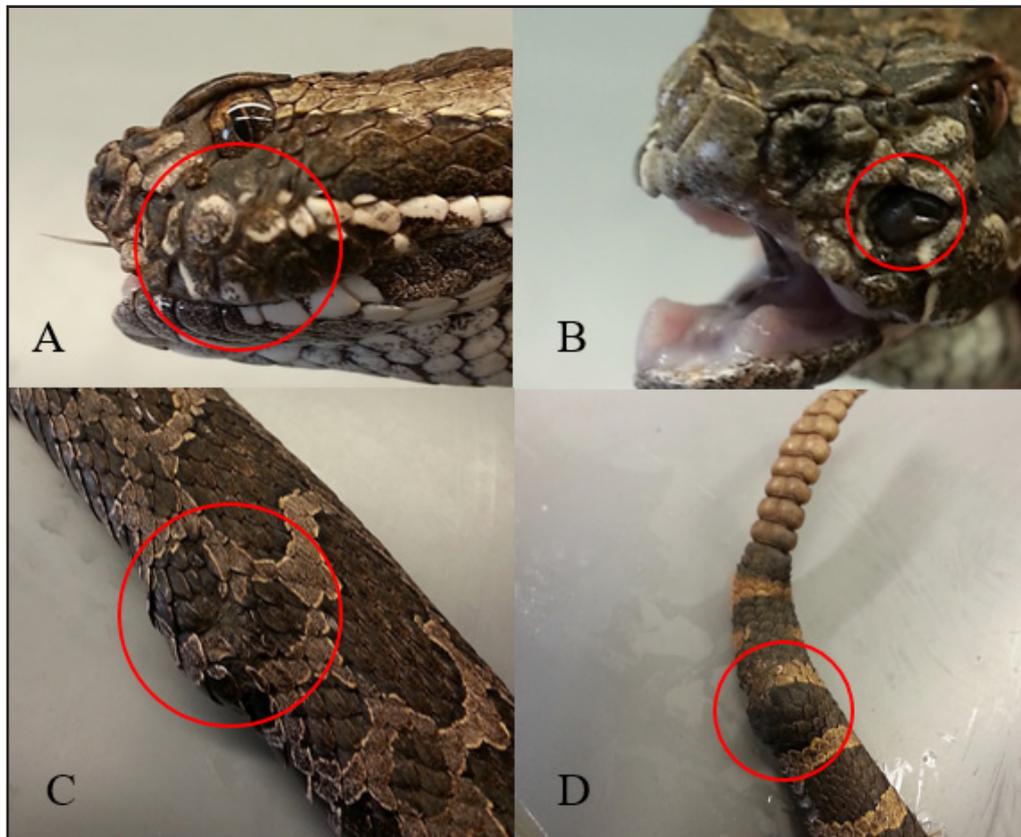


FIGURE 2. An anesthetized Massasauga (*Sistrurus catenatus*; ID #27) from Grayling, Michigan, USA, displaying clinical signs of snake fungal disease including facial swelling (A), obstructed left nasolabial pit (B), lateral lesion (C), and tail lesion (D) circled in red. All swab samples of these areas tested negative for *Ophidiomyces ophidiicola* using qPCR. (Photographed by Sasha Tetzlaff).

0.072, $P = 0.052$). Infected snakes were also more often completely concealed (i.e., not exposed) than uninfected ones ($\beta = 0.117 \pm 0.038$, $P = 0.006$; Fig. 3).

Thermoregulation.—We calculated 184 body temperatures for infected snakes and 745 for uninfected snakes. Body temperatures differed significantly based on the interaction of group by month ($\chi^2 = 14.10$, $df = 4$, $P = 0.007$). The predicted (least squares) mean monthly body temperatures of uninfected snakes ranged from 22.5° to 29.4° C (September and August, respectively), and that of infected snakes ranged from 28.0° to 30.6° C (September and May, respectively). Snakes in both groups maintained similar mean monthly body temperatures for most of the active season, but those of infected snakes were higher at the end of the active season in September ($\bar{x} = 28.0^\circ$ C, 95% CI: 24.7–31.4) than uninfected snakes ($\bar{x} = 22.5^\circ$ C, 95% CI: 20.3–24.7). Within each group, the mean monthly body temperatures of infected snakes did not differ between months, but uninfected snakes had lower body temperatures in September relative to all other months (Fig. 4).

Overwintering site selection.—Snakes arrived at their overwintering sites during September and October each year and emerged the following April to May. We observed strong fidelity to these sites for most snakes tracked more than one winter, and communal overwintering was common. Six uninfected snakes overwintered in forest and eleven did so in burned habitat. We removed two infected snakes tracked in 2013 from the field for captive treatment before overwintering commenced (Table 1). The six remaining infected snakes overwintered in burned habitat. Five overwintered in a localized (about 47 m²) area, including the individual (ID #27; Fig. 2) that had clinical signs of SFD yet tested negative for *O. ophidiicola*. Four snakes shared a single overwintering refugium in this location (Fig. 5).

DISCUSSION

In this first study comparing behavior of free-ranging Massasaugas with SFD or clinical signs of the disease to uninfected conspecifics, infected snakes moved less frequently, were less visible, and had higher body temperatures late in the active season than uninfected

TABLE 1. Descriptive summary of identity, infection status, sex, tracking duration, and fate of Massasaugas (*Sistrurus catenatus*) radio-tracked from May 2013 to May 2017 at Grayling, Michigan, USA.

ID	Group	Sex	Tracking start date	Tracking end date	Days known alive <i>in-situ</i>	Fate
1	Uninfected	M	18 May 2013	14 August 2014	454	Alive (transmitter failed)
2	Uninfected	M	18 May 2013	27 August 2014	467	Dead (2014–15 winter)
6	Uninfected	M	24 May 2013	1 May 2017	1439	Alive (at study conclusion)
7	Uninfected	M	9 June 2013	23 September 2016	1203	Alive (transmitter failed)
8	Uninfected	F	9 June 2013	15 September 2015	829	Alive (transmitter failed)
9	Uninfected	F	11 June 2013	19 October 2016	1227	Alive (transmitter failed)
10	Uninfected	M	9 June 2013	3 September 2016	1183	Dead (2016–17 winter)
11	Uninfected	F	17 June 2013	26 August 2014	436	Dead (2014–15 winter)
16	Uninfected	F	13 May 2014	11 July 2014	60	Dead (vehicle mortality)
17	Uninfected	F	16 May 2014	8 August 2014	85	Dead (cause unknown)
18	Uninfected	M	14 May 2014	6 August 2014	85	Dead (vehicle mortality)
19	Uninfected	F	14 May 2014	27 September 2014	137	Alive (transmitter failed)
21	Uninfected	M	21 May 2014	14 September 2015	482	Alive (transmitter failed)
22	Uninfected	F	3 June 2014	24 September 2014	114	Dead (2014–15 winter)
23	Uninfected	F	12 June 2014	28 September 2014	109	Alive (transmitter removed)
24	Uninfected	F	19 June 2014	24 September 2014	98	Dead (2014–15 winter)
26	Uninfected	M	23 July 2014	20 July 2015	363	Dead (predation)
3	Infected	F	18 May 2013	10 June 2016	1120	Dead (cause unknown)
12	Infected	M	28 June 2013	5 August 2013	39	Dead (euthanized)
13	Infected	M	28 June 2013	21 September 2013	86	Dead (in captivity)
20	Infected	M	21 May 2014	9 May 2015	354	Dead (predation)
27	Infected	F	24 August 2015	17 September 2015	25	Dead (predation)
28	Infected	F	13 May 2016	1 May 2017	354	Dead (in captivity)
29	Infected	M	13 May 2016	1 May 2017	354	Alive (at study conclusion)
30	Infected	F	17 June 2016	1 May 2017	319	Alive (transmitter removed)

snakes. The altered behaviors of infected snakes were likely interrelated through complex ecological tradeoffs, and our data provide a baseline for future studies investigating more consequential behavior. Massasaugas at Camp Grayling moved through a variety of habitats within an extensive and heterogeneous landscape to presumably satisfy habitat-dependent resource needs related to foraging, thermoregulation, reproduction,

and avoiding predators (DeGregorio 2008; Ravesi 2016). Conserving energy by reducing movement could assist with disease mitigation, but it is unknown if this affects fitness. For instance, males typically traverse long distances to find females (DeGregorio et al. 2011; Tetzlaff et al. 2017), but anecdotal evidence of infected males being sedentary during the breeding season (Tetzlaff et al. 2015) suggests SFD could have

TABLE 2. Identity of infected snakes, sample type used to test for *Ophidiomyces ophiodiicola*, qPCR results, when clinical signs were first observed, and severity of clinical signs for Massasaugas (*Sistrurus catenatus*) radio-tracked at Grayling, Michigan, USA, 2013–2017.

ID	Sample type	Initial sampling date	qPCR results	Clinical signs observed	Severity of clinical signs
3	Swab	11 May 2014	positive	20 September 2013	Single lesion
12	Biopsy	7 August 2013	positive	28 June 2013	Multiple lesions
13	Biopsy	25 September 2013	positive	31 August 2013	Multiple lesions
20	Swab	13 May 2014	positive	13 May 2014	Multiple lesions
27	Swab	29 July 2015	negative	27 July 2015	Multiple lesions
28	Swab	23 April 2016	positive	21 April 2016	Multiple lesions
29	Swab	23 April 2016	positive	NA	None
30	Swab	4 October 2016	positive	6 May 2017	Single lesion

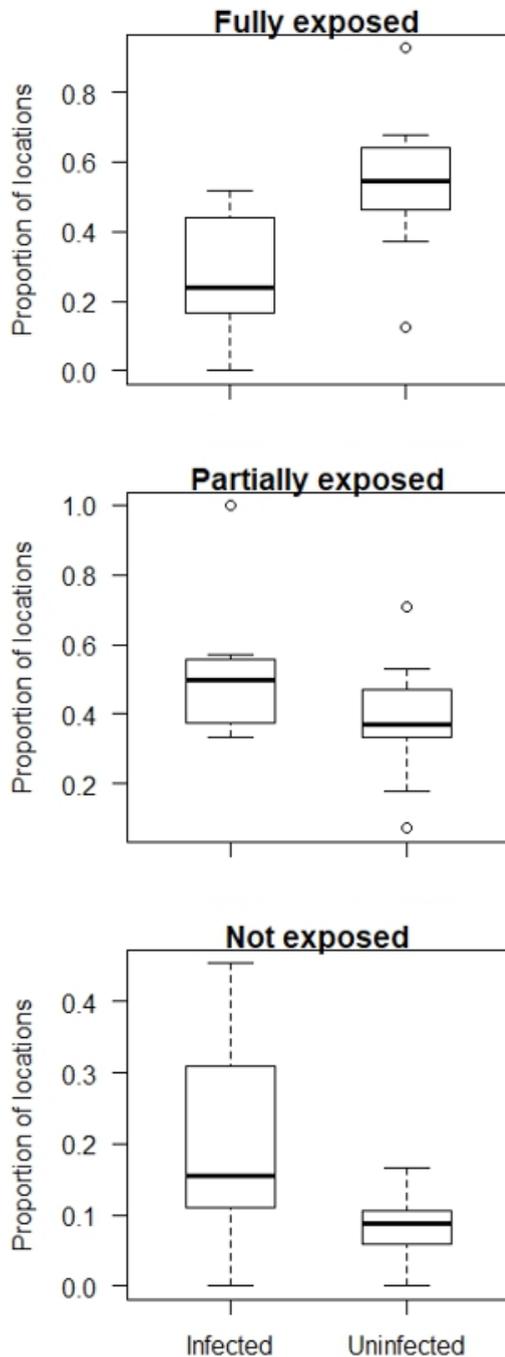


FIGURE 3. Categorical exposure levels of infected and uninfected Massasaugas (*Sistrurus catenatus*) at Grayling, Michigan, USA, 2013–2017. Bold lines are medians, boxes are the interquartile range (25% and 75% quartiles), error bars are 1.5 times the interquartile range, and open circles are outliers. Note the differing y-axis scales for each exposure category.

consequences for mate acquisition. Additionally, although reduced movement could be beneficial for ambushing prey, infected individuals may not have dedicated substantial time to foraging.

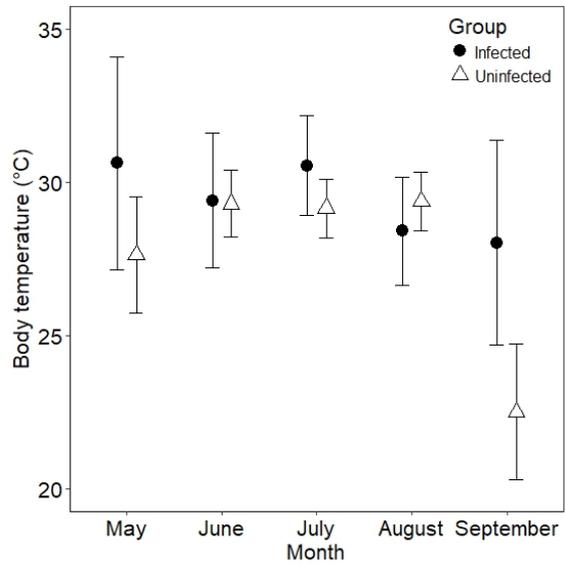


FIGURE 4. Mean monthly body temperatures with 95% confidence limits for infected (filled circles) and uninfected (open triangles) Massasaugas (*Sistrurus catenatus*) at Grayling, Michigan, USA, from 2013–2017.

Body condition is enhanced with greater food intake (Tetzlaff et al. 2017), which should assist immune function and benefit overall health. However, many infected individuals had poor body condition, particularly those with severe clinical signs, as was found for Pygmy Rattlesnakes (*Sistrurus miliarius*) with clinical signs of SFD (McCoy et al. 2017). Massasaugas are frequently exposed when ambush-hunting (Harvey and Weatherhead 2011), so the comparatively higher rates of hiding behavior exhibited by infected snakes may have precluded their ability or willingness to forage, given anorexia was observed in captive Corn Snakes with SFD (Lorch et al. 2015). However, behaviors other than foraging certainly influence visibility.

Warmer body temperatures can be a function of increasing exposure for Massasaugas (Harvey and Weatherhead 2010). Therefore, we expected infected snakes would dedicate much of their time to exposed basking to mitigate infection because similar behaviors have been observed for diseased snakes under laboratory conditions (Burns et al. 1996; Lorch et al. 2015). However, body temperatures of infected snakes did not differ from those of uninfected snakes across most of the active season. The lack of overall differences in body temperatures between snake groups could be a product of environmental temperatures at our northern latitude study site being overall uncondusive to promoting behavioral fever (Tetzlaff 2015). Together our findings suggest the energetic cost of frequent movements and potential risks (e.g., predation) of exposed basking and foraging may have been too great for infected snakes.

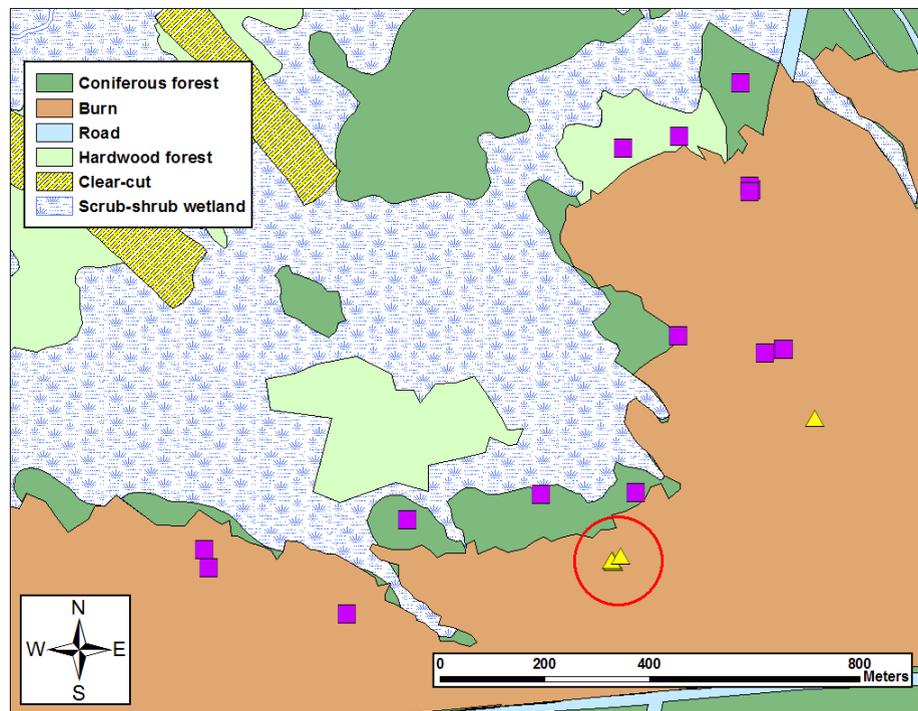


FIGURE 5. Known overwintering locations of infected (yellow triangles, $n = 6$) and uninfected (purple squares, $n = 17$) Massasaugas (*Sistrurus catenatus*) at Grayling, Michigan, USA, during the 2013–2014 to 2016–2017 winters. The points inside the red circle indicate an approximately 47-m² area where five of six infected snakes overwintered. Note some locations overlap for snakes communally overwintering in a given location.

Thus, an adaptive behavioral strategy for debilitated individuals during normally heightened activity periods may be to save energy and reduce non-vital movement, limit exposure, and cryptically thermoregulate during warm months.

Although we did not detect differences in body temperatures between snake groups from May to August, infected snakes had higher body temperatures than uninfected snakes in September. Infected snakes having higher late-season body temperatures than uninfected snakes is consistent with our observations of the infected snakes we studied, as well as non-telemetered individuals with clinical signs of SFD not part of the study, frequently engaging in late-season surface basking. We observed infected snakes basking near entrances to overwintering refugia during times when many (all in some years) uninfected telemetered snakes were below ground at their overwintering sites. Similar behavior has been reported for Timber Rattlesnakes (Clark et al. 2011; McBride et al. 2015), suggesting SFD could affect underlying acclimation physiology and/or infection may compromise typical behavioral responses to environmental cues promoting snakes to overwinter. Massasaugas at Camp Grayling overwinter for up to six months each year (Smith 2009). Thus, the need to bask at the end of the active season may instead be more pronounced for infected snakes if immune function

is reduced due to cooler environmental temperatures (Lorch et al. 2016) because opportunities for behavioral thermoregulation are essentially nonexistent for several months thereafter.

Many uninfected snakes died overwinter, but one infected individual survived three winters. All infected snakes overwintered in a large area of burned habitat, caused by an unintentional fire in 2010 (Ravesi 2016). Most infected snakes overwintered in a condensed location, with numerous individuals even occupying the same burrow, suggesting environmentally driven areas of high incidence of *O. ophiodiicola* may exist in the landscape. Environmental sampling (e.g., use of eDNA techniques) in future work will help clarify the distribution of the fungus. However, we cannot exclude the possibility that individuals became infected while traversing their active season ranges and simply transmitted the fungus to one another while overwintering nearby (Ravesi et al. 2015). It is unknown if *O. ophiodiicola* spores can be transmitted between individuals and cause infection, but the context of our observations underscores the need for research focusing on the relationship of disease transmission between individuals and the environment.

The continued impacts of habitat loss, road mortality, and persecution were reasons leading to the recent listing of Massasaugas as threatened under the U.S. Endangered

Species Act (U.S. Fish and Wildlife Service 2016). SFD is an additional potential threat to their persistence because the mortality rate of wild Massasaugas with the disease is over 90% for snakes brought into captivity for examination and treatment (Matthew Allender, unpubl. data). We acknowledge our sample size of only eight infected snakes is less than half of the number of uninfected snakes. However, obtaining markedly greater sample sizes for future studies will likely be challenging given concerns of surgically implanted radio-transmitters affecting infection susceptibility (Lentini et al. 2011; Hileman et al. 2017). Nevertheless, we suggest incorporating the behavioral impacts of SFD into future research efforts and management plans due to the altered behavior we documented for free-ranging infected Massasaugas. We also urge researchers conducting annual mark-recapture or similar studies to incorporate a sampling scheme for *O. ophiodiicola* into their protocols using swabbing methods and occupancy models leading to optimal detection probabilities and prevalence estimates (Hileman et al. 2017). Doing so could aid in determining if populations at sites known to harbor the pathogen are declining, stable, growing, or perhaps developing resistance over time, as has been noted for some frogs with chytridiomycosis (Scheele et al. 2017) and bats with white-nose syndrome (Langwig et al. 2017). Ultimately, linking snake behavior with the distribution of the fungus and habitat features at localized scales will lead to increased epidemiological knowledge of SFD, which could aid conservation efforts for imperiled snakes.

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