Disease ecology of ophidiomycosis in free-ranging snakes

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ABSTRACT

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Rachel M. Dillon

Ophidiomycosis (snake fungal disease) is caused by the pathogen *Ophidiomyces ophiodiicola*. Infected snakes exhibit dermal lesions, occasional systemic infections, and, in some cases, mortality. We studied snakes at Rondeau Provincial Park, Ontario, Canada, to explore whether ophidiomycosis develops during brumation or year-round. Throughout their active season, we quantified the prevalence of clinical signs of the disease on snakes and conducted qPCR of skin swabs to determine the prevalence of *O. ophiodiicola* on snakes. Prevalence of *O. ophiodiicola* and disease symptoms were highest on eastern foxsnakes (*Pantherophis vulpinus*) and very rare on other snake species. In *P. vulpinus*, pathogen and clinical sign prevalence was highest, directly after emergence from overwintering, with the majority of *P. vulpinus* being able to resolve clinical signs of ophidiomycosis by the return of winter. When we analyzed the survivorship of *P. vulpinus* we determined that the likelihood of a snake dying with ophidiomycosis is similar to a snake dying without ophidiomycosis. Given that *P. vulpinus* were the most affected species at our study site, ophidiomycosis does not appear to pose an imminent threat to our study population of *P. vulpinus* under current conditions.

Keywords: Eastern Foxsnake, *Pantherophis vulpinus*, ophidiomycosis, snake fungal disease, seasonal trends, fitness, body condition, qPCR

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Chapter 1 – General introduction: Ophidiomycosis

Emerging infectious diseases (EIDs) pose continuous conservation threats to wildlife populations. These diseases may seriously affect individual animal and human health, as well as have severe consequences for overall biodiversity. Emerging infectious diseases are defined as 1) infections that have newly appeared in a population or, 2) infections that have previously existed but are rapidly increasing in incidence or geographic range (Morse 1995). While many factors cause or increase instances of EIDs, those with the greatest over-arching influence on EID prevalence may be ecosystem alterations, movements of pathogens or vectors, and mutations in microbes (Williams et al. 2002). As humans continue to encroach on wild areas, there is a higher chance for EIDs to spread from domestic animals to wildlife populations (Daszak et al. 2000). EIDs can also be related to human intervention due to host or parasite translocations, often arising from the pet trade (Daszak et al. 2000).

Infectious diseases are caused by a variety of pathogens that include bacteria, viruses, fungi, and parasites. Several high-profile declines are specifically attributed to pathogenic fungi, many of which were previously unknown (Fisher, 2012). One such fungus is *Pseudogymnoascus destructans* – responsible for white-nose syndrome (WNS) in bats. WNS is the most devastating wildlife disease of mammals in recorded history (Bure and Moore, 2019), with up to 90% population decline in affected areas. Surpassing *P. destructans* in the amount of devastation it is causing, is *Batrachochytrium dendrobatidis*. This fungus causes chytridiomycosis in amphibians and has been implicated in the decline or extinction of up to 200 frog species (Skerratt

et al. 2007). In both cases, the fungi causing disease are highly pathogenic, virulent, and transmissible, leading to high mortality. Thus, conservationists were concerned with mentions of a potentially new fungal pathogen affecting snakes.

Disease ecology is complex, with multiple variables contributing to the contraction of a disease. The concept of the disease triangle, which shows the three-way interaction between the pathogen, host, and environment, is often used to disentangle these variables (Wobeser 2006, James et al. 2015). The theory of the disease triangle states that disease development requires the co-occurrence of a susceptible host, a virulent pathogen, and a suitable environment (Moore et al. 2011).

Ophidiomycosis, more commonly known as snake fungal disease, is caused by the fungal pathogen *Ophidiomyces ophiodiicola* (formerly *Chrysosporium ophiodiicola)* and is associated with skin infections. This disease was first implicated as a possible factor in the decline of a population of timber rattlesnakes (*Crotalus horridus*) in northeastern USA in 2011 (Clark et al. 2011). Since then, ophidiomycosis has been found in both wild and captive snake populations across the USA and into Ontario, Canada, as well as in Europe (Lorch et al. 2016; Franklinos et al. 2017; Allain & Duffus 2019). In addition to its varied distribution, which may in part be due to the international pet trade (Allender et al. 2015b; Lorch et al. 2016), a wide range of snake species have been reported to have the disease (Table 1). However, some groups of species were initially thought to be more frequently or more severely affected by ophidiomycosis than others, specifically vipers, though this could be a product of sampling efforts (Lorch et al. 2016). The reason for difference in disease prevalence between species is a yet unknown mechanism.

The fungal pathogen, *O. ophiodiicola*, is an environmental saprobe and thought to be ubiquitous in the environment. The Fisher model (Fisher et al., 2012), which is used to explain highly virulent fungal disease in animals, describes *O. ophiodiicola* as a generalist and opportunistic pathogen, using conidia and hyphae growing on detritus as propagules for rapid transmission (Allender et al. 2015b). *In vitro*, *O. ophiodiicola* grows on a variety of dead substrata and has the ability to utilize complex carbon, nitrogen, and sulfur resources found in a wide range of ecosystems (Allender et al. 2015b). This suggests that the fungus may infect snakes opportunistically, especially in warmer temperatures, as the optimal growth temperature for this fungus is 25° C (Allender et al. 2015b). A first line of defense for snakes, in the battle against *O. ophiodiicola* may be their unique skin microbial communities (Allender et al. 2018). One species of bacteria, *Morganella* spp., found on snake skin can even inhibit growth of *O. ophiodiicola,* which may play a role in an individual's ability to fight this disease (Hill et al. 2018).

Typical clinical signs of ophidiomycosis are scale crusts, ulcers, subcutaneous nodules, and swelling (Allender et al. 2011; Lorch et al. 2015). Often, lesions are considered minor to moderate in severity, but occasionally, individuals can present clinical signs that are much more severe, with the fungus invading the lower tissues and organs (Dolinski et al. 2014). Evidence suggests that ophidiomycosis may have an effect on snake behaviour as well. There were reports of increased basking as well as higher molting frequency in both wild and captive snakes with this disease (McBride et al. 2015; Lorch et al. 2015). These behavioural changes may have negative implications for snakes, and even cause death via a secondary method, such as depredation.

Fungal diseases often follow a seasonal pattern, as fungal growth rates and host immune response are dependent on temperature fluctuations, among other factors, that may be indirectly related to seasonality (Lugo et al. 2003; Pietikäinen et al. 2005). As ectotherms, snakes are particularly vulnerable to changes in temperature, especially when seasons change drastically, such as in more northern parts of North America. During colder months, snakes of all species brumate underground, either communally (intra- or inter-species) or alone (Gregory, 1982). This may have implications for transmission (e.g., close contact translates to higher rate of pathogen spread) of ophidiomycosis as well as the proliferation of *O. ophiodiicola*, resulting in seasonal trends of disease prevalence. Lesions indicative of ophidiomycosis are a strong predictor of *O. ophiodiicola* prevalence in the spring and summer months, with *O. ophiodiicola* being associated with skin lesions in 76% of snakes with histologically confirmed fungal dermatitis (McKenzie et al. 2019). It is important to note however, McKenzie et al. (2019) used a number of wild snake species in Kentucky, USA, where seasonal trends are not as drastic as the northern USA, or Canada. For example, Louisville, Kentucky, USA temperatures range from 0.5 °C to 26.6 °C, while Toronto, ON, Canada experiences temperatures of -10.2 °C to 23.1 °C (National Centers for Environmental Information, National Environmental Satellite, Data, and Information Service 2018a, 2018b). The snakes used in the McKenzie et al. (2019) study do not brumate for the same extended period as more northern snakes, and as such, the link between ophidiomycosis and brumation is still unknown.

Ophidiomycosis cause morbidity and mortality in both captive and wild snakes (Rajeev et al. 2009; Allender et al. 2011; Dolinski et al. 2014). Often times, death occurs

as a result of a secondary problem, such as anorexia or bacterial infection caused by a depleted immune response (Tetzlaff et al. 2017). However, whether these individual deaths are plentiful enough to affect snake populations as a whole, is still unknown. There are a few convincing cases of individual wild snakes dying as a direct result of ophidiomycosis Clark et al. 2011; Allender et al. 2011; Lorch et al. 2016). A declining population of rattlesnakes confirmed to have ophidiomycosis is often used as evidence to suggest that this disease can have detrimental effects on populations and even species persistence (Clark et al. 2011). That population of rattlesnakes in Clark et al. (2011) study however, had a plethora of other problems that may have contributed to its decline, including inbreeding and extensive habitat loss. As of yet, no other research has suggested that ophidiomycosis alone could cause large scale population declines in snakes.

Much about the ecology of ophidiomycosis and the snakes it affects is unknown. To understand this fungal disease's long-term implications, we must try to understand more about its mechanisms, including prevalence among species of snake and what times of the year infection is most likely to occur. Before coming to conclusions about the conservation consequences ophidiomycosis might have, we must begin to understand how this disease affects snake individuals and ultimately, populations.

The objective of my research is to begin to fill these knowledge gaps and to add to the growing amount literature in ophidiomycosis research. In Chapter Two, I conducted a field study of ophidiomycosis prevalence among a variety of snake species, using both *O. ophiodiicola* detection and clinical signs to predict the disease. I also further analysed seasonal trends of ophidiomycosis in a brumating species of

snake. In Chapter Three, I used radio telemetry data to answer questions pertaining to fitness of snakes living with ophidiomycosis, using body condition and movement trends as fitness proxies. I directly measured fitness through survivorship tests and rates of oviposition in diseased snakes. I also analysed snake mortality data to begin to understand the relationship between ophidiomycosis and others causes of mortality in snakes.

Table 1.1: List of wild snake species confirmed or suspected to have ophidiomycosis. * European species

Species	Taxonomic Family	Reference
Agkistrodon contortrix	Viperidae	(Lorch et al. 2016; McKenzie et al. 2019)
Carphophis amoenus	Colubridae	(McKenzie et al. 2019)
Coluber constrictor	Colubridae	(Lorch et al. 2016; McKenzie et al. 2019)
Crotalus horridus	Viperidae	(Clark et al. 2011; McBride et al. 2015; Lorch et al. 2016; Stengle 2018; McKenzie et al. 2019)
Diadophis punctatus	Colubridae	(McKenzie et al. 2019)
Farancia abacura	Colubridae	(Last et al. 2016)
Heterodon nasicus	Colubridae	(Lorch et al. 2016)
Lampropeltis getula	Colubridae	(McKenzie et al. 2019)
Lampropeltis nigra	Colubridae	(Lorch et al. 2016)
Lampropeltis triangulum	Colubridae	(Lorch et al. 2016; Ravesi et al. 2016; McKenzie et al. 2019)
*Natrix natrix**	Colubridae	(Franklinos et al. 2017; Meier et al. 2018)
*Natrix helvetica**	Colubridae	(Franklinos et al. 2017)
*Natrix tessellate**	Colubridae	(Franklinos et al. 2017)
Nerodia erythrogaster	Colubridae	(McKenzie et al. 2019)
Nerodia sipedon	Colubridae	(Glorioso et al. 2016; Lorch et al. 2016; McKenzie et al. 2019)
Pantherophis alleghaniensis	Colubridae	(Lorch et al. 2016)
Pantherophis spiloides	Colubridae	(McKenzie et al. 2019)

Pantherophis vulpinus	Colubridae	(Lorch et al. 2016)
Pituophis catenifer sayi	Colubridae	(Lorch et al. 2016)
Python bivittatus	Pythonidae	(Lorch et al. 2016)
Regina septemvittata	Colubridae	(Lorch et al. 2016; Price et al. 2016; McKenzie et al. 2019)
Sistrurus catenatus	Colubridae	(Allender et al. 2011, 2013, 2015a, 2016, 2018; Lorch et al. 2016; Tetzlaff et al. 2017; Hileman et al. 2018)
Sistrurus miliarius	Colubridae	(McCoy et al. 2017; Lind et al. 2018)
Sistrurus miliarius barbouri	Colubridae	(Cheatwood et al. 2003; Lorch et al. 2016)
Storeria occipitomaculata	Colubridae	(McKenzie et al. 2019)
Thamnophis radix	Colubridae	(Dolinski et al. 2014)
Thanmnophis sirtalis	Colubridae	(McKenzie et al. 2019)
*Vipera berus**	Viperidae	(Franklinos et al. 2017)

Chapter 2- Seasonal variation in ophidiomycosis and its resolution in wild snakes

Abstract

Ophidiomycosis, caused by the fungus *Ophidiomyces ophiodiicola*, is listed as a global conservation concern to snakes (Sutherland et al. 2014), but disease ecology of ophidiomycosis is not well understood. We studied snakes at Rondeau Provincial Park, Ontario, Canada, to explore whether ophidiomycosis is most prevalent directly after brumation or year-round. We collected skin swabs from snakes and conducted qPCR to detect the prevalence of *O. ophiodiicola* on snakes. We also evaluated the prevalence of clinical signs of ophidiomycosis on snakes. Prevalence of *O. ophiodiicola* and disease were highest on eastern foxsnakes (*Pantherophis vulpinus*) (*O. ophiodiicola* (qPCR detection): 21/96; Clinical signs: 112/324), and very rare on other species. Eastern foxsnakes may have a higher exposure risk due to their being the largest snakes in the park, as the longest snakes have the highest prevalence of *O. ophiodiicola* (t = -9.2347, df = 41.702, $p < 0.001$) and ophidiomycosis (t = -8.2621, df = 126.24, $p < 0.001$). *O. ophiodiicola* and lesion prevalence in foxsnakes decreased from April to October (*O. ophiodiicola*: SE = 0.003, df = 559, $p < 0.001$; Clinical Signs SE= 0.007, df= 135, $p < 0.001$), and we observed foxsnakes resolving their clinical signs and apparently recovering during their active season. This seasonal cycle of *O. ophiodiicola* ophidiomycosis is important to consider when determining what time of year to investigate ophidiomycosis in northern populations of snakes.

Keywords: Eastern Foxsnake, *Pantherophis vulpinus*, ophidiomycosis, snake fungal disease, seasonal trends, qPCR, overwintering, *O. ophiodiicola*

Introduction

Earth is currently experiencing a rapid loss of biodiversity (Hughes et al. 1994; Lydeard et al. 2004; Ceballos et al. 2017; Rosenberg et al. 2019); approximately two vertebrate species are lost per year (Ceballos et al. 2017). This decline in biodiversity, considered as Earth's sixth mass extinction, is attributed to habitat loss and destruction, climate disruption, overexploitation, and invasive species (Hughes et al. 1994; Ceballos et al. 2017), all of which can drive an increase in the number and prevalence of infectious wildlife diseases (Jones et al. 2008; Fisher et al. 2012). Disease can exacerbate other threats to wildlife resulting in an organism being unable to mount an appropriate response to risks. Emerging infectious diseases (EIDs) are defined as diseases that have recently increased in incidence or geographic range, recently moved into new host populations, recently been discovered, or are caused by newly-evolved pathogens (Daszak et al. 2000). Wildlife EIDs are contributing to biodiversity loss, and large-scale declines in wild populations have been attributed to emerging fungal diseases (Fisher et al. 2012). The spread of the fungal disease chytridiomycosis has caused the decline or extinction of more than 200 frog species, decimating the diversity of an already imperilled group of animals (Skerratt et al. 2007). Similarly, white-nose syndrome has caused a rapid decline in bat abundance, with some hibernacula experiencing declines of over 75% (Blehert et al. 2009), primarily across North America. Therefore, it is important to identify and understand pathogens and their effects on host species before they become a conservation concern.

Ophidiomycosis (snake fungal disease) is caused by the fungal pathogen *Ophidiomyces ophiodiicola* (formerly *Chrysosporium*). This is especially concerning as snakes belong to a taxa that are disproportionally at risk of decline, due to habitat loss

and degradation, introduced invasive species, environmental pollution, disease, unsustainable use, and global climate change (Gibbons et al. 2000). There has been a dramatic increase in the reports of ophidiomycosis in wild North American snakes since 2011 (Paré & Sigler 2016), which have caused many to classify this disease as "emerging" (Allender et al. 2011; Clark et al. 2011). Recent observations have been reported in the eastern United States (Rajeev et al. 2009; Dolinski et al. 2014; Guthrie et al. 2016; Lorch et al. 2016; Glorioso et al. 2016; Tetzlaff et al. 2017), though there are also cases of ophidiomycosis in a variety of wild snake species in Canada, Europe, and Australia (Vissiennon et al. 1999; Lorch et al. 2016). The prevalence of ophidiomycosis may vary among species, but the disease has been documented mostly in colubrids and vipers (Lorch et al. 2016). It is unclear what may make one snake species more susceptible than another, though likely there are a multitude of factors (i.e., immune function, environmental changes) that influence predisposition to the disease, including individual population dynamics (Lorch et al 2016).

The pathogenic fungus *O. ophiodiicola* is found on a variety of substrates in a range of environments and opportunistically infects snakes (Allender et al. 2011, 2015). *In vitro*, *O. ophiodiicola* grows well on dead organisms, and it can use multiple complex carbon and nitrogen sources and tolerate the wide range of pH (5 –11) and high sulfur levels most commonly found in the soil (Allender et al. 2015), suggesting it is an environmental saprophobe, which is an organism that derives its nutrients from nonliving or decaying organic matter. *Ophidiomyces ophiodiicola* also uses a compliment of enzymes for using many environmental carbon and nitrogen sources (Allender et al. 2011). This fungus grows well from temperatures ranging from 10°C –

35°C, where inhibition occurs. With increasing global temperatures, snake populations that experience milder winters could be disproportionately affected.

Clinical signs of ophidiomycosis include; crusty scales, superficial pustules, and subcutaneous nodules (Dolinski et al. 2014; Mcbride et al. 2015; Tetzlaff et al. 2015; Guthrie et al. 2016). Clinical signs are highly variable, from minor lesions to severe swelling of the face and invasion into the lungs and deep tissue (Figure 2.1). Ophidiomycosis has been associated with mortality and morbidity in some cases (Rajeev et al. 2009; Allender et al. 2011; Dolinski et al. 2014; Mcbride et al. 2015; Paré & Sigler 2016), but snakes are able to persist with the disease (Chandler et al. 2019).

Clinical signs consistent with ophidiomycosis have been documented in overwintering snakes for decades – before they were associated with this fungal disease (Clark et al., 2011). However, it is impossible to know if lesions were caused by *O. ophiodiicola* without having identified the pathogen. These lesions were referred to as hibernation/brumation sores or blisters (Paré & Sigler 2016), because they usually disappeared soon after emergence from dens (Clark et al., 2011). There has been an association between overwintering snakes and emerging from overwintering in poor condition, often exhibiting lesions or blisters (Paré & Sigler 2016). This same relationship is being noticed today with anecdotal observations suggesting that clinical signs of ophidiomycosis may be more common in snakes directly after emergence from brumation (Guthrie et al. 2016; Lorch et al. 2016). This may indicate that snakes are unable to mount an effective immune response to the fungus during overwintering, as has been observed in other animals that overwinter (Nelson & Demas, 1996; Bouma et al. 2010). Fungal infections that affect wild populations generally follow a seasonal cycle

due to varying rates of fungal growth in different environmental conditions, most often based on temperature (Kriger & Hero 2006; Berger et al. 2015). *Pseudogymnoascus destructans*, the fungal pathogen associated with white-nose syndrome in bats exploits its hosts during hibernation, and the success of this pathogen is directly associated with the hibernating physiology of these bats during overwintering (Cryan et al. 2010). Thus, *O. ophiodiicola* may affect snakes during their overwintering period, when they are most vulnerable due to their suppression of whole-body energy expenditure. Differing severity in clinical signs of ophidiomycosis across seasons has been noted in multiple studies that took place across a variety of latitudes in the US, though with conflicting results most likely due to the differing climates of the study areas (McCoy et al. 2017; Lind et al. 2018; McKenzie et al. 2019). There may be implications for testing for instances of *O. ophiodiicola* or disease, if they are most prevalent during certain times of the year. This may lead to sites being deemed "ophidiomycosis-free" if researchers test for the disease at the wrong time. Thus, we need to understand the seasonal dynamics of ophidiomycosis in snakes to assess its true conservation impact and investigate whether there is evidence for recovery of the disease in the wild.

We hypothesized that ophidiomycosis affects snakes on a seasonal cycle, developing during the snakes' overwintering period and resolving the following active season. We predicted that the prevalence of *O. ophiodiicola* and gross lesions consistent with ophidiomycosis would be highest on snakes directly after emergence from overwintering, and that *O. ophiodiicola* and/or clinical signs of ophidiomycosis would be detected on fewer snakes as the active season progresses. We also took the

opportunity to explore the variation in disease prevalence among species that occur in the same area.

Materials and Methods

We captured and examined snakes from April to October, 2013 – 2018, at Rondeau Provincial Park (RPP) in Morpeth, Ontario, Canada. Ophidiomycosis was present at the study site (CWHC, 2016) and eight species of snake were present at RPP, which allowed us to compare detection of *O. ophiodiicola* among species and the prevalence of ophidiomycosis.

Snakes were captured during weekly surveys of an established coverboard transect (N = ~ 350 boards). We also searched opportunistically in suitable habitats within RPP. We recorded sex, snout-vent length, tail-length, and mass. *Pantherophis vulpinus* (Eastern Foxsnake) and *Heterodon platirhinos* (Eastern Hognose Snake) were implanted with a uniquely coded Passive Integrated Transponder (PIT) tag, to allow us to recognize recaptures. Other species were not individually marked.

In 2017 and 2018 we also collected swabs from all captured snakes to test for *O. ophiodiicola* and examined each snake for clinical signs of ophidiomycosis. Each snake was swabbed twice with two separate swabs (Puritan 3" Sterile Standard Cotton Swab w/Semi-Flexible Polystyrene Handle, 2019), swabbing ventrally and dorsally along the length of the snake's body ("body" swabs). If a snake exhibited lesions consistent with clinical signs of ophidiomycosis, we collected two additional swabs, concentrating the swabs only on the dermal lesions ("lesion" swabs). We stored all swabs in lysis buffer at room temperature. Swabs were submitted to the Canadian Wildlife Health Centre (CWHC) offices in Guelph, Ontario for DNA extraction and a validated real-time

polymerase chain reaction (qPCR) assay targeting *O. ophiodiicola* was performed (Allender et al. 2015, Bohuski et al. 2015). The precision and detection limit of the assays were evaluated based on a ten-fold standard curve dilution series of gDNA from 5 to 50,000 fg of DNA. The cycle threshold (Ct) for this test was considered at 40 cycles (i.e., samples with lower Ct values contain more *O. ophiodiicola* DNA). Samples with a Ct value less than 40 were considered positive for *O. ophiodiicola* and coded with 1 for data analyses, whereas samples with a Ct value greater than or equal to 40 were considered negative for *O. ophiodiicola* (indicating the pathogen was absent or present in quantities below the detection limit of the assay) and coded with a 0 for data analyses. In R Studio version 1.0.153 (R studio Team, 2015), we used a Fisher's Exact test (*fisher.test*, Package *stats* version 3.5.1) to determine if there was a difference in *O. ophiodiicola* prevalence among species, followed by a Pairwise Comparison of Proportions test (*prop.test*, Package *stats* version 3.5.1), using Holm's p-value adjustment. In order to determine if length of a snake had any influence on their disease status, we analyzed the SVL of each *P. vulpinus* (as they are the largest snake at our site by far) and compared that with whether or not the snake had clinical signs or *O. ophiodiicola*, using a T-test (*t.test*, Package *stats* version 3.5.1). In addition, we also compared the CT values of positive swabs to the SVL of the associated *P. vulpinus* using a linear model (*lm*, Package *stats* version 3.5.1). A lower CT value indicates that there is a higher detection rate of the fungus meaning that we wanted to investigate if longer snakes had a higher detection rate.

We re-sampled individual *P. vulpinus* each time they were recaptured and used these data to test whether the prevalence of detected *O. ophiodiicola* on *P. vulpinus*

varied across the active season. We performed a logistic regression analysis of Julian date against presence (1) or absence (0) of *O. ophiodiicola* on each snake captured in 2017 and 2018, controlling for year and individual. If a snake had both "body" swabs and "lesion" swabs, the "lesion" swab result was used, as *O. ophiodiicola* could be concentrated in lesions (Bohuski et al. 2015). We ran the generalized linear mixed model (*Glmer*, Package *lme4* version 1.1-19) with a binomial distribution and a logit link function. We considered results of all analyses statistically significant with a threshold $\alpha < 0.05$.

For each *P. vulpinus* captured in the park from April – October of 2013 –2018, we recorded the presence or absence of clinical signs of ophidiomycosis: gross dermal lesions, including regional or local edema, crusts, ulcers, dysecdysis, and other forms of non-injury type damage to the dermis (Lorch et al. 2015; Guthrie et al. 2016). We collected biopsies under sterile surgical conditions to confirm the diagnosis through histology and qPCR. All snakes were released at their capture locations within 24 hours of capture. We performed a logistic regression analysis of Julian date against presence (1) or absence (0) of lesions on a snake, including individual and year as a random effect. We ran the generalized linear mixed model (*Glmer*, Package *lme4* version 1.1-19) with a binomial distribution and a logit link function. In snakes (n = 17) that were part of a related radio telemetry project (Chapter 3), we were able to follow the progression of lesions more closely, counting the number of lesions at least once, monthly. Linear models (*lm*, Package *stats* version 3.5.1) were then used to assess the trend of lesions over the active seasons (2017, 2018) for radio tracked snakes, controlling for individual.

To test whether the prevalence of clinical signs of ophidiomycosis (lesions) in *P. vulpinus* varied across the active season we performed a logistic regression analysis of Julian date against presence (1) or absence (0) of lesions on each captured snake, controlling for individual. We ran the generalized linear mixed model (*Glmer*, Package *lme4* version 1.1-19) with a binomial distribution and a logit link function.

To assess the concordance in *O. ophiodiicola* detection between "body" and "lesion" swabs from an individual, we counted the number of discrepancies in *O. ophiodiicola* detection between "body" swabs and "lesion" swabs in snakes exhibiting clinical signs of ophidiomycosis.

Results

Of 80 pairs of body and lesion swabs from all snake species, thirteen pairs (15.8%) included one swab that tested positive and one that did not (Table 2.1). Eleven pairs contained a positive lesion swab and a negative body swab, while two exhibited the opposite pattern (Table 1).

We collected 465 swabs from 314 individual snakes (Figure 2.2). *Ophidiomyces ophiodiicola* was not detected on *H. platirhinos* (n = 1) or *S. occipitomaculata* (n = 2), and we excluded these species from further analyses due to low sample size. *O. ophiodiicola* was most commonly detected on *P. vulpinus* (21/96) followed by *N. sipedon* (1/6) and *S. dekayi* (1/60), and finally, *T. sirtalis* (2/160) (Figure 2.2). *O. ophiodiicola* prevalence differed significantly among species ($p < 0.001$). *Pantherophis vulpinus* contributed to 64% of the *O. ophiodiicola* positive snakes sampled at RPP. Pairwise comparisons by proportions revealed that *O. ophiodiicola* prevalence on *P. vulpinus* differed from the *O. ophiodiicola* prevalence of *S. dekayi* ($p = 0.0037$), *T. s.*

sirtalis ($p < 0.001$), and *T. sauritus* ($p = 0.1203$), while the sample size for *N. s. sipedon* was too small to reliably detect a difference. *O. ophiodiicola* presence (n = 354) and clinical signs (n = 382) were positively associated with SVL. Longer *P. vulpinus* were more likely to test positive for *O. ophiodiicola* (Fig. 2.3; $t = -9.2347$, df = 41.702, $p = 1.224e-11$) as well as being more likely to have clinical signs (Fig. 2.3; $t = -8.2621$, df = 126.24, $p = 1.669e-13$). There was not a significant relationship between *P. vulpinus* CT values and SVL ($R^2 = 0.08$, df = 27, $p > 0.05$).

O. ophiodiicola presence was negatively associated with day of year (Fig. 2.4; SE= 0.007, df= 135, $p < 0.001$). We detected *O. ophiodiicola* most frequently on *P. vulpinus* directly after emergence from brumation (late April – early May; 19/38 snakes swabbed) and less frequently as the active season progresses. By the time the snakes re-entered overwintering sites (September – October), *O. ophiodiicola* was only detected on 1/30 *P. vulpinus* swabbed.

Clinical signs of SFD in *P. vulpinus* decreased in prevalence over the active season (Fig. 2.4; SE = 0.003, df = 559, $p < 0.001$). Clinical signs in a subset of 17 snakes radio-tracked and recaptured frequently over a 2-year period (2017 –2018), was also negatively associated with day of year and showed the seasonal nature of the disease in the same individuals. Snakes in 2017 that exhibited a large number of lesions at the beginning of the active season generally exhibited a decrease in lesion numbers within that year, with most snakes not experiencing any lesions by the end of the active season (Fig. 2.5; linear model; $R^2 = 0.15$, df = 32, $p = 0.0242$). The same trend was observed in 2018 (Fig. 2.5; linear model; $R^2 = 0.23$, df = 27, $p = 0.0081$) with 6 snakes from 2017 surviving through overwintering. Of the six snakes that were

tracked through overwintering, four individuals (67%) saw an increase in their number of lesions in the Spring of 2018, after going into brumation in the Fall of 2017 with no lesions at all.

Discussion

Ophidiomycosis is most prevalent in *P. vulpinus* and only affects a small number of individuals in other snake species (Fig 2.2). This may be due to *P. vulpinus* being the longest snake species in the park, and SVL being positively associated with *O. ophiodiicola* and disease presence (Fig. 2.3). In line with our hypothesis, ophidiomycosis observations are negatively associated with day of year (Fig. 2.4). *P. vulpinus* have the highest prevalence of *O. ophiodiicola* and disease directly after emergence from brumation and resolve most lesions by the end of the active season, consistent with our predictions of a seasonal trend (Fig. 2.4, 2.5).

Pantherophis vulpinus have the greatest detected prevalence of *O. ophiodiicola* (Fig. 2.2), even though they share hibernacula, coverboards, and habitat features with other snake species in RPP. This is interesting in that it leads to more questions as to how the pathogen causing ophidiomycosis persists in the environment and is transferred among individuals, if snakes living in the same areas are not infected equally. In laboratory conditions, the O. o grows on a variety of substrates, in a range of temperatures (optimal growth at 25°C) and pH values (Allender et al. 2015). *Ophidiomyces ophiodiicola* appears to tolerate a variety of environments, and we expected it would be equally prevalent on different species using the same immediate habitat. This is not the case at RPP, however, as *P. vulpinus* has the highest instances of *O. ophiodiicola* detection as a species. We investigated this trend further by analyzing

SVL and its relationship to both *O. ophiodiicola* and clinical signs. Longer snakes are more likely to test positive for *O. ophiodiicola* as well as be more likely to have lesions indicative of ophidiomycosis (Fig. 2.3). It is important to note that *P. vulpinus* are the longest snake species examined in our study, but even within their own species, longer snakes are more at risk for ophidiomycosis. It is possible that the longer a snake is, the more surface area can come into contact with *O. ophiodiicola*, thus leading to higher instances of clinical signs. SVL can also be used as a proxy for age, so it may also be a possibility that older (longer) snakes are more susceptible to ophidiomycosis. A recent study showed that the skin microbiome of a snake may influence susceptibility to *O. ophiodiicola* (Allender et al. 2018). Perhaps there is a difference in the skin microbiome of *P. vulpinus* in comparison to the other snake species in the park, which could explain why they are more likely to carry *O. ophiodiicola*. Alternatively, swabbing larger snakes means we could have sampled a larger area of skin, picking up more of *O. ophiodiicola* and are more likely to detect it with the qPCR. Next steps should include testing skin microbiomes of snake species that have been exposed the disease and begin to quantitate the fungal load on a snake by using the corresponding SVL and a standard curve.

We found that prevalence of both *O. ophiodiicola* (*O. ophiodiicola*; Fig. 2.3) and clinical signs of ophidiomycosis (gross lesions; Fig. 2.3), are most widespread in *P. vulpinus* directly after emergence from overwintering underground (May). In a study of wild snakes with ophidiomycosis in Virginia, USA, it was reported that a large proportion of snakes with skin lesions were captured in April and no snakes with skin lesions were captured after mid-July (Guthrie et al., 2016). This is similar to what we observed in

RPP snakes with clinical signs of the disease, though we took it a step further by linking seasonality to *O. ophiodiicola* detection. Both aspects of the disease – *O. ophiodiicola* detection and clinical signs –follow the same significant trend, with the majority of the *O. ophiodiicola* prevalence and lesions being detected in May, after overwintering, and then detection tapering off by the end of the active season, August – October (Fig. 2.3). This is also consistent with a study that took place in Kentucky in which ophidiomycosis was most prevalent in Spring and Summer, with 65.8% and 57.5% of snakes had a positive PCR, respectively (Mckenzie et al. 2019). There are two other studies (McCoy et al. 2017; Lind et al. 2018) that looked at the ophidiomycosis relationship with seasonality but these were done using pygmy rattlesnakes (*Sistrurus miliarius*) in Florida, where snakes do not have the overwintering period that more northern snake species deal with. Rattlesnakes snakes experienced the highest clinical sign prevalence in the months of January and February (McCoy et al. 2017), which is when our study species, *P. vulpinus*, are underground in brumation. This indicates that there are differing seasonal cycles of ophidiomycosis across study sites, depending on climate.

Many different factors connected with overwintering could contribute to these trends in disease seasonality. First, there is the potential that snakes only become infected with *O. ophiodiicola* within the underground hibernacula that northern snakes use for overwintering. This seems unlikely however, as *O. ophiodiicola* has been detected on snakes in the southern US, where snakes do not need to overwinter, though they could still use underground burrows during cold temperatures (Lips 1991; Lind et al. 2017). In addition to this, research suggests that *O. ophiodiicola* is a soil saprobe (Allender et al. 2015), indicating that it should not just be confined to a specific

hibernaculum, rather, the fungus should be ubiquitous in the environment. Another reason for this trend of increased *O. ophiodiicola* prevalence directly after emergence, could be that snakes are unable to mount an effective immune response during this brumation period. It has been noted that host response to infection with *O. ophiodiicola* included increased frequency of shedding (Lorch et al. 2015). Shedding of the skin presumably removes the fungus from the snake if it is only on the first layer of the skin, and research shows that lesions are largely resolved after shedding (Lorch et al. 2015). Snakes are unable to shed their skin during overwintering, which potentially allows for the infection to progress to more severe clinical signs, and into the lower dermal layers or potentially other organs, which could result in the increased number and severity of lesions on snakes after emergence from underground. It is also possible that UV exposure has an effect on *O. ophiodiicola* over time, which would explain higher infection in the spring with gradual disappearance over the summer (Fig. 2.3), as well as some snakes emerging from overwintering early to bask in the sun (Paré and Sigler 2016). While *O. ophiodiicola* has not been tested in the context of UV light, *Batrachochytrium dendrobatidis,* the causative agent of chytridiomycosis, has. UV light, however, was reported to be ineffective in killing *B. dendrobatidis* (Johnson et al 2003).

Snakes that emerge from overwintering with clinical signs of ophidiomycosis may succumb to the infection or recover during the summer (Guthrie et al. 2016). In the Guthrie et al. 2016 study, 73% of snakes (*Nerodia taxispilota, Farancia erytrogramma, Nerodia sipedon, Coluber constrictor, Thamnophis sauritus sauritus, Pantherophis alleghaniensis, Lampropeltis getula getula, Thamnophis sirtalis sirtalis*) swabbed in April had skin lesions, while 0% of snakes after mid- July had lesions. Many of the infected *P.*

vulpinus we observed with lesions persisted and recovered. In our study, the majority of snakes resolved all of their clinical signs by the time they entered brumation in the fall (Fig. 2.5). Not only were snakes able to persist with lesions, many were able to resolve them completely during their active season of ~ May to October (Fig. 2.4). The cyclic and seasonal pattern of ophidiomycosis becomes especially clear when we see snakes resolve all lesions completely by the end of an active season, only to emerge from overwintering the following season with lesions again (Fig. 2.4). This may be due to their ability to shed after emergence, ridding themselves of the infection via their skin, when they are unable to do so underground. For snakes that have a harder time resolving lesions, *O. ophiodiicola* may have already penetrated lower dermal layers and caused secondary problems for the snakes' health, such as bacterial infections or pneumonia (Rajeev et al. 2009; Allender et al. 2015, 2018; Lorch et al. 2016). It is important to note however that we were not set-up in the field to detect secondary problems that may have arose as a result of ophidiomycosis, unless there was a mortality and the snake could be sent away for necropsy.

Estimates of *O. ophiodiicola* prevalence based on qPCR of epidermal swabs are conservative because some disagreeing swab results from the same swabbing event. We observed 14 discrepancies between the qPCR results of "body" and "lesion" swabs taken from the same snake, on the same day (Table 2.1). Positive "lesion" swab and negative "body" swab, are much more common than negative "lesion" swab and positive "body" swab. Our results suggest that further confirmation of *O. ophiodiicola* is required (i.e., noting of clinical signs and/ or biopsies) due to the small proportion of discrepancies between body and lesion swabs. To further confirm the disease in a

snake, histology should be performed on biopsies of the snake, along with confirmation of *O. ophiodiicola* via qPCR and clinical signs exhibited by the snake. Unfortunately, the biopsy portion of confirmation of disease often presents logistical problems in the field as we are unable to take every snake encountered to an authorized veterinarian for biopsy and additionally, biopsies are invasive.

Future directions in researching ophidiomycosis and its persistence in snakes should include studies with a focus on snake hibernacula in northern populations, such as the ones in this study and even further north. Communally overwintering snake groups need to be further analyzed and compared for fungal pathogen prevalence. There seems to be a positive link between overwintering snakes and ophidiomycosis, but it could not be teased out entirely in this study because we need to test and analyze the substrates of a variety of hibernacula for *O. ophiodiicola* in areas where it is known to occur, along with the snakes that emerge from the hibernacula. This could have larger implications as climate change causes northern areas to warm, snakes may not need to overwinter for as long, changing the dynamics of the snake-disease pathosystem. More long-term tracking of individuals is also needed to further our understanding of the resolution of clinical signs. Our study fills fundamental knowledge gaps within research of ophidiomycosis, as we have provided evidence for the perseverance of snakes with ophidiomycosis over the active season. Ophidiomycosis may be more seasonally based than originally thought, as it affected snakes in our study in a yearly, cyclic manner. The time of year influences both *O. ophiodiicola* and disease prevalence and this may lead researchers potentially over- or under-estimating instances of the disease, which has implications for management of populations with ophidiomycosis.

Table 2.1: Swabs collected from a variety of snake species at Rondeau Provincial Park (2017 – 2018) to test for the pathogen causing ophidiomycosis, *O. ophiodiicola*. We examined each snake for clinical signs of ophidiomycosis. Each snake was along the length of the snake, both ventrally and dorsally ("BODY" swabs). If a snake exhibited lesions consistent with clinical signs of ophidiomycosis, we collected additional swabs, concentrating the swabs only on the dermal lesions ("LESION (LES)" swabs). Using quantitative PCR, each swab was analyzed to determine if *O. ophiodiicola* could be detected. Detection limit for this test is considered at an inverse cycle threshold (Ct) = 40 (i.e., samples with lower Ct values contain more *O. ophiodiicola* DNA). POS (+) samples indicate that *O. ophiodiicola* was detected below the (Ct) value of 40. NEG (-) samples indicate *O. ophiodiicola* was not detected at all.

# of swabs with ONLY BODY swabs (no clinical signs)		**# of swabs with BODY + LESION swabs (clinical signs)**			
POS (+)	NEG (-)	BODY (+) /LESION (+)	BODY (-)/ LESION (-)	BODY (+)/ LESION (-)	BODY (-)/ LESION (+)
10	295	12	54	2	12
Total = 305		Total = 80 (x 2 for paired swabs) = 160			
Total = 465					

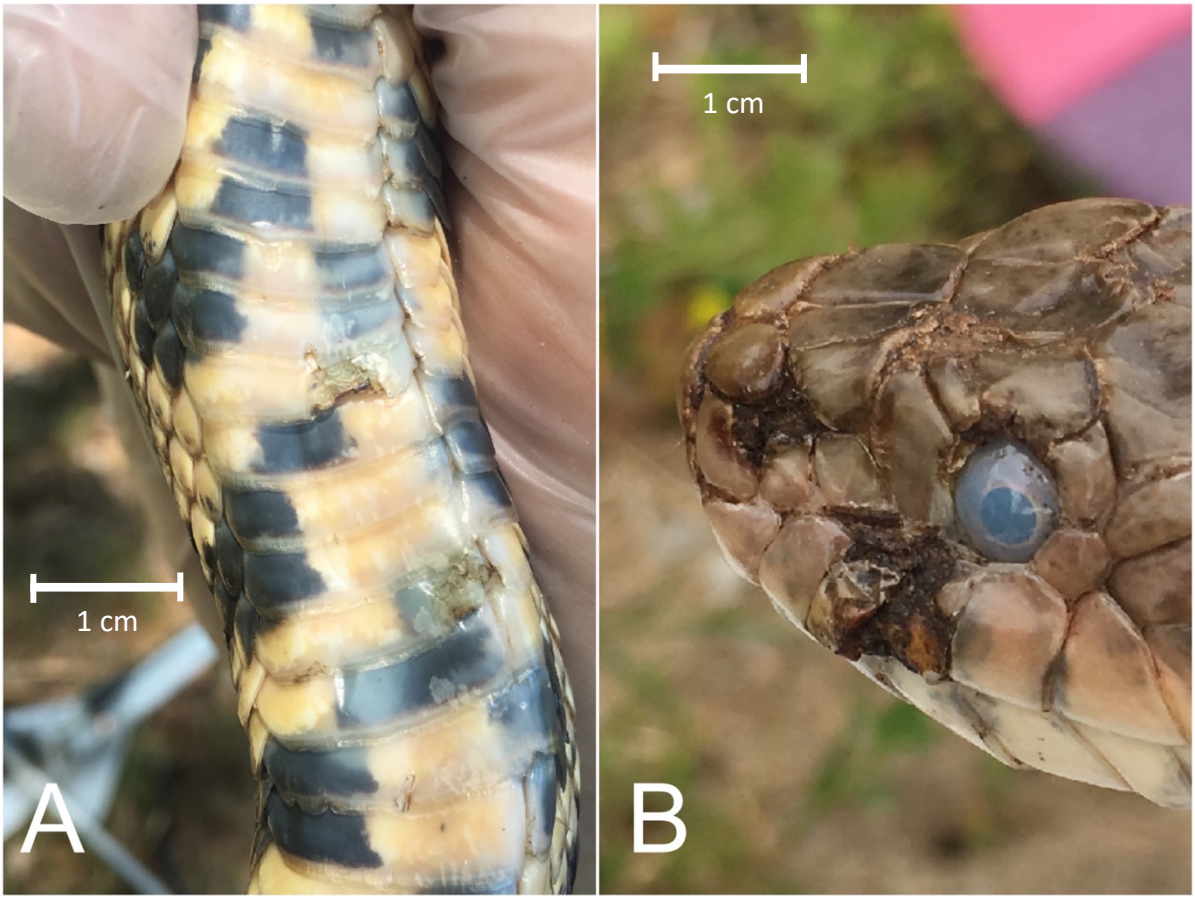

Figure 2.1: Two different *Pantherophis vulpinus* displaying clinical signs of ophidiomycosis cause by the fungus *Ophidiomyces ophiodiicola* in Ontario, Canada in 2017. 'A' depicts less severe clinical signs of the disease, slight crusting of the ventral scales. 'B' shows a snake dealing with much more severe clinical signs of ophidiomycosis in which the fungus is primarily affecting the face of the snake, invading lower tissue layers.

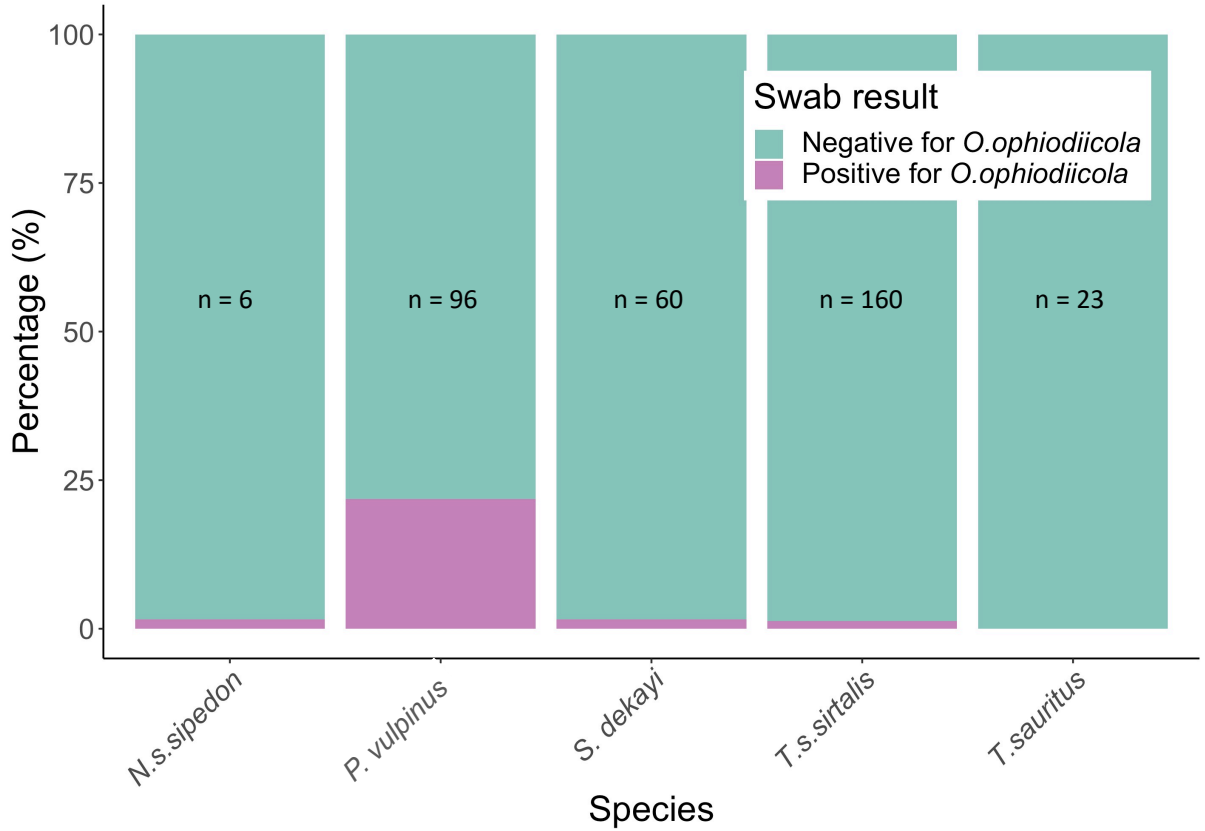

Figure 2.2: *Ophidiomyces ophiodiicola* prevalence in snakes at Rondeau Provincial Park (April – October, 2017 – 2018). Each observation represents a body swab (if the snake had a body and lesion swab, and there was a discrepancy between results from each swab, the positive swab was used) from a unique individual (*N* = 345).

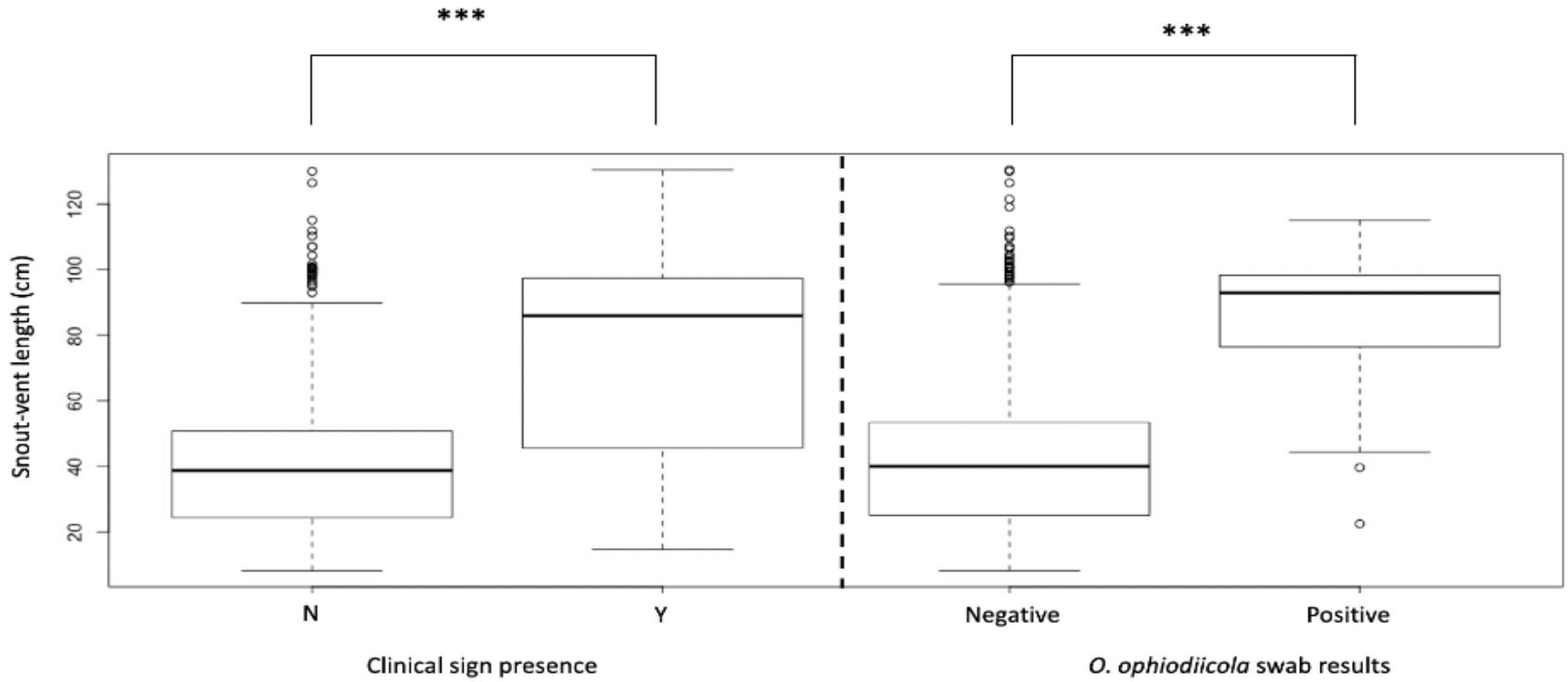

Figure 2.3: Boxplots illustrating snout-vent lengths of *Pantherophis vulpinus* and the presence of clinical signs of ophidiomycosis (n = 382) or the pathogen *Ophidiomyces ophiodiicola* (n = 354), sampled at Rondeau Provincial Park (April – October, 2017 – 2018). Each point represents an individual snake observation (clinical signs) or swab (*O. ophiodiicola*). *** indicates significance at $p < 0.05$

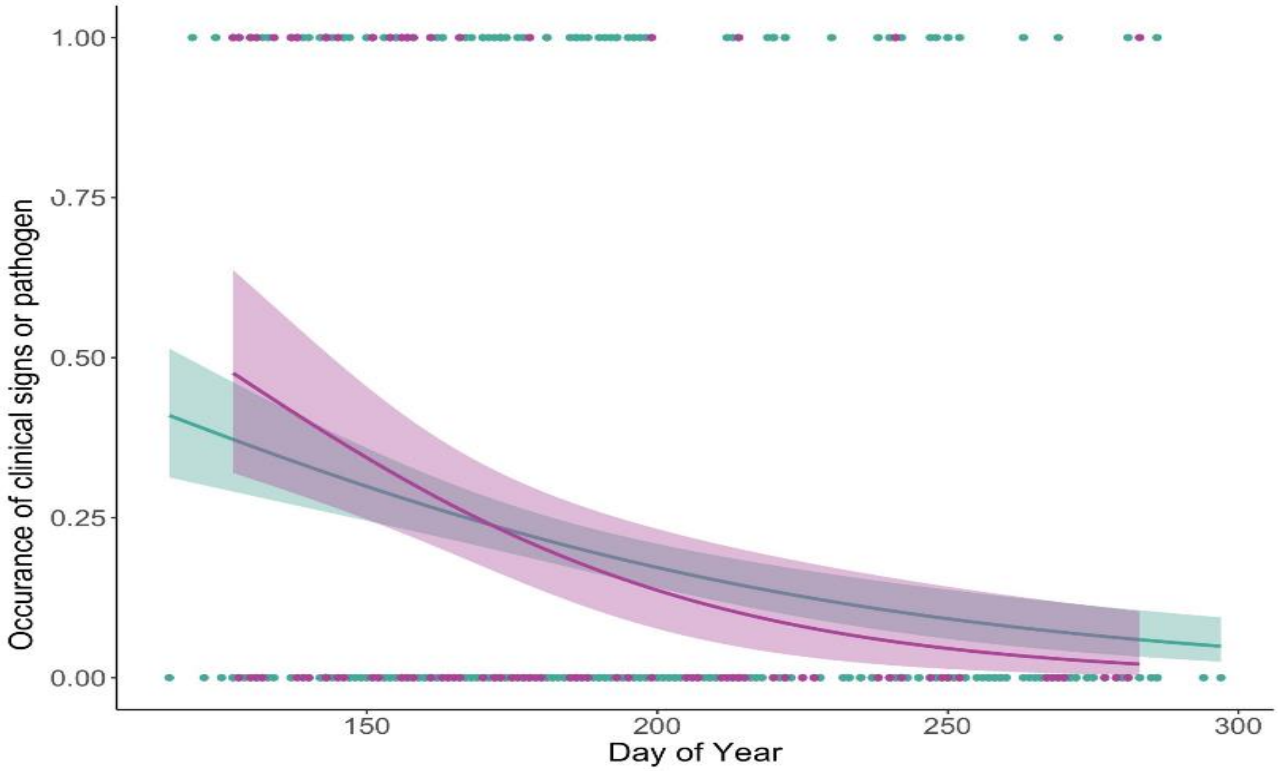

Figure 2.4: A binary logistic regression illustrating prevalence of clinical signs (lesions) of ophidiomycosis (n = 561; purple) and the pathogen *Ophidiomyces ophiodiicola* (n = 137; teal) in *Pantherophis vulpinus,* sampled at Rondeau Provincial Park (April – October, 2017 – 2018). The Y-axis represents the presence or absence of clinical signs (GLMM; SE = 0.003, df = 559, P < 0.001) or the pathogen, *O. ophiodiicola* (GLMM; SE= 0.007, df= 135, $p < 0.001$). Shaded areas represent 95% confidence intervals.

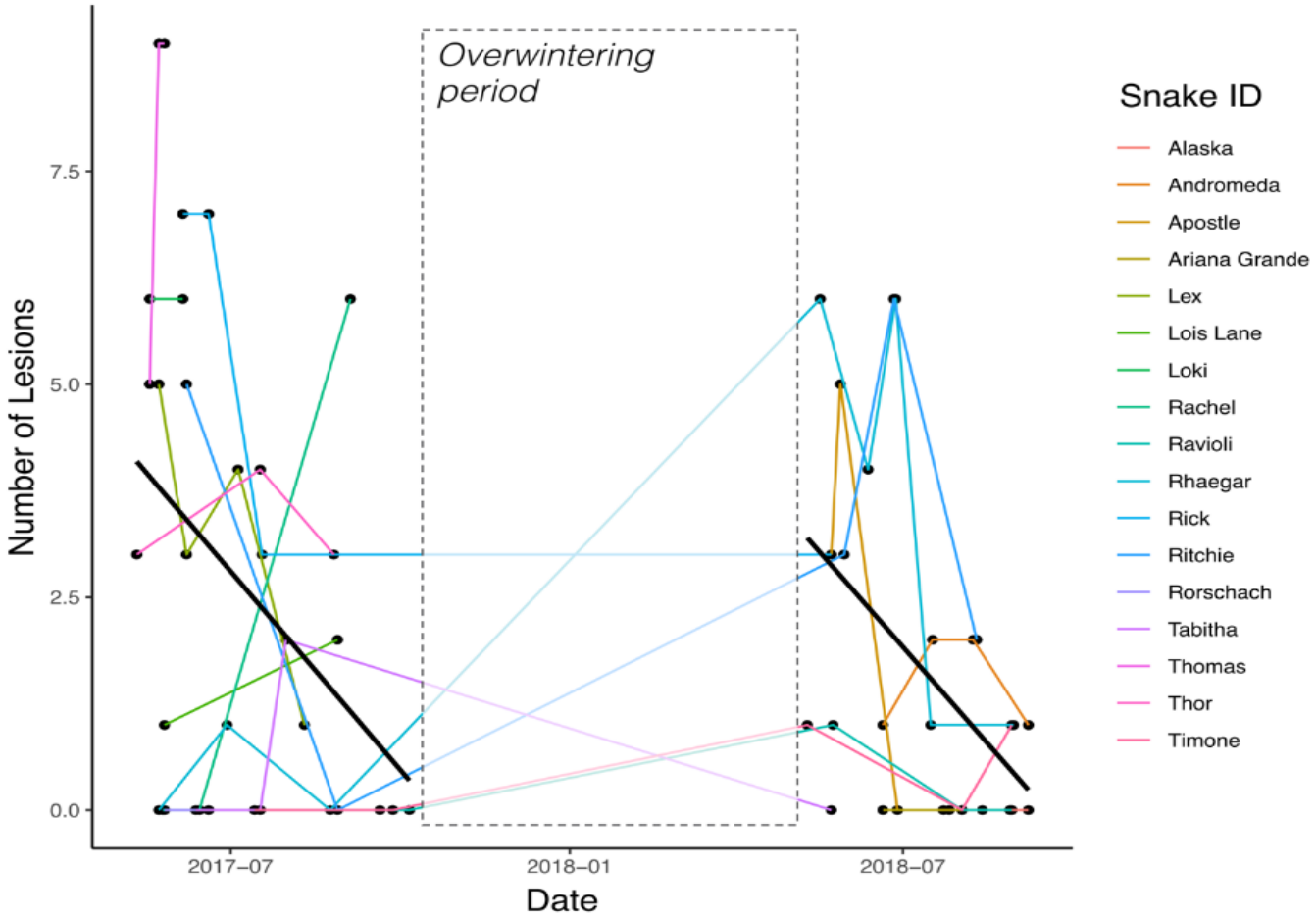

Figure 2.5: Regressions (bold black trend lines) illustrating a decline in number of lesions (clinical signs of ophidiomycosis) over the active season, for *P. vulpinus* individuals radio-tracked over 2 consecutive years at Rondeau Provincial Park, ON, Canada. Each coloured line represents an individual (n = 17), with 6 snakes surviving for 2 consecutive years. Each point represents the date of observation. The dashed line box represents the overwintering period, in which these snakes retreat underground and are unobservable. Bold black line, general trend: Linear Models (2017 Data, $R^2 = 0.15$, df = 32, $p < 0.05$ (0.0242)), (2018 Data, $R^2 = 0.23$, df = 27, $p < 0.01$ (0.008)).

Chapter 3 – Does ophidiomycosis affect *Pantherophis vulpinus* fitness?

Abstract

Ophidiomycosis (snake fungal disease) is caused by the fungal pathogen *Ophidiomyces ophiodiicola*. Infected snakes exhibit dermal lesions, occasional systemic infections, and in rare cases, mortality. To better understand the conservation implications of ophidiomycosis, we investigated its impacts on individual fitness in a population of endangered eastern foxsnakes (*Pantherophis vulpinus*). We tracked 30 foxsnakes over six years and quantified three fitness proxies: body condition, movement patterns, and ovipositioning. We observed similar body condition, distance travelled, and oviposition rates ($p > 0.05$) between snakes with and without ophidiomycosis. Interestingly, snakes with ophidiomycosis had larger home ranges (df = 20, W = 90, $p > 0.04$), suggesting that risk of infection may increase with exposure to a greater diversity of habitats. Of 19 snakes tracked from 2017 – 2019, 14 tested positive for *O. ophiodiicola* at some point during tracking. Using a multi-fate-multi-state survivorship model, we determined that *P. vulpinus* were not more likely to die while having ophidiomycosis (10.1%, UCL: 21.8%, LCL: 4.37%) compared to dying while otherwise healthy (3.3%, UCL: 12.2%, LCL: 0.83%), however infected snakes may be at a higher risk of predation than uninfected snakes. Overall, our results suggest that ophidiomycosis has only indirect effects on the fitness of eastern foxsnakes. Given that foxsnakes were the most affected species at our study site, ophidiomycosis does not appear to pose an imminent threat to our study population under current conditions.

Keywords: Eastern Foxsnake, *Pantherophis vulpinus*, ophidiomycosis, snake fungal disease, fitness, body condition, qPCR, survivorship

Introduction

Many reptiles and amphibians are imperilled (Gibbons et al. 2000; Sodhi et al. 2008; Todd et al. 2010; Böhm et al. 2013) because of threats such as introduced invasive species, global climate change, habitat loss, and disease (PARC 2007). The number of emerging diseases caused by fungi relative to other types of pathogens has risen (~7%) during the last two decades (Fisher et al. 2012). Several large-scale declines in wild populations and biodiversity have been attributed to fungal diseases, such as the chytrid fungus, *Batrachochytrium dendrobatidis* in amphibians (Skerratt et al. 2007), and the fungus that causes white-nose syndrome in bats, *Pseudogymnoascus destructans* (Blehert et al. 2009).

When populations are already imperiled, introduction of disease likely only exacerbates declines. For example, with chytridiomycosis, which has caused a global decline in already at-risk frog species, and even extinction in some species (Skerratt et al. 2007; James et al. 2009). Chytridiomycosis exemplifies how a fungal disease can result in a great loss of biodiversity in a relatively short time period, and thus, makes scientists wary of new emerging wildlife pathogens that then might cause similar declines in other species.

Ophidiomycosis (snake fungal disease) has been categorized as an emerging wildlife disease and was first described in 2011 in a population of eastern massasaugas (*Sistrurus catenatus*) in Illinois, USA (Allender et al., 2011). Since then, the disease has been reported in a variety of wild snake species across the USA, Canada (CWHC, 2016), and Europe (Allain & Duffus 2019). There is some debate as to whether ophidiomycosis is an “emerging” disease because clinical signs have been reported for

decades, particularly after a snake emerges from overwintering (Clark et al. 2011; Guthrie et al. 2016).

Ophidiomycosis is diagnosed following the identification of diseased skin infected with the fungus *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola* (Allender et al. 2011). Data suggests that the fungus infects snakes opportunistically and is found on a variety of substrates in a range of environments (Allender et al. 2015). Lesions, which must be associated with *O. ophiodiicola* to be considered ophidiomycosis, can be characterized by crusty scales, superficial pustules, and subcutaneous nodules (Dolinski et al. 2014; McBride et al. 2015; Tetzlaff et al. 2015; Guthrie et al. 2016). Clinical signs are highly variable, from minor lesions to severe swelling of the face and invasion of the lungs and deep tissue.

Ophidiomycosis has been associated with mortality and morbidity in some individual cases, but not all (Rajeev et al. 2009; Allender et al. 2011; Dolinski et al. 2014; Mcbride et al. 2015). Snakes often respond to *O. ophiodiicola* infection by increasing moult frequency, with some individuals being able to recover from the disease completely. Death caused by ophidiomycosis may arise from secondary complications such as anorexia, rather than direct fungal damage (Tetzlaff et al. 2017). Regardless how mortality occurs, it is important to consider infectious wildlife diseases at all scales (Tompkins et al. 2011), especially those considered ‘emerging’. Initial research often focuses on what a particular disease can do to an individual, but from a conservation perspective, how a disease may affect population dynamics is more important. Populations can also react to diseases differently (i.e., local adaptation), with some being more negatively affected than others due to environmental or constraints,

such as warmer temperatures (Harvell et al. 2002), or population dynamics, such as depleted genetic variance as a result of inbreeding (Acevedo-Whitehouse et al. 2003; Trinkel et al. 2011; Vander Wal et al. 2014; Lorch et al. 2016). This was the case in a population of Timber rattlesnake (*Crotalus horridus*), in which ophidiomycosis was thought have an influence in their decline, though there were other contributing factors, including higher than normal rainfall and genetic isolation (Clark et al. 2011).

Fitness can be defined in many ways, but one of its underlying concepts is the ability of an individual to survive and reproduce in a given environment (Demetrius & Ziehe 2007, Barker 2009). Proxies such as body condition or reproduction can be used to infer fitness in an individual. Fitness differences among individuals are necessary for selection to lead to change in a population. Thus, if an incoming disease negatively affects individual fitness and a large number of individuals are affected, the disease may cause population declines.

The conservation implications of ophidiomycosis are still largely unknown, though not undiscussed. Some studies indicate that ophidiomycosis poses serious long-term implications for populations of snakes, especially those already at risk (Allender et al. 2011) and ophidiomycosis was listed as a global conservation concern in 2014 (Sutherland et al. 2014). The purpose of our study was to characterize fitness impacts in a wild population of endangered *Pantherophis vulpinus* (Willson & Committee on the Status of Endangered Wildlife in Canada 2008) known to have ophidiomycosis. If ophidiomycosis negatively impacts host fitness, then snakes with ophidiomycosis, when compared to snakes without the disease, should have lower body condition, constricted movement patterns, and a reduction in egg laying throughout the active season –

especially directly before and after overwintering. In addition to these proxies, radio-tracking *P. vulpinus* allowed us to calculate the probability of survival for snakes in this population with ophidiomycosis.

Materials and Methods

We studied an endangered population of the *Pantherophis vulpinus* (Eastern Foxsnake) at Rondeau Provincial Park (RPP) in Morpeth, Ontario, Canada. *P. vulpinus* have been tracked using radio telemetry at RPP since May of 2013. Following animal care protocols and in collaboration with a qualified veterinarian, we surgically implanted transmitters (SI-2, HOLOHIL, Carp, Ontario, Canada) into a total of 44 snakes throughout the active seasons (May – August) of 2013 – 2018. These temperature sensitive transmitters (9 – 11g transmitter < 3% of the body mass of the snake) lasted from 12 to 18 months, requiring annual replacement (there is no mortality sensor). Snakes were tracked approximately once weekly from 2013 – 2015, and twice weekly in 2016 – 2018. Tracking took place from the time snakes emerged from overwintering in April – May to when they return to their hibernaculum sites for the winter (September – October). Each time a snake was tracked and located, GPS coordinates were recorded (UTM 17T, NAD83) as well as the pulse rate, which indicated snake body temperature. We also recorded habitat, and clinical signs of ophidiomycosis. Tracked snakes were processed once monthly. We recorded snout-vent length, tail-length, and mass. We also collected swabs from all captured snakes in 2017 and 2018 to test for *O. ophiodiicola* and examined each snake for clinical signs of ophidiomycosis (Table 3.1). Each snake was swabbed twice with two separate swabs (Puritan 3" Sterile Standard Cotton Swab w/Semi-Flexible Polystyrene Handle, 2019), swabbing ventrally and

dorsally along the length of the snake's body ("body" swabs). If a snake exhibited lesions consistent with clinical signs of ophidiomycosis, we collected an additional two swabs, concentrating the swabs only on the dermal lesions ("lesion" swabs). We stored all swabs in lysis buffer at room temperature. Swabs were submitted to the Canadian Wildlife Health Centre (CWHC) offices in Guelph, Ontario for DNA extraction and a validated real-time polymerase chain reaction (qPCR) assay targeting *O. ophiodiicola* was performed (Allender et al. 2015, Bohuski et al. 2015). The precision and detection limit of the assays were evaluated based on a ten-fold standard curve dilution series of gDNA from 5 to 50,000 fg of DNA. *Ophidiomyces ophiodiicola* was considered present on a snake if DNA amplification occurred within 40 cycles (cyclic threshold (Ct); Bohuski et al. 2015). If amplification did not occur within 40 cycles, the samples were considered negative for *O. ophiodiicola.*

Firstly, we investigated whether snakes with ophidiomycosis have smaller or larger home ranges (i.e., use smaller or larger areas throughout the active season) than snakes without ophidiomycosis. We created minimum convex polygons (100% MCPs) for each individual (n = 34) tracked from 2013 – 2018 based on telemetry data using the adehabitatHR package from R (Calenge 2006), following the recommendations of Row & Blouin-Demers 2006)). Snakes with < 5 relocations per year were removed from statistical analyses (n = 3). A home range was created for each year the snake was tracked, for example, if the snake was alive from 2015 – 2018 then it would have 3 home ranges. We compared the home range size of snakes that were or were not carrying the pathogen, *O. ophiodiicola*, as well as snakes with and without ophidiomycosis, using a Mann-Whitney U (Wilcoxon) test (Package *stats* version 3.5.1).

Clinical signs (lesions) are a strong predictor of *O. ophiodiicola* (Mckenzie et al 2019), and this field diagnosis was confirmed by histology and qPCR whenever possible, thus clinical signs are used as a proxy for the disease, ophidiomycosis.

Further, we examined the daily distance travelled by tracked snakes (trajectory) to determine if ophidiomycosis increases or reduces energy investment in movement, which is tied to foraging and mate-seeking. We used a linear mixed model (Package *lme4* version 1.1-19) with the daily distance travelled for each snake tracked between 2013 – 2018 (n = 32 snakes, 1605 relocations) as a function of clinical signs of ophidiomycosis and then *O. ophiodiicola* presence, using individual and year as random effects (Trajectory Model = lmer(Distance.per.Day ~ Clinical.Signs.or.Pathogen + (1|id) + (1|year)).

We also investigated the association between habitat use and detection of *O. ophiodiicola* on *P. vulpinus*. Each time a snake was swabbed (2017 – 2018) we recorded the habitat type that the snake was found in: anthropogenic (buildings, roads etc.), dunes, forest, marsh, or savannah. Only "body" swabs (n = 124) were used in order to not duplicate individuals that also had "lesion" swabs. We used a Fisher's Exact test to assess if swab pathogen result was independent of habitat type (*fisher.test*, Package *stats* version 3.5.1). In addition, we also compared CT values of positive swabs (n = 201) in each habitat to see if a particular habitat resulted in higher detection of the fungus, using a Fisher's Exact test (*fisher.test*, Package *stats* version 3.5.1).

Using body condition as a proxy for fitness, we calculated body condition for each tracked *P. vulpinus* from 2013 – 2018 using a body condition index (BCI), which is a reliable indicator of fat reserves for snakes (Bonnet & Naulleau, 1994). First, we used

log-transformed mass and average snout-vent length (SVL) to estimate the parameters of a linear model. We used all captures for each individual where a mass was taken. Second, we used the model parameters to calculate residuals of measurements for all captures of all snakes. To avoid confounding effects on mass caused by developing embryos, we separated male and female snakes for separate analysis. To compare BCI of snakes with and without clinical signs of ophidiomycosis we ran a Gaussian general linear mixed model (*Glmer*, Package *lme4* version 1.1-19) on male snakes and then female snakes, respectively (Model = lmer(eafo.residuals ~ eafo.Clinical.Signs + (1|ID) + (1|Year), data = eafoM)). When analyzing female BCI, gravid status was controlled for as a fixed effect. Ovipositioning in diseased female snakes was compared to non-diseased females (n = 15) using a Fisher's Exact test (*fisher.test*, Package *stats* version 3.5.1).

We analyzed the probability of a snake dying with and without ophidiomycosis. We did this by creating a multi-state-multi-fate model capture history in Rmark (Laake 2013). Using tracked snakes from 2017 – 2018 (n = 17), we assigned a state for each processing event, once per month for a total of 10 months (May – September, 2017, 2018). States were as follows: 0 = Unknown (i.e., before a snake was captured, as we did not know if a snake had lesions or not before first capture), D = Dead, L = Lesions (snakes with ophidiomycosis), H = Healthy (snakes without ophidiomycosis). This model provided us with the coefficient Psi, which is the likelihood of a snake transitioning from one state to another.

Further, we compared the cause of death between snakes with and without ophidiomycosis that died during the study period. Using necropsy results from the

Canadian Wildlife Health Cooperative we compared survivorship of snakes that were depredated or road-killed and used a Fisher's Exact test (*Fisher.test,* Package *stats* version 3.5.1) to determine if there was a difference in disease prevalence in each group. *P. vulpinus* found live under coverboards during the same time period were used as a random sample of the population. Finally, we assessed the concordance in *O. ophiodiicola* detection between "body" and "lesion" swabs from individual *P. vulpinus*.

Results

There was no difference in home range size between *P. vulpinus* individuals with or without clinical signs of ophidiomycosis for the years 2013 – 2018 (df = 57, W = 393, $p = 0.3291$). *Pantherophis vulpinus* individuals that tested positive for *O. ophiodiicola* did, however, have larger home ranges than snakes that tested negative for the pathogen for the years 2017 – 2018 (df = 20, W = 90, $p = 0.04$). There was no difference in daily distance travelled between *P. vulpinus* individuals with or without clinical signs of ophidiomycosis for the years 2013 – 2018 (df = 1604, t-value = 0.166, $p = 0.87$). There was also no difference in daily distance travelled between *P. vulpinus* individuals that tested positive for *O. ophiodiicola* and those that had no *O. ophiodiicola* detected on their skin for the years 2017 – 2018 (df = 603, t-value = 1.519, $p = 0.19$).

To investigate the prior result that snakes carrying the ophidiomycosis pathogen had larger home ranges than snakes without *O. ophiodiicola*, we looked at the types of habitats *P. vulpinus* were moving though. Swab result was not independent of habitat type ($p = 0.037$). Snakes that were swabbed in the marsh contributed to 52% of the positive swabs at RPP. When comparing CT values of positive swabs found in each habitat, marsh habitat has the widest spread of CT values (20 – 40). Marsh had the only

3 swabs positive for *O. ophiodiicola*, falling below a CT value of 25, which indicates a high level of detection. However, this difference in CT values was not significant among habitats ($p > 0.05$).

Of 54 paired body and lesion swabs, fourteen pairs did not match (i.e., one swab tested positive and the other did not (Table 3.1). Twelve pairs contained a positive lesion swab and a negative body swab, while two exhibited the opposite pattern.

The body condition index of male *P. vulpinus* were positively affected by ophidiomycosis during mid active season, ~ day 225 (df = 165, SE = 0.013, t-value = 3.919, $p = 0.000136$; Fig 3.3). However, at the two points of time that we are concerned with – the time directly after emergence from overwintering and the time directly before returning to overwintering – there was no difference in BCI between snakes with or without the disease (df = 165, SE = 0.0001, t = 1.482, $p = 0.140528$; Fig 3.3).

Ophidiomycosis did not affect BCI of female *P. vulpinus* (df = 104, SE = 0.032, t = 0.17, $p = 0.71$; Fig 3.3). Female snakes with and without the disease both emerged from and returned to overwintering at the same BCI, though mid active season (around day 200), snakes with clinical signs of ophidiomycosis had slightly higher BCI. We also observed successful oviposition in 3 of 9 female snakes showing clinical signs of ophidiomycosis, and in 2 of 6 female snakes that did not show clinical signs of the disease ($p > 0.05$).

A multi-state-multi-fate survivorship model was selected out of 3 models, using AICc values (Table 3.2). The model had a constant survivorship in a single state, with different likelihoods of transitioning. Results from the survivorship analysis show that *P. vulpinus* were not more likely to die while having ophidiomycosis (10.1%, UCL: 21.8%,

LCL: 4.37%; Fig 3.4) compared to dying while otherwise healthy (3.3%, UCL: 12.2%, LCL: 0.83%; Fig 3.4). There was also an equal likelihood of snakes transitioning from healthy to diseased, as transitioning from diseased to healthy (25%). The proximate causes of death observed in our study were road mortality and depredation. Of the 21 *P. vulpinus* that were found dead on roads between 2013 – 2018, only 2 individuals had ophidiomycosis (9.5%; Fig 3.5). In contrast, 6/8 depredated *P. vulpinus* had ophidiomycosis (75%; Figure 3.5). Snakes with ophidiomycosis were more likely to be depredated than snakes without ophidiomycosis ($p = 0.0023$).

Discussion

Snakes with ophidiomycosis are not more likely to die than snake without the disease and having ophidiomycosis does not negatively affect the fitness of *P. vulpinus* in such a way that might affect population dynamics. *P. vulpinus* with ophidiomycosis do not exhibit drastically different movement patterns than snakes without the disease. Snakes with ophidiomycosis also do not have lower body condition indices than snakes without ophidiomycosis. In fact, several female *P. vulpinus* successfully reproduced. Most importantly, snakes are not more likely to die with ophidiomycosis when compared to snakes without the disease. However, snakes with ophidiomycosis may be more susceptible to depredation than snakes without the disease.

We looked at MCP's of tracked *P. vulpinus*, comparing snakes with clinical signs of ophidiomycosis and snakes without (Fig. 3.1). Infected snakes do not have smaller or larger home range sizes compared to uninfected snakes. Supporting this, is our finding that ophidiomycosis also does not affect the daily distance travelled by *P. vulpinus* (Fig 3.2). This is a positive indication that fitness is not being affected, as it illustrates that

the disease is not changing the normal movement patterns of these snakes, suggestion that typical daily snake behaviour is occurring (i.e., foraging, searching for mates etc.). However, snakes on which *O. ophiodiicola* was detected (meaning that all snakes in this group did not necessarily have clinical signs) did have slightly larger home ranges than snakes that tested negative for *O. ophiodiicola* (Fig 3.1). Unfortunately, we are unable to completely disentangle if it is *O. ophiodiicola* that is causing snakes change their behaviours to have larger MCPs or if it is the larger MCPs of these snakes that are causing them to pick up *O. ophiodiicola* in their wider environment. Further studies should incorporate both specialist- and generalist- habitat snakes to compare prevalence of disease. Lab trials could also be designed to monitor snake movement in individuals before and after exposure to disease.

When analyzing *O. ophiodiicola* prevalence in habitat to further understand the relationship between movement and pathogen, we found that swabs from the marsh contributed to the majority of all swabs that were positive for *O. ophiodiicola*. It is possible that the snakes with larger home ranges tend to move through marsh habitats more, and the marsh is either more conducive to fungal growth, or the marsh just has more of *O. ophiodiicola* ubiquitous in the environment. This is similar to what was found in the Southern USA, in which snakes inhabiting more aquatic habitats (ex., *Nerodia sipedon*) were more likely to test positive for *O. ophiodiicola* (Mckenzie et al 2019).

Interestingly, *P. vulpinus* with ophidiomycosis have similar body condition to snakes without the disease. There was much overlap in the body conditions of both diseased and non-diseased individuals, especially in the specific time periods of interest – directly after emergence from overwintering, and directly before overwintering. When

looking at only male *P. vulpinus* snakes (Fig 3.2), body condition differed between diseased and non-diseased snakes midway through the active season during the time period for mating. At this point, male snakes with ophidiomycosis went from having a lower BCI than snakes that were otherwise healthy, to having a higher BCI during mid active season. Diseased male snakes could be more focused on molting to try to clear the fungus rather than expending energy looking for mates compared to healthy males. Mating usually happens late May – early June (~ Day 140 –161) (Willson & Brooks 2006), which is where the largest drop in body condition is for un-diseased *P. vulpinus.* Similarly, female snake body condition (Fig 3.3) differed between snakes with and without ophidiomycosis, only during the oviposition period, which occurs in July (~ Day 182 – 212) (Willson & Brooks 2006). Female snakes with ophidiomycosis did not experience the same drastic mass loss during the egg laying period and may indicated that the diseased snake are not carrying as many eggs and therefore have a higher BCI during this ovipositioning period.

We observed successful oviposition in 3 of 9 female snakes showing clinical signs of ophidiomycosis, and in 2 of 6 female snakes that did not show clinical signs of the disease. Successful reproduction is often a major indicator of fitness (Shine 2003), demonstrating that these diseased females were not affected by ophidiomycosis to such an extent that they were unable to mate and develop eggs. Unfortunately, we were unable to tell what happened to the eggs as many snakes laying their eggs did not coincide with our tracking schedule. This leaves room for uncertainty as infection may cause a reduction in the number of eggs produced or the size of the egg. Reduction in clutch number or size of eggs could have implications for the resulting fitness of the

offspring. However, a recent study found that Pygmy Rattlesnakes (*Sistrusrus miliarus*) are able to reproduce viable offspring while affected with ophidiomycosis (Lind et al. 2019). In contrast, there is also some evidence of vertical transmission of ophidiomycosis (Stengle et al. 2019), though this is only one laboratory test. This is an interesting avenue for further research and reproduction in diseased populations should be monitored.

It is important to note that our results using pathogen swabs may be slightly conservative, as we noticed discrepancies among swab *O. ophiodiicola* detection taken from the same individual at one capture event (Table 3.1). 'Positive "lesion" swab, negative "body" swab' are much more common than 'negative "lesion" swab, positive "body" swab'. In addition, the 14 paired swabs that fell into the category 'negative "body" swab and negative "lesion" swab' but did come from snakes with clinical signs of ophidiomycosis may represent snakes that have already cleared the pathogen, *O. ophiodiicola*, and have been able to mount a large enough shed response enough to clear the clinical signs of the disease (Lorch 2015), or perhaps they are just false negatives (Bohuski et al 2015).

P.vulpinus at RPP are not more likely to die if they have ophidiomycosis, compared to snakes without the disease. Analysis shows that ophidiomycosis is not affecting *P. vulpinus* survivorship in RPP and that snakes can persist with this disease. This contradicts other studies which gave evidence for widespread mortality and morbidity in free ranging snakes (Clark et al. 2011; Allender et al. 2011; Dolinski et al. 2014; Allender et al. 2015; Lorch et al. 2016). It is thought that the mechanism by which death occurs due to ophidiomycosis is multifactorial (Lorch et al. 2016), so it is possible

that these other populations that are seeing increased mortality may have another compounding variable(s) that is not currently affecting our study population in RPP. For example, the decline of a population of snakes thought to have ophidiomycosis was observed by Clark et al. (2011) and was pre-dated by high summer rainfall, so it is possible that high amounts of rainfall are linked to increased severity of ophidiomycosis. Ontario's northern climate may prevent ophidiomycosis from increasing in severity due to the fungus growing better in warmer climates, like in more southern studies. The probability of a snake transitioning from having ophidiomycosis to not having the disease was the same as the probability of a snake transitioning from not having the disease to having ophidiomycosis. This illustrates that it is just as possible for a snake to clear clinical signs of ophidiomycosis as it is to become infected. However, when comparing two causes of death: 1) road mortality and 2) predation, snakes with ophidiomycosis die from predation at a higher proportion than snakes without the disease (Fig 3.6). This may be caused by snakes with ophidiomycosis eliciting more risky behaviours, such as basking, which would make the snake more visible to predators such as racoons (Akcali et al 2019), particularly due to their high daytime presence at RPP. Interestingly, our movement results showed that snakes that test positive for *O. ophiodiicola* have larger home ranges, thus by moving through more new areas, snakes have a higher likelihood of coming into contact with predators. It is important to note *P. vulpinus* may not be found dead on roads quite as much as other snake species because *P. vulpinus* often climb trees to bask on limbs, rather than using the road for ambient heat like many other snake species (Willson & Committee on the

Status of Endangered Wildlife in Canada 2008). A different pattern may emerge if the study species was less arboreal.

While ophidiomycosis may not be affecting the RPP population of *P. vulpinus,* it is important that monitoring continues. There is evidence that ophidiomycosis prevalence and severity may be exacerbated by events such as a large rainfall or changing population dynamics like inbreeding. Intense weather events are more likely to happen in our changing climate (Rosenzweig et al. 2001), and this may impact the severity and speed at which ophidiomycosis affects snakes. This highlights the need for monitoring snake fitness and *O. ophiodiicola* prevalence, if not only after events such as an extremely warm winter or very wet spring. Furthermore, if human disturbances continue to threaten snakes at Rondeau Provincial Park and decrease their population size through persecution and habitat loss, we may see that ophidiomycosis begins to affect smaller populations differently and perhaps more severely (Pongsiri et al. 2009).
For now, however, ophidiomycosis does not seem to consistently pose a conservation concern to *P. vulpinus* in Southern Ontario. This is especially important considering this population of *P. vulpinus* is currently classified as endangered (Willson & Committee on the Status of Endangered Wildlife in Canada. 2008). Even though ophidiomycosis now affects this population, we now know that these snakes can endure this disease in a manner that is not currently causing them decline or negatively affect their fitness.

Table 3.1: Swabs collected from 84 individual *Pantherophis vulpinus* at Rondeau Provincial Park (2017 – 2018) to test for the pathogen causing ophidiomycosis, *O. ophiodiicola*. Some individuals were sampled more than once. We examined each snake for clinical signs of ophidiomycosis. Each snake was swabbed along its full length, both ventrally and dorsally ("BODY" swabs). If a snake exhibited lesions consistent with clinical signs of ophidiomycosis, we collected additional swabs, concentrating the swabs only on the dermal lesions ("LESION" swabs). Using quantitative PCR, each swab was analyzed to determine if *O. ophiodiicola* could be detected. Detection limit for this test is considered at an inverse cycle threshold (Ct) = 40 (i.e., samples with lower Ct values contain more *O. ophiodiicola* DNA). POS (+) samples indicate that *O. ophiodiicola* was detected below the (Ct) value of 40. NEG (-) samples indicate that *O. ophiodiicola* was not detected at all. Includes recaptures.

# of swabs with ONLY BODY swabs (no clinical signs)		**# of swabs with BODY + LESION swabs (clinical signs)**			
POS (+)	NEG (-)	BODY (+) /LESION (+)	BODY (-)/ LESION (-)	BODY (+)/ LESION (-)	BODY (-)/ LESION (+)
6	73	12	28	2	12
Total = 79		Total = 54 (x 2 for paired swabs) = 108			
Total = 187					

Table 3.2: Multi-state-multi-fate model selection for a *Pantherophis vulpinus* population (n = 17) at Rondeau Provincial Park exposed to ophidiomycosis. Variables: *S* = survivorship, *Stratum* = state that the snake is in (healthy, diseased, or dead), *Psi* = probability of a snake switching states (ie. healthy to diseased or diseased to dead), and *p* = probability of a snake staying in one state. The best model was selected using AICc values.

Rank	Model	AICc	Npar
2	S(~-1 + stratum)p(~1)Psi(~-1 + stratum:tostratum)	188.5649	7
1	S(~1)p(~1)Psi(~-1 + stratum:tostratum)	186.9161	6
3	S(~-1 + stratum)p(~1)Psi(~1)	196.0718	4

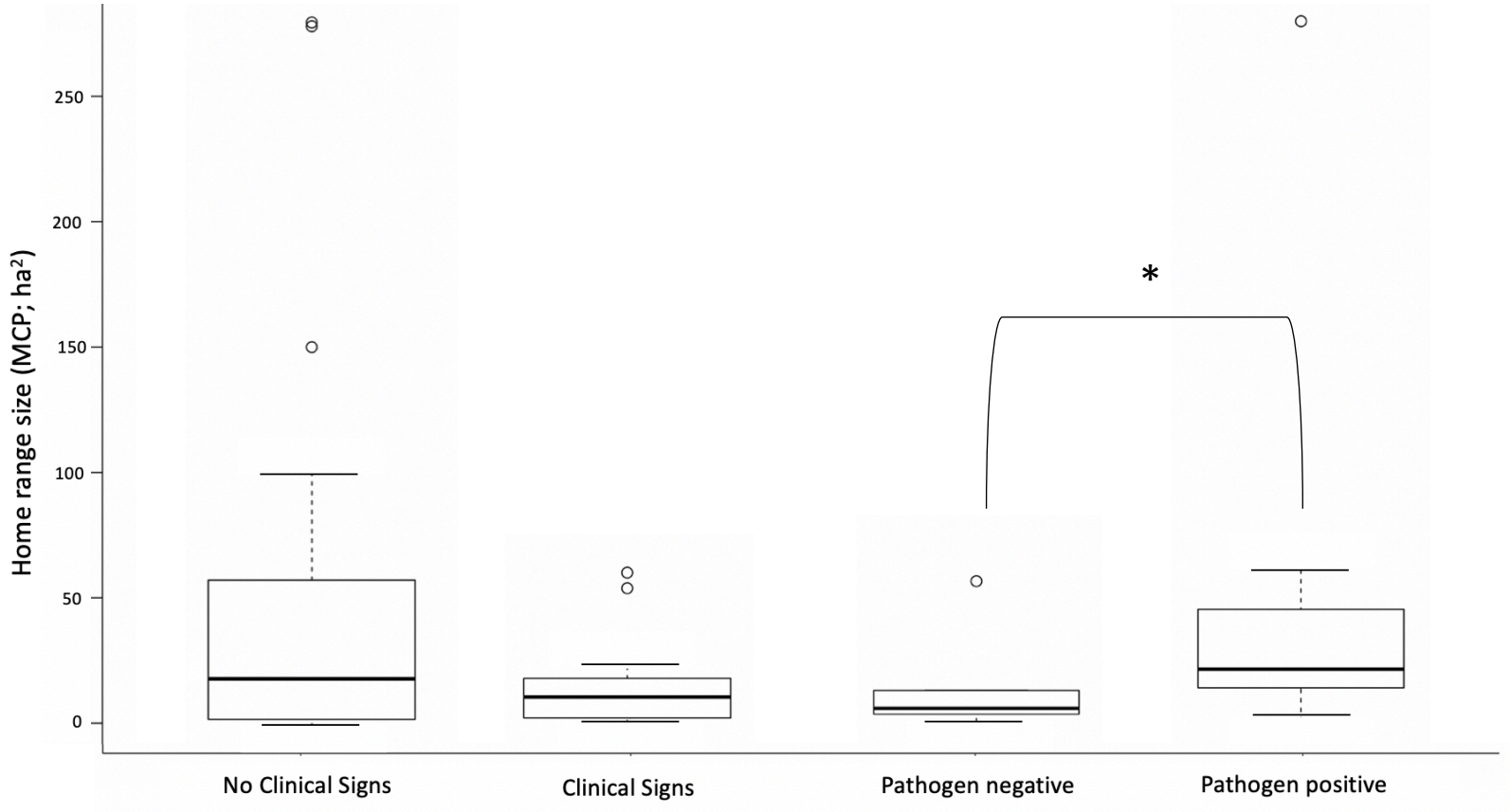

Figure 3.1: Boxplots depicting home ranges using minimum convex polygons (MCPs) of tracked *Pantherophis vulpinus*. Each boxplot represents a different category of tracked snakes based on *Ophidiomyces ophiodiicola* detection (n = 21) and clinical signs (n = 58). Year and individual were controlled for as random effect. Asterisk indicates significance ($p < 0.05$).

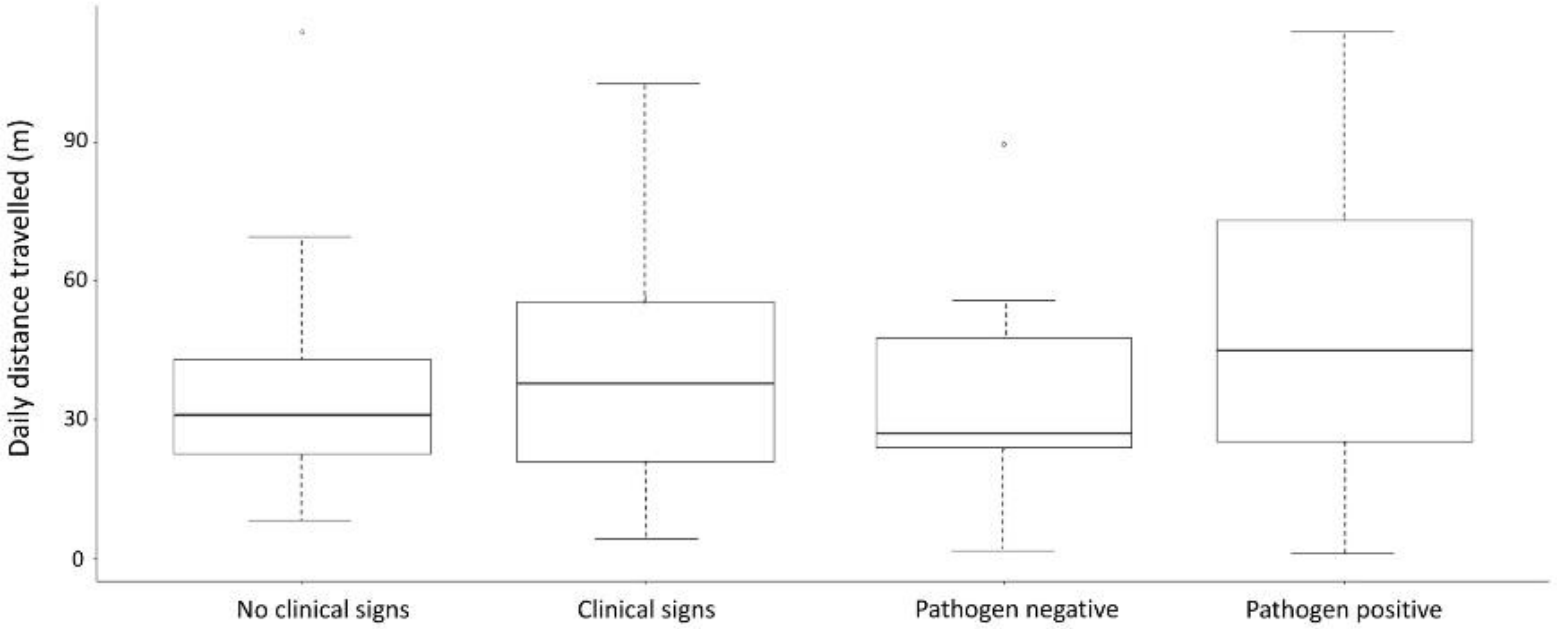

Figure 3.2: Boxplots depicting daily distance travelled (trajectories) of tracked *Pantherophis vulpinus*. Each boxplot represents a different grouping of tracked snakes based on their clinical signs and *Ophidiomyces ophiodiicola* detection. Year and individual were controlled for as random effects.

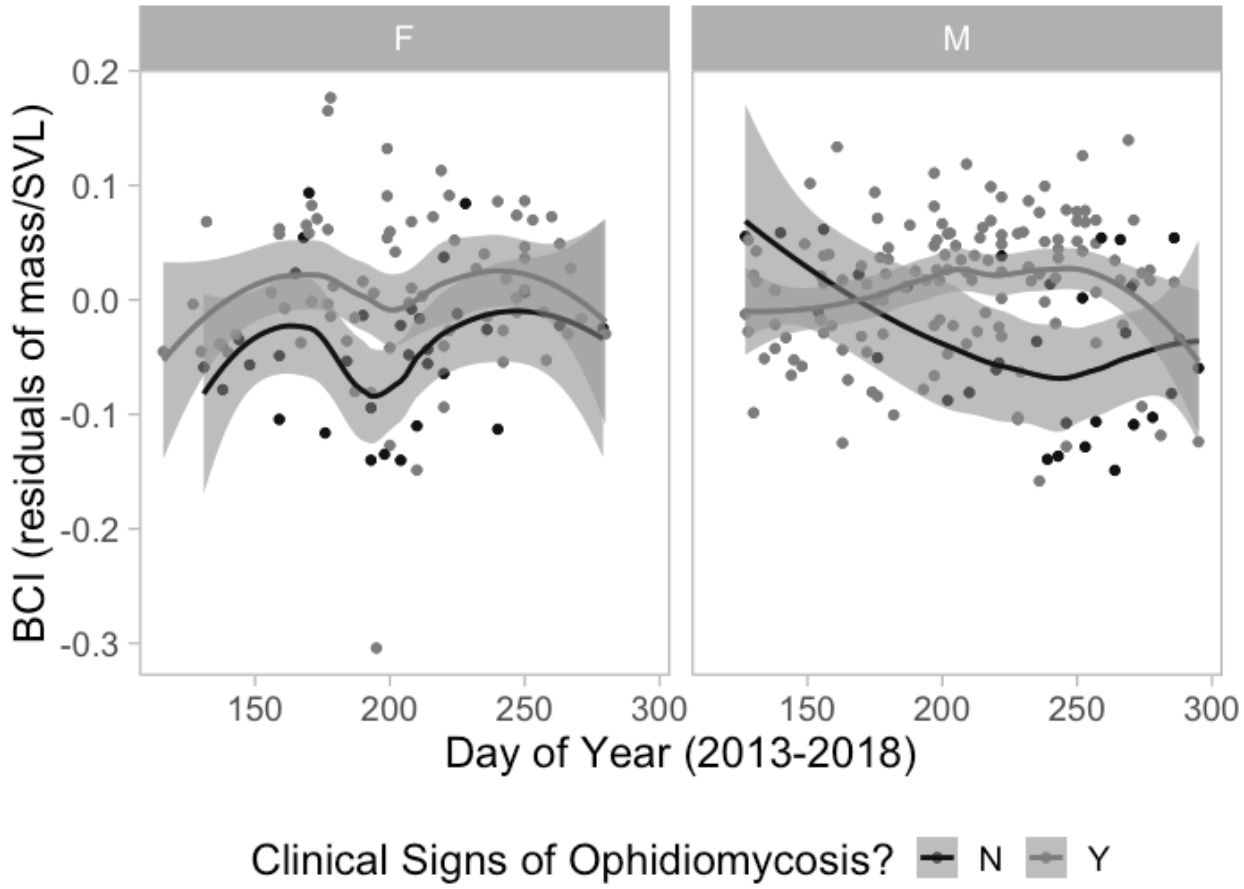

Figure 3.3: 'F' plot illustrates trends of Body Condition Index (BCI) in female tracked *P. vulpinus* throughout the active season (May – October) over 5 years. 'M' plot illustrates trends of BCI in male tracked *P. vulpinus* throughout the active season (May – October) over 5 years. Snakes were split into two categories based on presence of clinical signs of ophidiomycosis at the point of weighing. Year and individual were controlled for as random effects.

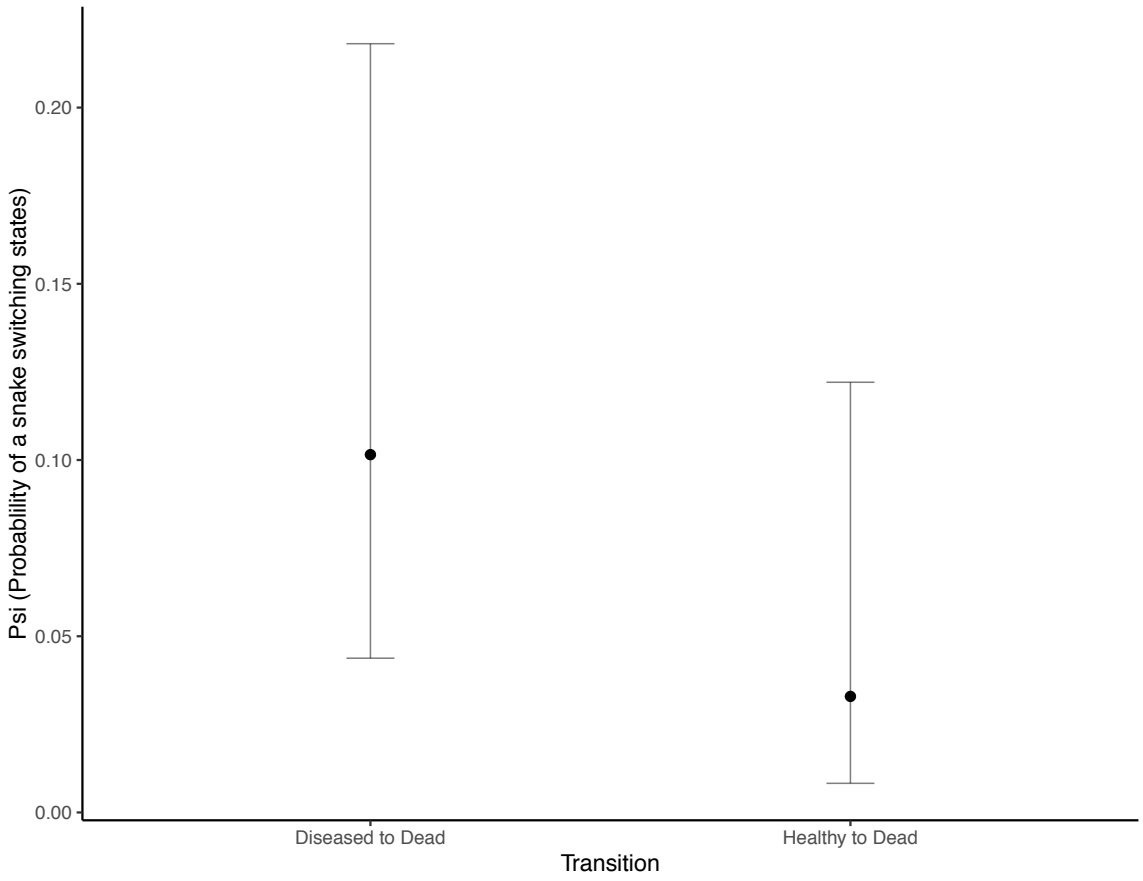

Figure 3.4: Multi-state-multi-fate model plot illustrating the probability (Psi) of a snake dying while having ophidiomycosis (“Diseased to Dead”) compared to dying while otherwise healthy (i.e., without ophidiomycosis clinical signs; “Healthy to Dead”). Error bars represent 95% confidence intervals.

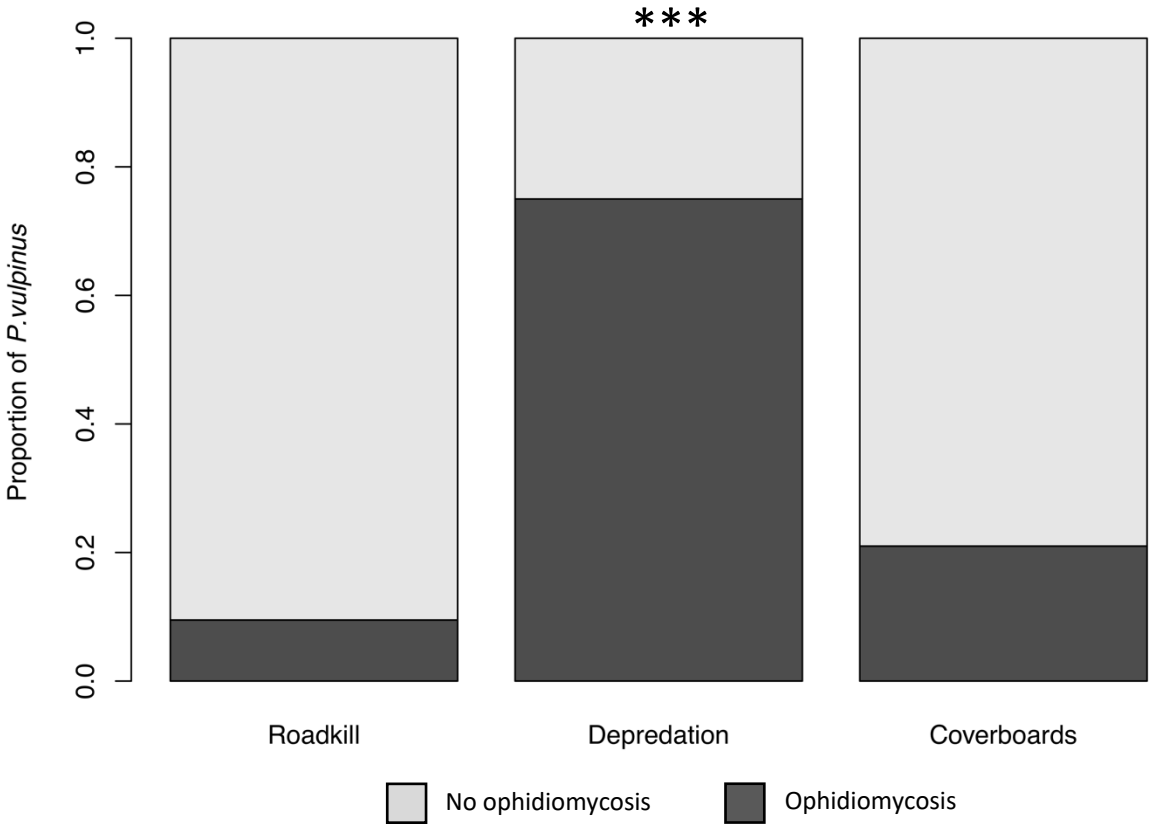

Figure 3.5: Prevalence of ophidiomycosis in road-killed, depredated, and live (coverboard detections) *Pantherophis vulpinus* at Rondeau Provincial Park, Ontario (2017 – 2018) *** indicates $p < 0.001$.

Chapter 4 – Conclusion

Ophidiomycosis is a fungal disease that causes mortality in some snakes but not all. Since being described as a conservation concern (Sutherland et al. 2014), there is no evidence to support that ophidiomycosis, caused by the fungal pathogen *Ophidiomyces ophiodiicola*, is a driver of snake population declines. Unlike populations of amphibians that have undergone population declines during outbreaks of chytridiomycosis (Bradley et al 2002, Skerratt et al 2007, Martel et al 2013), the population of *Pantherophis vulpinus* we studied in RPP did not experience a wide spread mortality event, even though many snakes had ophidiomycosis. This indicates that SFD may not be as large a conservation concern as initially suggested (Sutherland et al. 2014). Referring back to the disease triangle from Chapter 1, I used all three aspects of this model to address ophidiomycosis in Rondeau Provincial Park; host, pathogen, and environment (Wobeser 2006, James et al. 2015).

In Chapter 2, I found that *P. vulpinus*, an at-risk species in Ontario, has the highest prevalence of the pathogen, *O. ophiodiicola*, and clinical signs of ophidiomycosis. The eastern foxsnake is the most susceptible host in the park to ophidiomycosis, among 7 snake species. This did not mean that *P. vulpinus* have no chance for survival once they become diseased. In fact, my results show that *P. vulpinus*, can resolve not only lesions associated with the disease, but the disease-causing pathogen as well, over the active season. In addition, our study addresses the lack of multi-species assessments of ophidiomycosis, providing a wider understanding of species susceptibility. Further, Chapter 3 exemplified that ophidiomycosis does not

negatively affect the fitness of the host, *P. vulpinus*. Snakes with ophidiomycosis were not more likely to die than snakes without the disease. Ophidiomycosis also did not have a significant effect on the movement patterns, body condition, or oviposition of these snakes.

In Chapter 2, I analyzed the seasonal pattern of the pathogen that causes ophidiomycosis, *O. ophiodiicola*. I continued to support its seasonal pattern in relation to overwintering. Spring swabs (i.e., right after emergence from overwintering) consistently yielded higher *O. ophiodiicola* prevalence than any other time during the active season. This has monitoring and applied conservation implications, as swabbing for *O. ophiodiicola* is currently the predominant method for monitoring ophidiomycosis prevalence across North America and Europe (Hileman et al 2017). Depending on what time during the season someone swabs for *O. ophiodiicola,* detection may be either conservative or overinflated. I suggest monitoring throughout the active season for species that overwinter, emphasizing swabbing right after emergence and before returning to overwintering. Much more work needs to be done to understand the relationship between ophidiomycosis and overwintering. This chapter suggested that there may be some evidence of ophidiomycosis being transmitted between snakes during overwintering, exhibited by snakes that enter overwintering hibernacula without ophidiomycosis, only to emerge with the disease. Future work should include the testing of multiple hibernacula environments and emergent snakes for *O. ophiodiicola*.

Lastly, we investigated the environmental aspect of the disease triangle when analyzing movement patterns of our host snakes in Chapter 3. Once snakes were diseased, they did not have larger home ranges than snakes without the disease.

However, snakes that tested positive for *O. ophiodiicola*, meaning that they did not necessarily have clinical signs of the disease, did have larger home ranges. This presents opportunity for future research because we do not know if it is *O. ophiodiicola* causing snakes to change their behaviour and move more or if it is the larger movements that are exposing snakes to *O. ophiodiicola*. Preliminary results showed that marsh habitats may have higher prevalence of *O. ophiodiicola*, further suggesting, as McKenzie et al. (2019) did, a link between aquatic habitats and ophidiomycosis. Further distribution studies we be required to understand the relationship between environment and pathogen/host.

Ophidiomycosis does not represent a current threat to the endangered population of *P. vulpinus* at Rondeau Provincial Park. However, this population should be continued to be monitored for prevalence of this disease, and to ensure that much of the *P. vulpinus* population continues to resolve instances of the disease over the active season. As one of only a few *P. vulpinus* populations left in Canada (Willson & Committee on the Status of Endangered Wildlife in Canada 2008), it is of utmost importance to safeguard the biodiversity these snakes provide, especially in the current context of climate change. Any type of extreme weather event could trigger or contribute to occurrence of disease. As ophidiomycosis continues to be recognized in different snake populations worldwide, more long-term studies are needed, such as this one. We encourage the continued use of swabbing for *O. ophiodiicola* as well as confirming disease with clinical signs and histology. In addition, we recommend more than just one field season of studying a population for ophidiomycosis to determine fitness effects as disease prevalence changes cyclically. These types of longer term studies should be

implemented to understand the impact, if any, ophidiomycosis is having from a conservation perspective, so we can make proper management decisions and allocate resources to the most pressing conservation needs.

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Appendix

Table 1. *Ophidiomyces ophiodiicola* qPCR results from snakes swabbed at Rondeau Provincial Park, ON, Canada. Swabs/biopsies were processed at the Canadian Wildlife Health Cooperative in Guelph, ON, Canada and results were provided thereafter. Species codes are as follows: BRSN- Dekay's Brownsnake (*Storeria dekayi*), EAFO- Eastern Foxsnake (*Pantherophis vulpinus*), GASN- Eastern Gartersnake (*Thamnophis sirtalis sirtalis*), HOSN- Eastern Hognose Snake (*Heterodon platirhinos*), RISN- Eastern Ribbonsnake (*Thamnophis sauritus*), RNSN- Northern Ring-neck Snake (*Diadophis punctatus*), WASN- Northern Watersnake (*Nerodia sipedon sipedon*). *Ophidiomyces ophiodiicola* was considered present on a snake if DNA amplification occurred within 40 cycles (POS; cyclic threshold (Ct)). If amplification did not occur within 40 cycles, the samples were considered negative (NEG) for O. ophiodiicola. If a sample had a Ct value of 40, then it was deemed inconclusive.

LABEL	DATE	SAMPLE TYPE	RESULT (CT VALUE)
GASN9.5.17A	9-May-17	swab	not detected
GASN9.5.17B	9-May-17	swab	not detected
GASN9.5.17C	9-May-17	swab	not detected
GASN9.5.17D	9-May-17	swab	not detected
GASN9.5.17E	9-May-17	swab	not detected
GASN9.5.17F	9-May-17	swab	not detected
GASN9.5.17G	9-May-17	swab	not detected
GASN9.5.17H	9-May-17	swab	not detected
EAFO10.5.17A	10-May-17	swab	inconclusive
EAFO10.5.17A-LES	10-May-17	swab	POS (35.48)
EAFO-LALIB12.5.17 REMAINS/FECES	10-May-17	other	POS (37.37)
GASN11.5.17A	11-May-17	swab	not detected
GASN11.5.17B	11-May-17	swab	not detected
GASN11.5.17B-LES	11-May-17	swab	not detected
GASN11.5.17C	11-May-17	swab	not detected
GASN11.5.17D	11-May-17	swab	not detected
GASN11.5.17E	11-May-17	swab	not detected
GASN11.5.17F	11-May-17	swab	not detected
GASN11.5.17G	11-May-17	swab	not detected
GASN11.5.17H	11-May-17	swab	not detected
GASN11.5.17I	11-May-17	swab	not detected
EAFO12.5.17A	12-May-17	swab	not detected
EAFO12.5.17A-LES	12-May-17	swab	inconclusive
EAFO12.5.17B	12-May-17	swab	not detected
EAFO12.5.17B-LES	12-May-17	swab	POS (33.85)
EAFO12.5.17C	12-May-17	swab	inconclusive
EAFO12.5.17C-LES	12-May-17	swab	inconclusive
EAFO-NONON FROM VET SCALE PULLED FROM EYE 15.5.17	12-May-17	biopsy	POS (21.50)
GASN12.5.17A	12-May-17	swab	not detected
EAFO23.5.17C	17-May-17	swab	not detected
EAFO18.5.17A	18-May-17	swab	POS (37.96)
EAFO18.5.17A-LES	18-May-17	swab	POS (39.5)
EAFO18.5.17B	18-May-17	swab	inconclusive
EAFO18.5.17B-LES	18-May-17	swab	POS (36.2)
EAFO18.5.17C	18-May-17	swab	not detected
EAFO18.5.17C-LES	18-May-17	swab	POS (37.82)
EAFO18.5.17D	18-May-17	swab	not detected
EAFO18.5.17D-LES	18-May-17	swab	not detected
EAFO19.5.17A	19-May-17	swab	not detected

GASN19.5.17A	19-May-17	swab	not detected
EAFO20.5.17A	20-May-17	swab	not detected
EAFO23.5.17A	23-May-17	swab	POS (38.78)
EAFO23.5.17A-LES	23-May-17	swab	POS (33.95)
EAFO23.5.17B	23-May-17	swab	POS (37.23)
EAFO25.5.17A	25-May-17	swab	not detected
EAFO26.5.17A-LES	25-May-17	swab	POS (35.26)
GASN25.5.17A	25-May-17	swab	not detected
GASN25.5.17AA	25-May-17	swab	not detected
GASN25.5.17B	25-May-17	swab	not detected
GASN25.5.17C	25-May-17	swab	not detected
RISN25.5.17A	25-May-17	swab	not detected
EAFO26.5.17B	26-May-17	swab	not detected
GASN30.5.17A	30-May-17	swab	not detected
GASN30.5.17B	30-May-17	swab	not detected
GASN30.5.17C	30-May-17	swab	not detected
GASN30.5.17C-LES	30-May-17	swab	not detected
GASN30.5.17D	30-May-17	swab	not detected
GASN30.5.17E	30-May-17	swab	not detected
GASN31.5.17A	31-May-17	swab	not detected
GASN31.5.17B	31-May-17	swab	not detected
GASN31.5.17C	31-May-17	swab	not detected
GASN31.5.17D	31-May-17	swab	not detected
GASN31.5.17E	31-May-17	swab	not detected
GASN31.5.17F	31-May-17	swab	not detected
GASN5.6.17A	31-May-17	swab	POS (37.17)
EAFO1.6.17A	1-Jun-17	swab	not detected
EAFO1.6.17B	1-Jun-17	swab	not detected
BRSN1.6.17A	1-Jun-17	swab	not detected
BRSN1.6.17AA	1-Jun-17	swab	not detected
BRSN1.6.17AA-LES	1-Jun-17	swab	not detected
EAFO4.6.17A	5-Jun-17	swab	not detected
EAFO5.6.17A	5-Jun-17	swab	not detected
EAFO5.6.17B	5-Jun-17	swab	POS (37.92)
EAFO5.6.17B-LES	5-Jun-17	swab	POS (36.77)
EAFO5.6.17C	5-Jun-17	swab	not detected
EAFO5.6.17C-LES	5-Jun-17	swab	not detected
BRSN5.6.17A	5-Jun-17	swab	not detected
BRSN5.6.17B	5-Jun-17	swab	not detected
EAFO6.6.17A	6-Jun-17	swab	POS (37.39)

EAFO6.6.17A-LES	6-Jun-17	swab	POS (33.16)
EAFO7.6.17A	7-Jun-17	swab	not detected
EAFO7.6.17AA	7-Jun-17	swab	not detected
EAFO7.6.17AA-LES	7-Jun-17	swab	not detected
BRSN7.6.17A	7-Jun-17	swab	not detected
GASN7.6.17A	7-Jun-17	swab	not detected
GASN7.6.17B	7-Jun-17	swab	not detected
GASN7.6.17C	7-Jun-17	swab	not detected
RISN7.6.17A	7-Jun-17	swab	not detected
BRSN9.6.17A	9-Jun-17	swab	not detected
EAFO10.6.17A	10-Jun-17	swab	not detected
EAFO10.6.17B	10-Jun-17	swab	not detected
EAFO12.6.17A	12-Jun-17	swab	not detected
EAFO12.6.17B	12-Jun-17	swab	not detected
EAFO14.6.17A	14-Jun-17	swab	not detected
BRSN14.6.17A	14-Jun-17	swab	not detected
EAFO15.6.17A	15-Jun-17	swab	not detected
EAFO15.6.17B	15-Jun-17	swab	not detected
EAFO15.6.17C	15-Jun-17	swab	not detected
EAFO19.6.17A	19-Jun-17	swab	not detected
EAFO21.6.17AA	21-Jun-17	swab	not detected
EAFO21.6.17BB	21-Jun-17	swab	not detected
EAFO21.6.17CC	21-Jun-17	swab	not detected
EAFO21.6.17CC-LES	21-Jun-17	swab	not detected
BRSN21.6.17AA	21-Jun-17	swab	not detected
GASN21.6.17A	21-Jun-17	swab	not detected
GASN21.6.17AA	21-Jun-17	swab	not detected
GASN21.6.17B	21-Jun-17	swab	not detected
GASN21.6.17BB	21-Jun-17	swab	not detected
GASN21.6.17BB-LES	21-Jun-17	swab	not detected
GASN21.6.17C	21-Jun-17	swab	not detected
GASN21.6.17D	21-Jun-17	swab	not detected
RISN21.6.17AA	21-Jun-17	swab	not detected
RISN21.6.17AA-LES	21-Jun-17	swab	not detected
BIOPSYEAFO22.6.17A	22-Jun-17	swab	POS (25.92)
EAFO22.6.17A	22-Jun-17	swab	not detected
EAFO22.6.17A-LES	22-Jun-17	swab	not detected
EAFO26.6.17A	24-Jun-17	swab	not detected
EAFO27.6.17A	27-Jun-17	swab	POS (32.37)
EAFO27.6.17B	27-Jun-17	swab	POS (37.13)

EAFO27.6.17B-LES	27-Jun-17	swab	not detected
EAFO29.6.17A	29-Jun-17	swab	not detected
EAFO30.6.17A	29-Jun-17	swab	not detected
GASN29.6.17A	29-Jun-17	swab	not detected
EAFO29.6.17AA	29-Jun-17	swab	not detected
EAFO29.6.17A-LES	29-Jun-17	swab	POS (36.13)
GASN30.6.17A	30-Jun-17	swab	not detected
EAFO4.7.17A	4-Jul-17	swab	not detected
EAFO4.7.17A-LES	4-Jul-17	swab	not detected
EAFO5.7.17A	5-Jul-17	swab	not detected
EAFO5.7.17A-LES	5-Jul-17	swab	not detected
EAFO5.7.17B	5-Jul-17	swab	not detected
EAFO5.7.17C	5-Jul-17	swab	not detected
GASN5.7.17A	5-Jul-17	swab	not detected
GASN5.7.17AA	5-Jul-17	swab	not detected
GASN5.7.17B	5-Jul-17	swab	not detected
GASN5.7.17C	5-Jul-17	swab	not detected
RISN5.7.17A	5-Jul-17	swab	not detected
RISN5.7.17AMOUTHFLAKES	5-Jul-17	flake	not detected
EAFO6.7.17D	6-Jul-17	swab	not detected
EAFO6.7.17D-LES	6-Jul-17	swab	not detected
RISN6.7.17A	6-Jul-17	swab	not detected
RISN6.7.17A-LES	6-Jul-17	swab	not detected
EAFO12.7.17A	12-Jul-17	swab	not detected
BRSN12.7.17A	12-Jul-17	swab	not detected
BRSN12.7.17AA	12-Jul-17	swab	not detected
BRSN12.7.17B	12-Jul-17	swab	not detected
BRSN12.7.17BB	12-Jul-17	swab	not detected
GASN12.7.17A	12-Jul-17	swab	not detected
GASN12.7.17BB	12-Jul-17	swab	not detected
GASN12.7.17CC	12-Jul-17	swab	not detected
GASN12.7.17DD	12-Jul-17	swab	not detected
RISN12.7.17A	12-Jul-17	swab	not detected
RISN13.7.17A	13-Jul-17	swab	not detected
EAFO14.7.17A	14-Jul-17	swab	POS (38.99)
EAFO14.7.17A	14-Jul-17	swab	not detected
EAFO18.7.17A	18-Jul-17	swab	POS (38.91)
EAFO18.7.17A-LES	18-Jul-17	swab	not detected
EAFO18.7.17B	18-Jul-17	swab	not detected
EAFO18.7.17BFLAKES	18-Jul-17	flake	not detected

EAFO18.7.17B-LES	18-Jul-17	swab	POS (32.61)
BRSN18.7.17A	18-Jul-17	swab	not detected
BRSN18.7.17A-LES	18-Jul-17	swab	not detected
BRSN19.7.17A	19-Jul-17	swab	not detected
BRSN19.7.17AA	19-Jul-17	swab	not detected
BRSN19.7.17A-LES	19-Jul-17	swab	not detected
GASN19.7.17AA	19-Jul-17	swab	not detected
GASN19.7.17BB	19-Jul-17	swab	not detected
EAFO24.7.17A	24-Jul-17	swab	not detected
BRSN25.7.17A	25-Jul-17	swab	not detected
BRSN25.7.17B	25-Jul-17	swab	not detected
EAFO26.7.17A	26-Jul-17	swab	not detected
EAFO26.7.17B	26-Jul-17	swab	not detected
BRSN26.7.17A	26-Jul-17	swab	not detected
BRSN26.7.17AA	26-Jul-17	swab	not detected
GASN26.7.17A	26-Jul-17	swab	not detected
RISN26.7.17A	26-Jul-17	swab	not detected
BRSN26.7.17B	26-Jul-17	swab	not detected
EAFO31.7.17A	31-Jul-17	swab	not detected
EAFO31.7.17A-LES	31-Jul-17	swab	not detected
EAFO2.8.17B	2-Aug-17	swab	not detected
EAFO2.8.17B-BIOPSY	2-Aug-17	biopsy	POS (31.94)
EAFO2.8.17B-LES	2-Aug-17	swab	not detected
EAFO2.8.17A	2-Aug-17	swab	not detected
EAFO3.8.17A	3-Aug-17	swab	not detected
EAFO8.8.17A	8-Aug-17	swab	POS (26.78)
GASN16.8.17AA	16-Aug-17	swab	not detected
BRSN23.8.17A	23-Aug-17	swab	not detected
BRSN23.8.17B	23-Aug-17	swab	not detected
GASN23.8.17AA	23-Aug-17	swab	not detected
GASN23.8.17BB	23-Aug-17	swab	not detected
GASN23.8.17CC	23-Aug-17	swab	not detected
EAFO26.8.17A	26-Aug-17	swab	not detected
EAFO26.8.17A-LES	26-Aug-17	swab	not detected
EAFO28.8.17A	28-Aug-17	swab	not detected
EAFO28.8.17A-LES	28-Aug-17	swab	not detected
EAFO28.8.17B	28-Aug-17	swab	not detected
EAFO30.8.17A	30-Aug-17	swab	not detected
EAFO30.8.17A-LES	30-Aug-17	swab	not detected
RISN30.8.17A	30-Aug-17	swab	not detected

EAFO4.9.17A	4-Sep-17	swab	POS (34.11)
EAFO4.9.17A-LES	4-Sep-17	swab	POS (36.21)
EAFO4.9.17B	4-Sep-17	swab	not detected
GASN4.9.17A	4-Sep-17	swab	not detected
EAFO6.9.17A	6-Sep-17	swab	not detected
BRSN6.9.17A	6-Sep-17	swab	not detected
BRSN6.9.17B	6-Sep-17	swab	not detected
GASN6.9.17A	6-Sep-17	swab	not detected
GASN6.9.17A-LES	6-Sep-17	swab	not detected
GASN6.9.17B	6-Sep-17	swab	not detected
GASN6.9.17C	6-Sep-17	swab	not detected
GASN6.9.17D	6-Sep-17	swab	not detected
BRSN7.9.17A	7-Sep-17	swab	not detected
GASN7.9.17A	7-Sep-17	swab	not detected
RISN7.9.17A	7-Sep-17	swab	not detected
BRSN8.9.17A	8-Sep-17	swab	not detected
GASN14.9.17B	14-Sep-17	swab	not detected
WASN14.9.17A	14-Sep-17	swab	not detected
BRSN20.9.17A	20-Sep-17	swab	POS (37.13)
EAFO24.8.17A	24-Sep-17	swab	not detected
EAFO24.8.17AA	24-Sep-17	swab	not detected
EAFO25.9.17A	25-Sep-17	swab	not detected
EAFO26.9.17A	26-Sep-17	swab	not detected
BRSN26.9.17A	26-Sep-17	swab	not detected
GASN26.9.17A	26-Sep-17	swab	not detected
GASN26.9.17AA	26-Sep-17	swab	not detected
GASN26.9.17A-LES	26-Sep-17	swab	not detected
GASN26.9.17B	26-Sep-17	swab	not detected
EAFO27.9.17A	27-Sep-17	swab	not detected
EAFO27.9.17B	27-Sep-17	swab	not detected
EAFO27.9.17C	27-Sep-17	swab	not detected
EAFO27.9.17D	27-Sep-17	swab	not detected
BRSN27.9.17A	27-Sep-17	swab	not detected
BRSN27.9.17B	27-Sep-17	swab	not detected
GASN27.9.17A	27-Sep-17	swab	not detected
GASN27.9.17B	27-Sep-17	swab	not detected
RISN3.10.17A	3-Oct-17	swab	not detected
RISN3.10.17B	3-Oct-17	swab	not detected
EAFO4.10.17A	4-Oct-17	swab	not detected
GASN4.10.17A	4-Oct-17	swab	not detected

RISN4.10.17A	4-Oct-17	swab	not detected
RISN4.10.17B	4-Oct-17	swab	not detected
GASN5.10.17A	5-Oct-17	swab	not detected
EAFO6.10.17A	6-Oct-17	swab	not detected
GASN6.10.17A	6-Oct-17	swab	not detected
GASN6.10.17A-LES	6-Oct-17	swab	not detected
GASN6.10.17B	6-Oct-17	swab	not detected
GASN6.10.17C	6-Oct-17	swab	not detected
GASN6.10.17D	6-Oct-17	swab	not detected
GASN7.10.17A	7-Oct-17	swab	not detected
RISN7.10.17A	7-Oct-17	swab	not detected
EAFO8.10.17A	8-Oct-17	swab	not detected
EAFO8.10.17A-LES	8-Oct-17	swab	not detected
EAFO8.10.17B	8-Oct-17	swab	not detected
GASN8.10.17A	8-Oct-17	swab	not detected
RISN8.10.17A	8-Oct-17	swab	not detected
WASN8.10.17A	8-Oct-17	swab	not detected
WASN8.10.17B	8-Oct-17	swab	not detected
GASN9.10.17A	9-Oct-17	swab	not detected
RISN9.10.17A	9-Oct-17	swab	not detected
EAFO10.10.17A	10-Oct-17	swab	not detected
WASN 2.5.18A	2-May-18	swab	negative
WASN 2.5.18A LES	2-May-18	swab	negative
GASN 2.5.18A	2-May-18	swab	negative
RISN 2.5.18A	2-May-18	swab	negative
GASN2.5.18B	2-May-18	swab	negative
GASN2.5.18B LES	2-May-18	swab	negative
GASN2.5.18E	2-May-18	swab	negative
GASN2.5.18F	2-May-18	swab	negative
GASN2.5.18FLES	2-May-18	swab	negative
GASN2.5.18C	2-May-18	swab	negative
GASN2.5.18D	2-May-18	swab	negative
GASN 3.5.18A	3-May-18	swab	negative
GASN 4.5.18A	4-May-18	swab	negative
GASN 4.5.18A LES	4-May-18	swab	negative
BRSN 4.5.18A	4-May-18	swab	negative
BRSN 4.5.18A LES	4-May-18	swab	negative
EAFO 30.5.18A- RITCHIE OLD TRANS GOOP	4-May-18	debris	negative
GASN7.5.18A	7-May-18	swab	negative
FLAKING SCALES 17.5.18- RHAEGAR	7-May-18	flake	POS (28.48)

SK-M 18.5.18A	7-May-18	swab	negative
EAFO7.5.18A-RITCHIE	7-May-18	swab	Inconclusive (40.00)
EAFO7.5.18ALES	7-May-18	swab	POS (32.72)
EAFO 30.5.18A- RITCHIE BIOP	7-May-18	biopsy *	POS (24.27)
GASN8.5.18A	8-May-18	swab	negative
GASN8.5.18B	8-May-18	swab	negative
GASN8.5.18B LES	8-May-18	swab	negative
GASN8.5.18C	8-May-18	swab	negative
HOG8.5.18A	8-May-18	swab	negative
GASN8.5.18BB	8-May-18	swab	negative
EAFO 8.5.18A	8-May-18	swab	negative
EAFO 8.5.18A LES	8-May-18	swab	negative
EAFO 8.5.18B- RHAEGAR	8-May-18	swab	negative
EAFO 8.5.18B- RHAEGAR LES	8-May-18	swab	negative
RNSN8.5.18A	9-May-18	swab	negative
WASN9.5.18A	9-May-18	swab	negative
GASN9.5.18A	9-May-18	swab	negative
GASN9.5.18ALES	9-May-18	swab	negative
EAFO10.5.18A-TIMONE	10-May-18	swab	negative
EAFO10.5.18A- TIMONE LES	11-May-18	swab	negative
EAFO 11.5.18A	11-May-18	swab	negative
EAFO 11.5.18A LES	11-May-18	swab	negative
EAFO 11.5.18B	14-May-18	swab	negative
GASN 14.5.18A	15-May-18	swab	negative
GASN 14.5.18A LES	15-May-18	swab	negative
GASN 15.5.18A	16-May-18	swab	negative
GASN 15.5.18A LES	16-May-18	swab	negative
GASN 16.5.18A	16-May-18	swab	negative
EAFO24.5.18B- RAVIOLI LES	17-May-18	swab	negative
EAFO 7.5.18A- RITCHIE VENTR.	18-May-18	flake	POS (28.59)
BRSN 16.5.18A	21-May-18	swab	negative
BRSN 16.5.18B	23-May-18	swab	negative
GASN 21.5.18A	23-May-18	swab	negative
WASN 23.5.18A	23-May-18	swab	POS (38.47)
EAFO 23.5.18A-RICK	23-May-18	swab	POS (35.07)
EAFO 23.5.18A-RICK LES	23-May-18	swab	POS (35.60)
EAFO 23.5.18D APOSTLE	23-May-18	swab	POS (37.20)
EAFO 23.5.18D APOSTLE LES	23-May-18	swab	POS (30.69)
EAFO 23.5.18D BIOP APOSTLE	23-May-18	biopsy *	POS (20.49)
EAFO 23.5.18B	23-May-18	swab	POS (37.16)

EAFO 23.5.18B LES	23-May-18	swab	POS (31.41)
EAFO 23.5.18B BIOP	23-May-18	biopsy *	POS (19.49)
EAFO 23.5.18C	23-May-18	swab	POS (37.23)
EAFO 23.5.18C LES	23-May-18	swab	POS (33.89)
EAFO 23.5.18C BIOP	23-May-18	biopsy *	POS (24.68)
EAFO 23.5.18E	23-May-18	swab	negative
EAFO 23.5.18E LES	23-May-18	swab	negative
EAFO 23.5.18F	23-May-18	swab	POS (37.13)
EAFO 23.5.15F LES	23-May-18	swab	POS (36.15)
EAFO 23.5.18G	23-May-18	swab	negative
EAFO 23.5.18H	23-May-18	swab	negative
EAFO 23.5.18I TABITHA	23-May-18	swab	negative
EAFO 24.5.18B- RAVIOLI	23-May-18	swab	negative
GASN 23.5.18A	30-May-18	swab	negative
GASN 23.5.18B	30-May-18	swab	POS (36.59)
GASN 31.5.18AAA	31-May-18	swab	negative
GASN 31.5.18AAA LES	31-May-18	swab	negative
GASN 31.5.18A	31-May-18	swab	negative
BRSN 31.5.18A	31-May-18	swab	negative
BRSN 31.5.18BB	31-May-18	swab	negative
RISN 31.5.18AA	31-May-18	swab	negative
RISN 31.5.18A	31-May-18	swab	negative
BRSN 31.5.18B	31-May-18	swab	negative
BRSN 31.5.18C	31-May-18	swab	negative
BRSN 31.5.18AAA	31-May-18	swab	negative
RISN 31.5.18AAA	31-May-18	swab	negative
EAFO 31.5.18CC	31-May-18	swab	negative
EAFO 31.5.18BB	31-May-18	swab	POS (39.18)
EAFO 31.5.18BB LES	31-May-18	swab	POS (34.86)
EAFO 31.5.18AA	31-May-18	swab	Inconclusive (40.0)
EAFO 31.5.18AA LES	31-May-18	swab	POS (32.59)
EAFO 31.5.18BB-FLAKE	31-May-18	flake	POS (25.93)
EAFO 3.6.18A	3-Jun-18	swab	negative
EAFO 3.6.18A LES	3-Jun-18	swab	negative
RISN6.6.18A	6-Jun-18	swab	negative
BRSN6.6.18A	6-Jun-18	swab	negative
BRSN6.6.18B	6-Jun-18	swab	negative
GASN6.6.18A	6-Jun-18	swab	negative
BRSN 6.6.18AA	6-Jun-18	swab	negative
EAFO6.6.18A	6-Jun-18	swab	negative

EAFO6.6.18B	6-Jun-18	swab	negative
BRSN 13.6.18 AAA	13-Jun-18	swab	negative
GASN13.6.18AAA	13-Jun-18	swab	negative
GASN 13.6.18A	13-Jun-18	swab	negative
GASN 13.6.18B	13-Jun-18	swab	negative
BRSN 13.6.18 A	13-Jun-18	swab	negative
EAFO 13.6.18A	13-Jun-18	swab	negative
EAFO13.6.18B	13-Jun-18	swab	negative
GASN 22.6.18 AA	22-Jun-18	swab	negative
GASN 22.6.18 BB	22-Jun-18	swab	negative
EAFO 22.6.18 A	22-Jun-18	swab	negative
EAFO 22.6.18 B	22-Jun-18	swab	negative
EAFO 22.6.18B LES	22-Jun-18	swab	POS (36.56)
EAFO 22.6.18C	22-Jun-18	swab	negative
EAFO 25.6.18A-ALASKA	24-Jun-18	swab	negative
EAFO 25.6.18B- RAVIOLI	25-Jun-18	swab	negative
EAFO 26.6.18A RITCHIE	26-Jun-18	swab	POS (36.08)
EAFO 26.6.18A LES RITCHIE	26-Jun-18	swab	POS (33.14)
EAFO 27.6.18A	27-Jun-18	swab	negative
EAFO 27.6.18B- RHAEGAR	27-Jun-18	swab	negative
EAFO 27.6.18B LES- RHAEGR	27-Jun-18	swab	POS (34.86)
EAFO 28.6.18A -APOSTLE	28-Jun-18	swab	POS (37.13)
BRSN 29.6.18 JA	29-Jun-18	swab	negative
BRSN 29.6.18 JB	29-Jun-18	swab	negative
GASN 29.6.18 A	29-Jun-18	swab	negative
GASN 29.6.18 B	29-Jun-18	swab	negative
GASN 4.7.18 JA	4-Jul-18	swab	negative
GASN 4.7.18AR	4-Jul-18	swab	negative
GASN 4.7.18 JB	4-Jul-18	swab	negative
BRSN 4.7.18 C	4-Jul-18	swab	negative
BRSN 4.7.18 A	4-Jul-18	swab	negative
GASN 4.7.18 A	4-Jul-18	swab	negative
GASN 4.7.18 A LES	4-Jul-18	swab	negative
GASN 4.7.18 B	4-Jul-18	swab	negative
BRSN 4.7.18 B	4-Jul-18	swab	negative
EAFO 7.7.18A	7-Jul-18	swab	negative
BRSN 11.7.18 JA	11-Jul-18	swab	negative
BRSN 11.7.18 LES JA	11-Jul-18	swab	negative
GASN11.7.18 AR	11-Jul-18	swab	negative
GASN11.7.18A	11-Jul-18	swab	negative

GASN 11.7.18 BR	11-Jul-18	swab	negative
GASN11.7.18B	11-Jul-18	swab	negative
GASN 11.7.18 CR	11-Jul-18	swab	negative
GASN 11.7.18 JB	11-Jul-18	swab	negative
BRSN 11.7.18 JC	11-Jul-18	swab	negative
BRSN 11.7.18 LES JC	11-Jul-18	swab	negative
BRSN 11.7.18 JD	11-Jul-18	swab	negative
BRSN 11.07.18 A	11-Jul-18	swab	negative
BRSN 17.7.18 JA	17-Jul-18	swab	negative
GASN 17.7.18AR	17-Jul-18	swab	negative
GASN 17.7.18BR	17-Jul-18	swab	negative
BRSN 17.07.18 A	17-Jul-18	swab	negative
BRSN 25.7.18 JA	25-Jul-18	swab	negative
BRSN 25.7.18 JB	25-Jul-18	swab	negative
GASN 25.7.18 B	25-Jul-18	swab	negative
RISN 25.7.18A DOR	25-Jul-18	swab	negative
GASN 25.7.18A	25-Jul-18	swab	negative
EAFO23.7.18A	25-Jul-18	swab	negative
GASN26.7.18A	26-Jul-18	swab	negative
GASN 29.7.18A	29-Jul-18	swab	negative
GASN 30.7.18A	30-Jul-18	swab	negative
EAFO 17.7.18A- ARIANA GRANDE	30-Jul-18	swab	negative
GASN 31.7.18 BR	31-Jul-18	swab	negative
GASN 31.7.18RD	31-Jul-18	swab	negative
GASN 31.7.18CR	31-Jul-18	swab	negative
BRSN 31.7.18 JA	31-Jul-18	swab	negative
GASN 31.7.18 AR	31-Jul-18	swab	negative
EAFO 1.8.18 ANDROMEDA	1-Aug-18	swab	negative
EAFO 1.8.18 ANDROMEDA LES	1-Aug-18	swab	negative
EAFO 1.8.18A RHAEGAR	1-Aug-18	swab	negative
EAFO 2.8.18A RAVIOLI	2-Aug-18	swab	negative
EAFO 2.8.18C TIMONE	2-Aug-18	swab	negative
EAFO 2.8.18B ARIANA GRANDE	2-Aug-18	swab	negative
GASN 9.8.18 RA	9-Aug-18	swab	Inconclusive (40.0)
GASN 9.8.18 RA LES	9-Aug-18	swab	negative
BRSN 9.8.18 JA	9-Aug-18	swab	negative
GASN 9.8.18JA	9-Aug-18	swab	negative
GASN 10.8.18A	10-Aug-18	swab	negative
EAFO 10.8.18 RITCHIE	10-Aug-18	swab	negative
EAFO 10.8.18 RITCHIE LES	10-Aug-18	swab	negative

EAFO 13.8.18A ALASKA	13-Aug-18	swab	negative
GASN15.08.18A	15-Aug-18	swab	negative
GASN15.08.18B	15-Aug-18	swab	negative
GASN15.08.18JA	15-Aug-18	swab	negative
EAFO 15.8.18 A	15-Aug-18	swab	POS (37.04)
EAFO 15.8.18A TISSUE	15-Aug-18	liquid*	POS (24.73)
GASN21.08.18JA	21-Aug-18	swab	not detected
GASN 22.8.18 JA	22-Aug-18	swab	not detected
GASN 22.8.18 JB	22-Aug-18	swab	not detected
GASN 22.8.18 JC	22-Aug-18	swab	not detected
GASN22.08.18RA	22-Aug-18	swab	not detected
GASN22.08.18RB	22-Aug-18	swab	not detected
GASN22.08.18RC	22-Aug-18	swab	not detected
GASN22.08.18RD	22-Aug-18	swab	not detected
GASN 22.8.18 RE	22-Aug-18	swab	not detected
GASN 23.8.18RA	23-Aug-18	swab	not detected
GASN 23.8.18RB	23-Aug-18	swab	not detected
GASN23.08.18JA	23-Aug-18	swab	not detected
GASN 27.8.18A	27-Aug-18	swab	not detected
GASN 27.8.18B	27-Aug-18	swab	not detected
GASN 27.8.18C	27-Aug-18	swab	not detected
GASN 27.8.18D	27-Aug-18	swab	not detected
GASN 27.8.18E	27-Aug-18	swab	not detected
BRSN 27.8.18A	27-Aug-18	swab	not detected
EAFO 28.8.18A ARIANA GRANDE	28-Aug-18	swab	not detected
EAFO 29.8.18A TIMONE	29-Aug-18	swab	not detected
EAFO 29.8.18A LES TIMONE	29-Aug-18	swab	not detected
EAFO 29.8.18B RAVIOLI	29-Aug-18	swab	not detected
GASN 29.8.18 A	29-Aug-18	swab	not detected
GASN 29.8.18A LES	29-Aug-18	swab	not detected
EAFO 30.8.18A RHAEGAR	30-Aug-18	swab	not detected
EAFO 30.8.18A LES RHAEGAR	30-Aug-18	swab	not detected
EAFO 7.9.18A ALASKA	7-Sep-18	swab	not detected
EAFO 7.9.18B ANDROMEDA	7-Sep-18	swab	not detected
EAFO 7.9.18B ANDROMEDA LES	7-Sep-18	swab	not detected
EAFO 9.9.18A	9-Sep-18	swab	not detected
GASN 18.9.18A	18-Sep-18	swab	not detected
GASN 18.9.18B	18-Sep-18	swab	not detected
EAFO 26.9.18A	26-Sep-18	swab	not detected
EAFO 26.9.18A LES	26-Sep-18	swab	POS (37.63)

BRSN 26.9.18A	26-Sep-18	swab	not detected
EAFO HIBERNACULUM 7.5.18	31-May-18	swab	negative
EAFO HIBERNACULUM MARSH TRAIL 4.5.18	31-May-18	swab	negative
EAFO HIBERNACULUM SOUTH PT TR 7.5.18	31-May-18	swab	negative