Assessing the presence of *Ophidiomyces ophiodiicola* in New Brunswick and Nova Scotia

BY

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Abstract

Wildlife disease has been an increasing concern for ecologists as faunal populations continue to decline globally. Snake Fungal Disease (SFD), which is caused by *Ophidiomyces ophiodiicola*, was discovered in the mid 2000s in North America after reports of fatal skin infections in wild snakes. Since the description of this disease, monitoring has occurred to understand its distribution, but no studies have been completed on this fungus outside of the Great Lakes Region of Ontario and Québec within Canada. Thus, this research aimed to test for the presence of *O. ophiodiicola* in two previously unstudied provinces: New Brunswick and Nova Scotia. During this work, I sampled free-ranging snakes across their 2022 active season, and dead snakes collected between 2011 to 2022 and stored at museums and research institutions. In total, 158 samples from 6 different species of snakes and snake skins were sampled from the Maritimes and Maine, USA. Samples were analyzed at the Animal Health Laboratory using a standardized quantitative polymerase chain reaction (qPCR) assay and no *O. ophiodiicola* DNA was detected. Thus, we did not find any evidence that SFD is present in New Brunswick or Nova Scotia. Yet, I did want to investigate what could influence snake susceptibility to infection to inform management if this disease arrived. Epidermis damage may be a proxy of snake susceptibility because it could leave snakes vulnerable to infection. I found that epidermis damage decreased in free-ranging snakes throughout their active season, and additionally that snake body condition did not differ between individuals with and without epidermis damage. This suggests that snakes may be more at-risk of SFD infection at the start of their active season. Also, snakes with skin damage prior to SFD infection may not be more at-risk to this disease, if we can consider body condition a reliable metric of health. Overall, my research shows that SFD has not yet reached the eastern coast of Canada. My research highlights that rigorous monitoring is required throughout Canada to determine the current occurrences of *O. ophiodiicol*a because its presence may vary greatly by region. Additional information regarding the presence/absence of this disease can also help increase our understanding of the biology of *O. ophiodiicola* and the degree of threat it may pose to North American snake biodiversity.

Chapter 1: Introduction

Wildlife disease

Snake populations around the world are declining. It is currently estimated that 12% of snake species are at risk of extinction, based on a study that analyzed 17% (555/3346) of the world's snakes (Böhm et al. 2013). However, the lack of data on fossorial and cryptic snakes makes assessment difficult and suggests that the estimate of imperiled snakes is much lower than reality (Böhm et al. 2013). There are many current threats to snake population persistence (Reading et al. 2010). Agriculture and urban development transform the land, which can destroy or degrade the habitats of snakes that are specialists (Böhm et al. 2013). Semi- and fully aquatic snakes are also pressured from harvesting for food and from pollution (Böhm et al. 2013). Invasive species, such as the Cane Toad (*Rhinella marina*), are also a danger to terrestrial species (Phillips et al. 2003). These threats are magnified by the fact that snakes are often understudied, and a lack of empirical data limits our ability to monitor and conserve them properly. This lack of study can also leave new threats undiscovered, like novel pathogens that snakes could be encountering in their changing environments (Bonnet and Lourdais 2002). This danger of pathogens has recently been realized within North American snakes that now face challenges from a potentially novel disease. It was the discovery of fatal fungal skin infections in 2006 in wild snakes of the eastern USA that alerted scientists and conservation biologists to *Ophidiomyces ophiodiicola* (Clark et al. 2011; Lorch et al. 2016). Since then, our understanding of this fungus and the disease it causes, snake fungal disease (SFD), has greatly improved, yet there are still significant gaps in knowledge.

What is Ophidiomyces ophiodiicola

Ophidiomyces ophiodiicola is a keratinophillic fungus that can be found on the scaly skin of captive and wild snakes in North America (Allender et al. 2020) and Europe (Godwin 2021; Franklinos et al. 2017). It has also been found in the skin lesions of captive snakes in Australia and Japan (Takami et al. 2020; Sigler et al. 2013). *Ophdiomyces ophiodiocola* causes SFD and if this fungus comes in contact with a snake's dermal surface it may lead to an infection. Snakes could encounter this fungus via several routes, the most likely of which being when multiple snakes gather in a communal hibernaculum (Lorch et al. 2016). In this case, a host snake (i.e., with the fungus on its scales or infected with SFD) could spread *O. ophiodiicola* onto other snakes it encounters or through the soil it moves over. Soil has the capacity to be a reservoir of this fungus, especially in hibernacula, where *O. ophiodiicola* has less microbial competition and easy exposure to hosts (Campbell et al. 2021). This can result in many snakes emerging from hibernacula in the spring with mild *O. ophiodiicola* infections (Davy et al. 2021). Snakes can also encounter *O. ophiodiicola* by moving over contaminated snake sheds or other objects that have the fungus present on them. This risk increases when snakes live in environments used by humans (Campbell 2021; Pasmans et al. 2017). *Ophidiomyces ophiodiicola* can also spread by infected snakes being handled by humans that interact with other non-infected wild and captive snakes if proper sanitization protocols are not observed. After snakes encounter *O. ophiodiicola,* this fungus has the potential to cause a fatal infection in the individual snake. However, many factors must align for a spore on the snake to become a potentially lethal SFD infection, as *O. ophiodiicola* does not infect a snake as soon as it lands on the snake's scales.

An SFD infection can occur by the fungus bypassing the outer, scaly layer of skin through epidermal damage acquired by snakes as they move through their environment (Lorch et al. 2016). In addition, entrance through other vulnerable areas, such as the cloaca and nostrils, is possible (McCoy 2017). Once the infection reaches the superficial epidermis, it can easily spread into the mid-epidermis where this infection is commonly found (Lorch et al. 2015). If the infection reaches the muscle tissue, it can cause dermal and intramuscular inflammation which has been observed in infected snakes (Lorch et al. 2015). Observation of lesions in the lungs and esophagus of infected snakes (Lorch et al. 2015) could also imply that this fungus infects epithelial tissue. Once the infection has reached one of these tissues, it can result in host mortality (Lorch et al. 2016). Many treatments are available, although not all of them have been proven to be effective (Tetzlaff et al. 2015). These include triazole, which requires more refinement (Lindemann et al. 2017), and a mixed use of ciprofloxacin with itraconazole, which has been shown to help snakes recover from infection with lesions when administered to colubrids orally (Takami 2020). It is, however, important to note that there is a wide variety of different signs of SFD infections and are not limited to the ones mentioned above.

Biological factors like the health of the snake can impact the symptomatic expression of SFD with healthier snakes potentially having less symptomatic expression (Lorch et al. 2016)

Clinical signs of SFD, such as the formation of crusty scales, ulcers, or lesions on a snake's skin, can be due to necrosis at the site of infection and will often enlarge as the disease progresses. Though these crusty scales, ulcers, and lesions can form anywhere on the snake's body, the most observed locations are their ventral scales, cloaca, and face. Swelling can also occur due to local immune responses paired with ulceration in parts of the body where necrotic lesions have broken off (McCoy 2017). Premature and frequent ecdysis, as well as weight loss, are other signs often associated with SFD infection (McCoy 2017; Godwin 2021), with more frequent ecdysis observed in captive infected snakes (Lorch et al. 2015). This frequent ecdysis could be due to ecdysis giving snakes respite from mild infection because it can remove contaminated scale surfaces and crusty, injured scales (Lorch et al. 2016). In addition to morphological signs, there are also behavioral signs of an SFD infection. For example, early seasonal basking of severely infected snakes has been observed in wild populations (Godwin 2021). Captive infected populations of snakes are observed to spend more time in the open parts of their enclosures compared to non-infected snakes (Lorch et al. 2015). There are even effects of infection on reproductive success with SFD causing a decrease in sex hormones in both male and female snakes (Lind et al. 2019). Although it may seem like the signs of SFD are always present, infected individuals can also be asymptomatic for most of if not the entire period of infection (Bohuski et al. 2015). Therefore, the signs of SFD can vary depending on the entry point of the fungus and there is great variation in the consequences it has for snake behaviour and survival.

Eastern Ribbonsnakes (*Thamnophis sauritus*) have been tested in lab conditions to see how individuals differ in their signs and responses to SFD infection. With their first infection of SFD, Eastern Ribbonsnakes exposed themselves to higher temperatures while thermoregulating (Godwin 2021). Yet, snakes that were secondarily infected with SFD did not exhibit this behavioural response. This behaviour may be due to temperature being negatively correlated with the severity of SFD infection, with snakes infected in cooler temperatures retaining relatively good body condition due to these temperatures potentially limiting the progression of infection (McCoy 2017). This shows the potential importance of learning from prior infections to improve survival in free-ranging snakes (Godwin 2021). This behavior, however, has only been observed in the lab and it cannot be assumed that wild populations has this same learning behavior.

Previous research has shown that SFD has the capacity to have both individual and potential population-level effects on snakes in conjunction with other threats, like habitat loss. Research has shown that this disease can be deadly, with death rates averaging about 9.8% in wild snakes and 66.7% in captive snakes (Davy et al. 2021). In addition, SFD can be disastrous to snakes facing multiple threats as seen with rattlesnake populations in the USA, including Timber Rattlesnakes (*Crotalus horridus*) (Clark et al. 2011) and Eastern Massasaugas (*Sistrurus catenatus*) (Allender et al. 2016). Unfortunately, even small increases in mortality from SFD can be hazardous for snakes with spatially limited populations, like the Nova Scotian population of the Eastern Ribbonsnake, which only occurs in a small, southern part of the province and whose preference for moist, aquatic environments could lead to greater exposure to *O. ophiodiicola* (Parks Canada Agency 2012; Lorch et al. 2016). In addition, the existence of asymptomatic cases of SFD poses a risk of infection if escaped or released pets encounter wild populations of snakes (Lorch et al. 2016). SFD has the potential to be highly problematic for particular snake populations, as well local ecosystems, as snakes often play the role of keystone predators. This wildlife disease could negatively affect the balance of natural ecosystems and food webs wherever it is found.

Currently SFD occurs in free-ranging snake populations throughout continental North America and Puerto Rico (Allender et al. 2020). It is present in wild snake populations in 35% (n = 19/55) of USA states/territories. Louisiana documented its first case of SFD in 2015 (Glorioso 2016) and it was newly discovered in Idaho, Oklahoma, and Puerto Rico in 2018 (Allender et al. 2018). Occurrences of this disease are inconsistent across the southern and western United States; it was described in California in 2019 (Haynes et al. 2021) but other studies have failed to find infected snakes in the state (Allende et al. 2020). Recent reports have also detected SFD in the lower Rio Grande River in Texas (Zavala 2020), which is a state that had no positive tests for this disease in previous studies (Allender et al. 2020). Thus, there is the potential that SFD may be present in the portion of Mexico that borders the Rio Grande River. It is also unknown if this fungus is in Latin America, as it does not appear that any formal studies or monitoring programs for SFD have occurred in Central America.

Closer to home, current research shows *O. ophiodiicola* is prevalent within the Great Lakes region of the USA and Canada; specifically, this fungus has been found in an area spanning Detroit, NJ, USA east to Kingston, Ontario, Canada and northward to Sudbury, ON, Canada. In this area, 11.5% of snakes sampled across ~136,000 km^2 tested positive for *O. ophiodiicola* (Davy et al. 2021). The first confirmed case of SFD in Canada was from Ontario in 2015 and occurred in an adult female Eastern Foxsnake (*Pantherophis vulpinus*), but sampling of museum specimens has backdated its occurrence to 1986 (Davy et al. 2021). Due to its widespread distribution and duration of occurrences, it was hypothesized to be an endemic disease by Davy et al. (2021). Yet, outside of this region in Canada, including within Atlantic Canada, the occurrence of this fungus is largely unknown (Davy et al. 2021). Nearby in the United States, its presence extends up the eastern seaboard to New England, where snake populations could theoretically spread *O. ophiodiicola* to neighboring Canadian provinces (Allender et al. 2020). *Ophidiomyces ophiodiicola* also has the potential to enter Canada through Maine, because monitoring for this fungus has been limited to military installations within the state (no occurrences detected) so many snake populations remain untested (Allender et al. 2020). With recent reports of *O. ophiodiicola* being detected in nearby provinces and countries, it raises the question of whether this fungus is in Atlantic Canada, and, therefore, if it poses a threat to the understudied snakes of this region.

Research aims

I aimed to address this knowledge gap by testing for *O. ophiodiicola* and SFD in free-ranging populations of five snake species that occur in New Brunswick (NB) and Nova Scotia (NS). As *O. ophiodiicola* has been detected nearby in the United States and has been present in Canada (since at least 1986), I expected that it would also occur in both provinces I sampled. My original goal was to test for *O.ophiodiicola* and then send any dead snakes that tested positive for *O. ophiodiicola* for histopathology to diagnose *ophydiomycosis* (aka. SFD) using criteria currently applied by the Canadian Wildlife Health Cooperative (Davy et al. 2021). I had the secondary aim to assess whether SFD has a negative effect on snakes post-infection by comparing infected snake body condition to uninfected snakes to assess whether there are negative effects of this disease. However, I did not detect *O. ophiodiicola* in any of my samples. I therefore had to change my secondary aim after I had collected my data to focus on looking at the potential susceptibility of our native snakes to SFD if it were to arrive here in the future by using biometric data collected during the field season. So, my revised my research questions were: (1) does epidermis damage impact snake body condition, and (2) is there seasonality of epidermis damage in wild snake populations? It is important to understand the impact of epidermis damage and what affects its presence because epidermis damage increases the vulnerability of a snake to SFD infection. Overall, my research will determine if *O. ophiodiicola* and SFD is present in free-ranging snake populations in NB and NS, examine whether epidermis damage could put these snakes at risk of contracting SFD, and inform our epidemiological understanding of this wildlife disease.

Chapter 2: Methods

Study Species

There are four snake species that occur in NB. These are the Northern Ring-necked Snake (*Diadophis punctatus edwardsi*), Smooth Greensnake (*Opheodrys vernalis*), Red-bellied Snake (*Storeria occipitomaculata*), and Maritime Gartersnake (*Thamnophis sirtalis pallidulus*). Nova Scotia has the same species of snakes as NB with the addition of the Eastern Ribbonsnake (*Thamnophis saurita*) (Figure 1).

Figure 1. The snake species of NB and NS. On the upper left is an Eastern Ribbonsnake; on the bottom left is a Smooth Greensnake; in the middle of the figure is a Maritime Gartersnake; on the top right is a Red-bellied Snake; and on the bottom right is a Northern Ring-necked Snake. All the snakes present in NB and NS could potentially be impacted and infected by SFD.

Ophidiomyces ophiodiicola has been detected on 30 species of snakes across 6 different families (Lorch et al. 2016), but the risk of SFD infection has not been thoroughly assessed for all species in NB and NS. However, Eastern Ribbonsnakes have been used to study the behavior of snakes that have been infected by SFD, which indicates that the NS population of this species could be infected by this fungus if it is present in the landscape (Lorch et al. 2016). Additionally, *T. sirtalis* and Rough Greensnakes (*Opheodrys aestivus*), the closest living relative of the Smooth Greensnake, have been previously documented with SFD infections (Davy et al. 2021). However, little is also known about the populations of our native snakes in both provinces. In fact, the majority of these species' conservation status is unassessed: Red-bellied Snakes and Common Gartersnakes are a low priority candidates for assessment and Northern Ring-necked Snakes and Smooth Greensnakes are mid-priority (COSEWIC 2023). One species that has been assessed is the Eastern Ribbonsnake, which is listed as threatened (COSEWIC 2012).

Fieldwork Protocols

From May through October 2022, I surveyed for snakes within NB and NS in suitable habitat. During each survey, weather conditions like sun, rain, or clouds, were recorded, as well as the duration of the survey and the air temperature (°C) at the beginning and end of the survey. This data were used to inform our survey approach and maximize capture rates. During a survey, I and at least one other researcher would walk through a site looking for snakes within suitable habitat (Figure 2) and under possible shelter objects (e.g., rocks, logs, litter) (Boback 2003). In total, I found snakes in 60% (n = 9/15) sites in NS, and 58% (n = 18/31) sites in NB (Table 1). These sites sizes varied from the size of a residential lot to long logging roads and parks that stretched for tens of kilometers. I was able to survey a variety of habitats, including semi-aquatic, terrestrial and human-altered habitats across both provinces (Figure 2).

Table 1. Study sites wherein snakes were captured from May to October 2022. These are the study sites where I found snakes in NB (n = 18) and NS (n = 9) to test for the presence of *O. ophiodiicola*. I provide a general site name and its location data (censored for Eastern Ribbonsnakes, a sensitive species), and a description of its habitat.

Site Name	Latitude, Longitude	Habitat Description
(A) Nova Scotia		
Logging Road, Jordan Falls	43.8954, -65.2360	Road bisecting Acadian forest, clear cuts, and freshwater creeks
Sawmill, Lower Jordan Bay	43.6853, -65.2442	Active sawmill surrounded by a paved road, Acadian forest, bogs, and naturalized fields
Baseball field, Lower Jordan Bay	43.68411, -65.24394	Baseball field with long grass, paved road, and a parking lot
Private Land, Lower Jordan Bay	43.6841, -65.2439	Multiple buildings, gardens, shrublands, fields, wetlands, and a large pond
Riverview	45.7946, -63.7840	Acadian forest with creeks, wetlands, and shrubland
Point Pleasant Park, Halifax	44.6261, -63.5656	High human traffic park with hardwood forest
Caledonia	44.37785, -65.00756	Paved road surrounded by Acadian forest and lakes
RS Site 1	---	Marsh and lakes surrounded by Acadian forest
RS Site 2	---	Marsh and lakes surrounded by Acadian forest
(B) New Brunswick		
Millcreek Nature Park, Riverview	46.0557, -64.7694	Park with a large reservoir, creeks, Acadian forest, naturalized fields, gravel trails, and lawns
Riverview	46.0345, -64.8108	ATV trail through Acadian forest, fens and bogs, garbage dumping areas, and shrubland
Rockwood Park, Saint John	45.3185, -66.0670	Park with lakes, hiking trails through Acadian forest, buildings, and rocky escarpment
Fredericton	45.8505, -66.7318	Residential land with gardens, buildings, forest, and natural pools
Railway line in Aulac	45.8790, -64.2881	Railway line surrounded by agricultural fields, salt marsh, naturalized fields, rockpiles
Quarry in Aulac	45.8760, -64.2877	A water-filled quarry surrounded by young forest, shrubland, and dumping sites for garbage
Residential land in Aulac	45.8778, -64.2881	Buildings surrounded by forest, garbage piles, and gravel roads
Sackville Water Treatment Plant	45.9394, -64.4092	Man-made and natural ponds surrounded by Acadian forest, fields, rock walls, a private road
Tantramar Hiking Trails	45.9347, -64.4149	Hiking trails in hardwood forest
Sackville	45.8928, -64.3498	Residential buildings with gardens and forest
Quarry in Sackville	45.89877, -64.37951	A water-filled quarry surrounded by young forest and shrubland
Mount Allison Farm, Sackville	45.9036, -64.3845	Buildings, wetlands, naturalized fields, and forest
Atlantic Wildlife Institute, Cookville	46.0195, -64.3116	Buildings, ponds, stream, lawns, and forests
Memramcook	45.969, -64.43481	Dirt road near highway bisecting young forest and clear cuts
Synton	46.0111, -64.9592	Land with a river, agricultural hay fields, farmhouses, and Acadian forests
Residential land in Aulac	45.8810, -64.2948	Dirt road with dumped garbage and surrounded by young forest and naturalized fields
Mountview Road, Sackville	45.94848, -64.37407	Asphalt road
Walker Road, Sackville	45.93475, -64.39783	Asphalt road

Figure 2. Habitats surveyed for snakes during 2022 field season. This is a representation of different habitats surveyed: an Acadian Forest at the Tantramar Hiking Trails, Sackville, NB (top left); a marsh at Riverview, NB (top middle); scrubland from the Sawmill, Lower Jordan Bay (top right); a field with railway tracks from Aulac, NB (bottom left); part of a homestead/farm Lower Jordan Bay, NS (bottom middle); and a lake at Rockwood Park (bottom right).

If any snake were encountered, including non-native escaped pets (although none were found), then researchers would capture the snake by hand while wearing nitrile gloves. I then swabbed the snake's skin with a sterile cotton swab to sample for *O. ophiodiicola*. This was the first step in processing to maximise potential sample collection, because instruments and handling could unintentionally remove some of this fungus. I would swab the snake's skin by running a sterile cotton swab (Puritan®, 25-8062PD) along the ventral and dorsal sides twice. I repeated this with two different cotton swabs to collect duplicate samples. These 'body' swabs were then placed in separate vials containing 70% ethanol that were kept in a cooler with ice in the field until they could be placed and stored in a -20ºC freezer in the lab (typically between 2-14 h after fieldwork). I also visually checked for lesions or crusty scales, which are signs of *ophiodomycosis* (SFD) (Davy et al. 2021; Lorch et al. 2015). If any lesions were observed, two additional cotton swabs were used to specifically swab these lesions 15-20 times per swab. These were then stored the same way as the ‘body’ swabs above. Any signs of SFD observed were also photographed.

After swabbing live snakes, I identified the snake species and biometric measurements were taken, including age, sex, mass (g), snout-vent-length (SVL; mm), and total length (mm). A snake's age (adult, juvenile, or neonate) was determined by referring to previous research about the SVL of snakes at different life-stages from NS (Gilhen 1984). Any snakes classified as a juvenile or neonate were not probed to determine biological sex. I probed snakes using cloacal probes and water-based lubricant to determine the presence (or absence) of hemipenes (Jordan and Rodda 1994; Weatherhead et al. 1995). The mass of the snake was obtained by putting the snake in a pre-weighed plastic bag, then using a 300 g spring scale (Pesola, Medio-Line 40300). The snake's SVL and total length were measured by gently straightening a snake along a piece of cord and marking with a whiteboard marker the location of its cloaca (SVL) and the tip of its tail (total length) on the cord. I then placed the cord along a measuring tape to measure each length metric in mm) from the tip of cord where the snakes snout was to each of the markings. I also checked live snakes for signs of epidermis damage, which was classified as any scrapes cuts or gashes to the epidermis, eyes, mouth, tail, or cloaca of the snake. Lastly, I recorded the location of the capture using a GPS (Garmin, eTREX 32x), along with its accuracy in meters. During data processing, we took measures to minimize disease spread (Canadian Herpetofauna Health Working Group 2017) and prevent contamination between samples. Each snake was sampled with a pair of researchers (one as the handler and the other as the recorder), each snake was handled with a new set of nitrile gloves, and all equipment that touched a snake was disinfected with bleach wipes or 20% bleach solution (Canadian Herpetofauna Health Working Group 2017).

In addition to sampling live snakes, we also collected all snake skins that were found in the wild, as well as deceased snakes. Any dead snakes that were found or donated were swabbed using the same protocol as above, placed in a sterile plastic bag, and stored in a freezer. I was also able to sample dead snakes within the collections of the New Brunswick Museum and the Mersey Tobeatic Research Institute (NS). The samples from the Mersey Tobeatic Research were collected between June 2021 to October 2022 and were from NS south of Truro. The New

Brunswick Museum samples were collected between April 2010 and August 2022, with all samples being taken throughout NB and in Moosehorn National Wildlife Refuge in Maine where a Common Watersnake and Maritime Gartersnake where sampled. There were 16 specimens that were sampled with unknown capture dates from these institutions.

At the end of the field season, 16 snake skins and 159 body and lesion swabs were sent to the Animal Health Laboratory (AHL) at the University of Guelph to be tested for the presence of *O. ophiodiocola*. At AHL a qPCR assay was completed for each swab/skin to test for the presence of *O. ophiodiocola,* following the protocol described by Allender et al. (2015). Other studies in areas where SFD is known to be present have used the same assay and laboratory for their analyses (McKenzie et al. 2020a; McKenzie et al. 2020b; Davy et al. 2021; Dillon et al. 2022), which shows that this AHL laboratory assay is suitable for detecting *O. ophiodiicola* when fungal DNA loads are high enough. To determine if a sample has a positive test, it must produce the threshold of 10 copies/uL of *O. ophiodiicola* DNA during the qPCR analysis. AHL determined base detection range is sufficient to detect *O. ophiodiicola* using sensitivity analysis, and it is the same detection range that was used in Davy et. al. (2021) that detected *O. ophiodiicola* across Ontario (Davy et al. 2021). The lab has also assessed their assay prior to our work using a real-time qPCR assay with a panel of 22 different organisms, including fungus, bacterial pathogens, and DNA fragments specific for *Batrachochytrium dendrobatidis* and *B. salamandrivorans* (both chytridiomycosis agents) and where in positive tests only occurred with the detect *O. ophiodiicola*. Thus, this is a reliable test and detection range for determining the presence of this fungus.

I conducted this research under authorization from The Department of Natural Resources and Energy Development, the Government of New Brunswick (Scientific Permit No. SP22--001), and Natural Resources and Renewables, Wildlife Division, Government of Nova Scotia. I also sampled with permission at Nature Conservancy of Canada reserves. In addition, my protocols were approved by the Mount Allison University Animal Care Committee (ACC # 103184).

Mapping and Statistical Analysis

I visualised my data and performed statistical analyses using R software version 4.2.2 (R Core Team 2022). I mapped locations where samples were collected using the WGS84 reference frame, units in longitude and latitude, and using the '*world2'* basemap from the R package *'ggmap'* (Kahle and Wickam 2013). I also used the following R packages to create my maps and visualizations: '*rgdal*' (Bivand et al. 2023), *'dplyr'* (Wickham et al. 2023),'*terra* '(Hijmans 2023), '*maps'* (Becker et al. 2022),' *mapdata*' (Becker et al. 2022) and '*ggplot2*' (Wickham 2016).

I then analysed factors that may impact occurrence of snake epidermis damage, as well as whether epidermis damage impacted snake body condition. To analyse whether epidermis damage impacted snake body condition, I had to modify my dataset by removing all dead snakes, snake skins, and snakes without a determinable sex or age, resulting in a sample size of 79 individuals. This is due to dead snakes not being checked for epidermis damage during this study, as well as individuals with no determinable sex and ages not being able to be used in this analysis due to the inclusion of life stage within my model. For all statistical tests, α was set at 0.05. After models were run, I ensured that their assumptions were fulfilled (e.g., normality of residuals for fixed and random effects, homogeneity of variance, overdispersion) by using the *'check_model'* function from the R package *'performance'* (Lüdecke et. al. 2021). I performed a generalised linear mixed effect model (GLMM) with a binomial distribution, using the function *'glmer'* from the R package *'lme4'* (Bates et al. 2015) to test whether the presence of epidermis damage (binomial variable with 0 coding no, and 1 coding yes) is affected by the fixed effects of Julian date (Wilimovsky 1990), snake life stage (categorical variable with 3 levels: juvenile, adult male, or adult female), and species (categorical variable with 5 levels). I also included the random effect of study site in this GLMM because snakes sampled from the same study sites may be more similar to each other than between sites. If categorical variables were significant, I conducted post-hoc multiple comparisons among levels using the *'emmeans'* R function from the R package *'emmeans'* and corrected comparisons with a Tukey HSD multiplicity adjustment. Model results were also obtained from the output from a type III *'Anova'* function from the R package *'car'* (Weisberg 2019).

Secondly, I used a linear mixed effect model (LMM) from the function '*lmer'*, in the package '*lme4*' (Bates et al. 2015) to test whether body condition of snakes is impacted by the presence of epidermis damage (categorical variable with 2 levels: yes or no), snake life stage, and species. This LMM also included the random effect of site. The data in the LMM only included two species, Maritime Gartersnakes and Red-bellied Snakes, and any snakes who had recently eaten or were gravid were removed from the dataset. These species were selected due to having adequate sample sizes of both snakes for effective body condition estimation and power for analyses. I calculated the body condition of snakes using the scaled mass index (SMI) where I used separate mean SVL values for each of the species in the analysis. The output is a continuous, numerical body condition score with higher numbers reflecting more fat reserves (Peig and Green 2009; McGuire et al. 2018). SMI is a more accurate way to predict body condition in small mammals and snakes, compared to ordinary least squares regression of body mass against length (Peig and Green 2009). Model results are presented from the output from a type III *'Anova'* function from the R package *'car'* (Weisberg 2019). The same method was used to make post-hoc multiple comparisons as with the GLMM if necessary. Additional packages used in data wrangling and visualisation included '*ggplot2*' (Wickham 2016), '*lmertest'* (Kuznetsova et. al. 2017), '*tidyverse*' (Wickham et al. 2019), '*magrittr*' (Bache and Wickham 2022), '*data.table'* (Dowle and Srinivasan 2023), '*ggpubr*' (Kassambra 2023), '*cowplot*'(Wilke 2020) and '*patchwork*' (Pederson 2022).

Chapter 3: Results

Field results

My studies goal was to determine if *O. ophiodiicola* was present in New Brunswick and Nova Scotia. In my study, a total of 158 snakes and snake skins were sampled (Table 2). Maritime Gartersnakes were the most sampled snake in my study (51% of samples). The second-most common snake were Red-bellied Snakes (18% of samples). Northern Ring-necked Snakes and Eastern Ribbonsnakes were only sampled in NS. Locations of capture ranged from northern NB within the Kennedy Lakes Protected Area to the south shore of NS (Figure 3). Nine of the Eastern Ribbonsnakes, 5 Maritime Gartersnakes, 6 Red-bellied Snakes and four Northern Ring-necked snakes were sampled from the Mersey Tobeatic Research Institute while 22 Maritime Gartersnakes, 1 Common Watersnake and 3 Red-bellied and Smooth Greensnakes were sampled from the Museum of New Brunswick. Please note that our mapping did not include location data for Eastern Ribbonsnakes, as these are a sensitive species in NS. Results from the qPCR assay testing by AHL (Allender et al. 2015) also indicated that no swabs or snake skins tested positive for *O. ophiodiicola* nor had any presence of the fungus' DNA.

Table 2. The number of snakes swabbed for *O. ophiodiicola* from May to October 2022. The count of each species is separated into three groups: adult and juvenile snakes as determined by field measurements, and snakes categorized as 'other' are not measured due to sampling as frozen dead specimens during this study. Snake skins are presented in their own row because species and age cannot be easily identified from all shed skins.

Species	Adults	Juveniles	Other	Total
A) New Brunswick				
Maritime Gartersnake	29	16	23	68
Smooth Greensnake	2	1	3	6
Red-bellied Snake	10	5	5	20
Skin	-	-	-	16
B) Nova Scotia				
Maritime Gartersnake	4	-	7	11
Smooth Greensnake	1	2	-	3
Red-bellied Snake	3	-	6	9
Northern Ring-necked Snake	4	2	4	10
Eastern Ribbonsnake	4	-	9	13
C) Maine				
Maritime Gartersnake	-	-	1	1
Common Watersnake	-	-	1	1

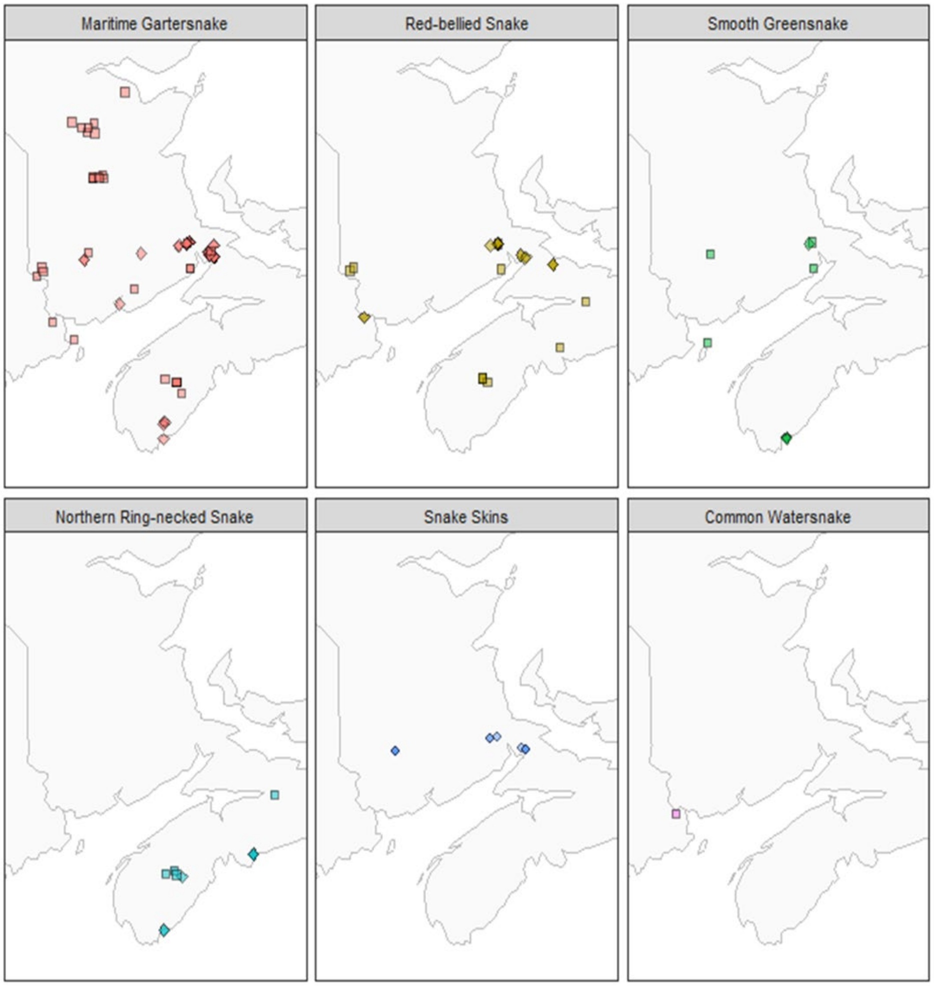

Figure 3. Maps of the location where each snake species was sampled, as well as snake skins, for *O. ophiodiicola* in NB and NS during fieldwork on wild populations in 2022 and specimens collected from 2011 to 2022. Circles represent snake skins, squares represent snake specimens collected dead or provided by the NB Museum and Mersey Tobeatic Research Institute, and diamonds represent live snakes sampled in the field. The one Common Watersnake included in our study was collected from Maine by the Museum of New Brunswick.

Epidermis damage analysis

Many examples of epidermis damage were observed in various species of live snakes during field surveys in the summer of 2022, including missing eyes, partially amputated tails, dorsal damage, and smaller scale injuries (Figure 4). A few of these instances of epidermis damage looked as though they could potentially be SFD lesions, so I swabbed these potential lesions on 14 Maritime Gartersnakes, 3 Eastern Ribbonsnakes, and 1 Smooth Greensnake, although none of these lesions tested positive for *O. ophiodiicola.* The species with the highest rate of epidermis damage was the Eastern Ribbonsnake, with 92% of individuals having epidermis damage, and the species with the least amount of epidermis damage was Red-bellied Snakes at 14%. The other species varied between 27-34% of individuals having epidermis damage. However some snake species like the Maritime Gartersnake had a higher live snake sample size (n=49) then the other snake species like the Red-bellied Snake(n=19) and the Eastern Ribbonsnake had the lowest sample size(n=4).

Figure 4. A visualization of epidermis damage observed during the 2022 field season. On the top left is a Maritime Gartersnake with a part of its tail missing; below this image is another Maritime Gartersnake with scale scarring on its ventral surface; in the middle is a Red-bellied Snake with a missing eye; and on the right is an example of dorsal damage on a Red-bellied Snake.

This led into my investigation of if seasonality affects epidermal damage and the health of snakes with epidermal damage. First, I found that there was a significant, negative relationship between (Julian) date of capture and epidermis damage ($\chi_{71,\,1} = 4.68$, $p = 0.03$) (Figure 5). There was not an effect of either species ($\chi_{71,\,4} = 6.63$, $p = 0.16$) or life stage ($\chi_{71,2} = 5.26$, $p = 0.07$) on the presence of epidermis damage. Second, I examined whether snakes with or without epidermis damage differed in their body condition (SMI). There was no relationship between the presence/absence of epidermis damage and snake body condition ($\chi_{56,\,1} = 0.17$, $p = 0.68$) (Figure 6). Snake body condition significantly differed between species ($\chi_{56,\,1} = 49.26$, $p < 0.01$); Maritime Gartersnakes were on average in a higher body condition than Red-bellied Snakes (Figure 6). Lastly, body condition did not differ across snake life stages ($\chi_{56,\,2} = 1.28$, $p = 0.53$).

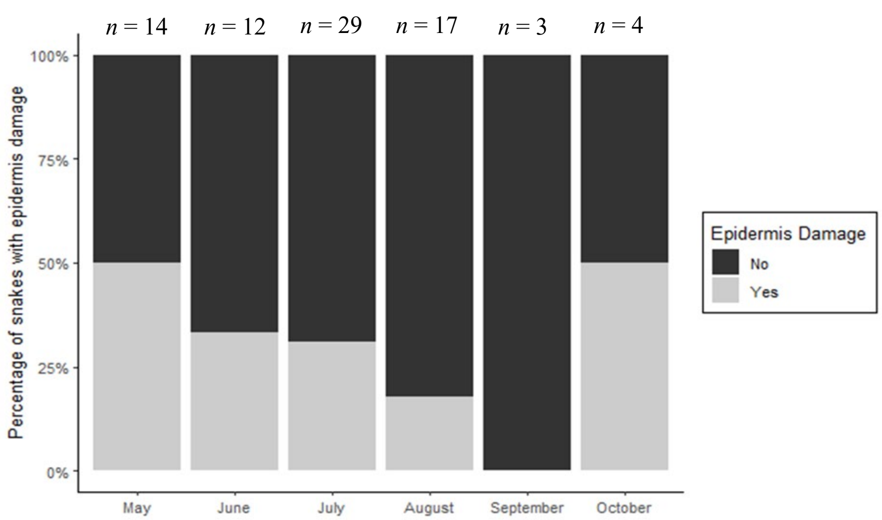

Figure 5. Percentage of snakes with and without epidermis damage caught monthly from May to October 2022 in NB and NS. The sample size of each month is represented by the *n* at the top of the graph. The light grey parts of the graph represent the percentage of total snakes caught that month with epidermis damage, while the black part of the graph represents the percentage of total snakes caught without epidermis damage. This is a representation of how (Julian) date affects the occurrence of epidermis damage, decreasing over the snakes' active season. October is an outlier, but it has a relatively low sample size compared to the other months, so it is likely an effect of our sampling approach rather than being biologically significant.

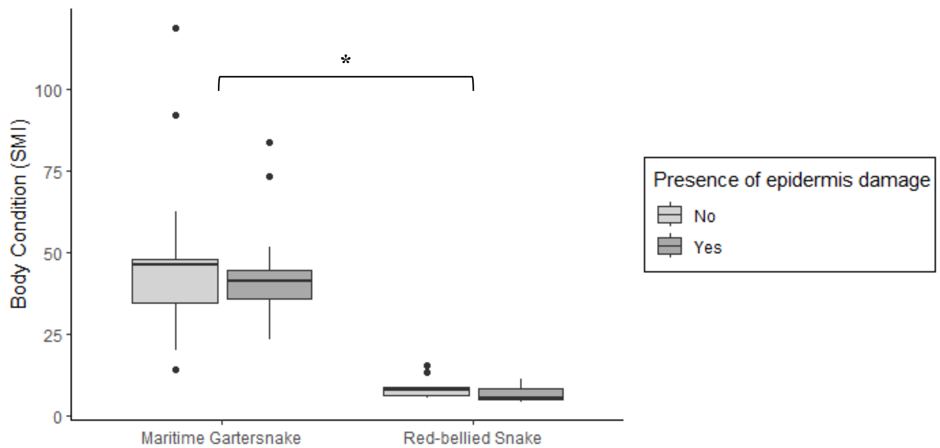

Figure 6. Body condition (SMI) of snakes with and without epidermis damage caught from May to October 2022 in NB and NS. The lines across the boxes in this boxplot represent the median body condition of snake with (dark grey) and without (light grey) epidermis damage. Each of the points represent outliers. The box represents the interquartile range of the data (IQR) and the lower and upper limits of the box are the first and third quantile, respectively. The bottom/ top whiskers that extend from the box are represent the minimum and maximum values of the data that are not considered outliers (1.5 times the IQR). There was no difference in body condition between snakes with and without epidermis damage. However, Maritime Gartersnakes had a significantly greater body condition than Red-bellied Snakes, as shown with an asterisk (*).

Chapter 4: Discussion

<u>*No SFD in the Atlantic Canada*</u>

The goal of my research was to conduct the first monitoring for *O. ophiodiicola*, the fungus that causes SFD, in Atlantic Canada. Overall, I did not detect the presence of *O. ophiodiicola* on any of the sampled snakes in NB and NS. I also had the opportunity to opportunistically sample two snakes from Maine, and no presence of *O. ophiodiicola* was found in those samples either. I also investigated factors that may locally affect a snake's chance of having epidermis damage, along with whether snakes with epidermis damage differ in their body condition, which may reflect a lower fitness and a higher risk level for disease. I found that it was more likely to observe snakes with epidermis damage at the beginning of the active season. Also, snake body condition did not differ among snakes based on the presence/absence of epidermis damage or their age. However, snake body condition differed between species;

Maritime Gartersnakes had a greater body condition than Red-bellied Snakes. Even though no *O. ophiodiicola* was found during this study, it does not definitively mean that this fungus is not present in the areas surveyed, as all I can say for certain is that I did not detect it within my initial surveillance of NB and NS.

The lack of evidence for *O. ophiodiicola* in Atlantic Canada did not support my hypothesis that SFD would be present in NB and NS. I expected that SFD would be present due to the commonality of positive cases in Ontario, New England, and the multiple points of entry for this fungus varying from large ports on Canada's east coast to the border with Quebec and the railway hub in Moncton, NB. I specifically targeted locations in NB and NS that had a high chance of *O. ophiodiicola* presence, such as snake populations along the Maine-NB border, the NB-Québec border, and major urban centers that may also be high-volume entry points (e.g., port cities like Saint John and Halifax, as well as cities like Fredericton and Moncton). Though there were areas that I was unable to cover (i.e., northern NS and northeastern NB), a large area was nevertheless sampled. Additionally, while I had good coverage across native species, there were a few species where sampling was limited, such as Northern Ring-necked Snakes in NB. Yet all snakes within this region can be infected by *O. ophiodiicola* and, thus, more commonly found species can serve as sentinels for other snake species. This is a common approach used in programs that monitor for diseases and their spread, like in Australia where a non-declining frog species, the Common Eastern Froglet (*Crinia signifera*), was used to monitor for chytrid fungus in areas with other more threatened frog populations with lower numbers (Brannelly et al. 2018). My research also has a comparable sample size to other SFD studies for an initial monitoring project. In my study, I collected approximately one snake per 811 km^2. This was estimated by taking the total area of both provinces and dividing it by my study's sample size. Similar studies have a lower sample sizes per area and still found snakes with SFD. For example, Davy et al. (2021) found one snake per 1,638 km^2 in the Great Lakes region, and a study in Virginia found one snake per 3,692 km^2 (Guthrie et al. 2016). It is thus unlikely that my sampling rate was too small to detect *O. ophiodiicola* if present. Although there were limitations to my research, my study design was likely sufficient as initial monitoring for this disease. It is, of course, impossible to prove a lack of presence of a disease through monitoring alone, as only one positive sample is evidence of presence, so all snakes within a population would need to be sampled to confirm absence and this is not logistically feasible. Regardless, this work has expanded our knowledge of SFD in a new region, indicating that this wildlife disease is either not present or present in very low numbers in NB and NS which can be used to advance our understanding of the epidemiology of SFD.

There are a few interpretations of this result. A lack of *O. ophiodiicola* detection could mean that *O. ophiodiicola* is an emerging pathogen that has not reached snake populations in NS and NB. This would be supported by the lack of detection of *O. ophiodiicola* in Maine by previous researchers (Allender et al. 2020) and the lack of positive results from the two snakes I sampled from Maine. However, this fungus has also been suggested to be an endemic rather than emerging pathogen in Canada (Davy et al. 2021). In both scenarios, it could simply be that the *O. ophiodiicola* population has not been supported in this region due to environmental factors, like climate, or that resistant snakes have limited disease spread (emerging) or occurrence (endemic) from central Canada. Notably, the region's climate may play a role in the lack of *O. ophiodiicola* occurrence in Atlantic Canada (Fontanella et al. 2008). Atlantic Canada in general has a harsher climate then other parts of southern North America (e.g., colder temperatures due to the gulf stream moving towards Europe) (Myers and Drinkwater 1989). This overall colder environment means that fewer snake species resides here, along with a lack of invasive reptiles (e.g., only the Red-eared Slider, *Trachemys scripta*, is documented in the region) (NB Invasive Species Council 2023) meaning there are fewer hosts for *O. ophiodiicola*. The regional climate may then be acting as a boundary from invaders, therefore reducing the potential for *O. ophiodiicola* to move into this region. Another possibility is that species-specific resistance to this fungus and SFD may affect its regional presence. To explore this possibility, I compared reported *O. ophiodiicola* detection for the 5 endemic snakes in the Atlantic Canada (Table 4), to snake populations present in central Canada and USA (Table 3). In contrast to Atlantic Canada, central Canada there has been documented prevalence and threat of SFD to its native snake populations.

The susceptibility to *O. ophiodiicola* for species occurring in Atlantic Canada (Table 4) varies from species who occur within the confirmed range of this fungus (Table 3). Specifically, the five species that occur in Atlantic Canada ranged in their susceptibility to *O. ophiodiicola* from 4 to 17% (with an average of about 10%). In contrast, the species that occur in central

Canada and USA exhibit greater variability in their susceptibility to *O. ophiodiicola* (ranging between 2 to 50% with an average of about 21%), but certain species within that locality have much higher susceptibility to *O. ophiodiicola*. Thus, the species composition at the regional scale may have the potential to impact the presence of *O. ophiodiicola* on the landscape. Looking specifically at the species occurring in Atlantic Canada, Maritime Gartersnakes have a low overall detection rate of ~ 4% (Table 4). Thus, Maritime Gartersnakes may be less susceptible to this fungus than other snakes. Interestingly, the potentially lower susceptibility to SFD of the snakes occurring in Atlantic Canada may serve to slow or to prevent the occurrence or spread of this fungus, as well as to buffer these snakes from the threats associated with this disease. This could be beneficial to snakes that are listed as at-risk in the region, such as Eastern Ribbonsnakes. However, reported susceptibility rates do not explain the lack of detection in our one Common Watersnake sample from Maine, as this species appears to be very susceptible to this fungus (a rate of ~ 49%; Table 3). While the low sample size is an issue here (n=1), other studies have also yet to document *O. ophiodiicola* in Maine (Allender et al. 2018). Current literature allows not only for interpretation of my findings but also my study design.

Reported data on species susceptibility allows for an increased understanding of the likelihood of documenting *O. ophiodiicola* regionally when considering species-specific sample sizes within my study. For example, using reported positivity rates of Common Gartersnakes in other regions (Table 4), I would have expected that 3 snakes should have tested positive based on my sample size for this species (n = 80). If we apply the same approach to the other snakes that occur in Atlantic Canada, if *O. ophiodiicola* is present in the region, we would have expected 5 Red-bellied Snakes, 1 Northern Ring-necked Snake, and 1 Eastern Ribbonsnake to have tested positive. The other study that sampled Smooth Greensnakes also did not detect *O. ophiodiicola*, however, their sample size is low (Allender et al. 2020). Based on these numbers, my study ought to have had a high enough sample size to detect *O. ophiodiicola* in the two most common species, Maritime Gartersnakes and Red-bellied Snakes, but potentially not the others. Knowledge of species-specific susceptibility is critical for both planning future studies, and interpretation of findings, yet it is important to note that this is not the only factor that can affect the occurrence of *O. ophiodiicola* and SFD on the landscape.

Table 3. Susceptibility to SFD of snake species that occur in central Canada and USA, a location where SFD is prevalent, as reported in literature. The general location of the study, its sample size (*n*), the number of samples that detected *O. ophiodiicola*, the percentage of snakes with SFD, and the citation are all reported. A weighted average for each species is also provided.

Common Name	Scientific Name	*n*	Detections	Proportion	Region	Source
Common Watersnake	*Nerodia sipedon*	19	9	0.47	Connecticut, USA	Licitra et. al., 2019
		72	38	0.53	Kentucky, USA	Mackenzie et al., 2019
		27	13	0.48	USA & Puerto Rico	Allender et. al., 2020
		24	5	0.21	Great Lakes region	Davy et. al., 2021
		6	1	0.17	Ontario, Canada	Dillon et al. 2022
		26	19	0.73	Maryland & Virginia, USA	Fuchs et al., 2020
		Weighted Average %		**48.85**		
DeKay's Brownsnake	*Storeria dekayi*	75	1	0.01	Great Lakes region	Davy et. al., 2021
		2	1	0.50	Maryland & Virginia, USA	Fuchs et al., 2020
		15	0	0.00	Tennessee, USA	Snyder and Sutton, 2020
		Weighted Average %		**2.17**		
Eastern Fox Snake	*Pantherophis vulpinus*	84	24	0.29	Ontario, Canada	Dillon et al. 2022
		2	2	1.00	USA	Lorch et al., 2016
		216	47	0.22	Great Lakes region	Davy et. al., 2021
		Weighted Average %		**24.17**		
Eastern Milksnake	*Lampropeltis triangulum*	3	0	0.00	Great Lakes region	Davy et. al., 2021
		4	3	0.75	USA	Lorch et al., 2016
		2	0	0.00	Tennessee, USA	Snyder and Sutton, 2020
		5	4	0.80	Kentucky, USA	McKenzie et al., 2019
		Weighted Average %		**50.00**		
Eastern Racer	*Coluber constrictor*	6	2	0.33	USA	Lorch et al. 2016
		13	6	0.46	Kentucky, USA	McKenzie et al., 2019
		57	7	0.12	Georgia, USA	Haynes et al. 2020
		Weighted Average %		**19.74**		
Gray Ratsnake	*Pantheophis spilodes*	9	6	0.67	Great Lakes region	Davy et. al., 2021
		10	0	0.00	Tennessee, USA	Snyder and Sutton, 2020
		2	1	0.50	Kentucky, USA	McKenzie et al., 2019
		Weighted Average %		**33.33**		
Massasauga	*Sistrurus catenatus*	83	3	0.04	Great Lakes region	Davy et. al., 2021
		102	5	0.05	Michigan, USA	Allender et al., 2016a
		5	5	1.00	USA	Lorch et al., 2016
		297	13	0.04	Michigan, USA	Hileman et al., 2018
		2	2	1.00	Michigan, USA	Tetzlaff et al., 2015
		Weighted Average %		**5.73**		

Table 4. Susceptibility to SFD of snake species that occur in Atlantic Canada as reported in literature. Of course, the species that are native to Atlantic Canada also typically occur in central Canada and USA too (i.e., they have greater overall biodiversity) and these studies are from these regions. The general location of the study, its sample size (*n*), number of samples that detected *O. ophiodiicola,* the percentage of snakes with SFD, and the citation are all reported. A weighted average for each species is also provided.

Common Name	*Scientific Name*	n	Detections	Proportion	Region	Source
Common Gartersnake	*Thamnophis sirtalis*	3	1	0.33	Connecticut, USA	Licitra et. al., 2019
		68	7	0.10	USA	Allender et. al., 2020
		8	4	0.50	Kentucky, USA	Mackenzie et al., 2019
		183	3	0.02	Great Lakes region	Davy et. al., 2021
		160	2	0.01	Ontario, Canada	Dillon et al. 2022
		Weighted Average %		**4.03**		
Red-Bellied Snake	*Storeria occipitomaculata*	2	0	0.00	United States	Allender et. al., 2020
		3	1	0.33	Kentucky, USA	Mackenzie et al, 2019
		1	0	0.00	Great Lakes region	Davy et. al., 2021
		Weighted Average %		**16.67**		
Northern Ring-necked Snakes	*Diadophis punctatus*	15	2	0.13	USA & Puerto Rico	Allender et. al., 2020
		9	3	0.33	Kentucky, USA	Mackenzie et al., 2019
		1	0	0.00	Great Lakes region	Davy et. al., 2021
		11	1	0.09	Georgia, USA	Haynes et al. 2020
		5	0	0.00	Tennessee, USA	Snyder and Sutton, 2020
		Weighted Average %		**14.63**		
Eastern Ribbonsnake	*Thamnophis saurita*	24	2	0.08	Great Lakes region	Davy et. al., 2021
		2	1	0.50	USA & Puerto Rico	Allender et. al., 2020
		23	0	0.00	Ontario, Canada	Dillon et al. 2022
		9	1	0.11	Maryland & Virginia, USA	Fuchs et al., 2020
		19	1	0.05	Georgia, USA	Haynes et al. 2020
		Weighted Average %		**6.49**		
Smooth Greensnake	*Opheodrys vernalis*	2	0	0.00	USA & Puerto Rico	Allender et. al., 2020
		Weighted Average %		**NA**		

Epidermis damage and body condition

As for my epidermis damage results, there are several potential reasons why epidermis damage in NB and NS snakes decreased as the active season progressed (from May to October), all of which could pose a problem if SFD were to be present in NB or NS. I could be observing greater epidermis damage early in the season due to two potential factors: a higher incidence of (1) predation within their hibernacula, and/or (2) early predation attempts occurring as snakes emerge from their hibernacula. In the spring, snakes are vulnerable to initial predation from predators that know the location of their hibernacula, snakes' antipredator behaviour may be impacted by requiring more time to reach their optimal temperatures during cold weather, and low amounts of vegetation at this time may also increase their conspicuousness to predators (Katzner et al. 2020; Charland and Gregory 1995). Another factor to consider is that during the winter, snakes are unlikely to shed due to their low metabolic rate. Shedding is crucial for the skin's healing process, so epidermis damage may be heightened at this time simply because it reflects a greater amount of time without renewal and there could be a greater cumulation of injury between sheds than is typical. However, these are only a few possibilities of why snakes may have increased epidermal damage at the beginning of their active season. It also means that if SFD was present in Atlantic Canada, snakes would likely be more vulnerable to infection in the spring when they are emerging from their hibernacula, and often congregating in large mating groups (e.g., *Thamnophis spp*.) (Shine et al. 2005), which could hasten disease spread.

In addition to seasonality, I investigated whether there is a relationship between a snake's body condition and the presence of epidermal damage. I found no evidence to support this relationship in my data. This suggests that snakes with epidermis damage may not be at a higher risk for mortality if infected, because they have similar fat reserves as snakes without damage. Another study on Corn Snakes (*Pantherophis guttatus*) found that lesions, swelling, and necrosis of skin were present on snakes infected with SFD, but all experimental groups (infected and non-infected snakes) had similar levels of fat stores (Lorch et al. 2015). However, wild Pygmy Rattlesnakes (*Sistrurus miliarius*) have been found to have decreased body condition when infected with SFD (McCoy et al. 2017). Overall, future work should advance our knowledge of the relationship between injury, SFD infection, and body condition in snakes in both lab and wild populations, allowing researchers to better understand the potential risks of epidermis damage of

SFD where to spread to an uninfected area. One means of increasing our knowledge of this disease is through a national monitoring program.

Future steps

Disease monitoring is important for the management of diseases that are emerging and understudied. If there is not constant monitoring for wildlife disease, there could be more occurrences of cases like White-nose Syndrome where the spread of this fungus (*Pseudogymnoascus destructans*) to a cave in New York state led to this disease spreading across eastern North America and decimating native bat populations (Puechmaille et al. 2011). Therefore a national monitoring program for *O. ophiodiicola* should be implemented across Canada, both in regions where this fungus is prevalent and in others where its presence is unknown or absent. There are similar on-going monitoring programs for other wildlife diseases in Canada, like SNAPS (https://snaps.amphibiandisease.org/) that annually monitors for the salamander chytrid fungus (*Batrachochytrium salamandrivorans*) across North America, prior to a potential introduction from Europe (Waddle et al. 2020). Ideally, monitoring of both captive and wild populations of snakes would occur on an annual basis to continue to monitor for the threat of *O. ophiodiicola* and SFD. Other studies have also suggested monitoring for *O. ophiodiicola* by integrating an annual monitoring program with a variety of on-going studies, such as radio telemetry, mark-recapture studies, and environmental sampling (e.g., soil sampling) (Allender et al. 2016). Joining forces with current snake research and adding monitoring for SFD to these projects would facilitate both collaboration and, hopefully, minimize logistical issues. In addition, regionally in Atlantic Canada, future monitoring should focus on areas with the greatest potential for disease spread, like in snake populations around Halifax, NS and regions of NB that border Maine and Québec to ensure early detection. Monitoring should also occur for the endangered Eastern Ribbonsnake population in NS. Annual population monitoring already occurs for this species and non-lethal skin swabbing could be easily and ethically integrated into their current management program. This recommendation fits under the Eastern Ribbonsnake Recovery Plan, which is to "identify population threats, evaluate their significance, and take steps to mitigate those threats" (Parks Canada Agency 2008). Currently, there is no mention of *O. ophiodiicola* and its potential risk to this population in the recovery plan (Parks Canada Agency 2008). If this population were to be exposed to this fungus, it could potentially have significant negative impacts, given that most of the population is already at risk of local extinctions due to its small size and isolated range in NS (Bell et al. 2007). Ensuring the persistence of the NS Eastern Ribbonsnake population is important because it is a distinct, geographically-isolated population that could not be replaced in the near future by emigration of other populations. Thus, a regionally-specific approach may be beneficial in some instances to augment a national monitoring program, thereby continuing to increase our understanding of SFD in Canada.

Overall, my research is the first completed survey in Atlantic Canada for the occurrence of the causal fungus of SFD: *O. ophiodiicola.* My finding that this fungus is not present in the region was unexpected and brings uncertainty to whether this fungus and disease is a novel and emerging (Lorch et al. 2016) or an endemic pathogen (Davy et al. 2021). Notably, my work suggests this pathogen may not be as widespread as it has previously been documented to be within more well-studied regions (Davy et al. 2021). Of course, there is still the possibility that this fungus is endemic to areas like Ontario and Quebec, but not the Atlantic coast of Canada as it could potentially be blocked by natural barriers such as the St. Lawrence River or the Appalachian Mountain range (Fontanella et al. 2008). These natural barriers may limit snakes from these different regions from encountering one another. Although *O. ophiodiicola* may not currently be in the Maritimes, it is well-known that the range and occurrence of wildlife diseases can quickly change, which we have previously seen with White-nosed Syndrome in bats (Puechmaille et al. 2011) and Chytrid fungus in frogs and salamanders (Brannelly et al. 2018; Waddle et al. 2020). While it is rare to have baseline data for regions prior to the occurrence of a disease, it is crucial to have a better understanding of the epidemiology, dynamics, and effects of pathogens. Thus, continued monitoring and surveillance of *O. ophiodiicola* in Atlantic Canada is essential to ensure for early warning of its presence, as *O. ophiodiicola* could become a potential threat to snake populations which in turn can have ecosystem-level effects. Snakes are important secondary predators in natural ecosystems, and generalist predators, like Maritime Gartersnakes, can help regulate a variety of prey populations such as amphibians, birds, invertebrates, and rodents. As a secondary predator, these snakes can also be important parts of the diet of larger predators, especially birds of prey, who have experienced decreasing populations within the last century (Gousy-Leblanc et al. 2021). A decrease in snake populations would result in all these species being affected. Overall, though this research increased our knowledge of *O. ophiodiicola* and SFD in North America, there is ample room left for future work to continues to investigate the range and dynamics of *O. ophiodiicola* over space and time, the factors that impact host susceptibility, and this fungus' potential to negatively impact threatened Nearctic snakes.

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