CANADIAN WILDLIFE HEALTH COOPERATIVE

# Snake Fungal Disease in Canada Rapid Threat Assessment

CREATING A WORLD THAT IS SAFE AND SUSTAINABLE FOR WILDLIFE AND SOCIETY

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# Snake Fungal Disease in Canada
# Rapid Threat Assessment

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**Prepared for Environment and Climate Change Canada**


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# Executive summary

Snake fungal disease (SFD) is an emerging and debilitating disease affecting a variety of free ranging and captive snake species across North America. It was first documented in Canada in 2015 in southern Ontario in the endangered eastern foxsnake. SFD has since been detected in the endangered queensnake in Ontario and its pathogen has been detected, without disease in Ontario in the eastern foxsnake, queensnake, eastern massassauga (all threatened-endangered), and 5 subspecies of gartersnakes (status not determined).

**The purpose of this assessment is:** to help the Wildlife Issues Unit, Wildlife Management and Regulatory Affairs, Canadian Wildlife Service determine actions to be taken on SFD to support the unit's mandate of preserving wildlife health in Canadian species and overall activities of policy development and implementation to that end.

**Snake fungal disease is an example of managing uncertainty.** The recent appearance of the disease, scant investment or time for research and investigation, lack of ongoing surveillance of snake health and general lack of ecological and monitoring information on Canadian snake populations precludes a quantitative risk assessment or a confident forecast of the future impacts and behaviour of SFD.

The Canadian Wildlife Health Cooperative created a decision tree to help transparently assess the credibility and seriousness of SFD as a threat to Canadian biodiversity and to inform the need or nature of risk management actions. From this we concluded:

1. **SFD is a credible threat to Canadian biodiversity but the magnitude of the harms caused by SFD cannot be estimated with confidence.** While there remain many uncertainties about the extent of population effects of SFD and the conditions under which these effects result in serious and irreversible harms, a precautionary approach would suggest it can be a significant additive stressor and should be considered serious for species at risk and/or for populations struggling with other cumulative stressors. There are analogous situations where emerging fungal diseases have had profound conservation effects on wild animals and plants. While it is not surprising that SFD is in Canada, we have little information with which to predict where SFD will spread in space, time or species and which populations are most vulnerable. We cannot be assured it is not in other locations or species due to the low level of submissions to diagnostic laboratories and comparatively low level of population monitoring and health assessment.

2. **It is not currently possible to forecast the epidemiological or ecological fate of SFD in Canada.** SFD is a newly described condition, involving an understudied pathogen occurring in species historically subject to little disease surveillance with almost no data on the association of SFD and population abundance and distribution. Estimates of SFD as a conservation risk and identification of high risk scenarios are, to date, largely opinion based, relying heavily on case studies and analogy. Impacts of SFD on individual animals, different populations and different species is variable or poorly documented under natural conditions. The casual mechanisms of SFD, especially the role of environment risk factors remain unresolved, precluding ranking of SFD risk factors.

3. **There are reasonable grounds to implement a management response to SFD.** Responding to SFD would be consistent with the mission of the Wildlife Issues Unit and would contribute to protecting species at risk and preparing them for further impacts from climate change. Canada has 26 species[1] of snakes. Eight are endangered, 5 are threatened, 4 are of special concern. Several threatened and endangered species in Canada are susceptible to the disease and some are already known to be infected or affected. Canada cannot be assured that SFD is new to the country and is restricted only to the two species discovered with SFD to date because of challenges in monitoring snake health and subjecting them to diagnostic assessments.

4. **There are limited options to directly manage SFD.** Deficiencies in knowledge about the transmission system for SFD plus the practical limitations to constraining the movements of free-ranging animals make isolation or quarantine impractical for wild snakes. Because of prevailing uncertainties, a precautionary approach would be to limit further release of the fungus associated with SFD into the environment through education campaigns to the pet trade and herpetologists to advocate and support biosecurity and to prevent unintentional movement of the fungus from a known SFD-positive area to areas of unknown or assumed negative status. There are no proven methods to prevent, mitigate or eliminate harms to populations from SFD through standard veterinary interventions. There has been some success in treating individual animals, an action that may be important in critically endangered species where each individual animal is required to maintain the genetic diversity of the population.

5. **A reasonable argument can be made to invest in a harm reduction approach focussed on increasing capacity to cope with SFD.** Reducing population vulnerability through a harm reduction approach would synergize with species recovery plans and attack plausible SFD risk factors. Vulnerability results from a combination of exposure, susceptibility, capacity to cope and cumulative effects of other stressors. As there are no current options to reduce exposure or SFD-specific susceptibility in free ranging populations, vulnerability reduction must focus on improving individual and population resilience and managing plausible environmental risk factors or causal co-factors. The suggested co-factors influencing the spread and effects of SFD overlap with the major challenges facing snakes in Canada (ex. climate, habitat loss, habitat degradation). Snake health management, with specific attention to SFD and other unknown diseases, should be integrated into species management plans due to synergies in current recovery plans and best evidence to deal with SFD. Efforts should be directed to identifying vulnerable populations by overlaying information on putative SFD risk factors and population status. Surveillance specific to SFD should target vulnerable populations and the current leading edges of the disease's known distribution in Canada. Outreach should encourage increased submissions of snake cases to the Canadian Wildlife Health Cooperative and linking those diagnostic results to snake population data to better estimate the role for diseases, including SFD, with population outcomes.

1 Canada has 26 species but some are split into subspecies for status reports, giving a total of 33

# Purpose of the assessment

The Wildlife Issues Unit, Wildlife Management and Regulatory Affairs, Canadian Wildlife Service, Environment and Climate Change Canada, requested a threat assessment on snake fungal disease to determine action to be taken on the disease to support the unit's mandate of preserving wildlife health in Canadian species and overall activities of policy development and implementation to that end.

The **objective of this threat assessment** is to determine the options to protect Canadian susceptible species from snake fungal disease (SFD) and its etiologic cause, *Ophidiomyces ophiodiicola*. It will focus on reviewing which species in Canada are vulnerable to the disease, what circumstances affect species vulnerability and Canada's capacity to identify, assess, and manage biodiversity and ecosystem risks from this disease. Unlike a risk assessment, the purpose is not to assess the probability or magnitude of effects, but rather to identify gaps and weaknesses in Canada's threat management capacity and thus to inform the need or nature of response to this emerging disease.

The **purpose of the report** is to assess the credibility and seriousness of snake fungal disease as a threat to Canadian biodiversity or other values and to inform the need or nature of risk management actions

## Rationale for the threat assessment

### Key Points

- Many Canadian snake species are confronting multiple stressors. The addition of a new disease would further complicate recovery plans
- SFD has been detected in Canada and its range is expanding in the eastern United States
- Emerging fungi have severely impacted other wildlife species, elevating emerging fungal diseases like SFD to be conservation concerns.
- There are multiple uncertainties preventing an evidence-based disease response or assessment of the ecological or population implications of SFD

### Details

Globally, nearly one in five reptile species are threatened with extinction, with another one in five species lacking sufficient information to assess their population status (Böhm et al, 2013). The breadth of species, geographic scope, and severity of declines in reptile populations is like those being experienced by amphibians. Canada has 26 species of snakes with 33 distinct subspecies. COSEWIC lists 4 as special concern, 5 threatened and 9 endangered in at least part of their range. The status of 12 has not been established. The cause of reptile population declines is known with certainty in some instances, suspected in many, and unknown in others (Gibbons et al, 2000). Habitat destruction and road mortality are problems for almost every snake species in Canada and they are factors that lead to small, isolated, genetically depauperate populations that are more prone to stochastic events, such as disease outbreaks.

In mid-March 2015, an adult female eastern foxsnake (*Pantherophis vulpinus*) with signs of dermatitis was accidentally disturbed while hibernating near Lake Erie in Ontario. This was the first documented case of SFD in Canada. The disease has since been diagnosed in the queensnake (*Regina septemvittata*) in Ontario and the fungus *Ophidiomyces ophiodiicola* (believed to be the proximate cause of SFD) has been detected in 3 other Canadian species (See table 1). Both the eastern foxsnake and queensnake are listed as endangered by COSEWIC.

SFD has been showing up with increasing frequency in snakes around the eastern and midwestern United States since 2006. Emerging fungal infections causing widespread population declines have increasingly been documented across diverse taxa from bats, and frogs, to corals and bees (Fisher et al, 2012). Emerging fungal pathogens and the re-emergence of previously uncommon fungal diseases in people has been associated with an increasing number of susceptible people due to concurrent immunosuppressive factors such as HIV, cancer therapy and other causes.

The biology of fungal pathogens provides them the ability to be a primary driver of population extinctions. The independence of many fungal pathogens from their hosts may promote their survival and virulence in new ecosystems and novel host species, precluding their attenuation to less virulent forms, as is often seen in bacterial and viral pathogens (IOM, 2011).

Given the severe conservation impacts of other emerging fungal diseases such as white nose syndrome in bats, salamander chytrid disease and frog chytrid disease, conservationists fear SFD could pose a similar threat to snakes.

## Format of the report

A decision framework was created to guide this assessment (appendix 1). The reader is encouraged to review this appendix prior to reading the report.

Part 1 of the report provides a brief history and overview of SFD.

Part 2 goes through the steps in the decision framework. **At each step, key points summarize the answers to the questions in the decision framework. More detailed information follows them.** Additional information on SFD is provided in the appendices.

The guiding framework consistent of six questions:

1. Are there species susceptible to this disease in Canada?
2. Was the disease expected?
3. Can the disease cause serious harm?
4. Are their opportunities for high levels and/or widespread exposure to the causative agent and/or risk factors?
5. Are there proven effective methods to respond to and eliminate or mitigate the threat?
6. Are there proven ways to contain or isolate the threat?

Under conditions of uncertainty we have taken a precautionary approach and assumed the answer in the decision tree questions to be yes when (i) analogous situations present a reasonable probability the correct answer is yes; (ii) the species at risk are highly valued; (iii) there are multiple species (including people) that may be at risk and/ or (iii) there are multiple possible harms.

# Part 1- Overview of snake fungal disease

### Key Points

- SFD is a newly described disease, but its fungal etiology is likely an endemic environmental organism that acts as an opportunistic pathogen under conducive conditions.
- SFD is present in Ontario as well as in 20 eastern states in the USA.
- The effects of SFD vary by species, population and individual but there are reports of this being a fatal disease linked to population declines.
- Environmental co-factors appear to play an important role in the emergence and impacts of SFD

### Details

## Brief history of the disease

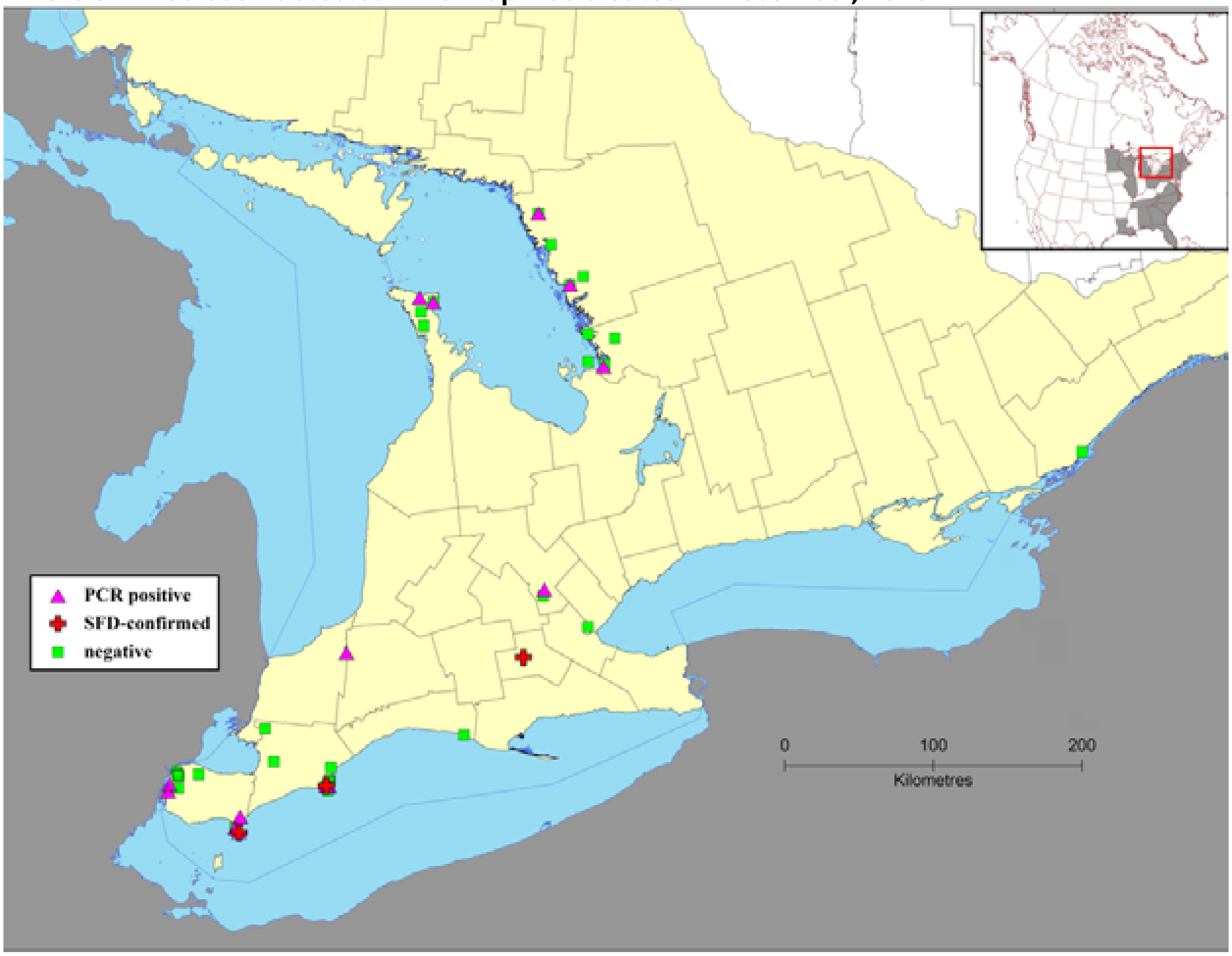
SFD is an emerging and debilitating skin disease syndrome affecting a variety of free ranging and captive snake species across North America. Disease reports in free ranging wild populations have generally been geographically restricted, with most cases being limited to the eastern United States. Some of the first SFD reports originated in New Hampshire, in 2006 where the disease was associated with a 50 % population decline in a population of timber rattlesnakes (*Crotalus horridus*) (Clark et al. 2011). SFD has now been identified in 20 U.S. states (Ohio, New Jersey, Massachusetts, New York, Florida, Alabama, Minnesota, New Hampshire, South Carolina, Wisconsin, Connecticut, Vermont, Virginia, Kentucky, Louisiana, Illinois, Pennsylvania, Georgia, Michigan, Tennessee) and Ontario. (Cheatwood et al. 2003; Rajeev et al. 2009; Clark et al.  2011; Allender et al. 2011; Allenear et al 2013; Fenton et al. 2015; McBride et al. 2015; Tetzlaff et al. 2015; Allender et al. 2016; Glorioso et al. 2016; Guthrie et al. 2016; Lorch et a.l 2016; Okhura et al. 2016; Ravesi et al. 2016).

Figure 1 presents SFD's geographic distribution.

## What causes SFD?

The name SFD has been proposed to exclusively refer to skin disease associated with infection only with the fungus *Ophidiomyces ophiodiicola* (Lorch et al. 2015). *O. ophiodiicola* was originally isolated and characterized from an abscess in a captive black rat snake (*Elaphe obsoleta obsolete*) (Rajeev et al. 2009). *O. ophiodiicola* has been demonstrated to result in the lesions of SFD in experimental infections (Lorch et al. 2015) and is believed to be the primary pathogen associated with SFD (Allender et al. 2015). However, there is currently no conclusive evidence demonstrating that it is the sole agent responsible for SFD (Tetzlaff et al. 2015). Co-infection with various other fungal agents is common. Opportunistic infection by a variety of other fungal pathogens can also result in similar skin disease and should be ruled out in any SFD case (Lorch et al. 2016). The genus *Ophidiomyces* is composed of a single species, *O. ophiodiicola*, and to date snakes have proven to be the only host of this fungus (Sigler et al. 2013).

**Figure 1: Geographic distribution of snake fungal disease in Ontario. The map insert shows US states where SFD has been detected. The map was created in December, 2016.**




*O. ophiodiicola* is occasionally found on the skin of snakes without any clinical signs of SFD or accompanying histological lesions (Bohuski 2015). Given that the fungus is more widespread than the disease and not all species of snakes in an SFD positive area are found with disease, it is likely that *O. ophiodiicola* is necessary for SFD but insufficient to causes disease on it own. It is possible that this fungus might represent a skin commensal or at least an unapparent subclinical infection under certain conditions (Lorch et al 2016). The fungus appears to have temperature dependent growth and this might allow it a specific niche in growing on external skin surfaces of hibernating snakes.

Environmental co-factors appear to be required for SFD to emerge. Climate change, particularly warmer hibernation temperatures and wet weather after hibernation have been linked to the outbreak of SFD in timer rattlesnakes in New Hampshire (Allender et al 2015, Clark et al 2011). However, temperature and moisture seem to not effect species equally as other species of snakes in the timber rattlesnake ecosystem were not seen to be affected by SFD at the time of the outbreak (Clark et al. 2011). Documented outbreaks of severe SFD have typically been in relatively small or isolated snake populations (although such populations may be under greater scrutiny than larger, less threatened populations). Habitat fragmentation and destruction as well as inbreeding depression have been suggested to be important co-factors (Lorch et al 2016). Despite these suppositions, there is no definitive information to identify, quantify and rank co-factors to direct management actions.

### What does SFD do to individual snakes?

*O. ophiodiicola* invades the superficial layer of skin where it subsequently causes thickening, crusting and death of the epidermis. When the crusts fall off, ulcerated and eroded skin is revealed. Cases of snakes with no apparent disease have tested positive for *O.phiodiicola* by PCR in the absence of mortality or noted population effects.  There are three courses of the infection; recovery, death from secondary disease processes; or death after the fungus invades muscles and deeper tissues.  See the section below addressing the question, “Does SFD causes serious harms?” for further details.

### What does SFD do to snake populations?

There has been inadequate integration of disease investigation information with ecological monitoring data to determine the population impacts of SFD. Lesions run the gamut from mild to severe/lethal and population-level effects have been severe in some cases but are not inevitable. Multiple factors (presence of an opportunistic pathogen, enhanced susceptibility due to genetic isolation and stochastic weather events) with interactive or synergistic feedbacks may combined to produce severe population level effects. While severe population effects appear to be exceptional at this time, the circumstances that are thought to produce them are becoming increasingly common. See the section below addressing the question, “Does SFD causes serious harms?” for further details.

# Part 2 – Answering questions from the decision framework

## Are there susceptible species in Canada?

**Key points**

- There are species in Canada that are susceptible to SFD and are vulnerable to additive effects of the disease due to concurrent stressors and because they are in small, isolated populations.
- SFD was first found in the endangered eastern foxsnake in Ontario. The disease has since been detected in the endangered queensnake in Ontario and the pathogen has been detected, without disease in Ontario in the eastern foxsnake, queensnake, eastern massassauga (all threatened-endangered), and the common gartersnake (status not determined).
- The number and distribution of susceptible species in Canada likely exceeds this list.
- To date, only snakes have been shown to be susceptible to SFD, but it has affected multiple species.

**Details**

## SFD in Canada

The first case examined by the CWHC as SFD-suspect was a skin sample from an eastern gartersnake collected in early June 2014 from Rondeau Provincial Park on the shore of Lake Erie in Ontario; however, culture of that specimen failed to demonstrate the presence of *O. ophiodiicola*. In mid-March 2015, an adult female eastern foxsnake with dermatitis was accidentally disturbed while hibernating at a location close to Lake Erie in Ontario. This became the first documented case of SFD in Canada. SFD has since been confirmed by histology in 3 eastern foxsnakes and one queensnake (Table 1).

**Table 1: Snake fungal disease status for Canadian snakes as of December, 2016**

| Species | COSEWIC Status | Disease Diagnosed | | Exposure determined by PCR |
|---|---|---|---|---|
| | | Free ranging in Canada | Free ranging in USA | Free ranging in Canada |
| Eastern foxsnake | Endangered | X | X | X |
| Northern watersnake | Not at risk | - | X | X |
| Queensnake | Endangered | X | X | X |
| Common gartersnake sspp[1] | Not determined | - | X | X |
| Eastern massasauga | Threated and endangered | - | X | X |

(1) Includes Valley, Maritime, Red-sided, Puget Sound and Eastern gartersnakes

The CWHC augmented passive surveillance with a parallel program using real-time PCR (qPCR) to screen apparently unaffected snakes for presence of the fungus in 2016, and began screening archived samples from cases going back to 2012. As of the end of November, 2016, the CWHC had examined 126 specimens comprising 9 species (Table 2). Diagnostic material included cases submitted for general scanning surveillance, cases submitted specifically for SFD testing, and opportunistic samples comprising road mortality, shed skins, and swabs from apparently asymptomatic snakes.

**Table 2: Results of enhanced surveillance for snake fungal disease and *O. ophiodiicola* in Ontario 2015-16**

| Species (Common name) | No. Tested | PCR Positive for O. ophiodiicola | SFD positive diagnosis | Known Mortality |
|---|---|---|---|---|
| Massasauga | 17 | 3 | 0 | 0 |
| Eastern foxsnake | 62 | 13 | 3 | 0 |
| Eastern milksnake | 2 | 0 | 0 | 0 |
| Eastern gartersnake | 13 | 1 | 0 | 0 |
| Northern watersnake | 2 | 2 | 0 | 0 |
| Queensnake | 4 | 4 | 1 | 0 |
| Butler's gartersnake | 3 | 0 | 0 | 0 |
| Dekay's brownsnake | 14 | 0 | 0 | 0 |
| Gray ratsnake | 1 | 0 | 0 | 0 |
| **TOTAL** | **126** | **23** | **4** | **0** |

Seven of the 25 snake carcasses submitted from 2012-2016 for general scanning surveillance were positive for *O. ophiodiicola* by qPCR, indicating exposure to pathogen but not necessarily presence of disease. The earliest positive sample was a massasauga submitted from the Parry Sound area in Ontario in 2012. There were no signs of dermatitis noted at necropsy and histology revealed no indication of fungal infection.

Twelve samples were submitted from live, apparently affected snakes specifically for SFD testing. Seven (58.3%) of those samples were positive for *O. ophiodiicola* by qPCR. Histology was available for 8 of those 12 cases, and SFD was confirmed with histology in 4 of those 8 cases. To date, all confirmed cases of SFD in free-ranging snakes in Canada have been diagnosed in living snakes. Many of these individuals are monitored closely and there have been no known deaths as of November 2016.

Of 89 opportunistic samples from apparently unaffected individuals, 9 (10.1%) were positive for *O. ophiodiicola* by qPCR.  Disease could not be confirmed because histological samples were not available; however, preliminary results suggest the fungus may already be widespread in southern Ontario although incidences of serious disease appears to be infrequent at this time.

It is reasonable to believe that the list of susceptible species extends beyond what has been documented so far in Canada because: (i) There has been a relatively low level and short time frame for surveillance and/or surveys for the disease; (ii) *"O. ophiodiicola* is widely distributed in eastern North America, has a broad host range, is the predominant cause of fungal skin infections in wild snakes and often causes mild infections in snakes emerging from hibernation"(Lorch et al, 2016), and (iii) SFD has been found in 30 snake species in the United States, many of which also reside in Canada.

To date *O. ophiodiicola* has been isolated from six families of snakes (Lorch et al. 2016). Affected free-ranging snakes in the Viperidae family include the pygmy rattlesnake, massasauga, timber rattlesnake and copperhead, (Cheatwood et al. 2003, Allender et al. 2011, Clark et al. 2011, Smith et al. 2013, Tetzlaff et al. 2015, McBride et al. 2015, Lorch et al. 2016,). Captive cases in Viperidae include cottonmouth (Lorch et al. 2016) and cases in eastern diamond-backed rattlesnake not classified as captive or wild (Sigler et al. 2013)

Non-crotalid species in which SFD has been observed include the northern watersnake, gartersnake, northern ribbonsnake, eastern foxsnake, queensnake, plains gartersnake, saltmarsh snake, racer, eastern milksnake, ratsnake, mudsnake, broad-banded watersnake, rainbow snake, eastern black kingsnake, bullsnake, Louisiana pinesnake and the brown watersnake (Cheatwood et al. 2003, Rajeev et al. 2009, Sigler et al. 2013, Sleeman 2013, Dolinski et al. 2014, Fenton et al. 2015, Price et a; 2015, Glorioso et al. 2016, Guthrie et al. 2016, Lorch et al. 2016, Ohkura et al. 2016, Ravesi et al. 2016)

Species that exhibit late maturity and slow reproduction are especially sensitive to mortality in adults in general, and especially to mortality in adult females. There is some evidence to suggest this group is hardest hit by disease. A study in Virginia found that female snakes were twice as likely to have skin lesions than males (Guthrie et al. 2016). The authors suggested that late season lesions found in gravid females may be evidence that the stress of pregnancy may predispose females to an increased risk of exposure or the inability to clear the infection. As more populations of snakes are studied across North America, the specific genetic, physiological, behavioural and ecological factors underlying any species differences in susceptibility will perhaps become clearer (Lorch et al. 2016).

## Was SFD expected?

### Key Points

- It was not surprising to find cases in Canada given; (i) the North American distribution, species affected and pattern of spread of SFD, (ii) previous evidence of a similar disease in archived samples in the USA and Canada and (iii) the increased frequency of emerging fungal diseases.
- Canada cannot be assured that this disease is new to the country and is restricted to two species due to challenges in monitoring snake health including the low level of submissions to labs and comparatively low level of population monitoring and health assessment and due to evidence of infection without disease in other species in Canada.
- Given the many stresses snakes face and the increasing role of fungi as emerging pathogens, it was not surprising that SFD could occur but there was no forewarning that this specific disease would emerge or where in Canada it would first appear or will subsequently be found.

### Detailed answer

The past two decades has seen an unprecedented number of fungal and fungal-like diseases linked with the most severe die-offs and extinctions ever witnessed in wild animals and plants (Fisher et al, 2012). Chytridiomycosis is believed to have contributed to the extinction of more than 100 frog species (Skerrat et al, 2007). Microsporidian fungi in the genus *Steinhausia* have been linked to the eradicated of their snail hosts (Gurr et al, 2011). White-nose syndrome of bats has killed millions of bats since first detected in North America in 2007, leading several species onto the endangered species list. Emerging fungal infections causing widespread population declines have increasingly been documented across diverse taxa including bats, frogs, soft corals and bees (Fisher et al, 2012).

The pattern of SFD spread is not consistent with a point source introduction and Lorch et al (2016) concluded that *O. ophiodiicola* has been present in North America for a long time but recent environmental changes are driving SFD emergence. Skin lesions described as hibernation ‘blisters’ or ‘sores’ have been described for decades in snakes emerging from hibernation, but their causes were rarely explored. Fungal infections have been described in snakes for many decades, and have been associated with some population effects for a couple of decades. Historical reports of free ranging snakes with similar skin lesions and disease also exist and molecular evidence suggest the presence of *O. ophiodiicola* in captive snakes in the eastern USA since at least 1986 (Sigler 2013). Molecular testing conducted by the CWHC (unpublished) of archived samples from massasauga rattlesnakes suggests that *O. ophiodiicola* was present in Canada in 2012.

The lack of detections of SFD or *O. ophiodiicola* in western North America may be due to survey bias and/or lower disease prevalence or severity (Lorch et al, 2016). The same can be said for Canada. Snakes are difficult to find and monitor when hibernating, which may be a vulnerable time for SFD. The documented geographical distribution of *O. ophiodiicola* is broader among captive snakes than wild snakes (Lorch et al, 2016). An exact originating timeline remains unclear and a more detailed mapping of the geographical distribution of this fungus is needed to further understand this emerging disease (Lorch 2016).

There has recently been an increase in the number of free-ranging snakes with fungal dermatitis submitted to some US wildlife diagnostic laboratories[2]. The CWHC receives very few snakes for diagnostic examination (tables 3 and 4).

**Table 3 – Summary of free-ranging snake diagnostic examination conducted by the Canadian Wildlife Health Cooperative 2005-November 2016**

| Province | Year(s) | Animals examined |
|---|---|---|
| Alberta | 2012 | 1 |
| British Columbia | 2015 | 2 |
| Saskatchewan | 2005-16 | 14 |
| Manitoba | 2011 | 100 |
| Ontario | 2006-16 | 129 |
| New Brunswick | 2010 | 5 |
| Nova Scotia | 2008-09 | 3 |
| Prince Edward Island | 2006-16 | 6 |
| Total | | 258 |

**Table 4 – Variety of snake species examined at the Canadian Wildlife Health Cooperative 2005-2016 November**

| Snake species/group | Count | Snake species/group | Count |
|---|---|---|---|
| Boidae (Boas And Pythons) | 2 | Butler's Garter Snake | 5 |
| Colubridae | 1 | Common Gartersnake | 106 |
| Common Watersnake | 3 | Eastern Gartersnake | 19 |
| Eastern Foxsnake | 65 | Western Gartersnake | 1 |
| Massasauga | 21 | Wandering Gartersnake | 1 |
| Pacific Rattlesnake | 1 | Plains Gartersnake | 6 |
| Eastern Hog-nosed Snake | 1 | Eastern Ribbonsnake | 1 |
| Milksnake | 2 | North American Racer | 1 |
| Northern Brownsnake | 13 | Queensnake | 5 |
| Red Cornsnake | 1 | Red-Bellied Snake | 5 |

There are few Canadian researchers working with snakes or snake infections. They have generally not reported their disease findings to the CWHC. Canada cannot say, with any confidence, that there has been sufficient surveillance of snakes to conclude that SFD is new to Canada, but the balance of evidence in North America would suggest it is an emerging disease or at least the re-emergence of an endemic fungus due to changing environmental co-factors.

2 https://www.nwhc.usgs.gov/disease_information/other_diseases/snake_fungal_disease.jsp

## Can SFD cause serious harm?

### Key Points

- The effects of SFD vary within and between species and individuals
- The infrequency of linking population monitoring data with disease surveillance information prevents generalized conclusions of the population effects of SFD although there are cases that have implicated SFD as a causes of severe population declines.
- There is no evidence that SFD is a risk to public health or a risk for domestic animals apart from pet snakes.
- There are experts who consider SFD to be a conservation emergency because of some cases of apparent population impacts and the severe population effects of other emerging fungal diseases of wildlife.
- While there remain many uncertainties about the extent of population effects of SFD and the conditions under which these effects result in serious and irreversible harms, a precautionary approach would suggest it can be a significant additive stressor and should be considered serious for species at risk and/or for populations struggling with other cumulative stressors.

### Details

#### Evidence specific to SFD

The exact expression of clinical signs and disease severity differs between individual animals and species. This variation may be due in part to the exact stage of infection at which an affected snake is captured. Most disease reports represent a single observation within the timeline of lesion development and lack follow-up with affected animals over its life course. Given the secretive nature of snakes, this disease is extremely difficult to study under natural conditions and information on free ranging snakes is, therefore, extremely limited. SFD outbreaks are probably only identified once snakes contain well developed and/or extensive skin lesions with many cases presenting in a state of advance illness or death. As such it has been difficult to reconstruct the exact pathogenesis for this disease in wild free ranging snakes and identify any pertinent causal or contributory history, specific behavioural adaptations or coping mechanisms, that put animals at risk and ultimately contribute to an individual snakes survival or death (Lorch 2015).

Mortality in severe infections might be directly related to the fungal infection itself (especially in the case of disseminated infection to vital organs); however, the chronic and slowly progressive nature of this disease suggests that the majority of negative effects are probably related to secondary complications such as (i) skin ulceration and secondary infections; (ii) obstruction of nares interfering with vomero-nasal organ, affecting olfaction, and hunting; (iii) associated discomfort and pain resulting in anorexia and inappetence, (iv) oral lesions obstructing or interfering with normal feeding and or hunting (jaw misalignment, oral obstruction); (v) behaviours that increase the risks of predation such as increased frequency of basking and (vi) excessive metabolic demand due to frequent skin moulting. Infection can result in behavioural alterations leading to snakes being observed in exposed sites.

In some populations, SFD has been associated with significant population declines but population and individual responses to SFD has been inconsistent across species and locations, ranging from inconsequential to catastrophic. Allender et al. (2011) reported 100% mortality in Illinois massasauga rattlesnakes that had SFD. Clark et al. (2011) documented over a 50% decline in a population of timber rattlesnakes following the appearance of clinical signs consistent with SFD.

Of the few studies in which populations were monitored and population-level effects can be evaluated, there is one case in which decline was noted (Clark et al. 2011), and three in which no decline was noted (Cheatwood et al. 2003, Allender et al 2011, Smith et al. 2013). In Ontario, snakes with no apparent disease have testing positive for *O. ophiodiicola* by PCR used at the CWHC (these may be subclinical cases or just exposed animals carrying spores on their skin). The CWHC has not yet seen cases of severe disease or death due to SFD.

### Case Study - Self resolving SFD

Eight timber rattlesnakes from 2 populations in Massachusetts were observed with mild-to-severe SFD affecting primarily the head, eye, pit organs, and mouth (McBride et al. 2015). None died as a direct result of infection, although one died under anesthetic to debride the lesions and obtain samples. Five showed improvement or resolution of their lesions without treatment. Self-limiting SFD with spontaneous recovery in timber rattlesnakes has been reported (Clark et al. 2011, Smith et al. 2013), indicating that not all mild or moderate infections will progress to severe disease and death in the viperidae.

As with crotalid snakes, lesions in non-crotalid species vary in severity, but severe disease and mortality have been reported less frequently in non-crotalid species. In 2014 a free-ranging plains gartersnake (*Thamnophis radix*) in Illinois was found to have severe disseminated ophidiomycosis. Systemic infections are very rare in the literature but this case was like a case of systemic ophidiomycosis reported in a captive gartersnake in Europe (Vissiennon et al. 1999, Sigler et al. 2013) suggesting the possibility that gartersnakes may be unusually susceptible to systemic infection (Dolinski et al. 2014).

Although rattlesnakes (*Crotalus* spp. and *Sistrurus* spp.) are often implicated in clinical disease reports and have often been considered to suffer from especially severe disease (Clark 2011; Allender 2011; McBride 2015) similarly severe disease outbreaks have been reported in other species (e.g. Lake Erie watersnake, eastern foxsnake, and garter snakes (Vissiennon 1999; Dolinski 2014, Lorch 2016)). The more frequent reports in rattlesnakes may reflect unique susceptibility or may be biased due to more active monitoring programs taking place in these high-profile species. The determinants of this variability in observed population impacts are currently unproven and there is little direct evidence to forecast which populations are most vulnerable to harm from SFD. There has been **insufficient time to determine if/how severe impacts are reversible in affected populations and little work linking disease investigations with population data**. Appendix 3 provides some details on well reported cases of SFD.

### Opinion specific to SFD

**Some experts viewing SFD as a conservation emergency** based on; (i) experience with frog chytrid disease and white-nose syndrome; (ii) the growth in number of emerging fungal diseases in general; (iii) the detection of some cases where SFD has had significant negative effects and; (iv) the apparent increase in distribution and abundance of SFD cases. Supporting these concerns are similarities between *O. ophiodiicol*a and the fungus associated with white-nosed syndrome which has killed millions of bats. Both occurs in the soil, seem to grow on a wide variety of substances, and possesses many of the same enzymes. The virulence, long-lived environmental stages and opportunistic and generalist nature of fungal pathogens can create unique challenges for their control.

Land development, especially transportation networks, is increasingly fragmenting and isolating snake populations and this poses an increasing risk of inbreeding depression and increased susceptibility to disease. If the interactive and synergistic effects of stochastic environmental events, low genetic diversity and presence of a pathogen can result in an "extinction vortex" (Gilpin and Soule 1986, Clark et al. 2011) then, for isolated populations lacking genetic diversity and facing increasingly extreme, variable and unpredictable weather patterns due to climate change, the presence of an opportunistic pathogen like *O. ophiodiicola* could have significant impacts on long term viability.

### Evidence from analogy

The past two decades has seen an **unprecedented number of fungal diseases in both animals and plants, many of which have caused some of the most severe die-offs and extinctions ever witnessed in wild species** (Fisher et al, 2012). Fungal diseases of wildlife are of management concern for 3 reasons:

1. They can cause severe population declines and extinctions.
    a. Best known are frog chytrid disease, salamander chytrid disease and white-nose syndrome of bats. Bees, sea turtles, crayfish and otters have also been challenged by fungal disease as have a wide variety of plants. Fungi can cause population limiting effects because of their life history characteristics including; long-lived infectious stages, survival not dependent on hosts due to free living stages; being generalists and thus able to infected tolerant hosts that can maintain and shed the pathogen; rapid reproduction rates and high virulence. Thus, much of past dogma on the inability of pathogens to drive populations to extinct because of host density regulating effects do not necessarily hold for fungi.
2. They are symptoms of stressed environments.
    a. Fungi are often opportunistic pathogens that usually infect compromised hosts. The expansion in numbers of immunocompromised people is an explanation for emerging of human fungal diseases. It has been postulated that climate change, pollutants, habitat degradation and competition with invasive species may be stressing wildlife, making them more susceptible to endemic, opportunistic fungi.

## Are there high levels and/or persistent exposure to the threat?

### Key Points

- *O. ophiodiicola* is an environmental opportunistic pathogen that can persist off its host in a wide variety of ecological conditions. Multiple snake species can harbour the fungus. The hazard is, therefore, present in multiple exposure settings.
- The distribution of the fungus is greater than the distribution of SFD buts its true range and ecologic niche is unknown.

- There are anecdotal reports of SFD in captive snakes in Canada including outside of Ontario.
- The distribution of SFD has been expanding in North America but the mechanisms of transmission and spread are unresolved.
- Exposure to plausible co-factors is pervasive, especially for species at risk and considering climate change

## Details

*O. ophiodiicola* can affect multiple snake species and can persists in the environment. The global distribution of *O. ophiodiicola* is unknown (Allender et al, 2015). Its geographical distribution is broader among captive snakes than wild snakes, including Australia, Germany and the United Kingdom (Lorch et al, 2016). The distribution of SFD and variety of species affected by the disease indicate that *O. ophiodiicola* is widespread in eastern North America. Several factors support *O. ophiodiicola* occurring as an environmental saprobe (Allender et al, 2016). Laboratory work suggests *O. ophiodiicola* has characteristics that allow this pathogen to survive in numerous ecosystems, and thus provide a widespread opportunity for snake exposure (Allender et al, 2016).

"The mode of transmission and the influence of environmental triggers on prevalence of this disease are not understood" (Allender et al, 2015). *O. ophiodiicola* is occasionally found on the skin of snakes without any clinical signs of SFD or accompanying histological lesions (Bohuski 2015) suggesting that this fungus might acts as a skin commensal or at least an unapparent subclinical infection (Lorch 2016). CWHC observations to date in Canadian cases support this possibility.

The CWHC is aware of some SFD suspect cases in captive snakes in British Columbia. One was a cluster of cases in a group of ball pythons (*Python regius*) and common boas/red tail boas (*Boa constrictor imperator*) in a large reptile rescue facility. The animals had clinical and pathological signs of fungal infections but with conflicting *O. ophiodiicola* PCR results; a Canadian lab detected the fungus by PCR but a US lab reported negative PCR results. Re-extraction and retesting of the original extract in Canada again tested positive suggesting that the fungus might have been present as a transient carrier or skin commensal in some sections of the skin. The second case was in an emerald tree boa (*Corallus caninus*) from an American Association of Zoos and Aquaria accredited facility. The snake had clinical and pathological signs of fungal infections. Fungal cultures yielded *Colonostachys* sp. and *Trichosporon* sp. and PCR testing was positive for *O. ophiodiicola*. Samples could not be forwarded to a US lab for specialized fungal culture or further PCR confirmation as no CITES permit was available at the time for export to the USA.

Confirming the presence of *O. ophiodiicola* in skin lesions or in the environment has been hampered by difficulty in isolating the fungus in culture and problems in identifying of *O. ophiodiicola* based on morphological characteristics. Recent advances in molecular diagnostics will be an important adjunct to research aimed at establishing the prevalence, distribution and transmission of this fungus (Bohuski et al, 2015).

The eastern foxsnake and queensnake are both designated by COSEWIC as endangered. Shared causes of their decline include habitat loss, impacts of housing and cottage development, and intentional and direct harm from people. The foxsnakes are further challenged by wetland drainage for agriculture, and road mortality while introduced species and a specialized diet create unique challenges for the queensnake. Other Canadian species known susceptible to SFD share similar challenges. Small population sizes and slow reproduction reduces many species capacity to cope with or recovery from additive mortality due to SFD. For isolated populations lacking genetic diversity and facing climate change, the presence of an opportunistic pathogen like *O. ophiodiicola* could have a major impact on long term viability. Vulnerability assessment for disease in general and SFD specifically have not been undertaken for Canadian snake populations, so the extent of these environmental stressors has not been documented or considered from a snake health perspective. The provincial recovery plan for eastern foxsnakes notes the lack of a comprehensive health and disease screening study as an important knowledge gap.

McBride et al. (2015) pointed out that increased cloud cover and humidity associated with high precipitation can be especially detrimental in SFD infected timber rattlesnakes because these conditions are correlated with reproductive failure in females (Martin 1993, 2002, Clark et al. 2011) and they may have facilitate infection with *O. ophiodiicola* since fungal disease in captive reptiles is often associated with inappropriate temperatures, high humidity, and stress-related immunosuppression (Paré et al. 2007, Mitchell and Walden 2013). Inbreeding depression caused by Isolation of populations can lead to increased population susceptibility to disease (Frankham et al. 2002, Ilmonen et al. 2008, Townsend et al. 2009).

## Are there known, effective means to prevent, mitigate or eliminate the harms from the threat?

### Key Points

- There are no proven methods to prevent, mitigate or eliminate harms to populations from SFD.
- There has been some success in treating individual animals, an action that may be important in critically endangered species where each individual animal is required to maintain the genetic diversity of the population.

### Detailed answer

The relative novelty of SFD results in a scant body of literature or research done on response options. We are left to work from first principles of disease control to explore management options.
There are 6 general ways to attack any disease; (1) treat affected individuals or populations to speed recovery and limit impacts; (2) promote sufficient immunological response (innate or acquired) to reduce disease susceptibility; (3) avoid exposure through pathogen exclusion, host isolation or depopulation; (4) exclude all susceptible host from the exposure area through quarantine or culling; (5) modify environmental and social risk factors, and (6) reduce population vulnerability

**Option 1 – Treatment (Not viable for populations but a potential option for individuals)**

Reports of the effectiveness of treatments for SFD have been variable. No clinical trials or treatment case series have been published to date. The feasibility of delivering treatments either through drugs or environmental modifications are limited to managing clinical disease in individual captive animals due to practical constraints to delivering drugs or chemicals to free-ranging, but cryptic animals. This option should be explored to manage disease in endangered species where individual animals are highly valued and important for species persistence and diversity, but is not a viable population management response.

**Option 2 – Promoting immunity (Potential utility by promoting general, innate immunity)**

A leading hypothesis for increased fungal disease in wildlife is increased host population susceptibility resulting from environmental stressors (Fisher et al, 2012). Vaccines are used to reduce disease susceptibility. However, vaccines of any form, including for SFD, are not available for snakes. There remain significant challenges to making vaccines for fungal infections including; lack of capital for research; the belief that patients with significant fungal diseases may be immunologically compromised and a lack of basic knowledge of the markers to target for fungal vaccines. Antibody production is temperature dependent in snakes, therefore, vaccination delivery would need to be linked to the optimal temperature for an immune response. Coupled with the challenges in delivering sufficient vaccine to enough of a population to prevent disease in wildlife, **vaccination is unlikely to be a viable option** in the near to medium term.

Age, nutrition, general health, ambient temperature and season affect the innate immune system of snakes. In temperate species, organs important for immune function regress seasonally. See option 6 for further discussion on improving resilience to increase innate resistance to infection.

**Option 3: Avoid exposure through pathogen exclusion, host isolation or depopulation (Potential to minimize anthropogenic spread, otherwise not viable)**

While culling has been used widely in domestic animals to remove susceptible hosts in the face of an outbreak, this is unlikely to work in snakes because; (1) a number of species have legal protection due to their conservation status; (2) the prevalence and role of asymptomatic hosts that may be reservoirs for infection is unclear; (3) there are no surveys and methods to differentiate preclinical infections from negative animals and (4) there are ethical issues and public perceptions that speak against culling as an option.

See the next section on the possibility of geographically isolating SFD

**Option 4: Remove all susceptible species from environments with the pathogen (Not viable)**

The mechanisms of transmission of *O. ophiodiicola* has yet to be defined and the environmental distribution of this pathogen is unknown. This prevents identification of geographic locations from which to exclude or remove susceptible species.

**Option 5: Modify social and environmental risk factors (Uncertain)**

While hypotheses exist regarding the role of risk factors in the genesis of SFD, well designed epidemiological studies are lacking, thus preventing assignment of priority to putative risk factors to target in management plans. At this time, definitive evidence that *O. ophiodiicola* is the sole cause of SFD is inconclusive as additional fungi are isolated from affected snakes. There are opinions and hypotheses that environmental changes may be a risk factor, particularly as they relate to habitat quality and quantity, and weather (temperature and humidity). Some of these changes can be linked to anthropogenic influences on habitat and to climate change. Specific risk management advice must await further research.

**Option 6: Reduce population vulnerability (Uncertain but synergistic with other management goals)**

See the answer to the question below on reducing vulnerability.

## Can SFD be geographically isolated?

### Key Points

- Deficiencies in knowledge about the transmission system for SFD plus the practical limitations to constraining the movements of free-ranging makes isolation or quarantine impractical for wild snakes.
- There are opinions and evidence that *O. ophiodiicola* is an environmental fungus already further geographically distributed than current reports of SFD in North America.
- Because of prevailing uncertainties, a precautionary approach would be to limit further release of *O. ophiodiicola* into the environment through education campaigns to the pet trade and herpetologists to advocate for and support biosecurity to prevent unintentional movement of the fungus from a known SFD-positive area to areas of unknown or assumed negative status.

### Details

A second hypotheses for the increase in fungal diseases of wildlife is increased anthropogenic transfer of fungi between locations (Fisher et al, 2012).

While it is unknown if *O. ophiodiicola* was introduced from outside of North America, the prevailing opinion is that this is an environmental fungus that exists in many locations in North America. SFD has been found in multiple but unconnected locations over the same relatively short timeframe, leading to the supposition that SFD is not an example of an introduced pathogen, but rather that something has changed in snake populations, making them more susceptible to this environmental fungal. This conclusion can be challenged by the lack of finding snakes suffering from other opportunistic or environmental pathogens. This may reflect a unique aspect of *O. ophiodiicola* or difficulties in detecting sick and dead snakes.

Border protection and management of the pet trade may prevent the introduction of new pathogens and susceptible hosts, but is unlikely to affect the progress of SFD now that it has emerged. Given that *O. ophiodiicola* is an environmental opportunist, that its distribution has yet to be linked to specific landscape features, that the mechanism of spread and transmission *of O. ophiodiicola* is unknown, and the challenge of constraining the movement of free-ranging wildlife, it is not likely that susceptible snakes could be isolated from the pathogen. Fisher et al (2012) opined that biosecurity efforts for wildlife pale in comparison to protection of agriculture assets because wildlife are not correctly valued economically.

The role of mechanical transfer on fomites such as boots and field equipment has not been determined. However, a reasonable precautionary approach to minimize further the probability of anthropogenic distribution of SFD could be achieved by advocating for or requiring; (1) permissions and permits to translocate snakes for research or conservation that create a potential to move pathogens between habitats; (2) people handling snakes adhere to biosecurity and disinfection protocols suitable for fungal pathogens and (3) people working in areas known to be positive for SFD follow disinfection and biosecurity protocols before moving to areas of unknown SFD status. Disinfection and biosecurity protocols will need to be based on basic principles and expert opinion until research is conducted to determine the optimal protocols.

## Can vulnerability be reduced to SFD?

### Key Points

- SFD has characteristics of an endemic disease that has occurred due to an increased in host range or pathogenicity due to changes in the pathogen, host and/or environment
- Strategies to combat endemic pathogens emphasize investigating and managing co-factors, synergies, and context dependencies
- There is evidence that environmental co-factors, especially climate change, and habitat loss and alteration may be important co-factors determining risk and impacts of SFD
- Given that vulnerability is the combined outcome of exposure, susceptibility, capacity to cope and cumulative effects of other stressors and given that there are no current options to reduce exposure or susceptibility, vulnerability reduction must focus on improving individual and population resilience and managing co-stressors
- The suggested co-factors influencing the spread and effects of SFD overlap with the major challenges facing snakes in Canada, therefore, SFD management should be integrated into species management plans due to synergies in current recovery plans and best evidence to deal with SFD.

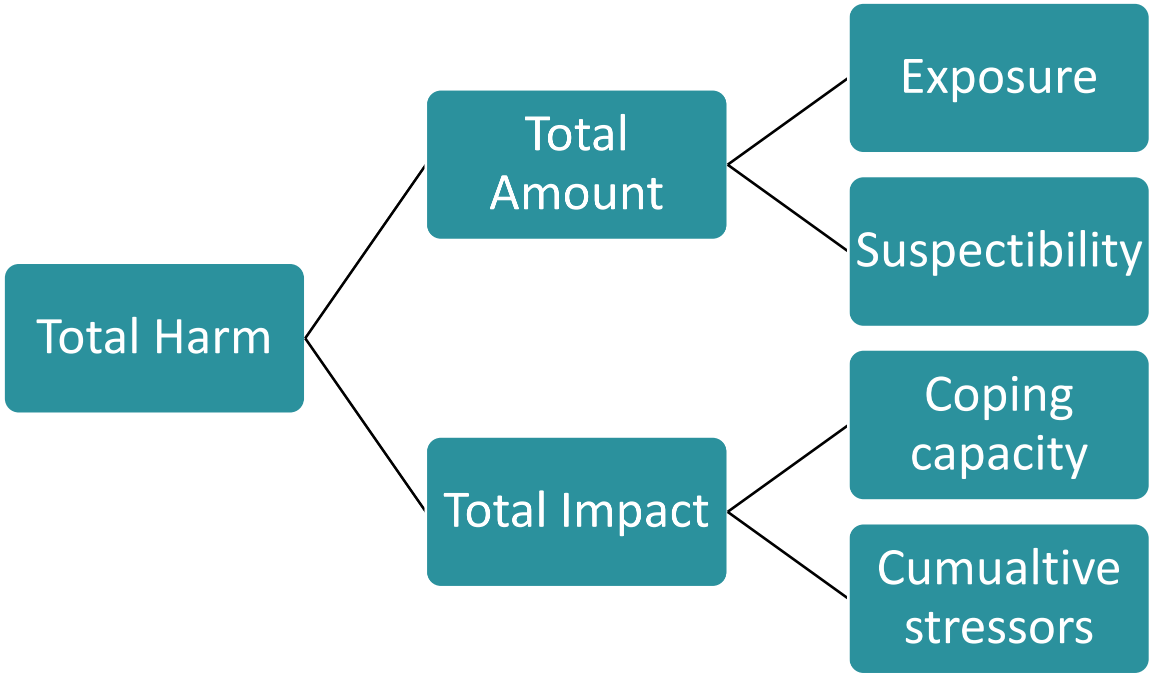
### Details

Vulnerability is the state of susceptibility to harm from exposure to stresses associated with environmental and social change and from the absence of capacity to respond to, and recover from stressful events (Adger, 2006). Answers to the preceding questions of the decision framework indicate that there are no options to promote acquired immunity to SFD and that non-specific promotion of innate immunity may most likely be achieved by securing appropriate habitat for thermoregulation, adequate prey of suitable quality and sufficient genetic diversity. The preceding answers also indicate that there are no known viable options to prevent wild snake exposure to *O. ophiodiicola* apart from generic recommendations on biosecurity and import controls. Vulnerability management for SFD seems dependent on promoting the ability of snakes and snake populations to respond to and recovery from the disease.

The first ever successful eradication of a fungal disease of wildlife through interventions on the chytrid-infected island of Mallorca provided enthusiasm for biomedical interventions against emerging fungal diseases or wildlife (Fisher et al, 2016) and several researchers are seeking similar approaches to diseases such as white nose syndrome and salamander chytrid infections. But challenges in delivering medications, vaccines or environmental disinfectants plus the persistence of *O. ophiodiicola*, and the presence of multiple and cumulative stressors affecting many snake populations argues for a pragmatic approach focused on helping species cope with SFD to foster long-term host–pathogen coexistence.

Harm reduction (Figure 2) aims to reduce the total amount of harm by reducing population vulnerability to the harms as well as reducing the total impact of the harms by promoting the populations capacity to cope with the specific harm and reducing the impacts of cumulative effects of other stressors. Harm reduction also tries to reduce the adverse consequences of a health threat without necessarily reducing that threat. Interventions may be targeted at the individual, the population, community or ecosystem. Its goal is to optimize population health within the current realistic circumstances.

**Figure 2 – Generic harm reduction model**




For any emerging disease, either the disease has recently spread into new geographic areas or it has been present in the environment but recently emerged. SFD has characteristics of the latter. Strategies for an introduced disease often focus on identifying and controlling agents of spread, whereas strategies for latter focus on minimizing co-factors affecting vulnerability or enhanced virulence (Rachowicz. 2005). Proposed co-factors to SFD are also critical threats to snake conservation in Canada. Vulnerability assessment and management are also fundamental components of climate change adaptation and preparedness. Therefore, co-benefits across programs could be achieved through a vulnerability focus on SFD management.

### Options to consider for vulnerability management

1. Reduce the amount of harm
   a. Reduce exposure
      i. Not viable (See preceding discussion on option 3 above)
   b. Reduce susceptibility
      i. Promoting specific immunity is not viable (See option 2 above)
      ii. Reptile immunity involves innate, cell-mediated and humoral compartments but, there is comparatively little known about immune function in reptiles. Resistance to pathogens is associated with fitness (Uivari and Madsen 2006) and stressful situation have been associated with lowered disease resistance in reptiles (Oppliger et al 1998). For reptiles in captivity, excessively high humidity, low environmental temperature, already having another disease, malnutrition, and stress from poor husbandry can affect the development of fungal diseases[3], suggesting landscape attributes, including habitat quality and quantity, climate, and prey availability may affect SFD susceptibility.
2. Reduce the cumulative effect of the harm
   a. Increase coping capacity.
      i. Coping strategies are closely related to resources and assets. For snake populations to cope with threats like SFD, it can be hypothesized that there must be either; (i) reproduction rates exceeding or equivalent to additional mortality from SFD; (ii) adequate genetic variability to allow populations to adapt to the disease if/when it evolves to a more benign manifestation; (iii) suitable habitat connectivity to allow animals to relocate to areas with low rates of exposure to the threat and/or (v) suitable habitat to meet snakes needs for daily living (food, shelter, security, appropriate weather)to support innate immune functions.
   b. Reduce other stressors causing adverse cumulative effects that reduce the diversity and numbers of animals needed to help populations withstand and recover from SFD
      i. Habitat loss and degradation, additional mortality or loss due to anthropogenic effects (ex. roads, intentional killing, collection for the pet trade) and climate change are common challenges to at risk snakes in Canada. Many species are exceptional sensitive to additional causes of mortality due to their low reproductive rate and prolonged developmental times. Reducing these and other stressors may have co-benefits of addressing putative SFD co-factors, addressing priorities in recovery plans and promote species resilience to both climate change and disease.

## Monitoring and Surveillance

### Key Points

- Evidence-based selection of priority areas and species to implement SFD management is complicated by the lack on routine snake disease surveillance and due to the lack of monitoring SFD plausible co-factors

3 http://www.merckvetmanual.com/pethealth/print/exotic_pets/reptiles/disorders_and_diseases_of_reptiles.html

- If SFD management steps are taken, monitoring, as a minimum, should be advocated to assist in adaptive management of SFD
- The CWHC has diagnostic capacity and expertise for snake disease surveillance but requires assistance from resource and wildlife agencies to encourage more submissions of snakes
- Efficiencies can be gained by integrating disease surveillance and SFD co-factor monitoring with ongoing population and habitat assessments being used to support species recovery plans.

### Details

The decision framework in appendix 1 identifies two situations where surveillance or monitoring is the principle response.

1. In situations where there are susceptible species but no evidence that an expected disease results in serious or irreversible harms, an appropriate action would be to monitor co-factors that could influence the populations vulnerability or alter important causal variables that affect the magnitude or likelihood of harm. This would be recommended in situations where; (i) the populations involved are highly valued or highly vulnerable to declines from other or cumulative effects; or (ii) when there are few options to avoid the harms from the disease if the epidemiological conditions change to favour disease outbreaks or changing virulence or impacts.

2. In situations where an unexpected disease affects susceptible species without evidence of harm, it is reasonable to undertake disease surveillance because of uncertainties about how an unexpected disease will affect populations of concern. Monitoring, rather than surveillance might be advocated for in situations where the affected populations are not at conservation-risk or there are viable options for rapid and effective interventions to mitigate or avoid harm. The secretive nature of snakes makes monitoring the effects of SFD difficult. Further complicating elucidation of SFD impacts are the clear variations in disease severity between different geographic regions which may be linked to strain differences in the pathogen, genetic composition of a given snake population, or environmental and/or behavioural factors that influence disease ecology.

#### SURVELLANCE VS MONITORING

**Monitoring** - intermittent performance and analysis of routine measurements and observations to detect changes in the environment or health status of a population, but without eliciting a response.

**Surveillance** - Systematic and continuous collection, analysis, and interpretation of data, closely integrated with the timely and coherent dissemination of the results and assessment to those who need to know so that action can be taken.

A third surveillance scenario occurs when a disease known to cause harm exists and an intervention is implemented. Surveillance allows managers to evaluate the impacts of interventions and provides ongoing feedback regarding the need to modify interventions based on changes in the effects and distribution of the disease.

A fourth scenario involves the need to implement targeted surveys to answer specific research questions including establishing the descriptive epidemiology of the disease (who is affected, where, when and what harms it causes) or to help provide foundational information to support causal research.

Selection of which form(s) of surveillance or monitoring is required/desired is predicated on management objectives, the cost: benefit of surveillance and the implications of being wrong if important changes to the disease behaviour are undetected. SFD is partially expected, may under certain conditions cause serious harms but lacks specific interventions to attack the disease. It, therefore, falls across a number of the monitoring and surveillance scenarios. The prevailing uncertainties about the disease suggest that scenario #4 may be advisable and that hypothesis driven surveillance at strategic sites is most appropriate.

Table 3 reveals that the CWHC has a very limited insight into trends in snake diseases due to the very low numbers of animals submitted to the laboratory. The CWHC-Ontario/Nunavut and Pacific regional centres have developed the necessary diagnostic capacity to detect SFD. CWHC-Ontario/Nunavut has launched small scale, local, cooperative scanning surveillance. Further surveillance initiatives in Ontario will require consultation with provincial partners.

## Conclusions and recommendations

Wildlife disease is now recognized as an increasingly frequent contributor to species decline and extinction and is becoming a more regular management concern (Dasak et al, 2000) It can be anticipated that with climate change, there will be growing needs to evaluate the emergence of new pathogens and parasites in wildlife (Stephen and Duncan, in press) and that in each of those situations, there will be high degrees of uncertainty. Not all emerging threats are of equal significance but there is no agreed upon means to rank relative importance of emerging disease threats in wildlife and to develop an explicit rationale for actions or inactions. In this report, we have used a decision framework to systematically consolidate the existing information on SFD and overlap it with basic disease control principles to assess SFD as a biodiversity threat and identify a reasonable course of action.

We have concluded the following.

### A management response to SFD is justifiable

- The Species at Risk Act notes – "the Government of Canada is committed to conserving biological diversity and to the principle that, if there are threats of serious or irreversible damage to a wildlife species, cost-effective measures to prevent the reduction or loss of the species should not be postponed for a lack of full scientific certainty." The Act directs managers to consider Canada's commitments to the conservation of biodiversity and to the precautionary principle when preparing recovery strategies.
    - SFD has the potential for serious harm in species already at risk. Despite significant scientific uncertainty, a precautionary approach can be justified as the harm reduction steps for SFD would complement actions recommended under species recovery plans and/or climate change adaptation planning.
    - The potential for harm is derived in this assessment in part from some reports of adverse

population impacts but more so from the finding that many species at risk in Canada are susceptible to SFD and are already over burdened with multiple other stressors.

- In the document, "Planning for a Sustainable Future: A Federal Sustainable Development Strategy for Canada 2013–2016," Target 4.7 includes an implementation strategy of providing information to reduce the risk of, and advice in response to, the occurrence of events such as wildlife disease events[4].
  - o The review has revealed weaknesses in disease surveillance for reptiles in general and SFD specifically as well as the need to coordinate information amongst stakeholders through information sharing.
- The Wildlife Health/Issues Section (Wildlife Management and Regulatory Affairs Division. ECCC) has responsibility to manage Canadian Wildlife Service environmental emergency preparedness policies and contingency plans for national consistency and effectiveness
  - o SFD should be considered a biotic emergency until evidence can be found to show it is not capable of severe population limiting effects. An initial response plan should define the threshold to 'de-classify" SFD as an emergency.

**Despite uncertainties, a response based on harm reduction principles is justifiable and can act in synergy with recommendations in some species recovery plans**

- Figure 3 summarizes a population health approach, adapted in brief to SFD. A SFD management program, rather than a single biomedical or biological intervention is recommended.
- Some monitoring and surveillance should be supported to start to fill some gaps on spread and distribution of SFD and to help identify most vulnerable populations upon which to strategically focus disease and risk monitoring.
  - o It would reasonable to advocate for increased scanning surveillance (achieved through efforts to encourage more snake submissions to the CWHC across Canada) and zonal surveillance targeting the edges of known SFD occurrence in Canada.

Figure 4 summarizes the answers to the questions outlined in the decision tree, supporting a recommendation to focus on reducing vulnerability as the principle strategy for SFD in Canada at the present time.

4 http://www.ec.gc.ca/dd-sd/default.asp?lang=en&n=CD4179F6-1

**Figure 3 – Application of population health principles for SFD harm reduction**

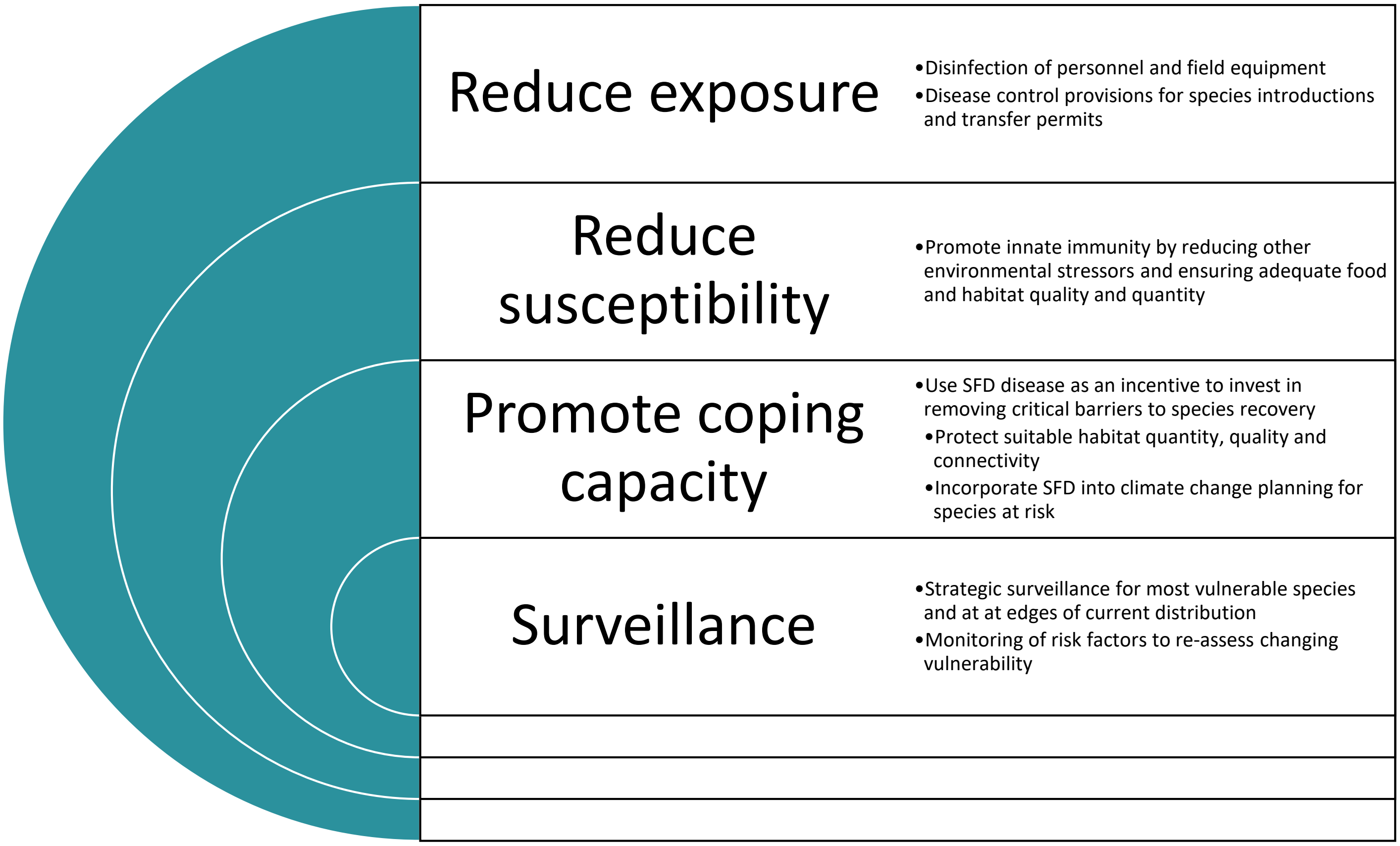
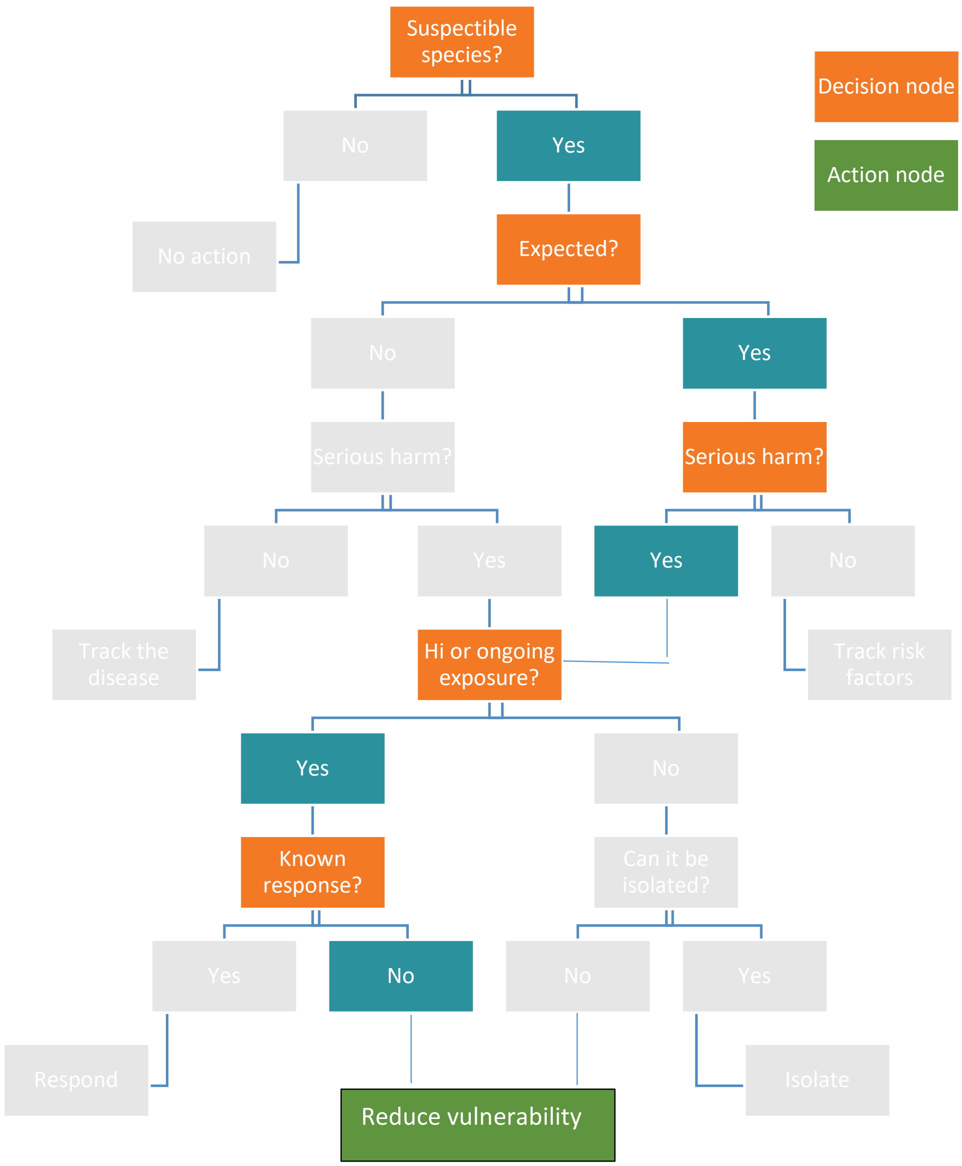


**Figure 4 – Application of the decision tree to snake fungal disease in Canada**




## Reduce exposure

Develop and distribute standardized recommendations for disinfection and handling of snakes, especially in or at the edges of known geographic distributions of SFD.

- **Disinfection**
  - SFD specific disinfection protocols are not yet available but should be pursued. ECCC should spearhead the development of generic protocols that are adaptable to various species and pathogens for use in all wildlife handling scenarios. Disinfection protocols recommended for the control of chytrid fungi in amphibians may act as interim recommendations.

- **Handling**
    - Experimental evidence suggests that breaks in the superficial keratin layer might play a significant role in early infection and the pathophysiology of the disease (Lorch et al, 2015). This could inform recommendations on the application of external markings or implantation of transmitters or other abrasive methods of identification that might put some snakes at an increased risk for developing SFD. Alternative methods of identification and monitoring should be considered in populations where SFD has already been identified.
- **Translocations**
    - Wild snakes should not be moved and released into new locations without assessment of SFD risks
    - Captive reptiles should never be released into the wild.
    - ECCC should keep up-to-date on emerging evidence that links SFD with the pet trade, but to date, there is no evidence to implicate pet importation for the introduction or spread of SFD.

### Reduce susceptibility

- SFD has characteristics of an endemic opportunistic pathogen that has been present in the environment but has increased in pathogenicity because of environmental changes or possibly, simply escaped previous human notice.
    - Strategies for these types of diseases focus on managing co-factors that may either increase exposure or increase susceptibility to infection or worsen the effects of infection (Rachowicz et al, 2005).
    - Immunosuppressive environmental stressors that may predispose snakes to *O. ophiodiicola* infection can be hypothesized to be like population stressors considered in species recovery plans associated with habitat abundance and quality, prey availability, and habitat connectivity to allow for movement and sharing of generic diversity.
        - Species recovery should account for SFD risk factors to address individual and population susceptibility

### Promote Coping Capacity

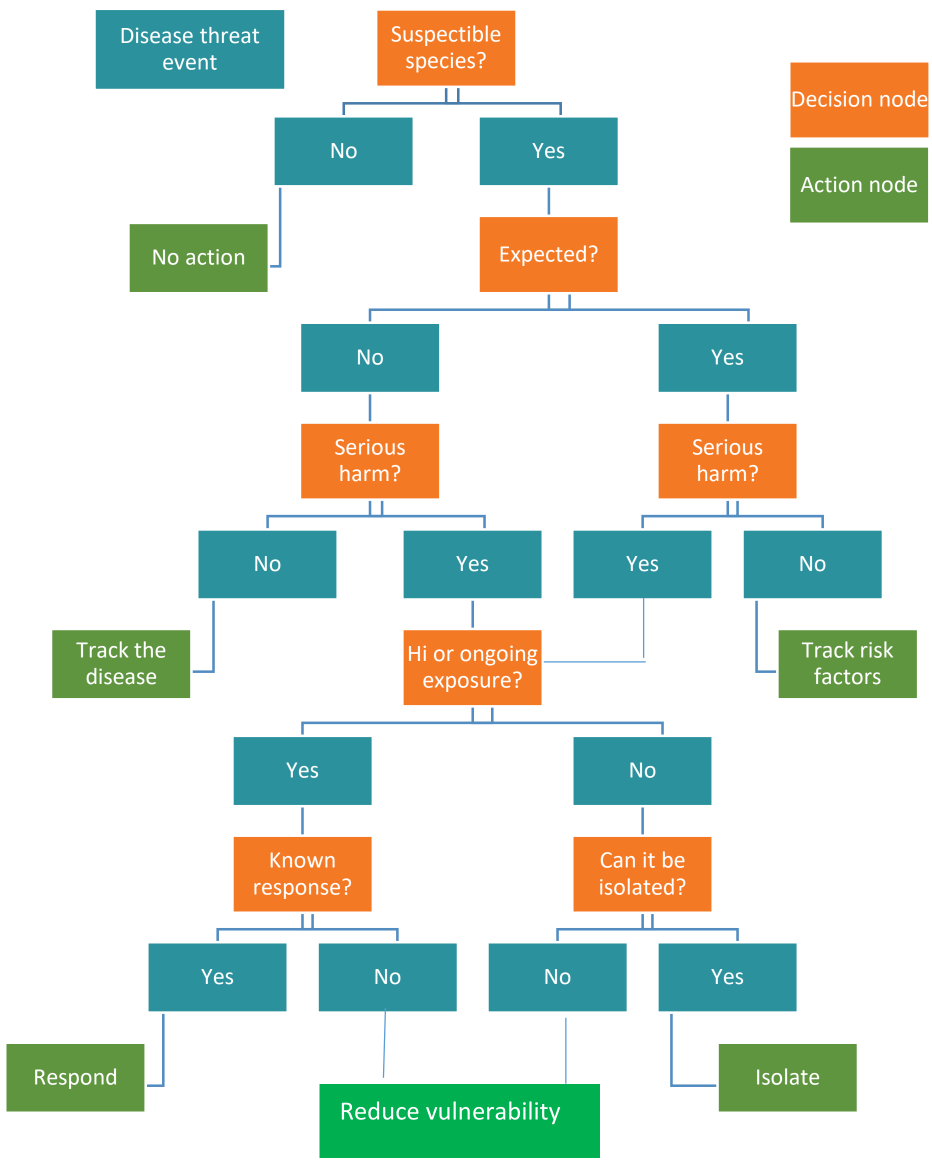
- Resilience can be defined as the capacity to respond to a disturbance by resisting damage and recovering quickly or as the capacity to absorb change while exhibiting a similar set of structures and processes. By adapting some concepts of managing for resilience from public health and conservation, we can conclude that a focus on coping capacity would need to include the following:
    - Adaptive management based on ongoing monitoring of factors that could affect functions or impacts of SFD in the foreseeable future
    - Investment in preparedness that identifies vulnerable populations in advance of harm and acts to reduce other impediments to recovery and ability to maintain function in the face of SFD emergence

- Ensure populations have the basic 'building blocks" of coping and recovery capacity by protecting abiotic determinants of health including habitat, generic diversity, population connectivity and species abundance and distribution.

**Surveillance and Monitoring**

- SFD surveillance, and snake disease surveillance more generally, should not be viewed as a stand-alone activity, but instead as a component of snake conservation management.  Monitoring to establish the descriptive epidemiology of SFD as well as to better associate environmental risk factors with impacts and susceptibility to SFD will help improve the biological understanding of SFD and better direct future management.
- The restrictions associated with shipping of medical samples for diagnostic testing of CITES classified animals will require that sufficient and reliable testing modalities be available within Canada. The CWHC has two regional centres with *O. ophiodiicola* PCR capacity and experienced diagnostic pathologists through all centres. ECCC should explore means to overcome these CITES associated constraints on wildlife diagnostic testing.
- There are unique challenges to undertaking surveillance in snakes due to their cryptic nature, the lack of investment in population monitoring and small the cohort of people with active interests in tracking snake populations
    - Active outreach to the herpatological community to advocate for increased submissions of snake samples to CWHC diagnostic labs is a short term, no-to-low cost methods to increase our understanding of snake diseases in Canada.
- Cooperation with provincial partners could foster low-cost epidemiological studies if more effective integration of disease surveillance information with population data was encouraged.

# Appendix 1: Decision tree framework for the threat assessment of SFD



## Using the decision tree

### Answer questions posed at decision nodes

### Decision Node D1- Are there species susceptible to the threat?

Susceptibility considers the extent to which individuals, populations or communities are negatively affected, influenced, or harmed when exposed to a threat. A species is considered susceptible if endogenous factors are sufficient to allow the threat to manifest as a disease process in individuals and/or if exogenous factors make a populations/communities or ecosystems more likely to experience harms from the threat.

A threat is subjectively scored higher in this decision node when:

- More than one species is susceptible
- Species in multiple genera (including people) are susceptible
- The species/population of interest is experiencing multiple stressors that might magnify or make more likely the additive harm from the threat.

### Decision node D2 – was the threat expected?

Unexpected threats are known to influence the response to a health threat as well as perceptions of risk. Decision makers at all scales are concerned about reducing the likelihood of surprises because surprises reduce our trust in the knowledge and people upon whom we rely to protect us. This typically requires a more precautionary approach to be taken when managing unexpected events until the level of uncertainty is reduced.

A threat is subjectively scored higher in this decision node when:

- there is analogy to indicate the threat is likely to cause harm
- the number of effected species/populations and geographic distribution rapidly increases.
- there is a diversity of species affected

### Decision node D3 – can the threat cause serious harm?

Final judgement of the threshold for to establish if a threat is serious can be subjective and based on risk perceptions. A threat will be considered able to cause serious harm if:

*Individuals*: (i) Animals - it causes morbidity or mortality that impedes the animal's ability to fulfill it expected ecological or economic function. (ii) People – it causes morbidity or mortality or causes notable economic or cultural impacts

*Populations*: the threat constrains the abundance and/or distribution of a population beyond capacity for the population to compensate and/or it reduces availability or safety of an ecological service provided by the population.

*Community*: Multiple populations are affected by the threat.

A threat is subjectively score higher in this decision node when:

- The harms are irreversible
- The population is unable to cope with or compensate for the harms
- More than one harm occurs

### Decision node 4 – is there high levels and/or persistent exposure to the threat?

Risk cannot exist if there is no exposure to the hazard causing the risk. Exposure is generally not homogenous over space and time or within populations and communities. Sub-populations with higher exposure are at higher risk, assuming equivalent susceptibility.

A threat is subjectively scored higher in this decision node when:

- The hazard can persistent in the environment
- The hazard is present in multiple exposure pathways
- There is a large amount of the hazard or high concentrations in the environment

### Decision node 5: Are there know, effective means to prevent, mitigate or eliminate the harms from the threat?

Risks can be eliminated, reduced or managed to tolerable levels if there are rapid, cost-effective means to reduce the exposure or harms from a hazard. This actions should cause as little damage as possible and not result in additional harms.

A threat is subjectively scored higher in this decision node when:

- There is little or no evidence with which to prescribe the most effective and efficient actions
- The cost of the actions exceeds the benefit and/or the actions are not practically or socially feasible or acceptable
- Collateral serious harms to the affected or connected species/populations may occur due to the risk mitigation actions

### Decision node 6: Can the threat or harm be isolated or contained to minimize its geographic distribution to tolerable levels?

The harms associated with a threat are more likely to irreversible or unacceptable when they can become dispersed over a larger geographic area, thus exposing more susceptible individuals and/or increasing the extent and duration of exposure opportunities. Spread of a disease increases the likelihood that vulnerable populations may become exposed.

A threat is subjectively score higher in this decision node when:

- There are no proven, feasible or socially acceptable means to isolate or contain a threat or vulnerable populations to prevent geographic spread
- Actions to isolate the threat cause unacceptable collateral damage to species or populations of concern
- The mechanisms of spread of the threat are unknown

## Dealing with uncertainty at a decision node

Under conditions of uncertainty we assume an answer is yes if; (i) analogous situations present a reasonable probability the answer could be yes; (ii) the species at risk are highly valued; (iii) there are multiple species (including people) that may be at risk and/ or; (iv) there are multiple possible harms

## Details and justifications of the action nodes

### Notes on interpreting the action nodes:

The nodes are not mutually exclusive. Most effective disease control programs require multiple strategies to be successful. The actions nodes reflect the major emphasis or goal to which control efforts should aspire but they do not prescribe the specific means, or combination of means, to achieve those goals.

### Action Node 1 – No action taken

Scenario - there is high confidence that no susceptible species or populations exist in plausible exposure pathways, indicating no risk exists. The goal in this step is to provide assurance of no harm through risk communication. If there is a high perception of risk about the disease event, research can be undertaken to confirm lack of susceptible populations and/or to determine and monitor the factors that could alter species or population susceptibility.

### Action node 2: Track the disease through surveillance or monitoring programs

Scenario - susceptible species/populations exist for an unexpected disease, but there is no evidence it can cause harm under the current conditions. The goal at this step is to provide assurance that the disease epidemiology is either not being inappropriately characterized as low risk and to contribute to preparedness plans in anticipation of changing disease epidemiology. Because the disease is new, emerging or re-emerging, there will be some uncertainty regarding how it may behave, especially under changing environmental conditions. If the susceptible populations are highly valued, efforts should be placed to track the disease, to monitor its spread in time and place, and evaluate if that spread is related to unanticipated serious harms. Whether this takes the form of systematic surveillance, periodic surveys and/or research depends on the value placed on the affected populations.

**Action node 3 – Monitor risk factors**

Scenario -an endemic of expected disease event occurs involving susceptible species, but the disease is not known to cause serious harm. Because disease ecology and epidemiology is not a fixed characteristic of a disease, the impacts of a disease can change with social and ecological change. The goal of this step is to maintain a situational awareness of an endemic risk to inform preparedness plans in response to changing risk factors. If the susceptible species are of high value or provide highly valued services, programs should be in place to review and/or monitor risk factors that may influence the epidemiology and impacts of the disease.

**Action node 4 – Implement disease response plan**

Scenario -a susceptible species is affected by an expected or unexpected disease that can cause harm and there are widespread or high levels of exposure. Actions should be taken to reduce the distribution and impacts of the disease. The goal of this step is to mitigate the harms from the disease. This action is taken when there is evidence of effective, cost-efficient means to mitigate the harms from the disease, prevent other populations from being effected and/or reduce the geographic spread of the disease.

**Action node 5 – Contain or isolate the disease and hazard to a restricted geographic area**

Scenario - a susceptible species is affected by an expected or unexpected disease that can cause harm but there is reasonable evidence that the geographic distribution is restricted to a focal location. The goal in this step is to restrict the harms to a specific geographic location. Options to restrict the movements of infected populations or the hazard should include human dimensions of species and hazards transmission and will require a good understanding of transmission dynamics and environmental persistence of the hazards.

**Action node 6 – Reduce the vulnerability of the populations at risk to the harms of concern**

Scenario - a susceptible species is affected by an expected or unexpected disease that can cause harm but there are no evidence-based, cost-effective means to mitigate the harm or isolate it to a specific location. The goal at this step is to help the species cope with and recovery from the impacts of the threat. Options to reduce vulnerability include; reducing exposure to the threat, reducing susceptibility, reducing other cumulative stressors and providing the intrinsic and extrinsic capacity to tolerate, cope and recover from harms.

# Appendix 2: Diagnosing snake fungal disease

**SUSPECT case definition** – that could be used as a tentative case definition based on clinical signs for use in the field.

### Grossly visible lesions of SFD

- Sign are typified by patchy areas of thick light brown to yellow scabs, crusty scales, patches of irregular skin thickening and pallor (hyperkeratosis), or superficial fluid filled blisters or pustules. Chronic cases often include firm, encapsulated, subcutaneous nodules which are indicative of deep dermal invasion, granulomatous inflammation, and fibrous encapsulation which often may result in swelling and distortion of the face and head. Lesions are especially prominent within areas of the skin that are typically exposed to natural abrasive forces such as the ventro-lateral aspects of the mouth, jaws, head, face, and nose tip, and the ventral scutes extending the entire length of the snake. Although these locations are the most typically affected areas, lesions may extend into adjacent surfaces or can be scattered anywhere along the skin surface of the snake. Affected snakes often also undergo frequent bouts of skin shedding/molting (premature separation of the superficial epidermis not within a normal molt period). Lesions often appear less apparent following shedding; however, affected foci often retain small patches of adhered skin shed (dysecdysis). Affected foci can often also be identified in the skin shed itself as irregular mottled, orange to brown, foci. Extension of the fungal organism into the specialized keratin layer overlying the eye (spectacle) often also results in edema and thickening which grossly appears as opaque cloudiness of the eye. Associated inflammation often results in subcuticular edema (anasarca) or fluid accumulation (fluid filled vesicles) between an impending skin shed and the newly formed epidermis. Immediately after infected snakes shed their skin, lesions may be less noticeable (at least temporarily).

**CONFIRMED case definition** if accompanied by characteristic clinical and histological lesions

### Histopathological lesions

- Histologically there is coagulative necrosis of the superficial keratin layers of the epidermis with epidermal thickening and hyperkeratosis and/or full thickness ulceration into the underlying dermis. Lesions often contain few to large numbers of fungal hyphae that are predominantly distributed along the skin surface and superficial keratin layers with extension of fungi along areas of necrosis and ulceration into the underlying dermis, subcutis and occasionally skeletal muscle layers and/or bone. In chronic cases, subcutaneous nodules are composed of central cores of eosinophilic necrotic debris with admixed fungal hyphae that are walled off by variable numbers of heterophils, macrophages, multinucleated giant cells, and peripheral layers of fibrous encapsulation (typical heterophilic granulomas). Hyphae are hyaline and clear, measure between 2.0 to 7.0 µm wide, contain parallel to slightly irregular sides, are occasionally septate, and exhibit rare dichotomous branching, and occasional characteristic rectangular arthroconidia are formed by surface hyphae (Rajeev 2009; Allender 2011; Latney 2013; McBride 2015). Hyphae can be accentuated with special histochemical stains (e.g. Periodic Acid-Schiff; Grocott's Methenamine Silver) which are especially helpful in chronic heterophilic granulomas where hyphae are often few in numbers and/or blended with necrotic debris. Although the histo-morphological features are often highly suggestive of *O. ophiodiicola*, it can be difficult to differentiate it from other common opportunistic fungal infections in snakes (e.g. *Trichophyton* spp., *Fusarium* spp. etc.) or to identify specific characteristic hyphal features in mixed fungal infections. Therefore, specific pathological criteria for the disease have yet to be established and additional ancillary diagnostic testing such as molecular PCR tests and/or fungal culture and identification is often necessary to ultimately confirm the diagnosis (Rajeev 2009; Allender 2015a; Bohuski 2015).

### Molecular diagnostics/PCR testing

- This is the most sensitive tests available. It should be considered that a positive test results does not confirm a diagnosis of SFD but rather confirms the detection of the presence of *O. ophiodiicola*.
    - Tests available
        - Allender et al. 2015a – currently available at Animal Health Laboratory, University of Guelph, Box 3612, Guelph, Ontario, Canada. Real-time PCR (qPCR) assay that targets the internal transcribed spacer 1 region between the 18S and 5.8S ribosomal RNA gene. Cross reaction with closely related fungal species not specifically investigated; however, sequence analysis of the targeted region should be specific for *O. ophiodiicola*.
        - Bohuski et al. 2015 PCR is available at United States Geological Survey (USGS) National Wildlife Health Center which has the mandate to test free ranging native snakes but will do captive or exotic trade snakes as part of research projects to establish the global distribution of the fungus. Consists of two real-time PCR (qPCR) assays, one that targets the internal transcribed spacer region (ITS) of the fungal genome, while the other targets the more variable intergenic spacer region (IGS). The NWHC has investigated the potential for cross reaction with 28 closely related fungal species and found none.
        - White 1990 – conventional PCR assay targeting fungus-directed 18s rRNA gene and used for general identification of various fungal species based on the generated DNA sequence. Although this is a functional assay for most fungal infections it has performed poorly with cases of *O. ophiodiicola* (personal communication Dr. Jeff Lorch USGS and personal experience with suspect SFD cases at Animal Health Centre British Columbia). Mixed infections also pose a significant hurdle and the mixed DNA sequence is often either impossible to decipher or the sequence from the most numerous or fastest growing fungus might dominate and overwhelm the sequences obtained from other fungi.

### Fungal culture and morphological identification

- The original description and characterization of this fungus outlines a variety of diagnostic morphologic features and biochemical test results for *O. ophiodiicola* (Rajeev 2009). The most distinct morphological features of this fungus on culture include the formation of abundant narrow, cylindrical-to-slightly clavate conidia. Cultures are accompanied by a strong pungent odor characteristic of onygenales (order of keratin lysing) fungi. Although highly effective if fungal growth is obtained, the most significant drawback of fungal culture is the extended time frame needed to reach a definitive diagnosis. *O. ophiodiicola* is also reportedly a fastidious and slow grower that is often outcompeted by other fungal organisms (either as co-infections or environmental contaminants) and specialized media is required (personal communication Dr. Jeff Lorch USGS).

# Appendix 3: Summary of key cases in SFD history

**Free-ranging snakes -- USA**

## The first documented report of fungal disease in free-ranging snakes in North America

Severe necrotizing fungal dermatitis, stomatitis and opthalmitis was diagnosed in 1997-98 in pygmy rattlesnakes from the Lake Woodruff National Wildlife Refuge in Florida (Cheatwood et al. 2003). In 1997-1998, in a population of roughly 600 snakes (May et al. 1996), 16 pigmy rattlesnakes with severe eye, head, mouth, and multifocal skin lesions were found during regular surveys of the study site, nine of which were either found dead in the field or were moribund (Cheatwood et al. 2003). At the peak of this outbreak, one gartersnake (*Thamnophis sirtalis*) and one ribbonsnake (*Thamnophis sauritis*) also were found with lesions consistent with SFD. A retrospective analysis of capture records between 1992 and 1997 revealed a further 59 pygmy rattlesnakes with signs consistent with fungal disease in this population, but these lesions all were characterized as focal to multifocal mild integumentary lesions without apparent mortality. It is not clear if this incident was a SFD outbreak. Fungal culture of samples from affected animals failed to grow *O. ophiodiicola*. The unusual mortality and six-fold increase in incidence of disease in 1997-1998 relative to the balance of the 1992-1999 interval support the characterization of this incident as an epizootic.

## The first case of population decline associated with SFD

In 2006, signs of fungal dermatitis were associated with the decline of an isolated population of the timber rattlesnake (*Crotalus horridus*) in New Hampshire (Clark et al. 2011). Other populations of this species monitored at the same time were apparently unaffected and disease was nominated as an important constituent of a suite of factors driving the observed decline (Clark et al. 2011). The effected timber rattlesnake population was exceptional in that it was a small, isolated, last known population of timber rattlesnakes in New Hampshire. The population had been constant at around 40 individuals from 1995-2005 (Taylor and Marchand 2006), and displayed signs of depauperate genetic diversity at both phenotypic (high proportion of dark and piebald morphs that are very rare or do not occur at all in other populations) and genotypic levels (significantly lower allelic richness, and, for remaining alleles, an excess of heterozygosity at neutral loci that is indicative of a recent population bottleneck) (Clark et al. 2011). The decline also coincided with a period of unusually wet weather in 2005 and 2006. Average monthly precipitation totaled for the state of New Hampshire for the May–October active season for 2006 was 101.3 cm, the highest on record from the United States National Climatic Data Center at nearly double the average 57.1 cm (McBride et al. 2015). The average for 2005 was 91.4 cm, the second-highest on record following 2006. The authors point out that increased cloudiness and humidity associated with high precipitation can be detrimental to timber rattlesnakes, as these conditions are correlated with reproductive failure in females (Martin 1993, 2002, Clark et al. 2011). In addition to a possible direct impact, the unusual and extreme weather may have facilitated infection with *O. ophiodiicola* since fungal disease in captive reptiles is often associated with inappropriate temperatures, high humidity, and stress-related immunosuppression (Paré et al. 2007, Mitchell and Walden 2013). Because of these concurrent environmental and population attributes, the extent of declines from SFD may not be generalized to all affected snake populations. While this level of mortality appears to be exceptional at this time, the circumstances that produced it are becoming increasingly common.

In the 2006 outbreak, many timber rattlesnakes displayed skin lesions around the head, chin, and body. One individual with severe mycotic stomatitis was found dead. Although timber rattlesnakes in this area typically enter hibernation during the first week of October (Clark et al. 2011), eight individuals remained visible outside of the hibenaculum area in October, and at least three of these lingered out of the den into the first week of November. Only one of those eight snakes was seen alive in spring of 2007, and it had a severely swollen eye, and has not been observed subsequently. Two of the remaining seven snakes were found dead in early spring of 2007; the others have not been seen since 2006. The role of SFD in late entrance to the hibernaculum is not clearly demonstrated here, but inappropriate basking associated with SFD was observed in infected timber rattlesnakes in Massachusetts where four infected snakes were observed basking in the sun during the winter months (December-March) (McBride et al. 2015). Population surveys from 2007-2010 observed only 19 individuals, indicating that the population declined by approximately 50% in 2006-2007 at the time the disease was observed.

### Outbreak in Carlyle Lake population of eastern massasaugas

*O. ophiodiicola* was confirmed as the cause of the severe fungal dermatitis that caused mortality in the Carlyle Lake population of eastern massasaugas (*Sistrurus catenatus*) beginning in 2008 (Allender et al. 2011). In 2008 three snakes (representing approximately 4 % of the population) with severe facial swelling and disfiguration died within 3 weeks after discovery (Allender et al. 2011). In 2010 a fourth snake with similar signs apparently survived with treatment.  No attempt was made to assess population-level effects. In 2011 the same population was surveyed and 0 of 34 apparently normal snakes were positive for *O. ophiodiicola* by PCR (Allender et al 2013), although three individuals were observed with subtle to mild lesions consistent with SFD.  There were no observations of severe infection or mortality during the 2011 survey. More recently, *O. Ophiodiicola* has been confirmed as the cause of mortality in two eastern massasaugas several hundred kilometres north of Carlyle Lake  Michigan in 2013 (Tetzlaff et al. 2015).

### Gartersnakes in USA

In 2012 a free-ranging plains gartersnake (Thamnophis radix) in Illinois was found to have severe disseminated ophidiomycosis (Dolinski et al. 2014).  Systemic infections are very rare in the literature but this case was similar to a case of systemic ophidiomycosis reported in a captive gartersnake in Europe (Vissiennon et al. 1999, Sigler et al. 2013) and a gartersnake in Pennsylvania in which necropsy revealed mycotic lesions in lung and pancreas (Ohkura et al. 2016) suggesting the possibility that gartersnakes may be unusually susceptible to systemic infection (Dolinski et al. 2014).

### Weather and SFD in Massachusetts rattlesnakes

The role of SFD in late entrance to the hibernaculum is not clearly demonstrated, but inappropriate basking associated with ophidiomycosis was observed in infected timber rattlesnakes in Massachusetts where four infected snakes were observed basking in the sun during the winter months (December-March) (McBride et al. 2015).

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