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Batrachochytrium salamandrivorans
A Threat Assessment of Salamander Chytrid Disease

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INTRODUCTION

Batrachochytrium salamandrivorans is a fungus known to infect salamanders in Asia and Europe. This pathogen has been associated with precipitous declines in European salamander populations. The objective of this threat assessment is to determine if *B. salamandrivorans* is a threat that warrants preventive steps in Canada and to provide some guidance on what those steps might be. The purpose of a threat assessment, in general, is to maintain readiness to predict and prevent disease impacts by assessing risk and recommending preventive actions. While a risk assessment tries to predict or quantify the impacts of an event/agent, a threat assessment focuses on means to avoid a known threat. This report will be concerned with answering four main questions:

1. What needs to be protected?
2. Is Canada vulnerable?
3. What can happen if the pathogen enters Canada?
4. What can be done to minimize exposure, loss or damage?

THREAT ASSESSMENT SUMMARY

Batrachochytrium salamandrivorans is a threat that requires response. European experience suggests that introduction to Canada could parallel the experience we are facing with White-nose Syndrome (WNS) in bats or the impacts of *Batrachochytrium dendrobatidis* (frog chytrid disease) on amphibians worldwide, both in terms of mortality rate and ecological effects. The capacity of WNS and frog chytrid disease to undermine the survival of affected species has been well documented. The ecological effects of catastrophic declines in both biodiversity and local populations from these diseases has yet to be fully realized, but they have inspired strong management responses to contain their spread and impact, including the need to protect species driven onto the endangered species list by these infections. *B. salamandrivorans* has shown to have the potential to cause similarly devastating population declines and increase the likelihood of extirpation and extinction of salamanders and newts.

Canada, along with the rest of North America, is a global centre of salamander biodiversity. Salamanders play important ecological roles. At the larval stage, salamanders are considered the major predator of invertebrates in some aquatic environments (ponds, swamps and forest streams), both in abundance and biomass. Canadian native salamanders include species known to be susceptible to *B. salamandrivorans* and species that are endangered, threatened or of special concern for which susceptibility to the fungus is unknown.

Canada is vulnerable to slow introductions of this pathogen by northward movement of species with territories overlapping Canada and the USA (assuming the pathogen was first introduced into the USA) as well as fast movements of infected/contaminated animals, water, soil or other fomites imported through global trade and movements of people and products. Evidence elsewhere implicates trade/human-mediated movements as the reason for spread from Asia to Europe. Known carrier species of salamanders are continuously imported to Canada as pets and research subjects. The cities of Toronto, Montreal and Vancouver may be locations of high pathogen entry possibility due to the high volume of trade that goes through their respective airports and the relatively large number of people who might own imported salamanders as pets. Native species could be exposed to the pathogen even if there was no release of an infected introduced animal. In addition to direct contact with infected salamanders, waterborne and other environmental routes of exposure provide avenues for successful escape and survival of the pathogen. It is likely that *B. salamandrivorans* could survive in the Canadian environment.

Changes in importation policy and practices plus elevation of the status of *B. salamandrivorans* to a named pathogen may be the ultimate means to protect Canada against this threat. Additional interventions could include supporting an “Alert and Awareness” campaign that engages the amphibian community, border services, diagnostic laboratories and citizens to watch for signs of salamander mortality to prompt early detection, response and, if possible, containment. Awareness should also highlight risks associated with importation of salamanders and newts and appropriate biosecurity. Investment in targeted surveys could help assess if the pathogen is present in Canada. Further susceptibility testing of Canadian species would assist in understanding the extent of Canadian risk.

OVERVIEW OF THE THREAT

The first evidence of infection with *B. salamandrivorans* in Europe was obtained while investigating the cause of the precipitous and dramatic decline of a population of fire salamanders, *Salamandra salamandra* in the Netherlands (47). The disease has caused a 96% decline in the affected population, bringing it to the brink of extirpation (30,47). This decline has made the diminished population more susceptible to environmental, demographic, and genetic stochasticity, thus further increasing its local extinction risk (47). Retrospective examination of thousands of amphibian samples from across the world supports the hypothesis that the pathogen was recently introduced to Europe from its original habitat in East Asia, specifically Thailand, Vietnam and Japan (30,31). While wildlife professionals and conservation groups in Europe were aware of the likely infectious cause of the decline since the die offs began in 2008, it was a report published in Science in 2014 highlighting “the coming salamander plague” that brought international media, scientific and management attention to this threat (48). The report summarized the work of researchers who unravelled the cause of the up to then enigmatic decline in fire salamanders, and described and named the new pathogen, *B. salamandrivorans* (30). Although based on the limited knowledge available, the pathogen seems thus far restricted to Asia and the outbreak locations in Europe (31). Prevailing gaps in biosecurity plus globalization in trade and movements can create avenues for introduction to susceptible salamander populations in North America. Recovery plans for some Canadian salamanders recognize diseases as a future potentially devastating threat for which action is necessary (34,46). These plans provide high level recommendations for action including: monitoring for infectious diseases; increasing awareness to reduce the likelihood of movement of the pathogen between locations through occupational or recreational means; and research on identification and containment of infectious threats. International amphibian experts urge preventive actions to protect naïve North American salamanders from what some refer to as “a new kind of amphibian apocalypse (48)”.

The pathogen

B. salamandrivorans is only the second species in the phylum Chytridiomycota known to infect and cause disease in vertebrates (30). The first pathogenic species of chytrid fungus described, *Batrachochytrium dendrobatidis*, was cultured from the skin a species of poison frog (*Dendrobates* sp) (28). Chytridiomycosis, the disease caused by *B. dendrobatidis*, has resulted in the decline and

extinction of more than 200 species of frogs and toads (order Anura) since it was first described in the late '90s (43). Chytrid fungi are widespread throughout the world, and are particularly abundant in high elevation soils (15). *B. dendrobatidis* was originally restricted to southern Africa where it causes a stable and endemic infection of the aquatic clawed frogs of the *Xenopus* genus (54). The international trade and exportation of *Xenopus laevis*, beginning in the 1930s, resulted in the concurrent global dissemination of the fungus and, eventually, the severe declines in wild amphibians first recorded in the 1970s (3,54).

Although *B. dendrobatidis* can infect amphibians in the Anura and Caudata (salamanders and newts) orders (eg 11,14), severe disease and mortality under natural conditions seem restricted to anuran species. Based on experimental infections conducted on selected species of each of the three amphibian orders, *B. salamandrivorans* seems incapable of establishing an infection in the skin of frogs (order Anura) and caecilians (order Caecilian). *B. salamandrivorans* has proved deadly to the majority of species salamander and newts in which experimental infections have been performed, but some appear to resist its lethal effects (31). Three species of Asian salamanders have been proposed as potential reservoirs: the blue-tailed fire-bellied newt (*Cynops cyanurus*), Japanese newt (*Cynops pyrrhogaster*) and Tam Dao salamander (*Paramesotriton deloustali*) (31).

Pathogen ecology

Chytrid fungi reproduce asexually by forming motile spores and require water to disperse (15,28). Chytrid fungi, including *B. dendrobatidis*, can live outside of a host (15). *B. dendrobatidis* remains viable for several days to weeks in water (23) and moist organic matter (24) even in the absence of nutrients. Although work specific to the *Batrachochytrium* genus still needs to be conducted, chytrid fungi are considered “extremophiles” – known to survive in even extreme environmental conditions, including high altitude and Canadian arctic winters (15,19).

Little is known about the ecology and transmission dynamics of *B. salamandrivorans*, creating the necessity to assume that its transmission and ecology of in the wild will be similar to what is known about *B. dendrobatidis*. It is likely, for instance, that *B. salamandrivorans* can also survive in moist environments, independent of an amphibian host. One of the few known differences between the two pathogens is their temperature tolerance: *B. dendrobatidis* achieves maximum growth between 17-25°C (37), whereas the optimal temperature for growth of *B. salamandrivorans* is between 10-15°C, with some growth in temperatures as low as 5°C and death at 25°C and above (30). There is an inverse correlation between the occurrence of *B. dendrobatidis* and a wide temperature range (32) that may at

least partly explain the limited impact of *B. dendrobatidis* amongst Canadian frogs. It could be postulated, based on the known impact of temperature on the occurrence of *B. dendrobatidis* (38), that this difference in environmental temperature requirements could affect the threat, and variations in the nature of the threat, for Canadian salamanders. It is uncertain whether the distribution and/or abundance of *B. salamandrivorans* in Canada would be enhanced or hindered by our weather. Salamanders tend to congregate only for a few days in breeding grounds (ponds and streams), preferring to live solitary lives in moist terrestrial environments during most of the year. Aquatic newts are permanent residents of water bodies except for the few years they spend on land as efts, a stage between metamorphosis and sexual maturity (50). In Canada, breeding occurs in the early spring when temperatures are usually above freezing. If the breeding time corresponds with the appropriate temperature, exposure to the fungus could be amplified and result in increased probability of infection and dispersal within the local salamander populations. The magnitude of such effects cannot be estimated with currently available information.

Pathogen movement

The global geographic distribution of *B. salamandrivorans* has been tentatively established through the enormous effort of multiple researchers who collected and tested thousands of skin swabs and tissue samples for *B. salamandrivorans* DNA (qPCR) (31). Current understanding indicates that the fungus is endemic in East Asia, has caused outbreaks in the Netherlands and Belgium, and has not been found in other sampled areas, including the USA (31). Retrospective examination of these samples from across the world supports the hypothesis that the pathogen was recently introduced to Europe from its original habitat in East Asia, specifically Vietnam, Thailand and Japan (30,31). No data exist for wild Canadian amphibians. So far, sampling for chytrid in North American amphibians has been limited to detection of *B. dendrobatidis* and has focused on frogs, with few efforts directed towards salamanders (eg 26,41,53).

Experimental infection with *B. salamandrivorans* through direct contact and contact with zoospore-containing water has been successfully performed (30,31). *B. dendrobatidis* transmission can similarly result from direct contact (skin to skin) with an infected individual, or from contact with organic matter (water, mud, leaf litter) where the fungus lives. It seems reasonable to speculate that transmission of *B. salamandrivorans* in the wild will be similar to what is known about *B. dendrobatidis*. Based on detection of *B. dendrobatidis* DNA (PCR) in feet and feathers from birds found in areas known

to be inhabited by the fungus, it has been proposed that waterfowl aid in long-distance translocation of chytrid fungi (17,56), but conclusive evidence is still lacking.

There is uncertainty as to the exact route of *B. salamandrivorans* spread to localized areas of the Netherlands and Belgium. The geographic confinement of outbreaks to specific European locations suggests to some that the spread was caused by human movement and not by animal migration or pathogen dispersion through the environment (31). Potential routes of introduction of *B. salamandrivorans* from East Asia to European environments include movement of human-associated movement of infected animals, contaminated soil, and contaminated water (30,31).

All Canadian salamanders need an aquatic environment to reproduce. Adult newts live permanently in water. The larvae of both groups spend months to years in forest streams or ponds (50). Thus, if *B. salamandrivorans* entered Canada, and became established in the moist environments rich in organic matter that constitute a salamander's natural habitat, all species in the exposure area could become infected both by motile waterborne spores and direct contact (during mating). Environmental temperatures at the time of most species reproduction are probably within the ideal replication range for *B. salamandrivorans* (30).

Host effects

The initial reports of mortalities in European fire salamanders to the regional herpetological association (the Reptile Amphibian and Fish Conservation Netherlands [RAVON, Dutch acronym]), were made in 2008. Members of the public were concerned about finding dead adult salamanders on popular footpaths in broad daylight (47). A thorough investigation yielded the diagnosis of fatal chytrid infection, but not caused by *B. dendrobatidis* (PCR negative) (30,48). *B. salamandrivorans* infection in field cases were later replicated in the laboratory, fulfilling well established criteria to establish that a particular pathogen is the cause of a specific disease (*ie* Koch's postulates - isolation of the pathogen from dead salamanders followed by culture in the laboratory and replication of clinical signs, disease and mortality by inoculation of healthy salamanders). This leaves little doubt that the newly discovered *B. salamandrivorans* was responsible for the mortalities and is now an emerging pathogen that can be fatal in salamanders (30).

Both *B. dendrobatidis* and *B. salamandrivorans* infect the skin of amphibians, neither infects deeper tissues or internal organs (3,30). Lesions are restricted to the epidermal layer of the skin and consist of multifocal erosion and ulceration, with variable superficial secondary bacterial

proliferation/infection (30), although many of the salamanders reported at the beginning of the European outbreak seemed to lack obvious external lesions (47). Experimental infections of fire salamanders caused death 12-18 days post-exposure, with the same clinical signs and pathological lesions found in the European outbreak (30)

While the information regarding the species of salamander first found to be fatally susceptible to *B. salamandrivorans* is well backed by experimental and epidemiological (30), nothing is known yet about influences of co-factors (environmental, predatorial, etc.) on disease ecology. The environment always plays a role in the ecology of a disease. It can, for instance, influence the fitness of pathogen making it more or less virulent (capable of causing severe disease), alter the fitness of the host (allowing it to avoid infection or fight it off once established), promote or limit the spread of disease, and/or make the host more susceptible to other pathogens or predators. The effect of infection with *B. dendrobatidis* on predator-prey relations has been studied, albeit in a limited way and with somewhat contradictory or difficult to interpret results (eg 21,36). For example, antipredator behaviour of infected tadpoles can vary between frog species and the effects of the pathogen on predation may be indirect and difficult to attribute (21,22,35,35). No studies on similar effects on infection caused by *B. salamandrivorans* have been performed. Several factors associated with species-specific characteristics like skin microbial flora (57), and environmental factors (eg temperature [3]) are thought to influence the clinical course and severity chytridiomycosis caused by *B. dendrobatidis*. Because no studies on similar effects on infection caused by *B. salamandrivorans* have been performed, the individual and environmental co-factors affecting disease caused by *B. salamandrivorans* in salamanders are unknown.

WHAT NEEDS PROTECTION

The risk of extinction in western North America is thought to be greater for amphibians than for birds or mammals (51). The term, the 'sixth mass extinction event' has been used to describe rapid and severe declines in species and populations of the entire class of amphibians (52). Although they are rarely the focus of popular attention, salamanders are challenged around the world and in Canada by a suite of threats such as climate change, introduction of diseases or predators, habitat loss, pollution, and road kills. For instance climate change is being blamed for long-term declines in stream salamander (*Gyrinophilus porphyriticus*) adults in New Hampshire. The 12-year study found that adult abundance was negatively related to annual precipitation which is predicted to increase with climate change (27).

Disease has been playing a significant role in amphibian population declines, especially chytrid disease due to *B. dendrobatidis* (43,52). Other diseases have also threatened amphibians such as ranaviruses, *Saprolegnia* spp fungi and *Roceirroia* spp. trematodes (40,48, 36). For example, researchers in Northeastern United States discovered a fungus-like mesomycetozoan parasite (*Amphibiocustidium* sp) new to North America that infects the red-spotted newt and associated with a recent decline in frogs in Italy. (36)

Canada is home to 26 species of salamanders, newts and mudpuppies (Table 1). There are many salamander species in Canada that are listed by COSEWIC (Committee on the Status of Endangered Wildlife in Canada) as endangered (n=6), threatened (n=3) or of special concern (n=3) due to a variety of factors such as restricted range, habitat loss and introduction of predator species (see Table 1 and Figure 1)(32). Seven species have been deemed to be not at risk while the status of the remaining 7 species has not been assessed either federally or provincially. Eight species are listed under the Species at Risk Act: 3 endangered (Allegheny mountain dusky salamander Carolinian population, small-mouthed salamander, tiger salamander southern mountain population), 3 threatened (Allegheny mountain dusky salamander Great Lake/St. Lawrence population, coastal giant salamander, Jefferson salamander) and 2 special concern (Coeur d'Alene salamander, spring salamander). Recovery strategies are in place for the SARA and COSEWIC listed species except for the Eastern tiger salamander prairie population, which only received COSEWIC status in November 2013 (45). Assessing the impacts of these plans on these species was beyond the scope of this project. Uncertainties regarding the ecological effects of salamander chytrid disease under Canadian conditions preclude a forecast of the effects of this disease on the status of these listed populations. However, given that diseases are recognized in species recovery plans as a threats, that diseases have been linked to large scale amphibian declines and that *B. salamandrivorans* effects in Europe have been significant, a reasonable precautionary assumption is that the introduction of this diseases into listed populations would cause a worsening of their population status.

Table 1. List of salamanders present in Canada, their distribution, conservation status based on the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and susceptibility to the chytrid fungus *Batrachochytrium salamandrivorans*.

Family	Latin Name	Common Name	Geographic Distribution	COSEWIC (a)	Threats (b)	Susceptibility, <i>B. salamandrivorans</i>		Screening, <i>B. salamandrivorans</i> (d)	
						Wild	Pet Trade	Captive	PCR
Ambystomatidae	<i>Ambystoma jeffersonianum</i>	Jefferson salamander	SC ON, SW QC	E	RR, HL, DT	Unk	3	2	ND
Ambystomatidae	<i>Ambystoma gracile</i>	Northwestern salamander	Coastal BC & Island	NAR	FR, RE	Unk		1	ND
Ambystomatidae	<i>Ambystoma laterale</i>	Blue-spotted salamander	ON, QC, SE MB	ND		Unk		1	ND
Ambystomatidae	<i>Ambystoma maculatum</i>	Yellow spotted salamander	NS to Lake Superior	ND		pRES (n=5)	8	20	9
Ambystomatidae	<i>Ambystoma macrodactylum</i>	Long-toed salamander	BC	NAR	HL	Unk		4	ND
Ambystomatidae	<i>Ambystoma mavortium</i>	Western tiger salamander (Southern mountain population)	BC	E	RR, HL, FS, DR, IPB	Unk			
Ambystomatidae	<i>Ambystoma mavortium</i>	Western tiger salamander (prairie/boreal population)	AB, SK, MB	SC		Unk		5	ND
Ambystomatidae	<i>Ambystoma texanum</i>	Small-mouthed salamander	ON	E	RR, IP	Unk	9		ND
Ambystomatidae	<i>Ambystoma tigrinum</i>	Eastern tiger salamander	MB	E	RR, SW	Unk		25	3
Dicamptodontidae	<i>Dicamptodon tenebrosus</i>	Coastal giant salamander	BC	T	HL, PD, LoR, LM, LG	Unk		3	ND
Plethodontidae	<i>Aneides vagrans</i>	Wandering salamander	Vancouver Island	SC	FO, LoR, PD, SH	Unk			
Plethodontidae	<i>Desmognathus ochrophaeus</i>	Allegheny mountain dusky salamander (Carolinian population)	ON	E	RR	Unk			
Plethodontidae	<i>Desmognathus ochrophaeus</i>	Allegheny mountain dusky salamander (Gt. Lakes/St. Lawrence pop.)	QC	T	RR, FO	Unk		1	ND
Plethodontidae	<i>Desmognathus fuscus</i>	Northern dusky salamander (Carolinian population)	ON	E	RR	Unk			
Plethodontidae	<i>Desmognathus fuscus</i>	Northern dusky salamander (QC, NB population)	QC, NB	NAR	HL	Unk		1	ND
Plethodontidae	<i>Ensatina eschscholtzii</i>	Ensatina	BC	NAR	HL, FO	Unk		2	ND
Plethodontidae	<i>Eurycea bislineata</i>	Northern two-lined salamander	ON, QC, NB	ND		Unk		2	ND
Plethodontidae	<i>Gyrinophilus porphyriticus</i>	Spring salamander (Adirondack/Appalachian population)	QC	T	DT, FO, FS	pRES (n=5)		1	ND
Plethodontidae	<i>Hemidactylium scutatum</i>	Four-toed salamander	ON, QC, NB, NS	NAR	HL, PD	Unk			
Plethodontidae	<i>Plethodon cinereus</i>	Eastern red-backed salamander	ON, QC, NS, NB, PEI	ND		Unk	346		ND
Plethodontidae	<i>Plethodon vehiculum</i>	Western red-backed salamander	BC	NAR	HL	Unk			
Plethodontidae	<i>Plethodon idahoensis</i>	Coeur d'Alene salamander	BC	SC	RR	Unk			
Proteidae	<i>Necturus maculosus</i>	Common mudpuppy	MB, ON, QC	NAR	PS	Unk		7	ND
Salamandridae	<i>Notophthalmus viridescens viridescens</i>	Eastern newt	ON, QC, NB, NS, PEI	ND		pLETH (n=5)	31	14	ND
Salamandridae	<i>Notophthalmus viridescens louisianensis</i>	Central newt	ON	ND		Unk			
Salamandridae	<i>Taricha granulosa</i>	Rough-skinned newt	Coastal BC and Island	ND		pLETH (n=4)		2	ND

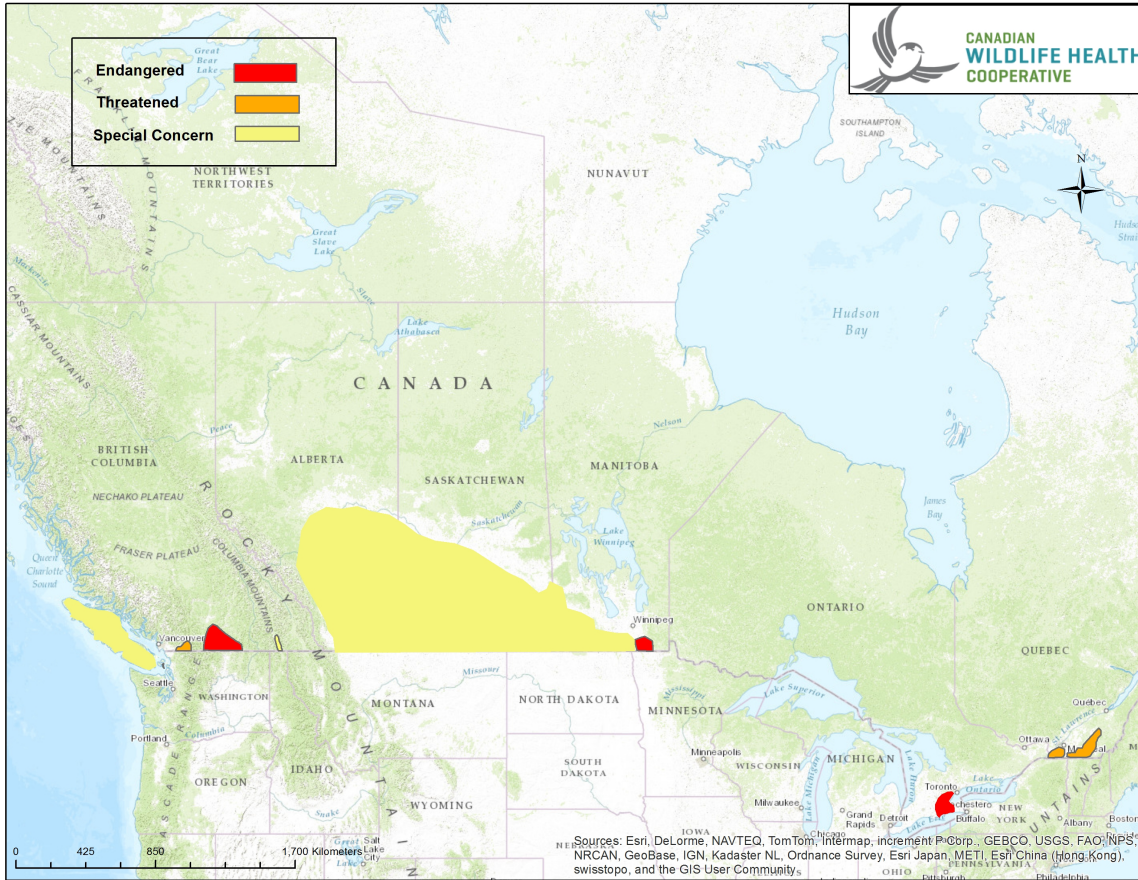
a) Conservation Status: E=Endangered, T=Threatened, SC=Special Concern, NAR=Not at Risk, ND=Not Determined

b) Threats: HL=Habitat loss, FR=Fragmented range, DT=Development, RR=Restricted range, FS=Fish stocking/Introduction of fish, RV=Ranavirus, IP=Introduction new predators, IPB=Introduction new predator: bullfrog, DR=Drought, FO=Forestry/Logging, RE=Reliance on ephemeral ponds, SW=Severe weather events, PD=Poor dispersal ability, LoR=Low reproductive rate, LM=Late maturity, LG=long generation time, LR=Long reproduction time, SH=Specific habitat requirements, PS=Pollutants and sedimentation

c) POTENTIAL susceptibility based only on one experimental exposure (Martel *et al.*, 2014): Unk=Unknown, pRES=probably resistant, pLETH=probably lethal

d) ND=not detected

Figure 1. Distribution of Canadian salamanders classified as Endangered, Threatened and of Special Concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC).



IS CANADA VULNERABLE?

Susceptibility

Little is known about the susceptibility of most North American salamanders. Only 4 species have been infected in experimental trials. Based on those results, there are at least two known susceptible species: the Eastern newt (*Notophthalmus viridescens*) and the rough-skinned newt (*Taricha granulosa*) (Figure 2). Both belong to the family Salamandridae and in preliminary infection trials have been found to be highly susceptible to the fungus with a 100% mortality rate (31). Due to their widespread distribution, migratory terrestrial stages, involvement in the pet trade and lethal susceptibility to the fungus, the Eastern and rough-skinned newts are two species of major concern in Canada.

It appears that the two other salamander species involved in the experimental infection trials and native to Canada, the yellow-spotted salamander (*Ambystoma maculatum*) and the spring salamander (*Gyrinophilus porphyriticus*), may be resistant to chytridiomycosis caused by *B. salamandrivorans* (31). We must note, however, that these are very preliminary results that must be further investigated before any species is deemed resistant to disease. Laboratory findings on species susceptibility do not always reflect susceptibility under natural conditions due to the artificial exposure scenarios and lack of environmental confounding factors in most experimental studies.

Many northern amphibians are at the edge of their distributions. Such edge species can be exhibited greater sensitivity to environmental changes (27). Many Canadian newts and salamanders are more abundant in southern Canada which is an area subject to more intensive anthropogenic changes including habitat alteration/loss, pollution, road traffic and other stressors

Canadian

Susceptible Species

Eastern newt: Ranges from Ontario to the Atlantic provinces and south to Florida. Its life cycle includes terrestrial and aquatic life stages and wide-ranging movement patterns. It is a keystone predator of aquatic invertebrates and has reduced the effect of invasive species (40). Its efts (juveniles, Figure 3) are sought after by the pet trade, so the species is one of many imported into Canada from the US (44).

Rough-skinned Newt. Ranges along the Pacific coast from Alaska to California and is wide spread throughout coastal British Columbia. It has a migratory terrestrial stage and returns to the aquatic environment after 4-7 years to metamorphose (1). The species is sought after in the pet market and is currently imported into Canada (18)

implicated as threats to Canada's endangered and threatened salamanders. The effects of the introduction of a new stressor in the form of *B. salamandrivorans* is unpredictable but based on first principles, it could be anticipated that the ability of native species to deal with an introduced pathogen would be compromised in areas already subject to additional stressors (27).

Figure 2. Canadian distribution of salamanders and newts known to be fatally susceptible to *Batrachochytrium salamandrivorans*.

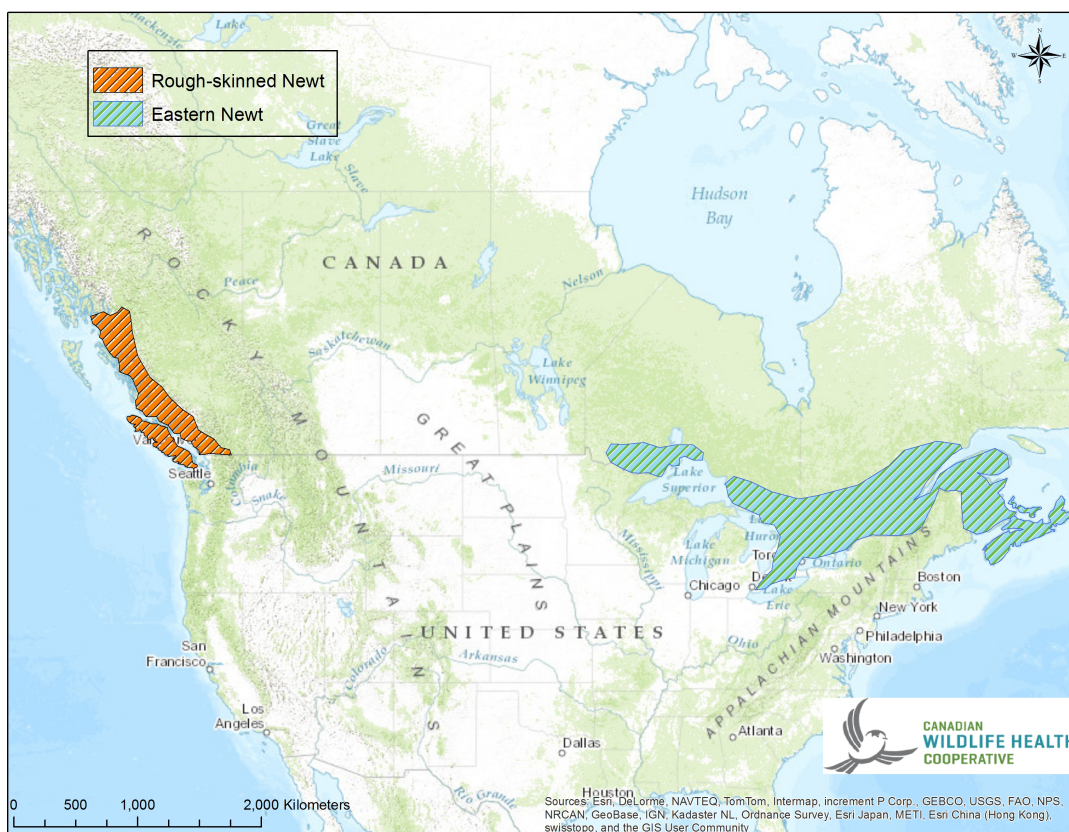


Figure 3. Colorful juvenile (eft) of the Eastern newt, *Notophthalmus viridescens*.

Photo courtesy of Patrick Maldowan,
Laurentian University



Exposure

Amphibians potentially infected with *B. salamandrivorans* and other known and unknown pathogens are regularly imported into Canada. While lack of information provided on documentation at the time of import makes it impossible to determine the exact reason for importation in most cases, the available information suggests that the majority of live salamander imports are for the pet trade. Very few salamanders are imported for consumption, bait, and other purposes (18, and personal communication H. Gerson, Feb 6 2015). Importation of live salamanders as bait is illegal in some provinces, such as Ontario; however, this is provincially regulated (33). We found little information on provincial regulation of salamander movement or uses. Provincial Wildlife Acts vary in their inclusion of salamanders as do provincial fishing regulations in terms of the use of salamanders as bait. Provincial programs seem guided by federal listings of salamander species.

Of the species known to be susceptible to or carry *B. salamandrivorans*, at least three are regularly imported to Canada and sold as pets (18,31) namely: the Japanese newt (*Cynops pyrrhogaster*) and the Tam Dao salamander (*Paramesotriton deloustali*) (both thought to be endemic hosts capable of asymptomatic carriage) and; the fire salamander (*Salamandra salamandra*) (decimated by the disease in the Netherlands and, thus, extremely susceptible to *B. salamandrivorans*)(30,26, 48). Although the USA could be said to be the largest source of live amphibians into Canada, most of those amphibians originate from other countries, and only pass through the United States in transit (H. Gerson, personal communication Feb 6, 2015). The length of time those amphibians spend in the USA while in transit to Canada varies. All imports from the US are accompanied by country of origin labelling from US Fish and Wildlife Service upon importation into Canada, whether the animals originate from the US or from another country (H. Gerson, personal communication Feb 6, 2015). However, the US often suffers from the same lack of background information as Canada.

Limitations in legislative requirements to record and track movements of imported amphibians complicate assessment of pathogen release likelihood or location. Legal importation for the pet trade requires some information on the number and species of salamanders entering Canada be recorded but the documentation is of limited use for risk or threat assessment as these documents frequently do not include total number of animals imported, there are occasions where the species is recorded incorrectly or salamanders are imported under fish or reptile categories (16). The records within the customs database are not readily searchable, making retrieval and summary of importation information extremely difficult. The final destination of the shipped salamanders is not recorded, so there is

currently no way of tracking these shipments within Canada (18, and personal communication H. Gerson, Feb 6 2015). To the knowledge of the Canada Border Services Agency (CBSA), illegal transport of salamanders into Canada occurs relatively infrequently and does not contribute significantly to the total volume of imports when compared with legal imports (H. Gerson, personal communication Feb 6, 2015)

Major hubs of importation such as Vancouver, Montreal and Toronto could be presumed to receive the largest shipments of infected salamanders, and soil or water contaminated with *B. salamandrivorans*. Their larger urban population may drive demands for the pet trade and pet ownership (39). For British Columbia, for instance, the pet trade and restaurant trades are the two most common routes by which exotic amphibians enter the province (51). The restaurant trade is almost exclusively for frog legs. There is no evidence that salamanders are not consumed in Canada (H. Gerson, personal communication Feb 6, 2015). While giant salamanders (*Andrias* sp.) are consumed in Asia, they are listed as CITES I species and, as such, require accompanying documentation on importation to Canada; these species were not included in published reports of imports into Canada (18). Twenty-one of Canada's 26 salamanders and newt species live within close proximity to Vancouver, Toronto or Montreal (Table 2).

Disposal of potentially infected water and substrate from aquaria housing infected salamanders released either directly into the environment or through wastewater systems is a plausible route of pathogen escape. The logistics of the pet industry can include repeated importation of salamanders and disposal of substrate and water in populated areas. Repeated environmental exposure has been shown to increase the likelihood of release of new pathogens and increase the probability of environmental establishment of an introduced pathogen (36). Vancouver and the lower mainland of British Columbia also have a large Asian community where the Buddhist ritual of captive animal release is practiced. There is a lack of systematic knowledge regarding the extent of this ritual or species released but it has been recognized as an ecological risk both globally and in British Columbia (42).

Table 2. Summary of salamander and newts species that live within approximately 100 km of major Canadian city.

Latin Name	Common Name	City*
<i>Ambystoma gracile</i>	Northwestern salamander	TO, V
<i>Ambystoma jeffersonianum</i>	Jefferson salamander	TO
<i>Ambystoma laterale</i>	Blue-spotted salamander	TO, M
<i>Ambystoma macrodactylum</i>	Long-toed salamander	V
<i>Ambystoma maculatum</i>	Yellow spotted salamander	TO, M
<i>Desmognathus fuscus</i>	Northern dusky salamander (Carolinian population)	TO
<i>Desmognathus fuscus</i>	Northern dusky salamander (QC, NB population)	M
<i>Desmognathus ochrophaeus</i>	Allegheny mountain dusky salamander (Carolinian population)	TO
<i>Desmognathus ochrophaeus</i>	Allegheny mountain dusky salamander (Great Lakes/St. Lawrence population)	M
<i>Dicamptodon tenebrosus</i>	Coastal giant salamander	V
<i>Ensatina eschscholtzii</i>	Ensatina	V
<i>Eurycea bislineata</i>	Northern two-lined salamander	M
<i>Gyrinophilus porphyriticus</i>	Spring salamander (Adirondack/Appalachian population)	M
<i>Hemidactylium scutatum</i>	Four-toed salamander	TO, M
<i>Necturus maculosus</i>	Common mudpuppy	TO, M
<i>Notophthalmus viridescens louisianensis</i>	Central newt	TO
<i>Notophthalmus viridescens viridescens</i>	Eastern newt	TO, M
<i>Plethodon cinereus</i>	Eastern red-backed salamander	TO, M
<i>Plethodon idahoensis</i>	Coeur d'Alene salamander	V
<i>Plethodon vehiculum</i>	Western red-backed salamander	V
<i>Taricha granulosa</i>	Rough-skinned newt	V

*TO=Toronto, V=Vancouver, M=Montreal

Not all routes of possible entry into Canada are anthropocentric. All 26 Canadian species of newts and salamanders found in Canada are also found in the United States. If the pathogen entered into the USA before into Canada, there is the possibility of entry via animal movements unassisted by people. While a single salamander may not cover a significant distance within a short time period, migrations over years, especially during breeding periods, may lead to slow but continual movement of *B. salamandrivorans* into Canada should salamanders in the United States become infected (1).

Although fish are not carriers of *Batrachochytrium* spp., shipment of hatchery-raised fish may serve as a way to disperse the fungus if amphibians infected with *B. dendrobatidis* are present at the source (20).

Uncertainties

The limited surveillance for, and novelty of, this pathogen results in inadequate data with which to conclude whether or not *B. salamandrivorans* is already in Canada. A review of the national wildlife disease database of the Canadian Wildlife Health Cooperative¹ found only 178 salamander or newt necropsy reports over the past 21 years (33% from Quebec, 29% from Saskatchewan and 29% from Ontario). A definitive diagnosis could not be made for half of the cases. One case of death due to chytrid infection and 7 reports of chytrid presence (but not the cause of death) were recorded. Six additional cases of epidermal or dermal ulceration or inflammation were reported. As testing was not available for *B. salamandrivorans*, these cases cannot be used as evidence of the presence/absence of this fungus.

The extent of scientific knowledge on *B. salamandrivorans* is sparse and virtually non-existent for North America (The references cited in this paper reflect the extent of published literature on this pathogen). Inadequate recording and tracking of sources or destinations of live amphibian imports prevent quantification of the likelihood of importation and spread of *B. salamandrivorans* in Canada. The validity of assuming that the pathology, epidemiology and impacts of *B. salamandrivorans* will mimic the same attributes of *B. dendrobatidis* is unknown.

What are the implications if the threat entered Canada?

In the northern hemisphere, to which salamanders are restricted, North America has the largest number of species, making Canada, the USA and Mexico global hotspots of salamander biodiversity (48,50). Salamanders play crucial ecological roles. David and Welsh (10) suggest that they can regulate food webs and contribute to ecosystem resilience in the following ways: (a) They provide direct and indirect biotic control of species diversity and ecosystem processes along grazer and detritus pathways; (b) their migrations connect aquatic and terrestrial landscapes; (c) their burrow systems contribute to soil dynamics; and (d) they supply high-quality and slowly available stores of energy and nutrients for tertiary consumers. They have been nominated as excellent ecosystem indicator species (55). For example, stream salamander monitoring and a composite stream salamander index of biotic integrity have been used to determine stream quality (45). Their life span, site fidelity and small territory, sensitivity to perturbations, tendency to occur in high density, tight physiological link to microclimate

¹ The Canadian Wildlife Health Cooperative maintains a national database that includes diagnostic results obtained through passive surveillance, surveys and investigations conducted by our key diagnostic partners in each of Canada's five veterinary colleges and the British Columbia Animal Health Centre.

and successional processes, and low sampling costs add to their value as indicator species for monitoring moisture cycling, food web dynamics, microclimate variability and environmental quality (55).

Salamanders have an important role in environmental conservation and natural history education. The past year, 2014, was designated as *The Year of the Salamander* by two of the most influential amphibian conservation groups in North America: the Amphibian and Reptile Conservation ([PARC](#)) and the Amphibian Survival Alliance (ASA, www.amphibians.org). The partnership between PARC and ASA resulted in a combined effort to offer materials online for educators and naturalists to raise awareness regarding the importance of salamanders in the natural world. Given their benign nature, small size and bright coloration, salamanders are ideal subjects to introduce children to the natural world and promote the importance of conservation.



Figure 4. Spring time in the Annapolis valley, Nova Scotia. Children explore a wetland where spotted salamanders, *Ambystoma maculatum*, lay their eggs every year.

Photo courtesy of Todd Smith, Acadia University.

While much has been written on the decline of amphibians due to chytrid infections, most has focused on frogs, much of that on tropical or temperate locations. Due to the novelty of salamander chytrid disease, there are no data to discuss its ecological effects. We were unable to find publications that established the ecological impacts of salamander and newt declines. Papers discussing declines of amphibians in general noted the challenges in extrapolating experimental data or observational studies to population level effects. Furthermore, dissecting out the effects of disease from other major threats to amphibians, such as habitat loss or pollution, has yet to be achieved. While it is generally thought that amphibian declines will have large-scale and lasting ecosystem-level effects (such as changes in algal

community structure and primary production, altered organic matter dynamics, changes in other consumers and predators, and reduced energy transfers between aquatic and riparian habitats) conclusive evidence of the magnitude and reversibility of ecological effects of *B. salamandrivorans* is lacking.

WHAT CAN BE DONE TO MINIMIZE EXPOSURE AND LOSS

Table 3. Recommendations in response to the outbreak of *B. salamandrivorans* in the Netherlands (25)

Disease Control Theme	Actions recommended
Control international translation of the pathogen	Recommend to the World Animal Health Organization (OIE) that <i>B. salamandrivorans</i> is declared a notifiable disease
	Prevent movement or entry through the pet trade by obligatory testing for known pathogens or a (temporary) ban of the salamander trade
Early detection to prevent spread in a country	Establish monitoring for pathogens in the pet trade
	Develop an early warning system for Europe for the quick detection
	Establish monitoring programs for salamander populations in areas of high risk.
	Develop emergency action plans that allow prompt responses should <i>B. salamandrivorans</i> approach high risk populations of salamander species
Promote procedures to reduce risk of moving the pathogen within the country	Standard operating procedure to increase biosafety during research on amphibians and other aquatic organisms.
	Increase biosafety during amphibian based conservation action
	Increase biosafety in captive collections of amphibians
Reduce uncertainties	Support research into the biology and epidemiology of <i>B. salamandrivorans</i>
	Support research on the conservation biology of salamanders.

Disease control

Given that *B. dendrobatidis* can easily become established if introduced in a propitious environment, and is impossible to eradicate even with direct human intervention (29), the preferred tool in avoiding the so-called salamander plague in Canada is to prevent introduction of *B. salamandrivorans* into naïve populations (48). Other disease control options are untested, limited in their ability to be implemented or not feasible. Mass depopulation of infected native salamanders or newts may not be possible either because the species are protected and/or because they are often cryptic and difficult to find. Isolating/quarantining their environments completely would unlikely be possible due to lack of enforcement capacity and ecological connectivity, but intense outreach to train and advocate for proper biosecurity and disinfection protocols could be mounted to reduce the likelihood of the pathogen being moved by people and/or equipment. Mass treatment or immunization are not available options.

Strategies proposed to mitigate the effect of *B. dendrobatidis* include the use of probiotics (bioaugmentation), but conclusive evidence of their effectiveness and an analysis of undesired effects to the natural environment are still lacking (4). Exposing *B. salamandrivorans* infected salamanders to 25°C for 10 days under controlled conditions resulted in complete clearance of infection and clinically cured all experimentally infected animals (6). The repeatability, species specificity, side effects and practicality of this approach are not known. No other interventions have been tested for *B. salamandrivorans*.

No systematic assessment of Canada's ability to respond to introduced/invasive amphibians was found, however a review of North American capacity to rapidly respond to introductions of non-native freshwater fish found a lack of legislative tools that would allow for rapid response (42). These authors found that the pet trade was poorly regulated for freshwater fish in most provinces in Canada (49). Most provinces, including British Columbia, Ontario and Quebec, are primarily concerned with regulating the two species of CITES-listed salamanders (*Andrias* and *Neurergus* spp) (12). Importation for the pet industry is largely unregulated and not monitored (18).

Import control

There is strong evidence that the amphibian trade contributed to the spread of *B. dendrobatidis* (13). Given the similarities between this fungus and *B. salamandrivorans*, it is reasonable to suggest that actions be taken to prevent the introduction of this pathogen into uninfected areas via international

trade. However, various free trade laws and statutory privileges associated with unrestricted commerce make regulation of the pet trade a challenging solution to this threat (39). Information regarding the economic benefits of the salamander pet trade compared with the economic and ecologic value of wild salamanders within Canada would allow for a systematic cost-benefit analysis for potential importation bans of salamanders. However, there is a lack of information readily available for such calculations. Waiting until economic calculations are completed before placing a precautionary ban may not be prudent given the potential impacts of these diseases and the anticipated delays in translating the ecological and social benefits of salamanders and newts into economic terms. Delays would leave Canada vulnerable to a disease for which there are few, if any, effective tools to contain its spread and impacts upon introduction.

Implementation of mandatory testing of imported specimens could increase the likelihood of pathogen detection before entry of imported animals into Canada, thus reducing the risk of native species becoming exposed to these pathogens (27). There is a specific and sensitive duplex real-time PCR that has been developed for the chytrid pathogens (7). However the cost of mandatory testing has not been established nor has the probability of false negative results. Sample sizes would need to be exceptionally high or 100% if the goal is to find a case in a single infected imported animal. A retrospective survey of animals derived from European pet stores, a pet exporter in Hong Kong and Heathrow airport found no positive animals among 542 tested salamanders and 1221 frogs using qPCR (26). The calculated probability of detecting one positive animal with those sample sizes, assuming a prevalence of 0.01 was approximately 0.97, suggesting either the prevalence is extremely low or zero in that cohort of animals. Among retrospectively surveyed captive amphibians outside of North America, 3 of 408 salamanders and 0 of 159 frogs tested positive for *B. salamandrivorans* using qPCR (26). These data suggest the prevalence of *B. salamandrivorans* in internationally moved and captive animals can be very low on average necessitating either near 100% screening of animals or improved risk-based surveillance to target animals imported from higher risk locations or circumstances.

Sampling strategies would benefit from knowledge on the number of imports and their susceptibility to *B. salamandrivorans*. For example, *Cynops orientalis* is one of the most commonly imported salamander species in the Canadian pet trade (18). While no individuals of this species have been found to be positive for *B. salamandrivorans* by PCR, other members of the genus were found to be reservoirs for the pathogen (31). Such details may help to focus targeted surveillance strategies.

In general, dead salamanders that arrive in Canada are disposed of by the Canadian Border Services Agency and are often not recorded and not tested if they occur in a small percentage of the shipped specimens. Shipments with high proportions of ill or dead salamanders may be reported as inhumane transport under the Health of Animals Regulations (18, and personal communication, H. Gerson Feb 6 2015). Diagnostic screening of dead salamanders from imported shipments would not only be useful for *B. salamandrivorans* surveillance, but also for examining other pathogens in imported species, many of which have limited data on their health and diseases.

Animal testing would not exclude the possibility of pathogen entry by contaminated water or soils. This suggests that border controls could not, on their own, assure protection of Canadian species.

Mandatory quarantine could be considered for species known to be susceptible to clinical disease or death after infection but would not be sufficient to ensure species that can be sub-clinical carriers are not released out of quarantine. The latter species would either need to be banned from importation or subjected to testing.

Awareness and preparedness

Reducing uncertainty and increasing preparedness

Improved understanding of vulnerability to inform deployment of outreach and surveillance efforts:

A more detailed evaluation of the geographic distribution of populations of salamanders and newts of concern and pathways of connection between those populations and likely routes of pathogen entry is required to better estimate locations where enhanced awareness and surveys for the pathogen should be targeted. More rigorous experimental testing is needed to understand the susceptibility status of Canadian species. Where endangered species are involved, alternative means such as field testing or review of retrospective archived samples to show evidence of infection, may be required.

Enhanced passive surveillance and targeted surveys to improve confidence in assertions that *B. salamandrivorans* is not present in Canada

It can only be assumed that *B. salamandrivorans* is not present in Canada. The lack of diagnostic capacity or diagnostic standardization; absence of a dedicated salamander chytrid surveillance program and low level of passive surveillance make it very unlikely this pathogen would have been detected even if it is present. Increasing diagnostic capacity and harmonization of diagnostic approaches in North

America would be an important first step to improving our ability to detect the pathogen. Active solicitation of samples of dead or ill salamanders to diagnostic centers of the CWHC or other diagnostic labs is needed to increase the number of animals tested per year. Elsewhere groups, such as Reptile Amphibian and Fish Conservation Netherlands (RAVON), have effectively engaged the Dutch and Belgian public in a voluntary surveillance effort that greatly relies on citizen science for detection of outbreaks in the salamander populations affected by *B. salamandrivorans*. Active surveys that could seek evidence of the fungus on alive salamanders may be of value but identifying priority/vulnerable locations and determining the intensity of sample should be undertaken before testing begins. Working with the amphibian research community to reduce costs while increasing geographic coverage may be a way to make active surveys more efficient. Given there is the possibility of natural movement of amphibians into Canada from the United States, it will be important to remain abreast of the *B. salamandrivorans* status of US amphibians and to focus some surveillance efforts along the Canada USA border.

Better knowledge of introduction pathways

A more detailed understanding of the salamander pet trade, including the distributions of pet salamanders and newts within Canada would be beneficial. To counteract problems in tracking amphibian importation, many countries are adopting a globally harmonized coding system for each imported species, which increases consistency and search access to records. The use of the Harmonized System developed by the Global Customs Organization is not mandatory but adoption and use of this system by the CBSA could allow for easier differentiation between fish, reptile and amphibian importation (18). This improved data collection would allow for better understanding of the salamander pet trade, which is required for an accurate assessment of risk of *B. salamandrivorans* introduction.

Promoting prevention

Increased awareness is critical to prime Canadian Border Services Agency and the Wildlife Enforcement division of Environment Canada about the need for border controls and inspections. Because amphibians are not covered under the Health of Animal Act, the Canadian Food Inspection Agency is unlikely to play a role in import inspections and testing. While health checks are occasionally performed on various species of ornamental fish, the few salamander species listed as CITES I are the only species that are required to be inspected upon entry into Canada (11). Because of this, inspection

of imported salamanders, performed by CBSA agents, are rarely done and are typically cursory (18, and personal communication, H. Gerson Feb 6, 2015).

The pet industry and pet owning communities should also be targeted in outreach programs that promote awareness of proper biosecurity and husbandry. Awareness campaigns on the suitability of salamanders or newts as pets, how pet choices can affect native wildlife and methods to reduce the risk of releasing pathogens through proper waste and water disposal could be subjects of messaging to pet owners. Reaching out to herpetologist groups, such as the [Canadian Herpetological Society](#) (formed by the merger of the Canadian Amphibian and Reptile Conservation Network and the Canadian Association of Herpetologists) with information about the threat, recommendations on field hygiene to avoid potential pathogen spread, and a request to report wild salamander mortalities and morbidity is critical to developing any form of surveillance for these cryptic species.

While no evidence-based guidelines may be available for waste management protocols, some general principles can be communicated along with information on the importance of this threat. Current national biosecurity standards and principles are focused on domestic species and deal with production limiting and foreign animal diseases of economic importance. There is a policy gap for similar standards and protocols in imported wild animals, as these are not covered under CFIA guidelines. Therefore, there are no required biosecurity standards or health certifications for salamanders and newts. More detailed biosecurity and response planning would need to be undertaken before implementing awareness campaigns.

Experience with *B. dendrobatidis* shows that there is capacity and willingness to mount collaborative efforts against an amphibian disease threat. The response from the general public, conservationists, researchers and wildlife professionals to the widespread frog declines caused by *B. dendrobatidis* was unprecedented and involved international organizations, governments, academic institutions, and private individuals. Education and outreach programs such as the [Save the Frogs!](#) initiative and captive holding and breeding 'rescue' projects such as [Amphibian Ark](#), developed cross-agency and cross-country collaborations and devoted funding and time to try to save threatened species. The effectiveness of these efforts has yet to be established but they may provide insights into public engagement and outreach methods for salamander chytrid disease prevention, preparedness and response.

Ensuring *B. salamandrivorans* is a priority issue for organizations working on alien invasive species may be another step forward.

Alert system

A community of practice should be supported with a means for rapid communication to alert local stakeholders to the detection of this pathogen as soon as possible as well as to receive information on *B. salamandrivorans* detection, salamander chytrid disease diagnosis, evidence of unexpected salamander or newt mortalities, or clusters of clinically compatible illness. This effort should be preceded by the development of surveillance case definitions that are useful both for laboratory-based etiological or pathological diagnosis as well as for field –based syndromic surveillance to ensure a consistent and sensitive system with actionable results.

Coordination and shared platforms for knowledge sharing

Lessons from North American experience with White-nose Syndrome in bats speaks to the necessity of a centralized coordinating body and shared platform for knowledge exchange when attempting to respond to a significant conservation disease in the absence of a suitable operational budget. Given the ecological connectivity across national borders and the importance of North America as a salamander biodiversity hotpot, a coordinate approach involving Canada, the Unites States and Mexico is warranted.

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