A North American Strategic Plan to Control Invasions of the Lethal Salamander Pathogen *Batrachochytrium salamandrivorans*

North American Bsal Task Force

Summary

Chytridiomycosis is a fungal disease of amphibians that has led to widespread mortality and extinctions. It is considered the greatest disease threat to biodiversity (Wake and Vredenburg 2008). In 1999, one pathogen species causing these population declines and extinctions was described: *Batrachochytrium dendrobatidis,* or Bd (Longcore et al. 1999). A second species that causes chytridiomycosis has been discovered more recently: *B. salamandrivorans,* or Bsal (Martel et al. 2013). Bsal has caused population extinctions of the fire salamander *Salamandra salamandra* in Europe, where it appears to have been recently introduced and its distribution is broadening (Spitzen-van der Sluijs et al. 2016; Stegen et al. 2017). Several lines of evidence support an Asian origin of Bsal (Martel et al. 2013; Laking et al. 2017; Nguyen et al. 2017; Yuan et al. 2018). Susceptibility trials of salamander species native to North America have revealed that some species are lethally affected including all tested species in the Salamandridae or newt family (Martel et al. 2014). Introduction of Bsal to North America, a hotspot of salamander diversity, could drastically reduce amphibian biodiversity and result in ecosystem effects (Gray et al. 2015; Yap et al. 2015; Richgels et al. 2016). To date, Bsal has not been detected in North America. Since effective mitigation strategies have not been developed to combat Bsal in the field, the best threat-abatement strategy currently available is to attempt to keep the pathogen from establishing in North America (Grant et al. 2017; Stegen et al. 2017). If detected, a rapid response plan is essential (Bsal Task Force 2018; **Appendix 4** below).

In addition to these two core needs of **preventing invasion** and being able to **respond quickly** if Bsal were to be detected in North America, it is vital to develop a more comprehensive network of strategic actions. A multi-pronged Bsal strategic plan developed by the North American Bsal Task Force includes the following components: improved **diagnostic tools** to identify the pathogen with accuracy and efficiency, including a network of diagnostic laboratories able to analyze for Bsal; **research advances** to better understand Bsal effects on North American species and how to potentially safeguard susceptible species; a **decision support** framework to aid science-policy prioritizations; a **data management group** that tracks Bsal inventory and monitoring activities, including records of no-detections from field and captive situations; a **response & management group** that facilitates development of mitigation actions; critical **surveillance strategies** to maximize the potential for early detection of the fungus; and improved **communication and outreach** pathways for rapid dissemination of new developments.

Plan Estratégico de Norte América para Controlar la Invasión del Patógeno Letal de Salamandras *Batrachochytrium salamandrivorans*

Grupo de trabajo Bsal en Norte América

Resumen

La quitridiomicosis es una enfermedad fúngica que afecta a los anifbios y es responsable de muertes masivas y extinciones de especies en todo el mundo. Por ende, esta enfermedad es considerada la mayor amenaza a la biodiversidad (Wake and Vredenburg 2008). En 1999, se describió por primera vez al hongo *Batrachochytrium dendrobatidis,* o Bd, como la especie responsable de colapsos poblacionales y extinciones en anifbios (Longcore et al. 1999). Más recientemente, se ha identificado una segunda especie de hongo patógeno que también causa quitridiomicosis: *B. salamandrivorans,* o Bsal (Martel et al. 2013). Bsal ha causado extinciones locales de la salamandra de fuego *Salamandra salamandra* en Europa, en donde aparentemente fue introducido de manera reciente y en donde su rango de distribución se está expandiendo (Spitzen-van der Sluijs et al. 2016; Stegen et al. 2017). Varias evidencias apoyan la hipótesis de que el Bsal tiene un origen asiático (Martel et al. 2013; Laking et al. 2017; Nguyen et al. 2017; Yuan et al. 2018). Experimentos de susceptibilidad a Bsal realizados en especies de salamandras nativas de Norte América, han demostrado que Bsal es letal en algunas especies, incluyendo todas las especies evaluadas de tritones (familia: Salamandridae) (Martel et al. 2014). La introducción de Bsal a Norte América podría reducir de manera dramática la biodiversidad de salamandras, asi como causar efectos graves en el ecosistema, ya que esta región del planeta consitutye el sitio de mayor diversidad de salamandras en el mundo (Gray et al. 2015; Yap et al. 2015; Richgels et al. 2016; Basanta et al. 2019). A la fecha, Bsal no ha sido detecado en el continente americano. Debido a que no se han desarrollado medidas efectivas para mitigar la infección por Bsal, la mejor estrategia para disminuir los riesgos de esta enfermedad, es evitar que el patógeno se establezca en Norte América (Grant et al. 2017; Stegen et al. 2017). Si Bsal es detectado, es necesario implementar un plan de respuesta inmediato (Grupo de trabajo Bsal 2018; ver **Apéndice 4** abajo).

Si Bsal es detectado en Norte América es fundamental, además de **prevenir la invasión** y **responder de manera inmediata**, el desarrollo de una red de acciones estratégicas. El plan estratégico desarrollado por el grupo de trabajo Bsal en Norte América se compone de los siguientes elementos: **herramientas de diagnóstico** para detectar a Bsal con precisión y eficiencia, incluyendo la existencia de una red de laboratorios de diagnóstico capacitados para detectar al patógeno; **avances en la investigación** para entender a profundidad los efectos de Bsal sobre las especies de anfibios de Norte América y cómo salvaguardar a las especies susceptibles; **un marco de apoyo para la toma de decisiones** para priorizar las políticas científicas; un **grupo de manejo de datos** que incluya todos los datos sobre Bsal, incluyendo registros de no-detecciones en el campo y en cautiverio; un **grupo de manejo y respuesta** que facilite el desarrolle de estrategias de mitigación; **estrategias de vigilancia** para maximizar el potencial de una detección temprana del hongo; y **vías de comunicación y divulgación** para la diseminación rápida de la información.

Plan stratégique nord-américain pour contrôler l'invasion par le *Batrachochytrium salamandrivorans,* un agent pathogène mortel pour les salamandres

Groupe de travail nord-américain sur le *B. sal.*

Résumé

La chytridiomycose est une maladie fongique qui a entraîné une mortalité à grande échelle chez les amphibiens et même la disparition d'espèces. Elle est considérée comme étant la maladie la plus menaçante pour la biodiversité (Wake et Vredenburg, 2008). En 1999, une espèce d'agent pathogène causant le déclin et la disparition de populations d'amphibiens a été décrite : le *Batrachochytrium dendrobatidis,* ou *B. d.* (Longcore *et al.*, 1999). Une deuxième espèce causant la chytridiomycose a été découverte plus récemment : le *B. salamandrivorans,* ou *B. sal.* (Martel *et al.*, 2013). Le *B. sal.* a causé la disparition de populations de salamandres tachetées (*Salamandra salamandra*) en Europe, où il semble avoir été récemment introduit ou où son aire de répartition s'élargit (Spitzen-van der Sluijs *et al.*, 2016; Stegen *et al.*, 2017). Plusieurs éléments de preuve indiquent que le *B. sal.* serait d'origine asiatique (Martel *et al.*, 2013; Laking *et al.*, 2017; Nguyen *et al.*, 2017; Yuan *et al.*, 2018). Des essais de sensibilité menés sur des espèces de salamandres indigènes en Amérique du Nord ont révélé que certaines espèces sont mortellement touchées, notamment toutes les espèces de la famille des Salamandridés (tritons) ayant fait l'objet des essais (Martel *et al.*, 2014). L'introduction du *B. sal.* en Amérique du Nord, une aire prisée par diverses salamandres, pourrait réduire de manière drastique la biodiversité des amphibiens et avoir des répercussions sur les écosystèmes (Gray *et al.*, 2015; Yap *et al.*, 2015; Richgels *et al.*, 2016, Basanta *et al*., 2019). Jusqu'à présent, le *B. sal.* n'a pas été détecté en Amérique du Nord. Comme aucune stratégie d'atténuation efficace n'a été élaborée pour combattre le *B. sal.* sur le terrain, la meilleure stratégie de réduction des menaces consiste à tenter d'empêcher l'agent pathogène de s'établir en Amérique du Nord (Grant *et al.*, 2017; Stegen *et al.*, 2017). Si le *B. sal.* est détecté, il faudra avoir un plan d'intervention rapide en place (Bsal Task Force, 2018; **annexe 4** ci-dessous).

En plus des deux besoins essentiels d'**empêcher l'invasion** et d'être en mesure d'**intervenir rapidement** si le *B. sal.* était détecté en Amérique du Nord, il est primordial d'élaborer un réseau plus exhaustif de mesures stratégiques. Le plan stratégique à volets multiples concernant le *B. sal.* élaboré par le Groupe de travail nord-américain sur le *B. sal.* englobe les éléments suivants : de meilleurs **outils de diagnostic** pour identifier l'agent pathogène avec exactitude et efficacité, y compris un réseau de laboratoires de diagnostic capables d'effectuer les analyses de détection du *B. sal.*; **des progrès en recherche** pour mieux comprendre les répercussions du *B. sal.* sur les espèces nord-américaines et les moyens qui pourraient protéger les espèces vulnérables; un cadre **d'aide à la prise de décisions** pour faciliter l'établissement des priorités en matière de science et de politique; un **groupe de gestion des données** qui assure un suivi des activités d'inventaire et de surveillance du *B. sal.*, et qui consigne les cas de non-détection chez les espèces sauvages et en captivité; un **groupe d'intervention et de gestion** qui facilite

l'élaboration de mesures d'atténuation; des **stratégies de surveillance** essentielles pour optimiser la capacité de détection précoce du champignon; de meilleures voies de **communication et de sensibilisation** pour diffuser rapidement les nouveaux développements.

Aperçu des mesures recommandées

1. Empêcher le *B. sal.* d'envahir l'Amérique du Nord en encourageant les parties intéressées à élaborer un programme de commerce propre pour les amphibiens qui certifie que les individus importés ne sont pas porteurs du *B. sal.*;
2. Mettre en œuvre un plan d'intervention contenu dans le présent plan stratégique, qui peut être adapté de manière à répondre aux besoins locaux, pour limiter une éclosion de chytridiomycose causée par le *B. sal.*;
3. Mettre au point un réseau de laboratoires de diagnostic qui peut effectuer des tests validés pour détecter la présence du *B. sal.* dans des échantillons prélevés chez des amphibiens ou dans l'environnement en temps opportun;
4. Évaluer la présence du *B. sal.* dans la nature et dans le commerce des amphibiens de compagnie aux États-Unis, au Canada et au Mexique, réduire le risque de propagation chez les amphibiens sauvages à partir d'amphibiens captifs, et réduire la probabilité que les humains jouent un rôle dans l'introduction accidentelle du *B. sal.* en Amérique du Nord;
5. Améliorer la compréhension du risque d'introduction du *B. sal.* en Amérique du Nord et évaluer le risque de maladie de cet agent pathogène mortel pour les amphibiens indigènes en Amérique du Nord par le groupement et la gestion de données antérieures et actuelles d'échantillonnage de la maladie dans un dépôt commun;
6. Concevoir des outils de prévention et d'atténuation de la maladie efficaces et rigoureux sur le plan scientifique pour contourner les infections et la mortalité liées au *B. sal.*;
7. Élaborer des mesures d'atténuation du *B. sal.* rigoureuses sur le plan scientifique, déterminer des voies rapides pour l'autorisation des mesures d'atténuation et faciliter le processus de consultation des exigences des politiques fédérales et des États concernant l'atténuation;
8. Collaborer avec des partenaires à la compilation et à la diffusion des résultats de suivi et des recherches effectués par le Groupe de travail sur le *B. sal.* et des collaborateurs par l'entremise des médias sociaux, des bases de données accessibles par portail Web et d'articles dans des bulletins;
9. Établir un réseau de partenaires pour communiquer les mises à jour sur les progrès réalisés concernant le *B. sal.* ainsi qu'un mécanisme efficace pour alerter le public et la communauté scientifique en cas de détection du *B. sal.* aux États-Unis, au Canada ou au Mexique.

Plan stratégique

Le plan stratégique présente une stratégie exhaustive sur les mesures à prendre pour détecter le *B. sal.* et prévenir l'établissement de l'agent pathogène en Amérique du Nord. Il débute avec des renseignements généraux sur le *B. sal.*, notamment l'importance écologique des salamandres dans les écosystèmes. S'en suit un résumé des options stratégiques visant à empêcher l'introduction du *B. sal.* La troisième section comporte les objectifs stratégiques de chaque équipe de travail du Groupe de travail nord-américain sur le *B. sal.* en ce qui concerne la détection, le confinement et l'atténuation du *B. sal.* Les travaux de ces équipes de travail portent sur les sujets suivants: **intervention et gestion, diagnostic, recherches, appui à la prise de décisions, gestion des données, surveillance ainsi que sensibilisation et communication**. Le modèle de plan d'intervention rapide se trouve à l'**annexe 4**.

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Overview of recommended actions

1. Prevent invasion of Bsal to North America by encouraging stakeholders to work toward a clean-trade program for amphibians that certifies that imported individuals are Bsal free.
2. Implement a response plan contained in this Strategic Plan, which can be customized to meet local needs, to contain an outbreak of Bsal chytridiomycosis.
3. Develop a network of diagnostic laboratories that can run validated tests to detect the presence of Bsal in amphibian or environmental samples in a timely manner.
4. Test for the occurrence of Bsal in the U.S., Canada and Mexico in nature and in the amphibian pet trade, reduce the risk of spillover from captive to wild amphibians, and reduce the likelihood of humans playing a role in the inadvertent translocation of Bsal within North America.
5. Advance understanding of the risk of Bsal introduction to North America and assess disease risk of this deadly pathogen to native North American amphibians through aggregating and managing past and current disease sampling data in a common repository
6. Develop effective, scientifically-sound disease prevention and mitigation tools to curb Bsal-associated infections and mortality.
7. Develop scientifically-sound Bsal mitigation actions, identify expedited pathways for permitting steps for actions, and facilitate the process of navigating the requirements for state and federal policy relative to mitigations.
8. Work with partners to compile and disseminate surveillance results and research conducted by the Bsal Task Force and collaborators via social media, accessible web portal databases, and newsletter articles.
9. Build a network of partners to communicate updates on Bsal developments, and an efficient mechanism for alerting the public and scientific community in the event of a positive detection of Bsal in the US, Canada, or Mexico.

Strategic Plan

The Strategic Plan presents a comprehensive strategy for what is needed to detect Bsal and to prevent its establishment in North America. It begins with background information on Bsal, including the ecological significance of salamanders in ecosystems. A brief review of policy options aimed at preventing the introduction of Bsal follows. The third section contains the strategic goals of each established working group of the North American Bsal Task Force as related to Bsal detection, containment and mitigation. These working groups are: **Response & Management, Diagnostics, Research, Decision Support, Data Management, Surveillance, and Outreach and Communication**. The Rapid Response Plan Template is included as **Appendix 4**.

I. Background

Batrachochytrium salamandrivorans

The fungus *Batrachochytrium salamandrivorans* (Bsal) is a member of the Chytridiomycota, which is an early evolved group of fungi characterized by production of zoospores with a single posteriorly directed flagellum (Powell 2016). Members of this group are largely decomposers, however the two species of the genus *Batrachochytrium* have adaptations to infect, grow and develop on amphibians. *Batrachochytrium dendrobatidis* is known to infect frogs, salamanders and caecilians as well as crayfish (McMahon et al. 2013) and has caused widespread population declines and extinctions, especially of frog species (Lips et al. 2006; Skerratt et al. 2007). However, some species can carry infections without showing signs of the disease chytridiomycosis (e.g., bullfrogs: Daszak et al. 2004), and some species are resistant to infection (Appendix 2). Bsal is a recently described, closely-related congener of Bd and has caused salamander population declines and extirpations in Europe (Stegen et al. 2017).

The life history of the Bsal pathogen is important to understand as it relates to its modes of transmission. Bsal reproduction consists of the asexual production of spores. A sexual stage is common in other chytrids (Powell 2016), but has not been observed in Bsal or Bd. However, evidence of past sexual reproduction (hybridization) has been detected in Bd (Schloegel et al. 2012; Jenkinson et al., 2016). Bsal has two types of spores, whereas Bd has one. Like all other members of the Chytridiomycota, Bsal produces flagellated zoospores, but it also produces unflagellated encysted spores (Stegen et al. 2017). Stegen et al. (2017) reported that the flagellated zoospores swim toward their potential host and can be consumed by micropredators, which are types of zooplankton. They found that encysted Bsal spores float on the water's surface, can persist in forest soils for a time, and adhere to amphibian hosts as well as to the feet of waterfowl, which could lead to widespread dispersal. When in water, the encysted spores were viable and infective for fire salamanders for at least 31 days and were more resistant to predation by zooplankton. Transmission of Bsal through contaminated forest soils occurred for up to 48 hours after the soil had been in contact with an infected salamander. Also, Stegen et al. reported that Bsal DNA could be detected from contaminated forest soils even after 200 days. The existence of a flagellated and an encysted spore is likely to increase within-population transmission rates above what would occur with a flagellated zoospore alone (Stegen et al.

2017). Amphibian host species that are lethally infected and species that are tolerant of infection (carriers) can contribute to transmission of the pathogen. Species that carry the infection but do not succumb constitute a reservoir for the pathogen. So far, only salamander species have been found to be lethally infected, whereas both salamander and frog species have been found to be carriers of Bsal (Martel et al. 2014; Stegen et al. 2017; unpublished data). A recent study found a surprisingly limited rate of dispersal of Bsal among populations, perhaps due to a fragmented landscape of suitable habitat types for salamanders (Spitzen-van der Sluijs et al. 2018).

Bsal evolved in Asia, where lethal infections have not been found, suggesting that a long co-evolutionary history has led to resistance or tolerance by amphibian species in Asia (Laking et al. 2017). Bsal has recently been discovered in Europe, and it appears to be spreading (Spitzen-van der Sluijs 2016). Infections were first observed in the Netherlands, followed by its discovery in Belgium and Germany. The lethal effect of Bsal on some European amphibian species suggests a recent arrival of a pathogen that encounters naïve hosts. The likely routes of within-continent spread are dispersal of infected amphibians among populations and possibly movement of spores by waterfowl, and by spillover of infected individuals once held in captivity (Nguyen et al. 2017; Yuan et al. 2018). Spread of Bsal between continents is likely due to importation of infected species from locations where Bsal in endemic (Nguyen et al. 2017). Indeed, anurans from Asia infected with Bsal have been found in a pet store in Germany (Nguyen et al. 2017; Yuan et al. 2018). In addition, Bsal was found on salamander species in China that are frequently imported. These studies suggest a role of trade markets in the between-continent spread of Bsal. In particular, the discovery that anurans can be infected opens up the possibility that trade in frogs for food, research, and pets can lead to between-continent dispersal.

Distribution of Bsal in the wild and in captivity

Bsal has been found in the wild and in captivity in Asia and Europe (Appendix 1). To date, 21 amphibian species in the wild have been found to be infected and at least 5 species in captivity have been found to be infected. In the wild, most infected species were in the family Salamandridae (17 species). One frog species found to be infected in the wild was *Bombina microdeladigitora* (Nguyen et al. 2017). This species is closely related to *B. orientalis* (Oriental fire-bellied toad) which is widely imported into the US in pet trade markets. An enormous number of *B. orientalis* were imported into the US between 2001-2009: 3.5 million individuals. Nguyen et al. (2017) concluded that Bsal was vectored in the wild in Europe via the pet trade. The five infected species found in captivity were in the families Salamandridae, Cryptobranchidae and Bombinatoridae.

Susceptibility of amphibian species to Bsal infection

The effects of Bsal infection vary with amphibian species. Some species, including those in the newt family Salamandridae, have been reported to be lethally infected by Bsal (Martel et al. 2014; Appendix 2). The fire salamander *Salamandra salamandra* is highly susceptible to Bsal and indeed once Bsal enters a population of fire salamanders, extirpation of the population has occurred rapidly (Stegen et al. 2017). However, other salamander species, such as the alpine

newt *Ichthyosaura alpestris*, appears to be tolerant of infection, in the sense that they can carry an infection without experiencing morbidity. Frog species, such as *Alytes obstetricans*, are also reported to be resistant or tolerant of Bsal infections. Unpublished susceptibility trials in the laboratory also have indicated that some species are resistant to infection, some species carry infections but do not succumb, and some species are lethally affected (Appendix 2). So far, nine species of salamanders develop chytridiomycosis from Bsal and succumb, especially when infected at a high dose (10^6 zoospores). The finding in nature and in the laboratory that frog species can carry infections demonstrates that the host range of Bsal is greater than initially thought based on the pioneering work of Martel et al. (2014).

Importantly, *Salamandrella keyserlingii* was found infected in the wild. This genus is a very early-evolved genus among the order Caudata. This suggests that the ability to be infected with Bsal is an ancestral trait and that unless shown otherwise, it is prudent to assume that all salamander species can be infected with Bsal.

Risk models

Three recent studies have explored the regions in North America that are most likely to be affected by the arrival of Bsal (Yap et al. 2015; Richgels et al. 2016, Basanta et al. 2019). Yap et al. (2015) used a Bsal habitat suitability model combined with a host-species richness map to identify four zones of high risk in North America: southeastern United States, western United States, the south coast of British Columbia, and the highlands of central Mexico. Richgels et al. (2016) used habitat suitability for Bsal and host richness in the US and included risk of introduction from the pet trade. Their model predicted three zones of high risk: the Pacific coast, southern Appalachian Mountains and the mid-Atlantic regions. These models assumed equal susceptibility of host species and had broadly similar conclusions. Basanta et al. (2019) used a habitat suitability model for Bsal and salamander distributions in Mexico to identify high-risk zones and potential hotspots areas to surveillance. This model predicted areas of Trans-Mexican Volcanic Belt, Sierra Madre del Sur, Sierra Madre Oriental, Northern Oaxaca, Mexican Gulf and the Yucatán Peninsula as risk zones suitable for Bsal in Mexico, and 13 hotspots with both high salamander diversity and suitable for Bsal. Current research into susceptibility of host species may refine these three models, which can indicate areas to focus Bsal surveillance efforts.

Ecological importance of salamanders

Salamanders play a vital role in aquatic and terrestrial ecosystems. In particular, they can be important in energy flow through ecosystems, suppression of leaf litter decomposition in terrestrial ecosystems which functions to sequester carbon from the atmosphere, and as keystone species that affect ecosystem biodiversity. Dramatic declines and extinctions of salamander species in North America could have important negative effects on both terrestrial and aquatic ecosystems.

In forested areas of North America, the population density of terrestrial salamander species can be very high. Recent estimates that take into account detection rate of a species indicate that that

surface populations are <20% of the total population (Bailey et al. 2004; Semlitsch et al. 2014). For example, if estimates are adjusted accordingly, then population densities of the Eastern red-backed salamander (*Plethodon cinereus*) in New Hampshire's Hubbard Brook Experimental Forest (Burton and Likens 1975a) were over $1/m^2$, and were up to $11/m^2$ in Virginia's Shenandoah National Park (Jaeger 1979). The population density of the related species *Plethodon serratus* was estimated to be about $1/m^2$ (Semlitsch et al. 2014). Even using estimates based on surface counts, the total biomass of salamanders was estimated to be 2.5 times the biomass of all breeding birds and equal to that of small mammals in the Hubbard Brook forest (Burton and Likens 1975a).

The large biomass of salamanders in forest ecosystems has several important implications. First, energy flow through or storage in salamanders can be large. In the Hubbard Brook forest ecosystem in New Hampshire, it was estimated that salamanders consume 5.8 kcal /m2 energy each year (Burton and Likens 1975b). The total amount of energy contained in soil invertebrates in a hardwood forest was estimated as 5.04 kcal m^2. Thus, salamanders' food requirements would require a complete turnover in the soil invertebrate community each year, although salamanders also can consume prey found above ground. It has been suggested that predation on woodland salamanders in the genus *Plethodon* is not a significant source of mortality (Hairston 1987). If so, then a large fraction of primary production is entering the salamander community though a pathway that starts with leaf litter and proceeds to soil invertebrates and onto salamanders. Thus, salamanders can act as an important store of energy in the ecosystem and as such might dampen fluctuations in energy flow. Elimination of a large fraction of terrestrial salamanders could magnify stochastic fluctuations in energy flow and would release soil invertebrates from predation, which can lead to large population sizes of these invertebrates.

Increased population sizes of soil invertebrates caused by reductions in woodland salamander populations could have drastic effects on CO_2 release into the atmosphere. An increase in leaf-shredding soil invertebrates and the resulting increase in leaf fragments would facilitate an increase in microbial decomposers. Total microbial respiration would increase as a result. Three studies have demonstrated that total leaf litter decomposition is suppressed when woodland salamanders in control plots were not experimentally removed (Wyman et al. 1998, Best and Welsh 2014, Hickerson et al. 2017). Wyman (1998) found that salamanders in New York suppressed decomposition by 11-17%. Preliminary calculations suggest that a suppression of leaf litter decomposition of 10%, if extrapolated over terrestrial ecosystems, keeps up to 10 GT of carbon per year from being released into the atmosphere. This is the same order of magnitude of carbon release from annual fossil fuel use. The implications for amplifying the greenhouse effect and climate change are profound. Another study found a similar effect and estimated that the species *Ensatina* across its range in California would prevent 72.3 metric tons of C from entering the atmosphere each year (Best and Welsh 2014). Whereas additional replication is required, these studies suggest that large declines of terrestrial salamanders could release large amounts of CO_2 into the atmosphere that otherwise would be stored in the soil ecosystem, with concomitant effects on climate change.

Furthermore, many amphibians have a key ecological functional role in transportation of reciprocal subsidies between aquatic and terrestrial ecosystems (Davic and Welsh 2004). As eggs are deposited in aquatic habitats and larvae develop there, they accrue aquatically derived

nutrients into their body mass. These energetic subsidies are transported to terrestrial ecosystems upon metamorphosis for many species, including salamanders. In terrestrial ecosystems, metamorphic and adult amphibians continue to grow, accruing terrestrial subsidies that are later brought back to aquatic habitats upon breeding. Such reciprocal subsidies effectively link aquatic and upland ecosystem energetics.

Salamanders are keystone species in temporary pond ecosystems meaning that their removal will have important impacts on the ecosystem. Experiments from the 1980s in experimental ponds demonstrated that removal of the keystone species *N. viridescens* caused changes in the anuran and zooplanktonic community structure (Morin 1983, Morin et al. 1983, Wilbur et al. 1983). Newts preyed upon the competitively dominant tadpole species, which allowed the persistence of competitively weaker species, such as the spring peeper *Pseudacris crucifer*. Based on recent Bsal susceptibility trials, newts are likely to be decimated across their range. Thus, changes in the community structure of frog species is a likely consequence.

II. Policy review

As noted by Stegen et al. (2017), there currently are no effective mitigation strategies to combat Bsal in nature. While it is important to continue research on mitigation strategies that can be effectively employed in the future, the only effective strategy at this time for North America is to keep Bsal out (Grant et al. 2017). Managers are considering proactive management actions that can be implemented ahead of an introduction, which may provide other options that can be used in concert with import control and reactive mitigation strategies to reduce the population-level risk. Current evidence suggests that a majority of amphibian species tested to date can be infected with Bsal and thus can act as carriers of the pathogen. The means to definitively accomplish the goal of excluding Bsal from North America include range of options: 1) a ban of importation of all amphibians; 2) protocols to ensure that any imported amphibians are free of the pathogen; 3) testing amphibians already in captivity in North America and removing Bsal from all infected individuals. Recently, the European Union (EU) enacted legislation to enact a clean-trade program for salamander species (EU Decision 2018). Canada has banned the free importation of all salamanders with any importation from the group of animals requiring a federal permit (CBSA 2018). So far, Mexico has not banned any amphibian imports and the country has trade programs with Asia and the US but not with Europe. The US has banned importation of 201 species of salamanders (USFWS decision 2016). The action by the EU can serve as a model for a clean-trade program in other countries and can be a model for broader legislation that could apply to pathogens of all wildlife species.

If a moratorium on importation of all amphibians is not possible, then a range of policy responses can be considered until such time as a viable clean-trade program is in effect:

- A moratorium on importation of all genera of amphibians shown to be infected in the wild or in captivity.
- A moratorium on importation of all genera of amphibians from countries where Bsal has been detected, either in the wild or in captivity.

While a partial salamander importation ban has been implemented in the US, a total salamander ban has been implemented in Canada, and a clean-trade protocol has been initiated in the European Union, to our knowledge no country has considered an importation ban on all amphibian species. It is now clear that some frog species can be carriers of Bsal. Frog species are imported into the US, Canada, and Mexico for food, research, pets, and as conservation-reliant species in rescue colonies, and represent a much larger fraction of amphibian imports than do salamanders. A ban on amphibian imports would not be necessary if a clean-trade program were effective. However, no single strategy is failure-proof, and therefore it remains imperative to develop and implement proactive management to mitigate risk of invasion, while pragmatically planning for the arrival of Bsal in North America.

III. The Bsal Strategic Plan for containment of a Bsal introduction into North America

The Strategic Plan is a product of the North American Bsal Task Force. Its purpose is to outline what is necessary for a successful response to the detection of Bsal in North America. The rotating chairs of the Task Force's Technical Advisory Committee since its inception in 2015 have been Dede Olson, Jennifer Ballard, Mike Adams, Reid Harris, Jake Kerby and Matt Gray.

History of the North American Bsal Task Force

At a 2015 workshop in Colorado, hosted by the USGS Amphibian Research and Monitoring Initiative and the USGS Powell Center, researchers and managers discussed approaches to learn more about Bsal and the related emerging infectious disease chytridiomycosis caused by it, and to forestall potential biodiversity losses in the Americas where it was not known to occur (Grant et al. 2016). The Bsal Task Force was initiated at this workshop (Figure 1, below). Seven interactive Working Groups (Figure 1B) were formed: 1) Surveillance/Monitoring, 2) Diagnostics, 3) Data Management, 4) Response, 5) Outreach/Communication, 6) Research and 7) Decision Support. Recently, a Management working group has formed to address mitigating actions in the event that Bsal were detected in North America and the Task Force is in the process of merging this with the Response working group. Annual reports are available at salamanderfungus.org.

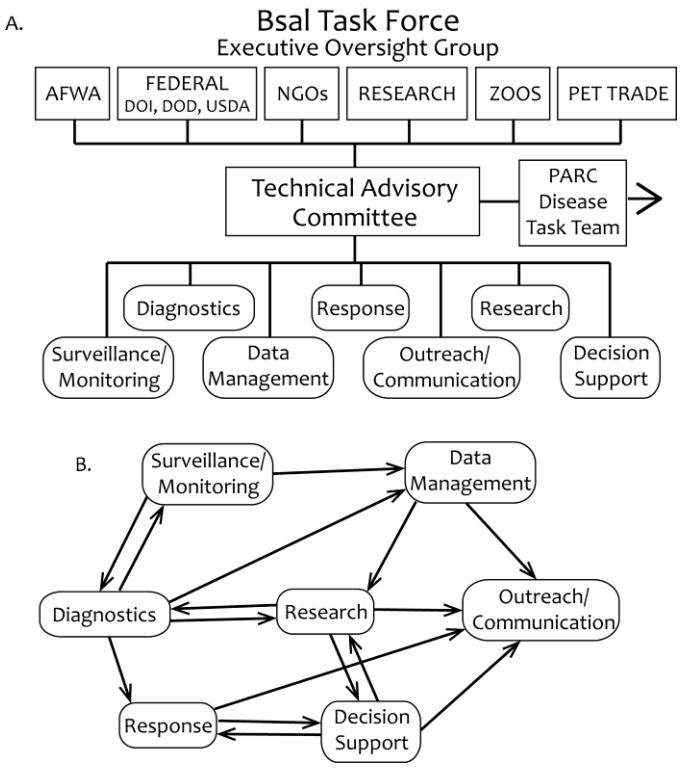

Figure 1 A. Structure of the Bsal Task Force. B. Initial Working Groups within the task force.

Working Groups were formed in June 2015. Since then, they have met via monthly conference calls to outline new tasks and discuss progress on existing efforts. Group membership is open and inclusive, but was initially founded with persons involved with disease research, natural resource management in state or provincial/territorial and other government agencies, environmental or conservation groups, non-governmental organizations, and the pet industry. Each group has 1-3 leads, who help to coordinate personnel, manage the workload, and report to the Technical Advisory Committee (TAC).

The TAC is populated by the Working Group leads and representatives from selected partner groups including government agencies, the IUCN Amphibian Survival Alliance (ASA), and the Pet Industry Joint Advisory Council (PIJAC). The TAC meets by conference call monthly, with a focus on new items and round-robin reporting by participants. New items have included tasks to be assigned or delegated to others, opportunities for products and grant proposals, and communication-outreach and networking needs. Monthly meeting notes are routed to TAC members, then to their working group members, to ensure communication. A lead for the TAC is determined by the TAC and is rotated each year. The incoming and outgoing leads serve as co-leads. Decisions of the TAC are made by consensus.

An Executive Oversight Group (EOG) was originally envisioned to be created as a mechanism to inform managers or leaders of new Bsal information or emerging Bsal topics at higher organizational levels, potentially including US Department staff, the Association of Fish and Wildlife Agencies

(AFWA), the Canadian Wildlife Service (CWS), and the Pet Industry Joint Advisory Council (PIJAC). The initiation of the Bsal Task Force EOG was proposed to national leaders at the North American Wildlife and Natural Resources Conference in March 2016. Discussion there expanded the need for such an oversight body not just for Bsal, but for other non-agricultural wildlife diseases with analogous task forces such as White-nose Syndrome in bats, as well as wildlife diseases without formalized task forces such as Sea-star Wasting Disease. An EOG for non-agricultural wildlife diseases is the topic of continued discussion. This topic segues to that of a recognized gap in laws for wildlife health in the US, Canada, and Mexico. Whereas the US Animal Health Protection Act (7 USC 109) covers agricultural wildlife health, there is no companion legislation for non-agricultural wildlife. Similarly in Canada, the Health of Animals Act is targeted towards agricultural animal health, so the Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act (WAPPRIITA) is used to control the flow of Bsal via controlling salamander imports instead. These are examples of what the EOG could address.

Sections of the Bsal Strategic Plan

The Strategic Plan presents a comprehensive strategy for detecting and preventing the establishment of Bsal in North America. An important goal of the Strategic Plan is to be able to use it to leverage funding for an effective response. The following sections detail the strategic plans of each working group. The format includes each working groups' goals and action items. A prioritization of goals and budget are included. The sections are: **Response, Research, Diagnostics, Decision Support, Data Management, Outreach and Communication, Surveillance, and Management**.

1. Response Working Group

(Priya Nanjappa, facilitator)

The **Response Plan** is found in **Appendix 4**. The Response Plan and its recommendation serve as a **template to be customized** by any agency or institution with management jurisdiction over wild or captive salamanders, respectively, when actions in response to a disease may be warranted. *This purpose statement may be further customized as needed for individual entities.* The Response Plan is provided as an outline and guidance for **local, rapid response** actions that could be triggered upon initial or subsequent detections of Bsal, in either wild or captive populations. The scenarios are based on what an entity using this plan might do after receiving information regarding Bsal detection status from a diagnostic laboratory with expertise in Bsal diagnostics. In other words, all recommended actions occur after the laboratory has made its determinations based on the Case Definition of Bsal (White et al. 2016). Also provided are considerations for *in situ* containment (i.e., in the existing location of the population) as well as establishment of *ex situ* populations (i.e., outside of the natural location, such as in captive assurance colony). Rapid containment and response measures may prevent broad impacts. The USGS Amphibian Research and Monitoring Initiative (ARMI) is also working to assist entities in making decisions regarding wildlife disease management, including the customization of this template. Contact ARMI Decision Support Lead, Dr. Evan Grant (ehgrant@usgs.gov) for

assistance. The Response Plan is considered a living document that will be updated as more information becomes available.

At the time of this writing, Bsal is not known to occur in North America and suggested responses are consistent with the high-alert condition of Bsal being yet undetected or rare in North America. This document is intended to be incorporated into a National Bsal Strategic Framework, where larger surveillance and monitoring strategies, research needs, policy needs, and related prevention strategies, along with public outreach and communication, are addressed.

2. Diagnostics Working Group

(Maria Forzan, facilitator)

The Diagnostics Working Group (DWG) is composed of professionals with expertise in the application and interpretation of an array of diagnostic tools. Our members work in academia, diagnostic laboratories and government agencies throughout North America and are involved in detection and reporting of amphibian diseases, including the salamander chytrid fungus, *Batrachochytrium salamandrivorans* (Bsal).

The main goals of the DWG are to assist with the promotion of consistent standards for diagnosis and reporting Bsal among the wildlife health community. We also serve as a forum to exchange ideas and work out the challenges involved in Bsal detection and to provide expert advice to the rest of the Bsal Task Force regarding the viability and pitfalls of traditional and new tools for Bsal detection and diagnosis.

Collaborations between members of the group has achieved several goals. In 2016, a pilot round robin proficiency test for Bsal detection by PCR was carried out (Forzán *et al.*, manuscript in progress).

Recently, an *in situ* hybridization protocol to detect Bd & Bsall cell in formalin-fixed paraffin-embedded tissues was developed (Ossiboff RJ, Towe AE, Brown MA, Longo AV, Lips KR, Miller DL, Carter ED, Gray MJ, Frasca Jr S. Differentiating *Batrachochytrium dendrobatidis* and *B. salamandrivorans* in amphibian chytridiomycosis using RNAScope® in situ hybridization. Frontiers in veterinary science. 2019;6:304). Definitive differentiation of Bd and Bsal in tissue sections of affected amphibians is impossible by fungal morphology based on routine histologic stains alone. As the case definitions for Bsal and Bd chytridiomycosis require both histologic and molecular evidence of infection, this new test to simultaneously screen for and differentiate the two fungal pathogens in tissue section is critical for accurate diagnosis.

A) **Goal 1.** Establish a long-term program for inter-laboratory quality control and evaluation of protocols for the detection of wildlife pathogens, particularly Bd and Bsal

Priority: Urgent

Rationale: Research and diagnostic laboratories throughout the world run PCR tests to detect wildlife pathogens. Standardization of methodologies is difficult, and even more difficult is it

for small research laboratories to acquire a certification granted by organizations such as the American Association of Veterinary Laboratory Diagnosticians or ISO committees. An option should exist to provide an accessible method for quality control/quality assurance that will allow participating laboratories to confidentially evaluate the quality of their own results. Based on a successful pilot round robin/ring test, a formal program to provide bi-annually quality testing to all volunteer laboratories can be established. The program would provide blind samples to participating laboratories, collate results, and provide feedback to all participants. Two things are crucial: providing the blind samples free of charge, so laboratories with limited budgets are not excluded, and maintaining the origin of results confidential so all participants can see where their results compare to the group but no one is able to match a set of results to a specific laboratory.

Action Items:

1) Determine a laboratory that can produce bi-annual sets of samples containing pre-determined concentration of inactivated Bd and Bsal zoospores in solution
2) Identify a group of laboratories willing to participate in testing blind samples and committed to reporting their results within a pre-determined period of time and following an established format including a minimum of methodological information
3) Develop a web-based platform for the collection of results and feedback to participating laboratories, as well as a deposit of information regarding recommended methodologies.
4) Provide a set of blind samples that include blanks, and one or both amphibian chytrid fungi (Bsal and Batrachochytrium dendrobatidis)
5) Collate reports from participating laboratories and provide feedback to all participants Produce a list of participating laboratories to agencies and other institutions interested in submitting samples for testing or collaborating in testing projects
6) Produce a list of participating laboratories to agencies and other institutions interested in submitting samples for testing or collaborating in testing projects

Estimated Time and Cost

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1-3)	Winter 2019, completed	$20,000	Funded by Environment and Climate Change Canada
(4-6)	TBD	$40,000	Not funded

B) **Goal 2**. Develop a standardized and replicable method that will allow comparison across studies and a reliable estimation of presence and/or prevalence and Bd and Bsal load in the wild

Priority: Urgent

Rationale: Numerous laboratories are already running Bsal PCR tests, both in native and exotic amphibians. Testing various protocols and establishing one that is most effective and that can fit the majority of technical settings would empower laboratories and provide an easier way to compare results amongst them. A common request from diagnosticians and researchers is the establishing of a set of recommended standards.

Action Items:

1) Establish a short list of protocols that are most likely to be used across agencies and institutions
2) Identify a group of laboratories willing to participate in testing blind samples following specific protocols – a subset of the round robin participants would be best
3) Provide detailed instructions on the protocols to test to participating laboratories
4) Define common metrics that laboratories should report to determine detection, quantification and variability of chytrid detection using molecular tools
5) Provide a set of blind samples that include blanks, and one or both amphibian chytrid fungi (Bsal and Batrachochytrium dendrobatidis)
6) Collate reports from participating laboratories and establish the protocol(s) that yielded most consistent results and determine variability
7) Establish a mechanism to provide laboratories with the standard(s) deemed most appropriate based on the round robin results

Estimated Time and Cost

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1)	TBD	$20,000	Not funded
(2)	TBD	$5,000	Not funded
(3)	TBD	$5,000	Not funded
(4)	TBD	$5,000	Not funded
(5)	TBD	$50,000	Not funded
(6)	TBD	$5,000	Not funded
(7)	TBD	$45,000	Not funded

3. Research Working Group

(Doug Woodhams, facilitator)

Urgent Research Needs

The goals of the Research Working Group of the North American Bsal Task Force are to facilitate communication and collaboration among scientists studying Bsal in North America and to ensure that high quality research on Bsal is produced rapidly. The Research Working Group is currently composed of >30 members, representing >20 organizations. Collectively, the group has decades of experience with amphibian (and other wildlife) pathogens and is qualified to make the following recommendations. The Research Working Group compiled a list of research needs for North America in 2016 and revised that list for this strategic plan based on recent publications. The priority of the research needs is ranked as **Urgent, High,** or **Medium**. We consider the research needs within each of these priority categories as having equal importance. Each year we intend to update this list as more information becomes available, and anticipate that the priorities will change.

The following research needs represent a comprehensive approach to advancing the understanding of Bsal's potential impact on amphibian host communities should it be introduced into North America. Although some proposed research is basic or theoretical in nature, all needs will lead to building foundational knowledge essential for responding to Bsal emergence. The Research Working Group urges organizations to dedicate financial resources toward these needs prior to the emergence of Bsal in North America because these research activities can require months or years to complete. Past experiences with emerging infectious diseases in wildlife populations have shown that preemptive and precautionary actions are essential to the success of containing and reducing the spread of novel pathogens (Langwig et al. 2015). Thus, the investment in Bsal research will provide an excellent case example of the usefulness of science-based preparedness in responding to novel pathogen introductions in the wild. The participating scientists pledge to collaboratively produce high quality research advances rapidly to prevent the loss of amphibian biodiversity in North America because of Bsal. The total estimated cost of the proposed research is **$8.9 million USD**, with approximately $2 million USD currently secured.

Research Needs

Each research need below is identified with an overall goal (A – H), priority ranking, research rationale, action items (i.e., objectives or independent studies), and estimated costs and timeline per action item. The estimated costs are direct costs and do not include facilities and administrative (i.e., indirect) costs that could be charged by organizations performing the research. Applied management relevance is indicated.

Research that informs management decisions will be used to evaluate optimal management strategies within the Decision Science Working Group. The Research Working group endeavors to reduce uncertainties that impede proactive and responsive strategies. Decision Support will consider multiple objectives, preferences and values of individual decision-makers, risk profiles, and current research frontiers and uncertainty. For example, identifying possible management interventions for infected habitats (goals D, G) can be evaluated in a decision analysis

framework to identify the optimal strategy, given species-specific susceptibility (goal C), and calculate the importance of reducing remaining uncertainties to improve decisions. Hence, it is essential that the Research Working Group interacts with the Decision Support and other working groups to produce research that has applied implications.

Summary Table:

A) Goal: Estimate the occurrence of Bsal in the U.S., Canada and Mexico amphibian pet trade, the risk of spillover to wild populations, and the likelihood of humans playing a role in the overland translocation of Bsal upon introduction.	
One study completed; (Klocke et al. 2017)	Estimate the occurrence and prevalence of Bsal in the North American pet trade through non-lethal surveillance of amphibians at ports of entry, wholesale distributors, and retail stores.
In Progress	Estimate the susceptibility of potential Bsal hosts (salamanders and frogs) in the pet trade (e.g., species commonly imported from Southeast Asia) .
	Characterize human behaviors for amphibian hobbyist and specialist groups to estimate the likelihood of Bsal spillover from consumers to wild populations and the acceptance of public outreach strategies designed to limit spread of Bsal.
B) Goal: Develop compartmental disease models, epidemiological tools using empirical data to identify critical transmission pathways and conditions under which Bsal is likely to emerge in amphibian host populations in North America.	
In Progress	Estimate latency period of infection and recovery rate for pre- and post-metamorphic amphibian hosts at biologically relevant temperatures.
In Progress	Estimate daily shedding and encystment rate of Bsal zoospores and the infectious dose (ID)-50 for pre- and post-metamorphic amphibian hosts at biologically relevant temperatures.
In Progress	Estimate daily contact rates of amphibian hosts at biologically relevant temperatures and densities when exposed to different complexities of habitat structure.
Within Spp: In Progress	Estimate probability of Bsal transmission between infected and uninfected amphibian hosts (within and between species) at different post-exposure durations and temperatures.
In Progress	Estimate the duration of Bsal zoospore persistence in water and soil given differences in various environmental conditions (e.g., temperature, micropredators, soil moisture, water chemistry, and bacterial presence).
	Estimate the influence of co-infection with other pathogens (e.g., *Bd*, ranavirus) on the likelihood of Bsal transmission and development of chytridiomycosis.
In Progress	Identify biological reservoirs for Bsal and determine the probability of transmission or translocation (on external surfaces or fomites) of Bsal by non-amphibian hosts (e.g., crayfish, waterfowl, humans).
C) Goal: Develop epidemiological tools (i.e., integral projection models) that enable objective classification of species tolerance to Bsal infection, which can be used to produce more informed Bsal risk models for North America.	

In Progress	Estimate the susceptibility (i.e., tolerance) of North American amphibians to Bsal infection and chytridiomycosis using standardized, dose-dependent experiments (suggestions for targeted taxa are provided in Appendix B).
In Progress	Estimate the impact of habitat characteristics (temperature, pH, salinity, zooplankton abundance, etc.) on Bsal infection risk.
In Progress	Develop Integral projection models (IPMs) that predict tolerance using temporal estimates of Bsal infection load and host fitness metrics (e.g., survival, disease ranking using microscopic and gross lesions).
In Progress	Using information developed in (1-3), map susceptibility indices on the geographic distributions of hosts and environmental suitability niches for Bsal to produce robust spatial predictions of Bsal risk in North America.
D) Goal: Identify effective methods to manage disease in captive and field settings.	
In Progress	Identify effective probiotic microbes and develop probiotic treatment methods to combat Bsal, including the exploration of host and environmental modes of treatment.
In Progress	Identify Bsal-consuming aquatic micropredators from natural habitats and test micropredator augmentation strategies.
In Progress	Evaluate novel vaccination methods as a possible disease mitigation tool and test different modes of delivery (e.g., different life stages, nasal associated lymphoid tissue vaccination, skin exposure).
	Explore the use of Bsal removal methods (e.g., attractants or traps).
In Progress	Explore the genetic correlates of disease resistance and the possibility of selective breeding hosts for Bsal resistance.
In Progress	Evaluate the potential use of disinfectants in the field to eradicate Bsal from a small area after a point source introduction (sensu Bosch et al. 2015).
	Determine minimal alterations to habitats that can promote disease risk reductions (e.g., increasing habitat temperature through shade reduction, altering pH or salinity, changing complexity of habitat structure to affect contact rates, dewatering habitats) or augmenting habitats with native anti-Bsal microbes.
In Progress	Determine the effectiveness of reducing host density or altering relative abundance of host species with different infection tolerances on invasion potential of Bsal.
E) Goal: Quantify innate and adaptive immune responses to Bsal as disease progresses under varying conditions.	
In progress	Determine whether amphibians are able to develop a lymphocyte-mediated immune response to Bsal, and how this and other responses compare among species, populations, life stages, and with environmental conditions.
In progress	Determine whether salamanders produce antimicrobial skin peptides or other antimicrobial compounds, and if skin toxins used for defense (e.g., tetrodotoxin, TTX) influence antimicrobial product production.
	Determine whether amphibians or symbionts produce antifungal compounds.

Within Spp: In Progress	Determine how the skin microbiome (e.g., bacteria, fungi, viruses) interacts with immune responses and influences disease susceptibility. Also, determine whether the skin microbiome can be manipulated and whether it is influenced by environmental conditions and host genetics.
	Establish hematological reference values and determine whether these parameters reflect immunity to Bsal infection in amphibian hosts.
In Progress	Determine whether protective immunity develops upon host clearance of Bsal and repeat exposure. Determine how protective immunity can best be established (e.g., vaccine, heat-clearing Bsal). Also, determine what immune responses are regulated by protective immunity (e.g., mucosal antibodies, skin defense compound expression, changes in microbiome).
F) Goal: Identify the mechanisms of Bsal pathogenesis	
In Progress	Quantify the changes in plasma electrolyte concentrations and other physiological parameters in Bsal-infected salamanders.
In Progress	Identify tissue tropism for Bsal-infected amphibians.
	Explore mechanisms of attraction to (e.g., chemotaxis) and physical binding of zoospores to hosts.
	Determine whether Bsal releases lymphotoxic or cytotoxic molecules
	Examine Bsal gene expression, molecular/hormonal cues affecting Bsal virulence factors
	Examine ecological factors influencing Bsal pathogenesis including temperature or other conditions that may influence Bsal zoospore morphology and infectivity.
	Determine whether Bsal releases lymphotoxic or cytotoxic molecules.
G) Goal: Establish effective methods for detecting and safely clearing Bsal infections.	
In Progress	Identify volatile organic compound (VOC)-producing, Bsal-inhibitory microbes and their inhibitory compounds.
In Progress	Test alternative antifungal compounds for use on a broad host taxonomic range and across life-history stages.
In Progress	Test the use of these microbes and/or compounds to clear existing infections, and minimize side effects.
H) Goal: Estimate the interactive effects of Bsal with natural and anthropogenic stressors.	
	Conduct susceptibility trials that include common natural and anthropogenic stressors (e.g., hydration, salinity, pesticides) to determine if outcomes following Bsal exposure are altered.
	Conduct susceptibility trials in complex settings that include community features such as predation and trophic interactions and changing habitat quality.

Detailed Goals

A) Goal: Estimate the occurrence of Bsal in the U.S., Canada and Mexico amphibian pet trade, the risk of spillover to wild populations, and the likelihood of humans playing a role in the overland translocation of Bsal upon introduction.

Priority: Urgent

Rationale: The most likely route of entry for Bsal into North America is unclean international trade of amphibians (Gray et al. 2015). Currently, animal health certificates for internationally traded wildlife are not required for most nations, including the United States. Although the U.S. Fish and Wildlife Service rule banning the trade of some salamander genera may have reduced the likelihood of infected animals entering the U.S. via trade (Grant et al. 2017), the ban does not include many taxa that are known suitable hosts (based on recent studies or unpubl. data), including frogs. Yuan et al. (2018) estimated that up to 66,000 salamanders infected with Bsal could have entered the U.S. in the past 10 years, and their estimates did not include frogs, which comprise 94% of imported amphibians. Indeed, Bsal has been documented in trade in Europe (Cunningham et al. 2015), and trade is hypothesized as the route of entry from endemic Asia to the European continent (Martel et al. 2014, Nguyen et al. 2017). Information on the occurrence of Bsal in the North American amphibian pet trade is needed. Klocke at al. (2017) performed preliminary surveillance for Bsal in the U.S. pet trade and did not detect it. However, their small sample size prevented detection of the pathogen at low prevalence (Yuan et al. 2018). No Bsal surveillance studies in the pet trade have been published for Canada or Mexico. In the case of Mexico, a formal petition has been made to the corresponding Federal Agency (SENASICA) so that amphibian imports are screened for Bsal, however the petition is still under evaluation. Additional information about the potential for commonly traded amphibian species to carry Bsal is also needed and will help guide surveillance efforts.

In addition to knowing whether Bsal exists and its prevalence in the North American pet trade, we need to understand the likelihood of consumers releasing unwanted pet amphibians or disposing their aquarium contents in the environment. This likelihood may be different between hobbyist and specialist amphibian consumer groups. If Bsal is detected in the pet trade or wild, it is important to know the willingness of consumers to participate in programs designed to modify public behavior in a way that will limit pathogen spread, such as providing unwanted pet amphibian amnesty programs and using disinfectants known to kill Bsal zoospores at home (aquaria) or in the field (recreational gear).

The studies below will use a combination of non-lethal testing of amphibians in the pet trade for Bsal infection and human dimension surveys to characterize public awareness, perceptions and behaviors associated with Bsal.

Action Items:

1) Estimate the occurrence and prevalence of Bsal in the North American pet trade through non-lethal surveillance of amphibians at ports of entry, wholesale distributors, and retail stores. This includes development of reliable diagnostic techniques with high sensitivity to detect Bsal in shipments.

2) Estimate the susceptibility of potential Bsal hosts (salamanders and frogs) in the pet trade (e.g., species commonly imported from Southeast Asia).

3) Characterize human behaviors for amphibian hobbyist and specialist groups to estimate the likelihood of Bsal spillover from consumers to wild populations and the acceptance of public outreach strategies designed to limit the anthropogenic spread of Bsal.

Management Relevance: Surveillance for Bsal in the pet trade is essential to know whether this foreign pathogen is in North America. Estimates of prevalence can be combined with shipping and distributor information to identify areas where spillover is most likely to occur, which can direct field activities. Understanding human behavior is also essential to assessing risk of human-mediated spillover or translocation of Bsal among sites and estimating public perceptions to future programs or regulations designed to thwart Bsal emergence.

Estimated Time and Cost:

Action Item	Estimated	Estimated Budget[1]	Funding Status
(1)	1 – 2 years (depending on scale of sampling)	$30,000 per state / province or port of entry $15,000 per distributor	Not funded but some previous work (Klocke et al. 2017, B. L. Talley, unpubl. data)
(2)	3 months per species	$15,000 per species	Partially funded
(3)	1 – 2 years (depending on scale of sampling)	$30,000 per state	Not funded

[1]Per state / province estimates are for sampling pathogens (1) and consumers (2) at up to 20 stores, which could be divided equally between hobbyist and specialist groups.

B) Goal: Develop compartmental disease models, epidemiological tools using empirical data to identify critical transmission pathways and conditions under which Bsal is likely to emerge in amphibian host populations in North America.

Priority: Urgent

Rationale: Identifying the importance of transmission pathways under varying conditions is fundamental to characterizing the epidemiology of host-pathogen systems and conceiving disease intervention strategies (Tien and Earn 2010, Langwig et al. 2015). Environmental transmission of Bsal can occur through water or soil, and it depends on various factors such as host shedding rates of the pathogen and pathogen persistence outside of the host (Nelson et al. 2009, Briggs et al. 2010). Transmission can also occur through direct contact between infected and uninfected individuals. The probability of transmission can change as disease progresses in the host (McCallum et al. 2001, Peace et al. 2019). We recommend development of Bsal epidemiology models for widely distributed, abundant host species in North America that are known to be susceptible to Bsal (e.g., *Notophthalmus viridescens*, *Taricha granulosa*) given their potential to maintain, amplify, and spread Bsal. The action items below outline the parameterization of models that can be used to identify key transmission pathways and conditions under which Bsal is likely to emerge. The proposed work involves a combination of controlled experiments and mathematical modeling following models developed for *Bd* (Briggs et al. 2010), ranavirus (Peace et al. 2019), and Bsal (Schmidt et al. 2017).

Action Items:

1) Estimate latency period of infection and recovery rate for pre- and post-metamorphic amphibian hosts at biologically relevant temperatures.

2) Estimate daily shedding and encystment rate of Bsal zoospores and the infectious dose (ID)-50 for pre- and post-metamorphic amphibian hosts at biologically relevant temperatures.

3) Estimate daily contact rates of amphibian hosts at biologically relevant temperatures and densities when exposed to different complexities of habitat structure.

4) Estimate probability of Bsal transmission between infected and uninfected amphibian hosts (within and between species) at different post-exposure durations and temperatures. Determine the role of dead individuals in the transmission and environmental persistence of Bsal.

5) Estimate the duration of Bsal zoospore persistence in water and soil given differences in various environmental conditions (e.g., temperature, micropredators, soil moisture, water chemistry, and bacterial presence).

6) Estimate the influence of co-infection with other pathogens (e.g., *Bd*, ranavirus) on the likelihood of Bsal transmission and development of chytridiomycosis.

7) Identify possible biological reservoirs for Bsal and the likelihood of non-hosts (e.g., waterfowl, humans) translocating Bsal on external surfaces or fomites. Model infection dynamics within realistic multi-host biotic communities.

Management Relevance: These predictive models can provide insight into transmission pathways, environmental conditions, and population characteristics that can be manipulated to reduce the impacts and persistence of Bsal at a site. For example, if direct contact between individuals is a key transmission pathway, intervention strategies that reduce contacts should be used, such as altering habitat structure or reducing animal density. If environmental transmission is a key pathway, strategies that change conditions that reduce zoospore persistence should be implemented. If non-amphibian hosts can contribute to the persistence of Bsal in the environment, strategies can be directed at managing these groups.

Estimated Time and Cost:

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1 – 5)	3 years	$1.5M	Partially Funded by NSF EEID[1]
(6)	2 years	$300,000	Not Funded but some previous work (Longo et al. 2019)
(7)	2 years	$300,000	Not funded

[1]Grant funded for *Notophthalmus viridescens* (National Science Foundation DEB EEID Grant #1814520) between species transmission and by dead individuals (portion of action item 4) not funded.

C) Goal: Develop epidemiological tools (i.e., integral projection models) that enable objective classification of species tolerance to Bsal infection, which can be used to produce more informed Bsal risk models for North America.

Priority: Urgent

Rationale: The likelihood of pathogen invasion is commonly modeled using risk analyses, which can be dependent on environmental conditions, host species distribution and susceptibility, and population characteristics (Václavík et al. 2010, OIE 2014). Preliminary Bsal risk models for North America based on environmental suitability indices for Bsal and salamander distributions suggest that the Southeast, Northeast, and Pacific Coast of the United States and south-central Mexico have high invasion potential (Yap et al. 2015, 2017; Richgels et al. 2016, Basanta et al. 2019). One limitation of these predictions is that little information was available for incorporating host susceptibility into the risk estimates. Since autumn 2015, the susceptibility of >30 North American amphibian species to Bsal has been estimated among several U.S. laboratories (Appendix 2). Integral projection models (IMPs)

can be used to categorize species susceptibility, considering their tolerance to infection (Wilber et al. 2016). Susceptibility indices can be combined with host species distributions and environmental niche data for Bsal to more robustly predict risk of pathogen invasion geographically. Biologists can use risk assessments to target locations for disease response and management actions. IMPs can also be used to classify the potential role of species during disease outbreaks (Wilber et al. 2016), which could range from resistant to reservoir to amplification hosts (Paull et al. 2012). Knowing the potential contribution of host species to community-level transmission can help direct disease intervention strategies, which can differ depending on host susceptibility (Streicker et al. 2013). The proposed work involves a combination of dose-dependent experiments and mathematical modeling to objectively categorize and rank species susceptibility.

Action Items:

1) Estimate the susceptibility (i.e., tolerance) of North American amphibians to Bsal infection and chytridiomycosis using standardized, dose-dependent experiments (suggestions for targeted taxa are provided in Appendix B).

2) Estimate the impact of habitat characteristics (temperature, pH, salinity, zooplankton abundance, etc.) on Bsal infection risk.

3) Develop Integral projection models (IPMs) that predict tolerance using temporal estimates of Bsal infection load and host fitness metrics (e.g., survival, disease ranking using microscopic and gross lesions).

4) Using information developed in (1-3), map susceptibility indices on the geographic distributions of hosts and environmental suitability niches for Bsal to produce robust spatial predictions of Bsal risk in North America.

Management Relevance: Comprehensive assessment of species susceptibility to Bsal in North America will produce robust Bsal risk maps (similar to Yap et al. 2015; Richgels et al. 2016, Basanta et al. 2019) in which pathogen surveillance and disease response actions can be targeted. Additionally, IPMs can lead to objective rankings of species susceptibility, and classifications of epidemiological roles (e.g., resistant, reservoir or amplification species), which provide insight into community-level impacts at sites. For example, communities dominated by carrier species (i.e., high Bsal tolerance) may experience minimal disease occurrence but high Bsal infection prevalence and be sites where the pathogen is maintained; whereas, sites dominated by amplification species (i.e., low Bsal tolerance) may experience rapid Bsal transmission, disease progression, and population declines.

Estimated Time and Cost:

Action Item	Estimated Timeframe	Estimated Budget	Funding Status

(1)	3 months per species	$15,000 per species[1]	Partially Funded[2]
(2)	1 year	$300,000	Partially Funded[3,4]
(3)	1 year	$300,000	Partially Funded[4]
(4)	1 year	$200,000	Funded[5]

[1]Completed species are listed in Appendix A; unfunded species are listed in Appendix B.
[2]BAND Foundation, Tennessee Wildlife Resources Agency, North Carolina Wildlife Resources Commission, U.S Fish and Wildlife Competitive State Wildlife Grant.
[3]Smith Conservation Fellowship,
[4]National Science Foundation DEB EEID Grant #1814520.
[5]U.S. Fish and Wildlife Service Competitive State Wildlife Grant.

D) Goal: Identify effective methods to manage disease in captive and field settings.

Priority: Urgent

Rationale: Managing disease threats like those posed by Bsal, are of the utmost importance for conservation. A proactive strategy for developing disease mitigation tools is imperative for having an effective rapid response if Bsal is introduced into North America (Grant et al. 2017). Priority for disease mitigation should focus on highly susceptible amphibian taxa as well as tolerant hosts that may act as Bsal reservoirs within the ecosystem. Mitigation strategies targeting the host, such as vaccination or probiotic bioaugmentation of the skin microbiota, or strategies targeting the environment, such as micropredator augmentation, are promising conservation frontiers for field-based mitigation (Bletz et al. 2013). We can use what we have learned from *Bd* as a foundation for developing and understanding potential disease mitigation and treatment strategies and also take advantage of novel directions. Within the amphibian-*Bd* studies, the addition of locally occurring protective bacteria to amphibian skin has effectively prevented *Bd*-associated chytridiomycosis in laboratory trials and a field trial (Harris et al.2009a,b, Vredenburg et al. 2011). Additionally, early studies suggest that adaptive immunity can be induced by a vaccination strategy (McMahon et al. 2014). Nasal delivery of vaccines against bacterial and viral infectious diseases has shown promising results in rainbow trout (La Patra et al. 2015), and may be an effective strategy for treating amphibian species. Furthermore, *Bd* infection risk has been correlated with environmental micropredators, and certain microeukaryotes can greatly reduce infection probability and reduce zoospore persistence in experimental contexts (Schmeller et al. 2014). Therefore, manipulation of micropredator communities could serve as a feasible strategy to minimize infection risk.

Action items:

1) Identify effective probiotic microbes and develop probiotic treatment methods to combat Bsal, including the exploration of host and environmental modes of treatment. Test non-target impacts of probiotics and examine potential for bacteremia through lesions.

2) Identify Bsal-consuming aquatic micropredators from natural habitats and test micropredator augmentation strategies.

3) Evaluate novel vaccination methods as a possible disease mitigation tool and test different modes of delivery (e.g., different life stages, nasal associated lymphoid tissue vaccination, skin exposure).

4) Explore the use of Bsal removal methods (e.g., attractants or traps).

5) Explore the genetic correlates of disease resistance and the possibility of selective breeding hosts for Bsal resistance.

6) Evaluate the potential use of disinfectants in the field to eradicate Bsal from a small area after a point source introduction (sensu Bosch et al. 2015).

7) Determine minimal alterations to habitats that can promote disease risk reductions (e.g., increasing habitat temperature through shade reduction, altering pH or salinity, changing complexity of habitat structure to affect contact rates, dewatering habitats) or augmenting habitats with native anti-Bsal microbes.

8) Determine the effectiveness of reducing host density or altering relative abundance of host species with different infection tolerances on invasion potential of Bsal.

Management Relevance: Disease response is essential to thwart pathogen outbreaks. Because amphibians have relatively low dispersal capability, host- and site-based management strategies can be effective, which has been demonstrated in some cases for *Bd* (Bosch et al. 2015, Vredenburg et al 2011). Upon identification of effective strategies, natural resource agencies will be equipped with the best practices to prevent (proactive) or reduce (reactive) Bsal chytridiomycosis in amphibian habitats and populations.

Estimated Time and Cost:

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1-3)	3 years	$400,000	Partially funded[1]

(4-6)	3 years	$400,000	Partially funded
(7)	2 years per strategy	$150,000 per strategy	Partially funded[2]
(8)	2 years	$200,000 density $200,000 composition	Partially Funded[3]

[1]$30,000 David H Smith Fellowship; 5,000 Foundation for the Conservation of Salamanders, and U.S. Fish and Wildlife Service Competitive State Wildlife Grant (disinfectants and plant-derived fungicides).
[2]National Science Foundation DEB EEID Grant #1814520 (habitat structure and temperature).
[3]U.S. Fish and Wildlife Service Competitive State Wildlife Grant (host density reduction for one species).

E) Goal: Quantify innate and adaptive immune responses to Bsal as disease progresses under varying conditions.

Priority: High

Rationale: Little is known about the immune defenses of salamanders against *Batrachochytrium* fungi. Preliminary research suggests that the fire salamander (*Salamandra salamandra*) – a European newt species in which exposure to a low dose of Bsal results in disease – has few effective immune defenses against Bsal infection (Martel et al. 2013, Van Rooij et al. 2015). Other salamander species appear to be more resistant to Bsal chytridiomycosis and several anuran species can clear infection (Martel et. al 2014, Stegen et al. 2017). Despite initial findings with species susceptibility trends (Goal C), the role of amphibian immune defenses in mediating host response to Bsal infection remains largely unknown. Immunocompetence in amphibians can differ among life history stages (i.e., age classes), among populations, and with changes in environmental conditions. In particular, amphibian immunity is influenced by temperature (Rollins-Smith 2017). Like other vertebrates, the immune system of amphibians is comprised of innate and adaptive components. For skin pathogens like chytrid fungi, antimicrobial peptides produced in the skin can be an important first defense (Holden et al. 2015).

Symbiotic microorganisms on amphibian skin can also contribute to their immunity through direct competitive interactions or by producing antimicrobial byproducts (Woodhams et al. 2018). Adaptive immune responses to Bsal are unknown. Understanding the mechanisms of host disease resistance can lead to the development of intervention strategies focused on host immunity, such as use of vaccines and bioaugmentation techniques. Because Bsal creates necrotic skin ulcerations that can extend through the epidermis (Martel et al. 2013), possible probiotic treatments need to be evaluated to ensure they do not contribute to bacteremia and sepsis (Bletz et al. 2018).

Action Items:

1) Determine whether amphibians are able to develop a lymphocyte-mediated immune response to Bsal, and how this and other responses compare among species, populations, life stages, and with environmental conditions.

2) Determine whether salamanders produce antimicrobial skin peptides or other antimicrobial compounds, and if skin toxins used for defense (e.g., tetrodotoxin, TTX) influence antimicrobial product production.

3) Determine whether amphibians or symbionts produce antifungal small molecule compounds.

4) Determine how the skin microbiome (e.g., bacteria, fungi, viruses) interacts with immune responses and influences disease susceptibility. Also, determine whether the skin microbiome can be manipulated and whether it is influenced by environmental conditions and host genetics.

5) Establish hematological reference values and determine whether these parameters reflect immunity to Bsal infection in amphibian hosts.

6) Determine whether protective immunity develops upon host clearance of Bsal and repeat exposure. Determine how protective immunity can best be established (e.g., vaccine, heat-clearing Bsal). Also, determine what immune responses are regulated by protective immunity (e.g., mucosal antibodies, skin defense compound expression, changes in microbiome).

Management Relevance: A mechanistic understanding of amphibian immune responses to Bsal will enable directed mitigation approaches. For example, determining how protective immunity can be established in salamanders may direct management toward vaccines or probiotic microbial therapy or other approaches to increase salamander resistance to chytridiomycosis. It also may be possible to alter habitat conditions to facilitate some host immune responses.

Estimated Time and Cost:

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1)	2 years	$200,000	Funded by NSF EEID[1]

(2)	2-3 years	$250,000	Partially funded by NSF EEID[2]
(3)	2-3 years	$250,000	Not funded
(4)	2 years	$200,000	Partially funded by NSF EEID[3]
(5)	2 years	$200,000	Funded by NSF EEID[1]
(6)	2 years	$200,000	Partially funded by NSF EEID[3]

[1]Grant funded for *Notophthalmus viridescens*.
[2]Grant funded for *Notophthalmus viridescens*; the role of TTX not funded.
[3]Grant funded for *Notophthalmus viridescens* for the first portion of this action item.

F) Goal: Identify the mechanisms of Bsal pathogenesis

Priority: High

Rationale: The mechanisms by which Bsal becomes a lethal pathogen are unknown (Van Rooij et al. 2015). Grossly and anatomically, chytridiomycosis due to Bsal develops differently in a host than *Bd*. *Bd* results in hyperkeratosis (i.e., skin thickening); whereas, Bsal causes ulcerative, necrotic skin lesions that can extend through the epidermis. The mechanisms of pathogenesis for *Bd* are compromised osmoregulation across the skin that leads to electrolyte imbalance in the blood (especially Na^+, K^+, and Ca^{2+}), which affects epidermal electrolyte transport, leading to asystolic cardiac arrest (Voyles et al. 2009).

The physiological mechanisms for Bsal pathogenesis may be similar to *Bd* (i.e., altered osmoregulation); however, electrolyte imbalance may be a consequence of skin destruction instead of hyperplasia. It is also possible that reduced cutaneous respiration could be a morbidity factor in Bsal-induced chytridiomycosis. In general, salamanders rely on cutaneous respiration more than frogs, especially species in the Plethodontidae (lungless salamander) family (Wells 2010). Bacteremia is another hypothesized mechanism of Bsal chytridiomycosis (Bletz et al. 2018).

The proposed work involves a combination of clinical and anatomical pathology to quantify structural and physiological changes in salamanders as Bsal chytridiomycosis progresses.

Additional areas of exploration will include the molecular pathways required for initial interactions between Bsal zoospores and their hosts. In particular, understanding how the Bsal zoospore is attracted to a suitable host (e.g., chemotaxis) and adheres are important areas for research, and represent possible opportunities for prevention or reduction of infection. Other areas of research focusing on Bsal biology that will provide important insight into pathogenesis include understanding how Bsal infection spreads through host tissue, and identifying molecular signatures specific to host infection.

Action Items:

1) Quantify the changes in plasma electrolyte concentrations and other physiological parameters in Bsal-infected salamanders.

2) Identify whether bacterial invasion from the skin via Bsal lesions and sepsis are contributing factors to pathogenesis.

3) Identify tissue tropism for Bsal-infected amphibians.

4) Explore mechanisms of attraction to (e.g., chemotaxis) and physical binding of zoospores to hosts.

5) Determine whether Bsal releases lymphotoxic or cytotoxic molecules.

Estimated Time and Cost:

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1)	2 years	$300,000	Not funded
(2)	1 year	$100,000	Not funded
(3)	2 years	$250,000	Not funded
(4)	2 years	$250,000	Not funded
(5)	3 years	$300,000	Not funded

Management Relevance: Understanding the pathology of Bsal will enhance our ability to predict susceptible species and provide the groundwork for making informed decisions about where and how to manage Bsal emergence.

G) Goal: Establish effective methods for detecting and safely clearing Bsal infections.

Priority: High

Rationale: Managing disease threats, like those posed by Bsal, are of the utmost importance for conservation. A first step is preventing entry of the pathogen into naïve regions like North America. Development of clearance strategies for traded amphibian species that can carry Bsal can allow trade to continue while minimizing the risk of Bsal introduction. Heat therapy and antifungal treatments have been found to be effective for one European salamander species (Blooi et al. 2015a,b). However, such treatments may not be suited for all amphibian species. Many species cannot tolerate elevated temperature and/or antifungal medications (e.g., itraconazole; Baitchman and Pessier 2013).

Action items:

1) Identify volatile organic compound (VOC)-producing, Bsal-inhibitory microbes and their inhibitory compounds.

2) Test alternative antifungal compounds for use on a broad host taxonomic range and across life-history stages.

3) Test the use of these microbes and/or compounds to clear existing infections, and minimize side effects.

4) Develop new diagnostic tools and improve existing tools.

Management Relevance: Clearing existing Bsal infections from captive-housed amphibians is critical for use in the pet trade and for captive management of critically endangered amphibians, or amphibians used in research. Improved methods may enable more effective policy recommendations, and make it easier to eliminate threats.

Estimated Time and Cost:

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1-3)	2 years	$300,000	Not funded
(4)	2 years	$50,000	Not funded

H) Goal: Estimate the interactive effects of Bsal with natural and anthropogenic stressors.

Priority: Medium

Rationale: Laboratory estimations of the susceptibility of amphibian species to Bsal are a good starting point for developing landscape risk models for Bsal emergence. However, amphibians have complex life histories and unique physiologies that make them particularly sensitive to stressors. Indeed, amphibians are heavily dependent on water, making them particularly sensitive to altered hydroperiod, desiccation, and decreases in water quality. Examples of impaired water quality include increased salinity, acidity, eutrophication, and pesticide contamination. In many cases, environmental stressors induce changes in host behavior and physiology that could potentially influence risk from Bsal. For example, changes in body condition or corticosterone (a hormone commonly elevated in response to stressors) can modulate immune function and possibly susceptibility to *Bd* (Tatiersky et al. 2015, Fonner et al., 2017). Similarly, physiological and behavioral responses to desiccation (e.g., changes in plasma osmolality, increased osmoregulatory behaviors) may influence infection dynamics and disease progression. These effects may be exacerbated or mitigated in more complex environments (e.g., mesocosms) by changes in community interactions and habitat quality.

Action Items:

1) Conduct susceptibility trials that include common natural and anthropogenic stressors (e.g., hydration, salinity, pesticides) to determine if outcomes following Bsal exposure are altered.
2) Conduct susceptibility trials in complex settings that include community features such as predation and trophic interactions and changing habitat quality.

Management Relevance:

Understanding how environmental and community conditions modulate susceptibility to Bsal will help predict invasion risk. In addition, if stressors are identified (e.g., pesticides), management strategies can be implemented to reduce the effect of the stressor.

Estimated Time and Cost:

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1)	3 years	$150,000 per stressor	Not funded
(2)	3 years	$300,000	Not funded

4. Decision Support Working Group

(Evan Grant, facilitator)

Decision Support Needs

The goal of the Decision Support Working Group (DSWG) of the North American Bsal Task Force is to support management decisions regarding Bsal through the facilitation of decision-making processes, identification and collation of information needed to make decisions, development of models to predict the outcomes of different management options, and the evaluation of tradeoffs and risk to overcome impediments to optimal decision-making. The DSWG is currently composed of six members, representing three organizations (USGS, USFWS, and Pennsylvania State University). Collectively, the group has decades of experience in decision science, amphibian and pathogen ecology, research, mathematical modeling, and working directly with managers.

Emerging diseases have the potential to affect social, economic and ecological interests of North American resource managers, who are entrusted by society to manage protected areas and wildlife populations. Although preventing the arrival of a pathogen is most effective for controlling emerging infectious diseases, prevention is not failsafe. Resource managers must consider multiple social, economic and ecological objectives, which result in difficult trade-offs for any given disease management strategy (i.e., an optimal action for managing a wildlife disease may result in declines in recreational or economic values). Complexity arises in balancing numerous and competing demands on land managers, and this effectively limits our ability to identify and implement proactive management - representing a major challenge for developing management strategies for Bsal and other emerging infectious diseases. To date, there are no viable treatment options available for Bsal, which limits the alternatives available for managers until effective treatments are identified (n.b., the Research Working Group has identified research priorities to address this knowledge gap). Much uncertainty remains, which also makes choosing an (untested) management action challenging. Decision science represents a framework for developing strategies and determining a course of action in the face of uncertainty. Additionally, even if treatments are identified, implementation may still be delayed if other management objectives are predicted to suffer; decision analysis helps identify optimal solutions across potentially competing management objectives.

Management Relevance

Despite calls for improved responses to emerging infectious diseases in wildlife, management is seldom considered until a disease has been detected in a population. Reactive approaches often limit the potential for control and increase the total cost of a response. By using the tools from decision science and behavioral psychology to facilitate conversations between researchers and wildlife managers and identify optimal management strategies, the DSWG can help navigate the common pitfalls of developing and implementing proactive management solutions for Bsal ahead of an invasion, and plan for thoughtful responsive management once Bsal arrives in a

population. Acting under high levels of uncertainty is a hallmark of wildlife disease management, and the use of formal decision analytics (e.g., multi-criteria decision analysis, risk analysis, cost-benefit analysis within a structured or adaptive management framework, and portfolio decision theory) is increasing among natural resource agencies as a rational and transparent framework for managing diseases. Decision analytic approaches can examine trade-offs between managing despite uncertainty and delaying action to gain additional disease information. In addition, this framework can identify key trade-offs among competing objectives which are often ignored, but which can be highly influential in the final decision-making process, and in optimizing management responses.

Some major challenges to Bsal management include: limited control options for the initial introduction of disease, widely dispersed populations over multiple states and regions, fragmented management authority by diverse agencies (state or provincial/territorial, federal, and non-profits), and deep uncertainties in ecological characteristics of the pathogens, populations, and effectiveness of potential treatments. The DSWG priority goals (outlined below) are designed to respond to these challenges. Each need below is specified as an overall goal, rationale, and a list of action items. We also include a timeline and budget for each goal listed.

A) Goal: Improve ability to manage impacts of Bsal to native amphibian populations by developing a process for identifying critical research needs. Develop a process for identifying critical research that will lead to an improved ability to manage Bsal.

Priority: High

Rationale: Identify critical research needs that impede decision-making for responsive and proactive management. The collaborative development of research priorities between land managers and researchers is an integral component for creating and evaluating effective and efficient management solutions.

Action Items:

1) Coordinate a Bsal "science experts" workshop to collaboratively create a system diagram to help identify areas of greatest research need (i.e. regions within the system diagram that may facilitate the development of proactive management strategies). System, or influence, diagrams map ecological system components and relationships that lead to defined outcomes. Research priorities are generated for those areas of the system diagram where improved knowledge will have the greatest contribution to selecting optimal management actions, and can be formally assessed using decision-analytic tools. This work will be conducted in collaboration with the Research Working Group.
2) Hold a series of structured decision making workshops with managers (i.e., USFWS refuge biologists, regional land managers, Canadian Fish and Wildlife biologists, etc.) to identify where proactive management can be implemented and what the barriers to implementing proactive management are across regions and management entities.

Estimated Time and Cost:

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1)	1.5 - 2 years	$200,000	
(2)	1.5 - 2 years	$300,000	

B) Goal: Identify approaches to improve proactive management for Bsal when competing objectives or risk are impediments to action.

Priority: High

Rationale: The proactive implementation of management ahead of an introduction to reduce the future severity or duration of a disease outbreak is difficult for several reasons. First, management for one aspect of a system may induce trade-offs in the ability to fully support another part of the system (e.g., culling a population ahead of an outbreak to reduce transmission reduces the immediate population size). Second, intervention is often associated with risks that cannot be reliably forecasted (e.g., the probability of pathogen invasion and the resulting severity are uncertain). Finally, any decision must incorporate uncertainty about whether a management action will be successful in a novel system.

Action Items:

1) Use simulation, modeling, and optimization techniques to identify optimal actions given various impediments.
2) Using these models, evaluate possible trade-offs of action versus inaction, and estimate costs of delaying action. Evaluate risk tolerance and effect on optimal actions under different levels and sources of uncertainty.

Estimated Time and Cost:

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1)	2.5 years	$380,000	
(2)	1 year (in addition to above; can be done simultaneously)	$120,000	

C) Goal: Conduct and update risk assessments.

Priority: High

Rationale: Based on recent risk assessments, amphibian importation restrictions were instituted in the US and Canada in response to the threat of Bsal invasion. Unfortunately, the banning of salamander imports is unlikely, by itself, to completely mitigate the risk of introduction and spread (OiE Guidelines for Wildlife Disease Risk Assessment) of this disease. For example, restrictions on the movement of domestic birds in 2015 failed to prevent highly pathogenic avian influenza outbreaks, which were attributed to poor or incomplete adherence to biosecurity recommendations. In addition, Bsal has recently been detected on several commonly imported anuran species in addition to urodeles, and the complete range of Bsal amphibian hosts is unknown. Thus, while the USFWS and Environment and Climate Change Canada decisions are an excellent first step to protecting North American salamander species from Bsal introduction, here we further explore the effectiveness of the possible combination of prevention strategies for mitigating risk from an emerging pathogen, using Bsal as a case example.

Action Items:

1) Estimate the residual risk to populations after implementation of strategies (e.g., importation ban, clean-trade certification, or other trade-based strategies) designed to reduce risk of introduction of Bsal into wild populations of amphibians in the U.S., Canada, and Mexico
2) Identify how other actions, in combination, may further reduce risk to native amphibians. Remaining risk will be calculated for combinations of pre-introduction (proactive) and post-introduction (responsive) management actions.

Estimated Time and Cost:

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1)	1 year	$80,000	
(2)	1.5 years	$160,000	

D) Goal: Frame Bsal management problems at regional and resource manager levels

Priority: High

Rationale: This goal is the bulk of the work needed to plan and develop implementation strategies for management of Bsal risk. Several managers are working with the DSWG to frame and evaluate their decision options for proactive Bsal management. Framing management problems as decisions can enable managers to identify possible proactive solutions. This approach recognizes context-specific constraints, such as agency mandates, trade-offs among other mission elements, and relevant uncertainties that must be accommodated in developing a response.

Action Items:

1) Engage resource managers at multiple scales (e.g., single protected area, regional, national) to develop decision frameworks, and specific and relevant measurable attributes, for their particular jurisdictions (e.g., all State forests, or provincial/territorial parks, a single National Park, National Wildlife Areas, Wildlife Refuge or Forest). Particular emphasis should be made to include objectives and metrics to understand tradeoffs among habitats, amphibian populations, and pathogen occurrence and prevalence.
2) Work with managers with complementary or spatially proximate at-risk populations to develop decision frameworks for linked decisions (i.e., actions chosen by one decision maker may affect the available actions to another decision maker). An example would be identifying proactive management with and without importation restrictions, or identification of optimal control strategies for neighboring protected area populations.

Estimated Time and Cost:

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1)	3 years	$380,000	
(2)	2 years	$300,000	

E) Goal: Use risk assessments to inform surveillance design and identify whether management should consider proactive, reactive, or a combination of management strategies, dependent on the presumed presence and spatial distribution of Bsal.

Priority: High

Rationale: A number of sampling designs may be useful for detecting the presence of the pathogen within and among populations, and work is underway to improve predictions of areas which may be at highest risk for declines should the disease be discovered. Data from a surveillance program without an associated state-dependent management plan (i.e., conditional on the state of the disease) of the appropriate scale that matches the scale of a management decision, is of limited use; the design of an optimal program must consider the possible management responses for various scenarios. This work will be conducted in collaboration with the Surveillance and Research working groups.

Action Items:

1) Incorporate information from surveillance work into current risk assessments for Bsal, and adjust surveillance efforts accordingly to incorporate prior expectation of Bsal occurrence and observations from a designed surveillance program.
2) Previous risk assessments used a limited number of criteria to identify high risk areas; determine if other criteria can be included to improve risk assessments.

Estimated Time and Cost:

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1)	2 years	$250,000	
(2)	1 year	$110,000	

5. Management Working Group

(Laura Sprague, facilitator)

Summary & Mission Statement:
Managers are often faced with making rapid decisions. The process can be quite overwhelming and confusing when dealing with multiple factors like an emerging pathogen, differing state or provincial/territorial, and federal jurisdictions, regulations, policy and permitting. The Bsal Management group was formed to bridge the gap between identifying and implementing scientifically-sound Bsal mitigation actions by proactively designing guidelines, identifying permitting steps, and facilitating the process of navigating the requirements for state or provincial/territorial and federal policy. Simply put, our purpose is to ***facilitate efficient and rapid response to*** **Bsal** ***invasion***.

The management working group has significant overlap between several other working groups. In general, the research working group focuses on testing possible disease management options, the decision working group helps biologists decide upon a course of action given likelihood of success, and the management working group assists biologists with implementing management strategies. In addition, the management working group can help in customizing a rapid response plan using the template developed by the response working group.

Note: Portions of this section refer explicitly to US laws, regulations, procedures and agencies, and do not apply broadly to Canada or Mexico. Work is ongoing to better integrate Canada and Mexico in the near future.

Background*:*
Bsal introduction and disease outbreaks in North America could happen at anytime. Managing disease threats like that posed by Bsal, are of the utmost importance for conservation. A proactive understanding of the steps and required approvals for rapid response if Bsal is introduced into North America will facilitate effective implementation of management strategies and reduce impacts on native salamander populations. Priority for management should focus on preventing outbreaks and minimizing the potential spread across the landscape. Focal hosts include highly susceptible amphibian taxa as well as tolerant hosts that may act as Bsal reservoirs within the ecosystem.

Mitigation strategies can target the host or environment. We can use what we have learned from *Bd* as a foundation for developing and understanding potential disease mitigation and treatment strategies and also take advantage of novel directions as new, innovative ideas are discovered

through research. Host-directed strategies are mitigation tools aiming to foster disease resistance or tolerance, such as skin probiotics (Bletz et al. 2013, Harris et al. 2009a,b), vaccinations (McMahon *et al.*, 2014, LaPatra *et al.*, 2015), and antifungal medications (Hudson *et al.*, 2016; Hardy *et al.*, 2015; Bosch *et al.*, 2015). Environment-directed strategies include micropredator manipulations (Schmeller *et al.*, 2014; Buck *et al.*, 2011), salt augmentations (Stockwell *et al.*, 2015, 2014), environmental probiotics (Muletz et al. 2012), habitat alterations and removal of infection hosts. These strategies have potential for mitigating Bsal's impact on North American salamander biodiversity.

To implement many of these management actions on the ground, government agencies may be required to follow national and/or statewide policies related to the potential environmental impacts associated with these conservation strategies.

US Federal Lands

US Federal agencies are required to follow the policies of the National Environmental Policy Act (NEPA) of 1970. The NEPA process or "Environmental impact assessment process" applies when a Federal agency has discretion to choose among one or more alternative means of accomplishing a particular goal. It requires agencies to determine if their proposed actions have significant environmental effects to land and water, protected wildlife and plants, historic properties, cultural resources, and other interests, as well as to consider the environmental and related social and economic effects of their proposed actions. NEPA's procedural requirements apply to a Federal agency's decisions for actions, including but not limited to permanent or temporary construction projects, limiting public access to public lands, chemical or biological treatments, funding, assisting, conducting, or approving projects and permitting of private actions.

Private and State entities will often become involved in the NEPA process when applying for permits if they will be using public land access or public waters in their actions.
The NEPA process is generally a long drawn out process and can be difficult to navigate through if you are not familiar with it and can take years to accomplish, but must be completed before Federal management decisions are made.

The Council on Environmental Quality (CEQ) oversees the NEPA process with the help of the Environmental Protection Agency (EPA) who issues permits for chemical and biologic use based on the Clean Water Act and Clean Air Act.

Once a proposed action has been developed, and agency can pursue one of two paths:

A. Environmental Assessment (EA)- Determines the significance of the effects and to find alternative measures
B. Environmental Impact Statement (EIS)- must be accomplished if an action significantly affects the quality of the human environment

If an action may occur more than once or routinely and will not have a significant impact on the human environment (either positive or negative), the agency may seek a categorical exclusion

(CE) from CEQ that precludes the need to prepare an EA or EIS for future actions. However, the process for obtaining approval from CEQ for a CE is lengthy and complex. The need must be carefully justified and CEs are rarely granted.

However, on rare occasions, CEQ may exempt an action from NEPA under the following circumstances:

A. If the agency needs to take an action that would typically require preparation of an environmental impact statement in response to an emergency, and there is insufficient time to follow the regular NEPA process, then the agency can work with CEQ to develop alternative arrangements for compliance with NEPA (40 C.F.R. §1506.11) and proceed immediately to mitigate harm to life, property, or important resources.
B. The NEPA analyses and document may involve classified information. If the entire action is classified, the agency will still comply with the analytical requirements of NEPA, but the information will not be released for public review. If only a portion of the information is classified, the agency will organize the classified material so that the unclassified portions can be made available for review (40 C.F.R. §1507.3(c))

US State Lands

There are 16 states with Environmental Quality Acts that require that state and local agencies to perform Environmental Impact Statements (EIS) or at least Environmental Reviews (ER) before performing actions and applying for permits. Please See Appendix 3 for a list of states, the Act in which they are bound to, and the governing body of the act.

Goals:

A) Goal: Facilitate and improve a natural resource agency's ability to take proactive and reactive actions to prevent introduction and spread of Bsal.

Priority: High

Rationale: Should Bsal invade North America, it is imperative to not only have a selection of effective mitigation options to counter the threat, but understand the steps needed to implement such actions.

Action items:

1) Define a list of proactive and reactive actions and tools available that can be taken by managers to prevent the introduction and spread of Bsal
2) Define and outline justifications for management actions, including effectiveness and possible (or lack of) environmental impacts for applying for Cat Ex approval.
3) Develop "blanket documents" for exemption requests
4) Develop "blanket documents" for permitting
5) Identify a list of contacts for rapid submission to relevant permitting agencies.
6) Develop a communication chain for expediting processes.

7) Explore mechanisms to set up an emergency response fund and how to disperse such funds.

Management Relevance: Proactive management and efficient response to outbreaks by natural resource agencies can be hindered by lengthy approval processes and lack of clarity surrounding the necessary steps. Our actions will offer guidance and tools to biologists enabling them to efficiently implement strategies on the ground, ultimately fostering persistence of our native salamander diversity.

Estimated Time and Cost

Action	Estimated Timeframe	Estimated Budget	Funding Status
(1)	Continuous living document	None at this time	NA
(2)	3-6 months	None at this time	NA
(3)	3-6 months	None at this time	NA
(4)	3-6 months	None at this time	NA
(5)	3-6 months	None at this time	NA
(6)	3-6 months	None at this time	NA
(7)	6 months - 1 year	None at this time	NA

B) Goal: Brief and train (as necessary) natural resource agencies about the North American Bsal Task Force and available Management/Mitigation options at a Regional Level.

Priority: High

Rationale: Local management will be the "first" to know of any potential detection and to be proactive should be well informed. Keeping all levels of management informed will help to expedite any processes.

Action items:

1) Provide briefing to natural resource agencies about Bsal Task Force, the Strategic Action Plan, and available management tools.
2) Distribute a Bsal informational brochure/white paper to local field offices of federal, state, tribal and local agencies that may have vested interest in the detection and mitigation of Bsal.
3) Provide training workshops which could be done in-person or remotely targeting local management groups (may vary by region or state)

Management Relevance: Local managers can only effectively response if they are provided with the needed information and understand the possible actions and steps needed to implement actions.

Estimated Time and Cost

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1)	1-2 years	None at this time	NA
(2)	3 months	None at this time	NA
(3)	3-6 months	anticipated	Not funded

C) **Goal**: Provide information and build understanding of the Bsal, the Bsal task Force, the Strategic Action Plan, and available Management/Mitigation options at headquarters (HQ) for Federal Agencies.

Priority: High

Rationale: Assistant Deputy Secretaries, including Policy and Budget, DOI, DOD, DOA, Water and Science, etc. are essential in implementing rapid response and should be prepared in a proactive plan.

Action items:
1) Develop a Bsal informational White Paper to distribute to HQ of government agencies that may have vested interest in the detection of Bsal or are critical in the permitting process of implementing management actions.
2) Develop traveling presentation targeted for Federal HQ.

Management Relevance: Rapid response will require fast permitting and approvals for management actions. Understanding at the HQ of Federal agencies will foster rapid action.

Estimated Time and Cost

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1)	3-6 months	??	Not funded
(2)	3-6 months	??	Not funded

6. Surveillance Working Group

(Michael Adams, facilitator)

Mission

Our mission is to facilitate and coordinate the surveillance of Bsal in North America.

Management Relevance

The purpose of this surveillance effort is to detect the initial introduction of Bsal in North America, thereby allowing for a more effective response. Management and conservation actions cannot proceed without the fundamental information about when and where Bsal is introduced to North America. While opportunistic Bsal sampling improves the odds of detecting Bsal over not sampling at all, this haphazard approach is unlikely to detect Bsal at the onset of its invasion. Instead, our vision is the early detection of Bsal to allow for an effective and rapid response, with the ultimate goal of conserving biodiversity.

Background

Achieving a broad and robust surveillance network is difficult and expensive due to the labor involved. No single entity has been identified that has this capacity. Instead, the emphasis has been on coordinating and encouraging sampling for Bsal such that something closer to a reasonable level of surveillance is achieved. This has not been sufficient and the current approach is to build a network of undergraduate teaching institutions to engage students in surveillance. This will increase both awareness and the amount sampling.

In the US, the only major sampling was a one time effort by the US Geological Survey Amphibian Research and Monitoring Initiative (ARMI). This effort sampled across the U.S. and allocated resources according to estimated risk of Bsal as per Richgels et al (2016). Over 10000 amphibians (mostly salamanders) were sampled. Bsal was not detected. ARMI continues to sample as a very low level in select areas where resources allow. USFWS has done some sampling using their Fish Health Laboratories. There is an ongoing effort to sample in Appalachia which is one of the high risk areas. In addition to sampling, in the US an iNaturalist site was set up as way to gather observations from the public of sick or dead amphibians that might need follow-up investigation. Similarly, the Partners for Amphibian and Reptile Conservation Herp Disease Task Force set up a Herp Disease Alert System (HDAS) that provides another way to gather observations of sick or dead amphibians that might not otherwise be reported. In both cases, observations are typically forwarded to the relevant state biologist but in some cases, when deemed necessary, members of the HDAS can use their personal networks to help facilitate further investigation.

In Canada, the provincial governments are the lead jurisdiction for amphibian disease surveillance. The provinces of B.C. and Ontario have conducted the most intensive chytrid monitoring programs to date. In Ontario, over 900 amphibians were sampled opportunistically along a latitudinal gradient over a 4-year period (2014-2017). All samples were tested for Bsal and all tests were negative (Chistina Davy, unpubl. data). In 2016, provincial biologists in B.C. sampled for Bsal within a small number of wild Rough-skinned Newt (*Taricha granulosa*) and captive (pet store) salamander populations on the south coast—one of the high-vulnerability zones identified by Yap et al (2015). Bsal was not detected by qPCR analyses in any swabs from

the 82 wild newt and 15 captive salamanders sampled (Govindarajulu et al 2017). In many provinces, such as Alberta, Saskatchewan, Québec and Newfoundland, the current approach is one of passive surveillance in which Bsal investigations are triggered by unusual or mass amphibian mortality events. However, Ontario is considering low-level opportunistic sampling over the short-term, as resources allow, and given the concerns about pet trade as a vector for Bsal seized amphibians from the illegal pet trade will be tested for Bsal in B.C. The Canadian public can submit reports of sick or dead amphibians to the Canadian Wildlife Health Co-operative (CWHC). The CWHC is able to advise on the collection of carcasses for follow-up investigation and screens samples for diseases and parasites to assess the health of wild populations (CWHC 2019). Canadian provinces and territories may have additional reporting tools for sick or dead amphibians, such as the Government of British Columbia's "Frogwatching" site, which is monitored by the provincial amphibian specialist.

In Mexico, surveys directed to identify Bsal in natural populations have been conducted by members of Dr. Gabriela Parra-Olea's research lab. So far 119 individuals of 41 species (frogs and salamanders) have been sampled and Bsal has not been detected by qPCR analyses in any of the swabs (Parra-Ole, unpubl data). Next year (2020) additional surveys will be conducted by Dr. Eria Rebollar and Dr. Gabriela Parra-Olea in plethodontid salamanders and Ambystoma species across the Trans-Mexican Volcanic Belt. Additionally, we are starting efforts to certificate both research labs so that legal amphibian imports in Mexico can be screened for Bsal.

Goal 1: Establish a wide-reaching, ongoing, coordinated and sustainable Bsal surveillance program.

Priority: Urgent

Rationale: A robust surveillance network is needed for early detection of Bsal upon introduction. The earlier Bsal is detected, the better the chance of containment and of limiting negative consequences for amphibian biodiversity.

Action Items:

1. Develop the Student Network for Amphibian Pathogen Surveillance (SNAPS), a program that accomplishes surveillance objectives by engaging students.
2. Develop learning modules for SNAPS that incorporate Bsal surveillance as an active learning component in the curriculum. These modules would be compatible with conservation biology, ecology, disease ecology, microbiology, and other related courses. They should also include those that are appropriate for introductory biology or environmental science courses that may recruit a greater number of faculty participants. Other learning modules can focus on disciplines outside of the natural sciences to recruit even broader participation (e.g., policy, environmental education, and statistics).
3. Test learning modules, protocols, data entry, and other processes during the current 2019 – 2020 academic year. Improve upon where needed ahead of broader recruitment for the 2020 – 2021 academic year.

4. Coordinate with the Data Management Working Group to establish a SNAPS portal on amphibiandisease.org for SNAPS data entry and management.
5. Secure animal care authorization for SNAPS.
6. Secure ongoing funding.
7. Adopt a visual identity for SNAPS and establish a website.
8. Develop recruitment and/or instructional videos.
9. When ready, recruit more broadly to scale-up for wider geographic and taxonomic coverage.

Goal 2: Identify Bsal sampling efforts that are occurring outside of SNAPS and the Bsal Surveillance Working Group.

Priority: Ongoing

Rationale: Researchers and managers are conducting their own Bsal surveillance across North America, but these efforts are not coordinated. Therefore, this Working Group should at least contact these PIs, and catalogue their efforts to maintain an ongoing account of the entire Bsal surveillance effort.

Action Items:

1. Coordinate with the Bsal Research Working Group to identify PIs who are conducting Bsal surveillance as part of their broader research programs.
2. Identify other PIs across North America who may be sampling for Bsal and encourage them to input their efforts into amphibiandisease.org.

Goal 3: Support and facilitate sampling of amphibians in the pet trade.

Priority: Ongoing

Rationale: Bsal is likely to be introduced to North America through the amphibian pet trade. Therefore, surveillance among captive amphibians is a logical priority for the early detection of Bsal. Furthermore, detection of Bsal in the pet trade prior to its introduction in the wild, will provide conservationists and managers with the opportunity to contain the pathogen and prevent it from affecting wild populations.

Action Items:

1. Coordinate with the newly established Pet Industry/Trade Working Group to support and facilitate Bsal sampling of amphibians in the pet trade.

Goal 4: Facilitate and support Bsal surveillance in Mexico and Canada.

Priority: Ongoing

Rationale: Bsal threatens salamanders across North America, not just the United States. Mexico in particular has a very diverse salamander assemblage, such that the global priority for salamander conservation must include Mexico. Unfortunately, current efforts

of the Bsal Surveillance Working Group have only focused on the U.S. with a need for expanding into the rest of North America.

Action Items:

1. Recruit colleagues from Mexico and Canada for the Bsal Surveillance Working Group.
2. Communicate with colleagues in Mexico and Canada to understand the extent of their current surveillance efforts and identify actions that the Bsal Surveillance Working Group should take to support their efforts.
3. Recruit SNAPS participation in Mexico and Canada.

Budget Summary

Goal	Estimated Timeframe	Estimated Budget	Funding Status
(1)	2019 – 2021: Development of SNAPS program 2021 – 2023: Broader recruitment outside of the Bsal Surveillance Working Group 2023 – 2025: Scaling up for effective, ongoing surveillance effort	$15,000 per year $100,000 per year $200,000 per year	Initial funding provided by the USGS Amphibian Research and Monitoring Initiative (ARMI). The rest is currently unfunded.
(2)	Ongoing	NA	
(3)	Ongoing	NA	
(4)	Ongoing	NA	

7. Data Management Working Group

(Michelle Koo, Deanna H. Olson, facilitators)

A) **Goal.** Comprehensive Data Management of Bd and Bsal samples for Archived, Aggregated Monitoring and Analytic Modeling

Priority: Action items are classified as urgent or ongoing below.
Rationale: For effective monitoring and for understanding disease dynamics of chytridiomycosis, comprehensive data management is needed for Bd and Bsal samples. The Amphibian Disease portal (https://amphibiandisease.org) meets these goals and addresses the needs of researchers for private and public datasets in a standards-compliant, web-accessible portal hosted by UC Berkeley. Through its website, the Amphibian Disease portal could be an effective outreach and technical interface for the research community, which its Data

Dashboard demonstrates. Further, web services to other scientific portals such as AmphibiaWeb help extends its reach to the other audiences in education and conservation.

Action Items:

1) ***Urgent***: Bd-maps.net transition to amphibiandisease.org
    a. US Forest Service data management and data update, Dede Olson and Kathryn Ronnenberg. FS salary covering this.
    b. Coordination with PhD student in Germany who is also working on an independent update.
    c. Web programming needed to transition Bd data into new portal format, existing funding from US Forest Service to UC Berkeley is being used for this.
2) ***Ongoing/Urgent***: Database/ Web programmer: Portal development, maintenance and troubleshooting; this includes further integration with relevant portals such as AmphibiaWeb (reciprocal links on species-specific pages) and other enhancements = $10k/yr
3) ***Ongoing***: Interface with other Bsal Working Groups, especially the Surveillance and Monitoring Working Group
    a. This could be accomplished by a Bsal workshop, face-to-face in person or remotely.
        i. If face-to-face in person, could accomplish additional objectives and would need travel stipends. e.g., 30 people @ $1k = $30k
    b. Could do at a society meeting to multitask but due to our multidisciplinary nature difficult to find a meeting where even half would attend
        i. If remote, a video conference would be best. Perhaps Communications Working Group could help organize or a project coordinator (small stipend to facilitate, ca. $800/yr).
4) ***Ongoing***: Bsal and Bd surveillance data aggregation and input to amphibiandisease.org from world surveillance and literature
    b. Outreach to educate researchers to archive and share their data; outreach to journals to include the Portal as a resource for archiving and sharing their data prior and post publication
    c. Some data will still be needed to be gathered from the primarily literature; student hired to conduct this work.
    d. UC Berkeley student stipend, 1 term/year at 0.3 FTE plus computer access = $10k/yr

5) ***Urgent***: Built-in Analytic and research support from the Disease Portal. Currently data are all georeferenced and well suited for correlating with other web-accessible spatial datasets, such as climate, habitat, etc. to enable analytic functions useful for monitoring and predicting disease outbreaks, for example. Requires web programmer ($10-25,000 for 6 mos.) and data scientist programmer (approx. $50,000 for 1 year)
6) ***Urgent***: Fully support a customized version for **SNAP: Student Network for Amphibian Pathogen Surveillance,** a collaboration with the Surveillance Working Group to archive disease data from the efforts of course-based student sampling efforts. SNAP specific portal pages and summary statistics would have

be an important showcase for student-based efforts and allow future student-led research projects. Requires web developer and modest database modifications (up to $30,000)

Estimated Time and Cost

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
(1)	***Urgent***: Bd-maps.net data migration and integration into Amphibian Disease portal – in progress	$5000	Funded, current FS cooperative agreement, with AmphibiaWeb discretionary funds
(2)	Amphibian Disease Portal development and maintenance - ongoing	$10,000/year	Not adequately funded (AmphibiaWeb has some discretionary funds to cover up to

			$3000, inclusive of #1)
(3)	Working Group interactions – Summer 2018	$30,000 (dedicated meeting) to $800	Not Funded
(4)	Coordinator/ Data wrangler to help solicit and aid researchers to share their data in the portal including scanning the new literature	$10,000/year	Not Funded
(5)	Analytic support and modeling platform for Disease Portal	Up to $75,000	Not Funded
(6)	SNAPS support and customized portal	$15,000 - $30,000	Not Funded

8. Outreach and Communication Working Group

(Mark Mandica, facilitator)

Mission and Summary

The Outreach and Communications Working Group (OCWG) produces a variety of Bsal-related outreach communication materials that includes web presence, fact sheets, press releases and briefs. The intended audience of these digital and print publications is diverse and split into two groups: the scientific community and the general public. These communications include lay and scientific articles, blog posts, updates to the official website (salamanderfungus.org), and building a network on social media (Facebook and Twitter). OCWG's purpose is to work with the groups within the National Bsal Task Force to disseminate research published by the group and others.

In order to increase the efficacy of dissemination, the OCWG continues to build an online network via social media, increasing followers and directing them to the website, which serves as a hub and repository for published developments relating to issues, detections and research regarding Bsal.

While not tasked with conducting or publishing research on the topic, the OCWG does synthesize findings and communications within the National Bsal Task Force for the purposes of producing lay articles meant to educate the public, and highlighting key elements in social media posts as well. OCWG focuses on national coverage, as salamanders are at risk throughout the United States.

Finally, the Outreach and Communications Working Group organizes, designs and publishes the Annual Report for the National Bsal Task Force. This annual report summarizes advancement within all working groups and the current status of the Bsal fungus. This report is published on the Salamander Fungus website, and available to both the general public and scientific community.

Goals

Goal #1. Work with partners to disseminate research published by the National Bsal Task Force via social media and newsletter articles.

Priority: Ongoing
Rationale: Build a network of partners to publish updates on Bsal developments, and an efficient mechanism for alerting the public and scientific community in the event of a positive US detection of Bsal.

Goal #1

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
Identify outlets for publication	Ongoing	No Funding Necessary	NA
Develop publication partner relations	Ongoing	No Funding Necessary	NA
Produce general public articles	Annual	No Funding Necessary	NA

Goal #2. Continue to build a network on social media to communicate developments within the National Bsal Task Force.

Priority: Ongoing
Rationale: Build a network of followers on social media to publish updates on Bsal developments, and an efficient mechanism for alerting the public and scientific community in the event of a positive US detection of Bsal.

Goal #2

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
Build Social Media Presence	Ongoing	No Funding Necessary	NA
Summarize Publications for Newsletters/Blogs	Ongoing	No Funding Necessary	NA

Goal #3. Produce short Public Service Announcement video on the presence and implications of Bsal.

Priority: Ongoing
Rationale: A short (less than 5 minute) public service video has been identified as an effective way to communicate to the general public: the importance of salamanders, the potential devastating impacts of Bsal arriving in the US, what the current response plan is, and how everyone can help.

Goal #3

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
Identify Videographer	Late 2018	No Funding Necessary	N/A
Develop material content with TAC	Late 2018 - Mid 2019	No Funding Necessary	N/A
Produce Video	Late 2020	$10,000	Not funded

Goal #4. Produce a flyer illustrating the implications of Bsal.

Priority: Ongoing
Rationale: A multipurpose flyer which can inform the general public as well as concerned professionals would be a key educational tool. This flyer would be produced inexpensively, and delivered to professional agencies around the US who can then deliver them directly to their audiences, and display them in highly trafficked areas.
Goal #4

Action Item	Estimated Timeframe	Estimated Budget	Funding
Identify Printer	Late 2018	No Funding Necessary	N/A
Develop material content with TAC	Late 2018 - Mid 2019	No Funding Necessary	N/A
Produce Flyer	Mid 2020	$3,000	Not funded
Ship to partners, agencies and TAC	Late 2020	$200.00	Not funded

Goal #5. Update Bsal Task Force Website.

Priority: Ongoing
Rationale: Update and modify salamanderfungus.org website to make navigation easier and improve dissemination of task force information. Work with partners at Amphibian Survival Alliance to streamline future updates and management of website.

Goal #5

Action Item	Estimated Timeframe	Estimated Budget	Funding Status
Gather Ideas from TAC	Early 2019	No Funding Necessary	NA
Develop material content with ASA	Mid 2019	No Funding Necessary	NA
Update and Publish New Website	Late 2019	No Funding Necessary	NA
Continuing Publishing New Documents and Links to Website	Ongoing	No Funding Necessary	NA

Appendices

Appendix 1. Records of Bsal in nature and in captivity

Species Common Name	Species Scientific Name	Family	Location	Disposition (Wild/Captive)	Citation
Chinese Giant Salamander	*Andrias davidanus*	Cryptobramchidae	China	Captive	Yuan et al. 2018
Many-webbed Fire-bellied Toad	*Bombina microdeladigitora*	Bombinatoridae	Germany	Captive	Nguyen et al. 2018
Four species of urodele*	--	--	UK	Captive	Cunningham et al. 2015
North African Fire Salamander	*Salamandra algira*	Salamandridae	Germany	Captive	Sabino-Pinto et al. 2015
Fire Salamander	*Salamandra salamandra*	Salamandridae	Germany	Captive	Sabino-Pinto et al. 2015
Vietnamese Crocodile Newt	*Tylototriton vietnamensis*	Salamandridae	Vietnam	Captive	Martel et al. 2014
Many-webbed Fire-bellied Toad	*Bombina microdeladigitora*	Bombinatoridae	Vietnam	Wild	Nguyen et al. 2018
Chuxiong Fire-bellied Newt	*Cynops cyanurus*	Salamandridae	China	Wild	Yuan et al. 2018
Chinese Fire-bellied Newt	*Cynops orientalis*	Salamandridae	China	Wild	Yuan et al. 2018
Dayang Newt	*Cynops orphicus*	Salamandridae	China	Wild	Yuan et al. 2018
Clouded Salamander	*Hynobius nebulosus*	Hynobiidae	Japan	Wild	Martel et al. 2014
Alpine Newt	*Ichthyosaura alpestris*	Salamandridae	Netherlands	Wild	Spitzen-van der Sluijs et al. 2016
Alpine Newt	*Ichthyosaura alpestris*	Salamandridae	Belgium	Wild	Spitzen-van der Sluijs et al. 2016
Alpine Newt	*Ichthyosaura alpestris*	Salamandridae	Netherlands	Wild	Martel et al. 2014
Smooth Newt	*Lissotriton vulgaris*	Salamandridae	Netherlands	Wild	Spitzen-van der Sluijs et al. 2016
Japanese Clawed Salamander	*Onychodactylus japonicas*	Hynobiidae	Japan	Wild	Martel et al. 2014
--	*Pachytriton wuguanfui*	Salamandridae	China	Wild	Yuan et al. 2018

--	*Paramesotriton aurantius*	Salamandridae	China	Wild	Yuan et al. 2018
Hong Kong Warty Newt	*Paramesotriton hongkongensis*	Salamandridae	China	Wild	Yuan et al. 2018
Tam Dao Salamander	*Paramesotriton deloustali*	Salamandridae	Vietnam	Wild	Martel et al. 2014
Tam Dao Salamander	*Paramesotriton deloustali*	Salamandridae	Vietnam	Wild	Laking et al. 2017
--	*Paramesotriton sp.*	Salamandridae	Vietnam	Wild	Laking et al. 2017
Fire Salamander	*Salamandra salamandra*	Salamandridae	Netherlands	Wild	Spitzen-van der Sluijs et al. 2016
Fire Salamander	*Salamandra salamandra*	Salamandridae	Belgium	Wild	Spitzen-van der Sluijs et al. 2016
Fire Salamander	*Salamandra salamandra*	Salamandridae	Germany	Wild	Spitzen-van der Sluijs et al. 2016
Fire Salamander	*Salamandra salamandra*	Salamandridae	Belgium	Wild	Martel et al. 2013
Fire Salamander	*Salamandra salamandra*	Salamandridae	Netherlands	Wild	Martel et al. 2014
Fire Salamander	*Salamandra salamandra*	Salamandridae	Belgium	Wild	Martel et al. 2014
Siberian Salamander	*Salamandrella keyserlingii*	Hynobiidae	Japan	Wild	Martel et al. 2014
Black Knobby Newt	*Tylototriton asperrimus*	Salamandridae	China	Wild	Laking et al. 2017
Black Knobby Newt	*Tylototriton asperrimus*	Salamandridae	Vietnam	Wild	Yuan et al. 2018
Chiang Mai Crocodile Newt	*Tylototriton uyenoi*	Salamandridae	Thailand	Wild	Martel et al. 2014
Himalayan Newt	*Tylototriton verrucosus*	Salamandridae	China	Wild	Yuan et al. 2018
Vietnamese Crocodile Newt	*Tylototriton vietnamensis*	Salamandridae	Vietnam	Wild	Laking et al. 2017
Ziegler's Crocodile Newt	*Tylototriton ziegleri*	Salamandridae	Vietnam	Wild	Martel et al. 2014
Ziegler's Crocodile Newt	*Tylototriton ziegleri*	Salamandridae	Vietnam	Wild	Laking et al. 2017
Sword-tailed Newt	*Cynops ensicauda*	Salamandridae	--	Wild*	Martel et al. 2014
Summary Captive:	*N = 5 (plus possibly 4 more) species*	***Summary Wild:***	*N = 21 species*		

Appendix 2. Results of Bsal Susceptibility Trials

Species that developed Bsal chytridiomycosis

Desmognathus auriculatus	Plethodontidae
Pseudotriton ruber	Plethodontidae
Eurycea wilderae	Plethodontidae
Aquiloeurycea cephalica	Plethodontidae
Chiropterotriton spp.	Plethodontidae
Notophthalmus viridescens	Salamandridae
N. perstriatus	Salamandridae
N. meridionalis	Salamandridae
Taricha granulosa	Salamandridae

Species that are Bsal carriers

Ambystoma opacum	Ambystomatidae
A. laterale	Ambystomatidae
A. mexicanum	Ambystomatidae
Desmognathus ocoee	Plethodontidae
D. aeneus	Plethodontidae
Eurycea cirrigera	Plethodontidae
Plethodon metcalfi	Plethodontidae
P. cinereus	Plethodontidae
Cryptobranchus alleganiensis	Cryptobranchidae
Anaxyrus americanus	Bufonidae
Lithobates chiricahuensis	Ranidae
Hyla chrysoscelis	Hylidae
Scaphiopus holbrooki	Scaphiopodidae

Species that are resistant

Desmognathus conanti	Plethodontidae
D. monticola	Plethodontidae
Plethodon shermani x teyahalee	Plethodontidae
Hemidactylium scutatum	Plethodontidae
Eurycea lucifuga	Plethodontidae
Necturus maculosus	Cryptobranchidae
Lithobates sylvaticus	Ranidae

Appendix 3. List of US States with Environmental Quality Acts and governing body to contact for information, and list of Provincial amphibian-reptile specialists in Canada

State	Governing Act	Governing Body	Notes:
California	California Environmental Quality Act (CEQA) 1970	Attorney General	Require EIS for Local Projects, requires ER for individual businesses for agriculture, requires statements for potential impact on Climate change
Connecticut	Connecticut Environmental Policy Act (CEPA) 1973		
Georgia	Georgia Environmental Policy Act (GEPA)1991		Require EIS for local governments If the cost is greater than $250k or the state pays for more than 50% of action
Hawaii	Hawaii Environmental Policy Act (HEPA) 1974	Hawaii Office of Environmental Quality (OECQ)	
Indiana	Indiana Environmental Policy Act (IEPA) 1972	Indiana Department of Emergency Management	
Maryland	Maryland Environmental Policy Act (MEPA) 1973	Maryland State Legislature	Only required when it's a legislative action (gov pays for it)
Massachusetts	Massachusetts Environmental Policy Act 1977		
Minnesota	Minnesota Environmental Policy Act 1973		Requires EIS for local governments, requires ER for agriculture actions
Montana	Monatan Environmental Policy Act 1971	Montana Environmental Quality Council	
New Jersey	Executive Order 215 (1989)	New Jersey Department of Environmental Protection	
New York	NY State Environmental Quality Review Act (SEQR) 1978	State and Local Government	Require EIS for local governments. Requires ER for agriculture, State and Local gov permits are required. Citizens may sue the state if an action if "harmed" by an action. Require an EIS for climate change
North Carolina	North Carolina Environmental Policy Act 1971	North Carolina Department of Environmental and Natural resources	
South Dakota	South Dakota Environmental Policy Act 1974		
Virginia	Virginia Code Sections 10.1-10.1188 (1973)	Virginia Department of Environmental Quality and other state agencies	More than one agency may b required to permit depending on the action

Washington	Washington Environmental Policy Act (SEPA) 1971	Washington Department of Environmental Quality	Require EIS for local governments
Wisconsin	Wisconsin Environmental Policy Act(WEPA) 1974	State Controller and Wisconsin Department of Natural Resources	

Council on Environmental Quality, A Citizen's Guide to the NEPA, 2007

List of Provincial Amphibian-Reptile Specialists in Canada

Alberta

Margo Pybus
Provincial Wildlife Disease Specialist
Fish and Wildlife Policy Branch
Alberta Environment and Parks
6909-116 Street
Edmonton AB T6H 4P2

Telephone: (780) 427-3462
Email: margo.pybus@gov.ab.ca

Robin Gutsell
Wildlife Status Biologist
Fish and Wildlife Policy Branch
Alberta Environment and Parks
9920-108 Street
Edmonton AB T5K 2M4

Telephone: (780) 644-1154
Email: robin.gutsell@gov.ab.ca

British Columbia

Purnima Govindarajulu
Amphibian and Small Mammal Specialist
Ecosystems Branch
Ministry of Environment
P.O. Box 9338 Stn Prov Govt
2975 Jutland Rd.
Victoria BC V8W 9M1

Telephone: (250) 387-9755
Email: purnima.govindarajulu@gov.bc.ca

Helen Schwantje
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Fish, Wildlife, and Habitat Branch
Ministry of Forests, Lands and Natural Resource Operations
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Manitoba

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Manitoba Conservation and Water Stewardship
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Newfoundland and Labrador

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Appendix 4. Response Working Group Rapid Response Template

Last revised January 28, 2019

PLEASE NOTE: Within this document are **explanatory notes and questions to stimulate discussion** to help clarify the intent of the information provided for end users and to facilitate their customization of the template. These are **placed throughout the text in *blue italics*** thus distinguishing these notes from other guidance provided for the purpose responding to a detection or outbreak of Bsal.

Purpose: This document and recommendations that follow serve as a **template to be customized** by any agency or institution with management jurisdiction over wild or captive salamanders, respectively, when actions in response to a disease may be warranted. *This purpose statement may be further customized as needed for individual entities.*

Herein is provided an outline and guidance for **local, rapid response** actions that could be triggered upon initial or subsequent detections of Bsal, in either wild or captive populations. *The scenarios are based on what an entity using this plan might do after receiving information regarding* Bsal *detection status from a diagnostic laboratory with expertise in* Bsal *diagnostics. In other words, all recommended actions occur after the laboratory has made its determinations based on the Case Definition of* Bsal *(White et al. 2016).* Also provided are considerations for *in situ* containment (i.e., in the existing location of the population) as well as establishment of *ex situ* populations (i.e., outside of the natural location, such as in captive assurance colony). Rapid containment and response measures may prevent broad impacts. *The USGS Amphibian Research and Monitoring Initiative (ARMI) is also working to assist entities in making decisions regarding wildlife disease management, including the customization of this template. Contact ARMI Decision Support Lead, Dr. Evan Grant (ehgrant@usgs.gov) for assistance.*

This template was produced by the **Bsal Response Working Group** as part of their work with the **Bsal Task Force's Technical Advisory Committee** (see www.salamanderfungus.org for additional information), and is considered a living document that will be updated as more information becomes available.

At the time of this version, Bsal is not known to occur in North America and suggested responses are consistent with the high-alert condition of Bsal being yet undetected or rare in North America. This document is intended to be incorporated into a National Bsal Strategic Framework, where larger surveillance and monitoring strategies, research needs, policy needs, and related prevention strategies, along with public outreach and communication, are addressed.

***Batrachochytrium salamandrivorans* (Bsal)**
Rapid Response Template

Preliminary definitions and resources

This section sets forth how terms are defined within the rest of the document. These definitions will also occur in the larger Action Plan. However, entities customizing this template should add other definitions as they deem appropriate.

Definitions:

1) Bsal-*susceptible host species* – We use this phrase broadly to indicate both species for which Bsal can be fatal and species that can be infected by Bsal but not develop disease symptoms, hence may serve as reservoirs and carry Bsal. At the time of this version, experimental evidence suggests that anurans may carry it (Stegen et al. 2017). Thus, we assume that all amphibian species *may* be susceptible to Bsal or be carriers of Bsal unless it is demonstrated that a species cannot be infected. Bsal *has been termed the "salamander fungus" because it was described from infected fire salamanders in Europe and has been shown to either infect or to be carried by several other salamander species (Martel et al. 2014). New evidence suggests that some anurans can also be infected and carry the pathogen, potentially without developing clinical signs of infection (Yuan et al. 2018; Stegen et al. 2017). This template and this definition will be updated when new evidence of species-specific susceptibility becomes available.*

2) *Wild host population* – Free-ranging population of Bsal-susceptible species.
   a. Naïve (no prior Bsal detections known at a given site)
   b. Exposed (prior Bsal detections documented at a given site)
   c. Unknown (no or insufficient Bsal surveillance has been performed to know the status)

3) *Captive host population* – Any population that is not free-ranging, including outdoor enclosed spaces or fenced runs where contact with wild amphibians or disease vectors may be possible (e.g., zoo, aquarium, research facility, university).
   a. Naïve (no prior Bsal detections known from the captive location)
   b. Exposed (prior Bsal detections documented from the captive location)
   c. Unknown (no or insufficient Bsal surveillance has been performed to know the status)

4) *Mortality event*, wild – Death of one or more free-ranging amphibians in the environment, whether or not the Bsal pathogen has been detected.

5) *Mortality event*, captive – Death of one or more amphibians in a captive environment, whether or not the Bsal pathogen has been detected.

6) *Eradication* – The assumed elimination of Bsal from individual amphibians *in captivity* based on four (4) consecutive, negative PCR tests, each one week apart, per individual, as described in Blooi et al. (2014).

7) *Participating Laboratory*. The particular laboratory that has been engaged during a testing or response effort; see also Resources below regarding the Diagnostic Laboratory Network.

8) *Reporting Individual(s)*. The individual(s) who submitted the sample(s) (e.g., swabs, carcasses, live animals) to a laboratory for diagnostics. This is the person(s) the laboratory is to contact to provide results. In some cases, this may be a scientific researcher. *At the time of this version, the* Bsal *Task Force is working to develop a statement and working with key scientific journal editors to ensure that sharing of scientific findings with management agencies in order to facilitate early detection and rapid response actions will not diminish the value or integrity of the scientific findings or the person(s) involved.*
9) *Core Response Team* (CRT). The group of authorized professionals, and other parties involved in the initial discovery, that evaluates the situation and makes recommendations for next steps. The CRT may include other trusted parties, as appropriate, where information can be securely shared, and will not compromise scientific integrity (see suggested composition in Resources)**.** *We reference the use of such a team as part of the recommended actions in the response scenarios described in this template. We suggest that certain members of this team be identified in advance, to facilitate a rapid response. Below, we offer additional suggestions regarding role and composition. However, the use or role of the team is ultimately at the discretion of the entity customizing this template.*

Resources

1) **Diagnostic Laboratory Network.** A consortium of participating laboratories equipped to handle Bsal testing requests, and to employ specific protocols (as recommended by the Bsal Task Force's Diagnostics team) for Quality Assurance and Quality Control (QA/QC). Assists with coordination of sample handling. The list of known labs capable of Bsal testing is provided on the Bsal Task Force Website: www.salamanderfungus.org/resources/labs. *Entities customizing this template may benefit from contacting their nearest laboratory(-ies) to understand their sample submission protocols, fees for services (as applicable), and any other requirements to collaborate in the event of a disease outbreak (whether* Bsal *or other pathogen).*

2) **Core Response Team (CRT).** *As noted above, we reference the use of such a team as part of the recommended actions in the response scenarios described in this template. Here, we offer suggestions on the charge and composition of the team. However, the use or role of the team is ultimately at the discretion of the entity customizing this template.*
    a. **Purpose**. The CRT is an advisory group who discusses the specific scenario and helps to make initial decisions regarding response actions and related communications. Any member of the CRT is expected to keep the shared information **confidential** until the **management agency or entity with jurisdiction** (i.e., the authority to make decisions about the species or the lands affected) indicates how, where, when information may be shared.

    b. **Composition**: ***The composition of this team may change depending on the specific circumstance. A brief explanation on the suggested composition: a)***

Individual who discovered the mortality event or was involved in research that led to a Bsal*-positive detection may have ability to assist in response-related actions or follow-up work at the site; b) Agency with management jurisdiction, or land manager will be able to confirm actions that can or cannot be taken; c) The state or provincial/territorial fish and wildlife agency is the primary management authority for amphibians & can assist with appropriate species management actions on non-federal lands; d) Amphibian experts can advise on most current science. Specifically,* ***the* Bsal *Technical Advisory Committee was formed to include appropriate expertise in the event of a* Bsal *outbreak,*** *and is at your disposal for confidential advisory assistance.*

i. Reporting Individual(s)
ii. Agency or entity with jurisdiction over the affected species or lands
iii. Land or Facility manager(s)/owner(s) where samples were collected, if different from the entity in (2)(b)(ii)
iv. State agency personnel in charge of amphibians
    1. NOTE: The Association of Fish and Wildlife Agencies' Amphibian & Reptile Program Manager, Priya Nanjappa (pnanjappa@fishwildlife.org), can assist in determining the appropriate state contacts.
v. Key amphibian expert scientists who can provide recommendations, **in a confidential consulting capacity**, for short and long-term responses based on best available science:
    1. NOTE: Please consider contacting the Bsal Technical Advisory Committee leadership (response@salamanderfungus.org); one or more members will be available to assist in a confidential advisory capacity.

3) **Points of Contact (PoCs):** *Entities customizing this template should populate with preferred PoCs.*
    a. Provide a list of key contacts in a given state, federal agency, or management unit (e.g. unit director or manager; staff veterinarian, lead herpetologist or wildlife biologist) to inform when there is a positive/after CRT and Reporting Individual.
    b. Include Permit coordination contacts (state, federal, ESA, etc.)

4) **Wildlife Health Expert Networks.** Qualified wildlife experts to assist in treatment of captive or privately-owned animals, issuing health certifications or other documentation to verify animal health, emergency responses, etc., may be found via:
    a. The Diagnostic Laboratory Network established via the Bsal Task Force's Diagnostics Working Group (see www.salamanderfungus.org).
    b. Veterinary experts:
        i. Association of Reptile and Amphibian Veterinarians (ARAV);
        ii. Board-certified zoological medicine veterinarians; or

iii. The American Association of Wildlife Veterinarians (AAWV)
iv. The Canadian Wildlife Health Cooperative (CWHC)

c. Wildlife Epidemiologists or Wildlife Disease Ecologists

5) **Facilities.** A list of available captive housing or breeding facilities, with contacts (e.g., Amphibian Ark (AArk), Canada's Accredited Zoos and Aquariums (CAZA), Association of Zoos and Aquariums (AZA)-accredited zoos, other local facilities)
   a. Treatment. *Entities customizing this template should identify secure, emergency facilities in their network to temporarily house moribund (dying, unable to right themselves) or sick but potentially treatable animals.*
   b. Rescue colonies. *Entities customizing this template should identify facilities to house rescued animals or those collected for the purpose of captive breeding and reintroduction.*
   c. Museums or other storage facilities. *Entities customizing this template should identify facilities for vouchered animals, or archived tissue samples, swabs or extracted DNA.*

   Questions: *What AArk or AZA facilities are local? Are you familiar with the appropriate contacts there? What local museums are able to accession animals? Can they also accession tissues, swabs, DNA?*

6) **Protocols.** *Along with those below, consider also other protocols that may be useful, e.g., data submission or management protocols.* Recommended guidance can be found at the Bsal Task Force website, www.salamanderfungus.org, via the Diagnostics or Research pages, but see also Pessier & Mendelson (2017), including:
   a. Biosecurity protocols for field, lab, use of live cultures, etc.
   b. Swabbing and storage (and transportation) protocols
   c. **See also Appendix I**, where pertinent portions of the guidance manual have been included and adapted for quick reference.

RESPONSES

NOTE: The scenarios below pertain to mortality or PCR detection events (and subsequent confirmation of causative agent), however, any suspicious-appearing amphibians should be investigated. Examples of suspicious-appearing amphibians would be sick or lethargic individuals, those with black circular or oblong lesions, or inability to right themselves. In this heightened state of awareness, all such amphibians should be reported. The Partners in Amphibian and Reptile Conservation (PARC) Disease Task Team has established an alert system to help connect people to the appropriate experts and authorities quickly; please see the PARC Disease Task Team website for information and for how to send a report to herp_disease_alert@parcplace.org.

Scenario 1: Mortality event, cause unknown; Wild

Mortality events may be due to any number of causative agents. The actions below include collection of samples to confirm a diagnosis and activities to be considered while results are pending. These should be implemented at the discretion of the jurisdictional management unit depending on the level of response they are able to take to help minimize potential impacts. Contact [your local amphibian expert or member of a Veterinary Expert Network] to assist. *Entities customizing this document should identify appropriate amphibian experts local to your jurisdiction.*

When uncertain how to proceed or whom to contact, the Partners in Amphibian and Reptile Conservation (PARC) Disease Task Team has established an alert system to help connect people to the appropriate experts and authorities; send a report to herp_disease_alert@parcplace.org.

Actions recommended (one or more, items 2-6 in no priority order and as feasible):

1) **Notification to agency with management jurisdiction.** To facilitate a Bsal early detection and rapid response, contact the management agency with jurisdiction where the mortality event occurred (which may be your own agency) to ensure they are aware of the testing event and impending results. ***Important --*** *Given the heightened state of alert for* Bsal *and the critical nature of early detection and rapid response, when customizing this template, please consider including this recommended action of contacting the management agency with jurisdiction where the mortality event occurred, even if this may be your own agency, to be sure they are aware that a mortality event and testing is underway, while results are pending.*

   Questions: *Do you know the appropriate contacts for disease response in the agencies with management jurisdiction in your state? (If not, the PARC Disease Task Team may be able to assist; send a message to* herp_disease_alert@parcplace.org *requesting information on the appropriate contacts.)* ***For management agencies:*** *Are there other partners that you need to engage and if so, should it be at this stage or after results are received?*

2) Tissue collection for diagnostics.
   a. Collect any live but apparently moribund (dying, unable to right themselves) or lethargic animals, using humane euthanasia procedures, as applicable (**see Appendix I, Section A**); submission to Participating Laboratory. Swabs alone are insufficient to confirm a Bsal diagnosis.
   b. Carcass collection, fresh-dead (see **Appendix I, Section A**), for diagnostic necropsy and submission to Participating Laboratory.

c. Sampling of other live amphibians (e.g., swabbing skin for use in a PCR assay), if area is high risk and if feasible (**Appendix I, Section B**).

3) Biosecurity protocols, as established (**Appendix I, Section A(3)**), implemented for all field gear especially as part of implementing #2 above, and also upon leaving die-off site.
Questions: *Have you considered establishing an approved set of biosecurity protocols for sampling or surveillance in a disease-affected site?*

4) Heightened alert considerations.
   a. Increased surveillance
   b. Local personnel notification. *It may be helpful to form and consult the CRT (see Resources above) or to assess notifications at this stage, and could be handled on a "need to know" basis.*

5) Containment considerations. The following are options that might help prevent spread of pathogens.
   a. Restricted public access to the exposed area(s).
   b. Signage at or around the exposed area(s).
   c. Local personnel notification and access restrictions to the exposed area(s). *Again here, it may be helpful to consult the CRT or to assess notifications at this stage, and could be handled on a "need to know" basis.*

6) See below for "Definitive detection, Wild" for additional response

Scenario 2: Mortality event, cause unknown; Captive

Mortality events may be due to any number of causative agents. The actions below include collection of samples to confirm a diagnosis and activities to be considered while results are pending. These should be implemented at the discretion of the captive management facility depending on how conservative or comprehensive of a response they are able to take to help minimize impacts. Contact *[your local amphibian expert or member of a Veterinary Expert Network]* to assist.

When uncertain how to proceed or whom to contact, the Partners in Amphibian and Reptile Conservation (PARC) Disease Task Team has established an alert system to help connect people to the appropriate experts and authorities; send a report to herp_disease_alert@parcplace.org.

Actions recommended (one or more, items 2–6 in no priority order and as feasible):

1) **Notification of state or provincial/territorial fish & wildlife agency.** To maintain transparency and open communications regarding Bsal and to facilitate early detection and rapid response, we recommend contacting the state or provincial/territorial fish & wildlife agency where the mortality event occurred to ensure they are aware of the testing event and impending results. ***Important --*** *Given the heightened state of alert for* Bsal *and the critical nature of early detection and rapid response, when customizing this template, please consider including this recommended action of contacting the state or provincial/territorial fish & wildlife agency where the mortality event occurred, to be sure they are aware of the mortality event and that testing is underway, while results are pending. This allows them to consider additional surveillance or management actions to further protect wild populations.*

   Questions: *Do you know the appropriate contacts for disease response in the agencies with management jurisdiction in your state or province/territory? (If not, the PARC Disease Task Team may be able to assist; send a message to* herp_disease_alert@parcplace.org *requesting information on the appropriate contacts.)* ***For management agencies or industries:*** *Are there other partners that you need to engage and if so, should it be at this stage or after results are received?*

2) Tissue collection for diagnostics.
   a. Collect tissue and/or moribund (dying, unable to right themselves), abnormally behaving, or co-located live animals, as feasible and using humane euthanasia procedures, as applicable (**Appendix I, Section A**); submission to the facility's pathologist, where applicable, or, after confirming closest lab that is able to handle the specific case, to a Participating Laboratory (see also the Diagnostic pages of www.salamanderfungus.org).
   b. Carcass collection, fresh-dead animals for diagnostic necropsy; submission to Participating Laboratory (**Appendix I, Section A**).
   c. Consider collecting swabs from living animals without symptoms contained in the same enclosures or nearby.

3) Biosecurity protocols, as established in Pessier & Mendelson (2017), implemented for:

a. Disinfection of captive caging/housing facilities and materials prior to reuse for treated or new animals.
b. Treatment and disinfection of water prior to disposal.
c. Treatment of plant or soil substrate materials prior to disposal.

Questions: *Have you established/considered establishing an approved set of biosecurity protocols for disease-affected population/housing materials in captivity?*

4) Containment considerations. For exposed, captive animals that remain living, we suggest the following:
   a. Individual quarantine for all potentially exposed animals until causative agent is determined.
      i. Consult with your local amphibian or veterinary expert and consider prophylactic treatments, and post-treatment testing and monitoring, as per guidance in Blooi et al. (2015a, b).
   b. Halt transport/commerce of exposed, co-located, co-shipped, or all amphibians until health conditions and pathogen eradication can be verified.
   c. Retrieve chain-of-contact/custody information (i.e., individuals or entities throughout the history of possession of the animals or lot in which the affected amphibians originated).
      i. Inform and recommend to all potential points of transmission to follow quarantine, testing, and treatment recommendations.
   d. Ensure biosecurity standards have been met (see #3) prior to resumption of any transport or commerce of animals or caging materials, in accordance with existing federal, state or provincial/territorial, or local laws.

5) See below for "Definitive detection, Captive" for additional response.

Scenario 3: Detection of Bsal Presence, by Polymerase Chain Reaction (PCR) (Wild or Captive)

This scenario is defined as: Detection of *B. salamandrivorans* DNA, as determined by a Participating Laboratory, based on swab or tissue samples of individual amphibians or from the environment (e.g., environmental DNA sampling), using PCR testing. *Ideally, the Participating Laboratory will have also verified the result by a second Participating Laboratory.*

This scenario indicates potential presence of Bsal, but is NOT considered a "definitive detection" of Bsal until additional evidence of Bsal has also been determined. However, in the heightened state of alert, the guidance below is to facilitate early detection, rapid response efforts, while confirmation of Bsal presence is pending.

A detection of Bsal *presence via PCR could occur a) in an instance where no clinical sign or histopathologic evidence, nor evidence of a current mortality event, exists that is indicative of an active* Bsal *outbreak, or b) as an outcome of Scenarios 1 or 2 above, or c) may arise independently via surveillance or research of wild or captive populations*.

Actions recommended (one or more as feasible):

1) Initial diagnostic results communicated by Participating Laboratory to:
   a. Reporting Individual(s), who in turn informs:
      i. Detection site landowner/manager
      ii. Wildlife agency or entity with management authority

2) Agency or entity with management authority forms and convenes the Core Response Team (CRT). *Some entities customizing this template may consider developing an Incident Command System to help coordinate across other agencies or stakeholders.*
   a. Consider also engaging the Bsal Task Force Technical Advisory Committee leadership (response@salamanderfungus.org), who are available to assist by advising on resources and responses, and will keep the information confidential. *Through the Task Force's working groups, additional assistance can be provided on next steps following a PCR detection.*
   b. Consider developing a communications plan that facilitates internal agency and Core Response Team communications to external stakeholders and the public (including signage for affected sites, intended visitor behavior modifications). *These are potential, suggested components of a communications plan; customized actions may differ.* ***Questions:*** *Is there any cultural or archaeological significance of the site? Is it a popular visitor site that may require a visitor management plan, or additional staffing to advise the public and help avoid disturbance or public contact with affected areas?*

3) Further investigation. Additional diagnostic testing should be conducted as feasible (e.g., sequencing and phylogenetic analyses, isolation by fungal culture, necropsy and histopathologic examination of associated dead animals or tissues where applicable) by a Participating Laboratory for a definitive diagnosis (White et al. 2016).

4) Management Actions, **Wild populations**

a. Biosecurity protocols, as established (**Appendix I, Section A(3)**), implemented for all field gear used at the Bsal-positive site.
b. Increased surveillance at Bsal-positive site.
    i. If available, test any archived amphibian tissues from the site of detection for Bsal.
    ii. Evaluate known amphibian species composition at the site, with special consideration for presence of federally-listed, state-listed, and at-risk salamander species.
        1. If listed and/or at-risk species are present, evaluate need and opportunity available for taking healthy individuals from the wild and placing them in captivity for establishment of a breeding (captive assurance) colony.
    iii. Conduct additional sampling of amphibians and water at the site of detection.
    iv. Evaluate movements of other animals in or out of the site
c. Heightened awareness by managers at the Bsal-positive site.
    i. Collect any morbid or dead amphibians at that site and submit to Participating Laboratory for testing.
    ii. Review any existing data from vicinity of site for evidence of population or mortality trends.
    iii. Initiate population monitoring of affected amphibian species to determine if stable or declining.
d. Containment Considerations. Consider options that might help prevent the spread of Bsal:
    i. Restricted public access to the exposed area(s).
    ii. Signage at or around the exposed area(s).
    iii. Local personnel notification and access restrictions to the exposed area(s).
    iv. Direct actions, when evaluating risk and with an abundance of caution. ***Questions****: Is drying or treating the site an option? Is the harm of taking an extreme action greater than doing nothing?*

5) Management Actions, **Captive populations**

a. Containment
    i. Ensure no shared water sources or water flowing out of the animals' caging/housing.
    ii. Individual quarantine. Isolate affected animals, including any that were housed with affected individuals.
        1. Perform additional diagnostics on co-located individuals.
        2. Eradicate Bsal sources.

a. For live, captive animals whose samples return a positive Bsal result, eradication may be attempted:
    i. For failsafe eradication, we recommend humane culling or euthanasia, and either:
        1. Preservation of infected individuals, per **Appendix 1(A),** for further histological analysis (consult with your CRT and your Participating Laboratory to confirm necessity).
        2. Disposal of infected individuals using strict biosecurity protocols. See Section 8.6 in Pessier & Mendelson, 2017, or humane methods in accordance with the *AVMA Guidelines for the Euthanasia of Animals: 2013 edition* (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf).
    ii. If there are reasons to maintain the animals, eradication of Bsal may be possible and has been demonstrated in published literature (Blooi et al. 2015a; Blooi et al. 2015b). *There may be reasons to maintain and treat animals, e.g., with threatened or endangered species. However, there may also be reasons to maintain infected animals, e.g., for additional diagnostics or research. Consult with the CRT and your Participating Laboratory to determine options.*
    iii. As such, we suggest the following:
        1. Treat per guidance in Blooi et al. (2015a, b). *As new treatments and research are being investigated, we will update this template.* ***Please note:*** *the methods tested to date only are confirmed in Fire Salamanders (Salamandra salamandra); keep in mind that species differences may come into play with respect to treatment validity and effectiveness. This is why multiple swabs for PCR testing over time are necessary to confirm eradication.*

2. Swab treated animals post-treatment (see Appendix I, Section B) and submit repeat samples to a Participating Laboratory to confirm Bsal eradication.
3. Repeat treatment regime(s) and post-treatment swabbing until confirmation of Bsal eradication.

b. Disinfection, per Pessier & Mendelson (2017):
   i. All caging/housing materials and equipment prior to reuse.
   ii. All water prior to disposal.
   iii. All plants, soils, or other organic materials prior to disposal.
c. Captive population monitoring. Evaluate the exposure to other co-located amphibians, including:
   i. Determine other places it could be in the facility, and disinfect these areas.
   ii. Assess other potential sources of spread or origin of the pathogen, including through shared water sources or uses and movements, and quarantine or disinfect these sources.
   iii. Assessment across the collection to determine whether it is clinically stable or if there is a trend of increasing morbidity and mortality.
d. Reporting, and additional testing, throughout the chain of custody (i.e., individuals or entities throughout the history of possession of the animals or lot in which the affected amphibians originated).
   i. At minimum, swab amphibians for PCR analysis throughout the chain of custody.
   ii. Consider additional monitoring, as in 5(c) above.

6) Document Bsal treatment. Prior to resumption of transport or sale (in accordance with existing federal, state, or local laws), consider obtaining a health certificate or other documentation from a member of one of the Veterinary Expert Networks verifying Bsal treatment and eradication for *each individual animal* that tested positive for Bsal and was treated and for which Bsal was shown to be eradicated. *Entities customizing this template should keep in mind that each state may or may not have specific laws regarding "official" health certifications or alternative options; it is important to consult your state fish and wildlife agency and state department of agriculture regarding either the recommendation being an official or unofficial form of documentation.*

7) Additional management guidance via CRT
a. Messaging considerations.
   i. CRT will advise on and assist in development of preliminary detection messaging for the *Reporting Individual(s)* or the agency/entity with management jurisdiction over the site of detection to disseminate.

b. Movement restrictions, voluntary or mandatory, implemented by landowner/manager, captive population owner, or agency with jurisdiction over the captive animals, to reduce further transmission (e.g., prohibitions on collecting wild salamanders from the wild site; temporary moratorium on movement or sale of salamanders from the captive facility until further information is known). *Entities customizing this template may consider including additional guidance for tracking animals that were documented to be infected and then treated, including reporting or other requirements upon relocation to new jurisdictions.*

6) Subsequent communications:

a. If the Bsal Technical Advisory Committee has not been engaged in prior steps, consider contacting them regarding the findings and actions (response@salamanderfungus.org).
b. Internal communications as required by *Reporting Individual's* agency/organization.
c. Internal communications within the agency or entity with management jurisdiction of the detection site as management decisions are made, on a need-to-know basis.
d. Local stakeholder and chain-of-contact/custody outreach.
e. No further communications until detection status is definitive. *Limiting communications to a "need to know" group of people may help until confirmations of* Bsal *(or other pathogen) detection is received, to avoid unnecessary attention or public reaction.*

Scenario 4: Definitive detection, Wild

This scenario is defined as: Evidence of *both* 1) the presence of Bsal, as determined by the Participating Laboratory through either PCR-testing, or through isolation of a Bsal fungal culture as identified with genetic sequencing; *and* 2) signs of infection, as determined by the Participating Laboratory based on either clinical signs of disease in individual animals, or by histopathological characterization consistent with Bsal infection. *Evidence of presence without evidence of infection is not enough to determine definitive detection of* Bsal *(see Iwanowicz et al. 2017). Laboratory determinations are based on the Case Definition for* Bsal *chytridiomycosis (White et al. 2016), accepted by the Diagnostic Working Group of the* Bsal *Task Force.*

Actions recommended (one or more, as feasible):

1) Results communicated by Participating Laboratory to:
   a. Reporting Individual(s), who in turn informs:
      i. Detection site landowner/manager
      ii. Wildlife management agency with jurisdiction over species and/or land

2) Agency or entity with management authority forms and convenes the Core Response Team (CRT)
   a. Consider also engaging the Bsal Task Force Technical Advisory Committee leadership (response@salamanderfungus.org), who are available to assist by advising on resources and responses, and will keep the information confidential.

3) Subsequent Communications (in order of priority)
   a. Internal communication as required by the Reporting Individual's agency/organization.
   b. If the Bsal Task Force Technical Advisory Committee leadership has not yet been informed, notify them of the findings (response@salamanderfungus.org).
   c. Formal stakeholder notifications (e.g., partner institutions or agencies).
   d. Public announcement/press release as appropriate.
   e. Local stakeholder outreach (e.g., public groups who use the affected sites and could be asked to either disinfect gear and to report observations of dead amphibians).
   f. Scientific publication outlet.
   g. Bsal reporting database.

4) Emergency Meeting convened among parties identified in 2a, and possibly 3a–b, above to discuss:
   a. Risk/threat assessment. *Some areas to assess for potential risk include species movements, people's activities, water movements, etc., and risk level to co-occurring species.*
   b. Management actions and considerations:
      i. Containment of mortality/detection site:
         1. Landowner/manager restrictions on public access to site, except for approved personnel.

2. Strict use of approved biosecurity protocols (Appendix I, Section A(3)) for all personnel, their gear, vehicles, etc. when exiting site.
    a. Establish dedicated equipment/gear including nets, footwear, etc. for the site.
3. Deployment of fencing or other containment measures to reduce or prevent spread by other wildlife.
4. Demarcation of the affected area(s) to minimize or prevent trespass by personnel or public.

ii. Establishment of *ex situ* colony(-ies):
1. Engage additional partners (Amphibian Ark, CAZA, AZA, American Association of Zoo Veterinarians, etc.) to assist.
2. Initiate rescue/captive assurance populations:
    a. Based on conservation status (e.g., federally or state/provincially-listed).
    b. Based on proportion of local population affected and proportion of total population represented locally.
    c. As an attempt to salvage/save affected, but treatable, individuals.

iii. Priority surveillance:
1. Detection site
    a. Sampling of other amphibian species at the detection site, particularly any within those families shown to be susceptible in Martel et al. 2014 and Stegen et al. 2017 (or more recent publication, if available).
    b. Additional sampling of exposed amphibian species or substrates.
2. Non-independent sites (e.g., potential transmission pathways of water bodies connected to the detection site by permanent or ephemeral water flow or watershed considerations, and adjacent terrestrial areas).
3. Adjacent waters or lands within natural movement distances of the affected species.
4. Nearby sites that may serve as refugia for translocating uninfected salamanders.

iv. Movement restrictions and prohibitions on collections of wild salamanders from affected site.

v. Other interventions as feasible, e.g., antifungal treatments for surviving animals, as described by Blooi and colleagues (2015b), or possibly habitat treatments or disinfection. *As new information becomes available on pending research and mitigation strategies, we will update this template.*

Preliminary data show some habitat treatments may be effective in eradicating the related pathogen, Batrachochytrium dendrobatidis *(Bd; Bosch et al. 2015). In the early stages of* Bsal *detection and rapid responses, these may be the best opportunities to address site-level habitat treatments as part of containment and eradication.*

1. *Culling/Euthanasia*
2. *Bleaching site*
3. *Draining*
4. *Site closures (including physical barriers)*
5. *Signage or additional staffing to address desired visitor behavior modifications*

Questions: *Whom might you contact for each of the above possible actions? Is there an "expert team" you could develop and have on call for the different actions above? The* Bsal *Task Force can assist in identifying a few national contacts, and perhaps also some local contacts, as a start.*
What local, state or provincial/territorial, or federal resources are there to accomplish the actions above (e.g., labs, chemical application or water draining equipment)?
What local, state or provincial/territorial, and federal laws may apply for environmental compliance? Do agency or local law enforcement contacts need to be informed or engaged?

Scenario 5: Definitive detection, Captive
This scenario is defined as: Evidence of *both* 1) the presence of Bsal, as determined by the Participating Laboratory through either PCR-testing, or through isolation of a Bsal fungal culture as identified with genetic sequencing; *and* 2) signs of infection, as determined by the Participating Laboratory based on either clinical signs of disease in individual animals, or by histopathological characterization consistent with Bsal infection. *Evidence of presence without evidence of infection is not enough to determine definitive detection of* Bsal *(see Iwanowicz et al. 2017). Laboratory determinations are based on the Case Definition for* Bsal *chytridiomycosis (White et al. 2016), accepted by the Diagnostic Working Group of the* Bsal *Task Force.*

Actions recommended (one or more, as feasible):

1) Results communicated by Participating Laboratory to:
   a. Reporting Individual(s), who in turn informs:
      i. Captive animal owner/captive facility manager or veterinarian
      ii. State or provincial/territorial agency(-ies) with jurisdiction over captive animal health and movement (e.g., wildlife management agency, or state/provincial/territorial department of agriculture)

2) Agency or entity with management authority forms and convenes the Core Response Team (CRT)
   a. Consider also engaging the Bsal Task Force Technical Advisory Committee leadership (response@salamanderfungus.org) is available to assist by advising on resources and responses, and will keep the information confidential.

3) Subsequent Communications (in order of priority)
   a. Internal Reporting agency/organization (if applicable)
   b. Pet store, or importer, or zoological institution where animals were acquired

8) Chain-of-contact/custody stakeholders (i.e., individuals or entities throughout the history of possession of the affected amphibians, and other associated individuals or entities).
   c. Formal stakeholder notifications (per CRT guidance)
      i. State veterinary health official
      ii. AZA Taxonomic Advisory Group or Species Survival Plan contacts
   d. Scientific publication outlet.
   e. Bsal reporting database.
   f. Public announcement/press release as appropriate (and in collaboration with captive animal/facility owner).

4) Emergency Meeting convened among parties identified in 2 and possibly 3(a–c) above to discuss:
   a. Risk/threat assessment.
   b. Management actions.
      i. Containment.

1. Ensure no running water out of the housing of the animals
2. Eradicate Bsal sources.
    a. For live, captive animals whose samples return a positive Bsal result, eradication may be attempted:
        i. For failsafe eradication, we recommend humane culling or euthanasia and disposal of infected individuals using strict biosecurity protocols. See Section 8.6 in Pessier & Mendelson, 2017, or humane methods in accordance with the *AVMA Guidelines for the Euthanasia of Animals: 2013 edition* (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf).
        ii. If there are reasons to maintain the animals, eradication of Bsal may be possible and has been demonstrated in published literature (Blooi et al. 2015a; Blooi et al. 2015b). *There may be reasons to maintain and treat animals, e.g., with threatened or endangered species. However, there may also be reasons to maintain infected animals, e.g., for additional diagnostics or research. Consult with the CRT and your Participating Laboratory to determine options.* As such, we suggest the following:
            a) Treat per guidance in Blooi et al. (2015a, b). *As new treatments and research are being investigated, we will update this template.* ***Please note:*** *the methods tested to date only are confirmed in Fire Salamanders (*Salamandra salamandra*); keep in mind that species differences may come into play with respect to treatment validity and effectiveness. This is why multiple swabs over time are necessary to confirm eradication.*
            b) Swab treated animals post-treatment (see Appendix I, Section B) and submit samples to a Participating Laboratory to confirm Bsal eradication.
            c) Repeat treatment regime(s) and post-treatment swabbing until confirmation of Bsal eradication.

ii. Quarantine. Isolate any potentially affected individual animals, including any that were housed nearby or co-located with affected individuals.
    1. Perform additional diagnostics on quarantined, co-located individuals.
    2. Employ strict use of biosecurity protocols (see Pessier & Mendelson, 2017), for all people/personnel handling the affected species, particularly prior to exiting quarantine area.
iii. Disinfection, per Pessier & Mendelson (2017):
    1. All caging/housing materials and equipment prior to reuse.
    2. All water prior to disposal.
    3. All plants, soils, or other organic materials prior to disposal.
iv. Captive population monitoring. Evaluate the exposure to other co-located amphibians, including:
    1. Determine other places it could be in the facility, and disinfect these areas.
    2. Assess other potential sources of spread or origin of the pathogen, including through shared water sources or uses and movements, and quarantine or disinfect these sources.
    3. Assessment across the collection to determine whether it is clinically stable or if there is a trend of increasing morbidity and mortality.
    4. Evaluate other sources of infection, including new acquisitions.
v. Reporting, and additional testing, throughout the chain of custody (i.e., individuals or entities throughout the history of possession of the animals or lot in which the affected amphibians originated).
    1. At minimum, swab amphibians for PCR analysis in either direction throughout the chain of custody.
    2. Consider additional monitoring, as in 4(b)(iv) above.
vi. Voluntary surveillance of affected populations.
    1. Additional sampling of affected species and captive environment (plant and other substrates).
    2. Sampling of all other amphibian species in the facility.
    3. Sampling of stock of original importer or zoological collection
        a. Exposed animals
        b. Other co-located animals
    4. Sampling throughout the chain-of-contact/custody of exposed individual animals.
vii. Voluntary movement restrictions/prohibitions of movement or sale of affected species.

1. Place a temporary moratorium of sale or movement of all salamanders from same zoological collection, captive breeder, pet supplier, or importer. *The entity customizing this document can determine whether to qualify this action as "mandatory" or "required" or other descriptor. When there is a definitive detection of* Bsal*, we suggest the strongest possible measures to reduce risk of spread and facilitate containment.*
2. Document Bsal treatment. If animals are treated prior to resumption of transport or sale (in accordance with existing federal, state, or local laws), consider obtaining a health certificate or other documentation from a member of one of the Veterinary Expert Networks verifying Bsal treatment and eradication for *each individual animal* that tested positive for Bsal and was treated and for which Bsal was shown to be eradicated. *Entities customizing this template should keep in mind that each state may or may not have specific laws regarding "official" health certifications or alternative options; it is important to consult your state fish and wildlife agency and state department of agriculture regarding either the recommendation being an official or unofficial form of documentation.*

APPENDIX I.

PROTOCOLS AND PROCEDURES FOR SAMPLING FROM MORTALITY EVENTS, AND FOR SAMPLING FROM LIVING ANIMALS, FOR DIAGNOSTIC TESTING

Text adapted, with permission, from:
Pessier, A.P. and J.R. Mendelson (eds.). 2017. A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs, Ver. 2.0. IUCN/SSC Conservation Breeding Specialist Group: Apple Valley, MN.
NOTE: There is a new version of this manual, published in 2017. This Appendix will be updated to reflect any new information in 2018; in the meantime, the updated manual can be found here**.**

A. TISSUE COLLECTION DURING MORTALITY EVENTS. Mortality events where multiple animals are found dying or dead are observed in amphibian survival assurance colonies as well as wild amphibian populations. Although well-known infectious diseases of amphibians (e.g., chytridiomycosis or *Ranavirus* infection) may be strongly suspected, it is important to keep an open mind and always consider other potential causes. Many different disease conditions can initially look very similar and require laboratory investigation to achieve a definitive diagnosis.

It is always advisable to contact the lab where you intend to send samples and discuss with them their preference on how to prepare and ship the animals. *If possible, well in advance of a mortality event, consider contacting your nearest diagnostic laboratory to find out their preferences for preparing and shipping animals in various scenarios of a mortality event.*

The initial goal of investigating mortality events is to collect and preserve representative samples that can be used for the different types of laboratory techniques that may be needed.

Complex protocols can be designed for sample collection during mortality events—especially if veterinary guidance is available—however, a simple and basic approach is also sufficient for most situations.

- If wildlife health expert guidance is not available or if animals are small:
    - Perform the carcass-fixation necropsy method (see Chapter 9 in Pessier & Mendelson, 2017) on one-half to two-thirds of the dead animals.
    - For the remaining animals, freeze the carcasses whole as soon as possible and label with the species name, individual identification number and date.
        - For freezing of entire carcasses or individual tissue samples, ultracold temperatures (–70°C or below) or in liquid nitrogen are preferable. However, regular household freezer temperatures (–20°C) are sufficient for short-term storage.
        - As a last resort, if a freezer or liquid nitrogen is unavailable, fixation of carcasses or tissue samples in 70% ethanol (instead of formalin) may still allow application of some molecular diagnostic techniques.
- If wildlife health expert guidance or an individual experienced with amphibian anatomy is available, perform the dissection necropsy method (see Pessier & Mendelson, 2017) on the dead animals.
- In addition to saving samples from all major organs in fixative solution for histopathology, freeze additional samples of individual organs.
    - Suggested samples for freezing include skin, liver, kidney, lung, intestine, brain and any tissue thought to be abnormal during dissection (e.g., enlarged or discolored organs or organ nodules). In addition, stomach contents, coelomic fat bodies and skeletal muscle can also be saved, especially if exposure to a toxic substance is a possibility.
    - Organ samples are saved in sterile Whirl-Pak® style bags (Nasco, USA, www.enasco.com) or cryovials such as Nunc CryoTubesTM or Vangard CryosTM (Sumitomo Bakelite Co., Ltd. Japan, www.sumibe.co.jp/english/).
    - Containers should be labeled with the species name, individual animal ID number, specimen type, date, and county and state where collected.
- If moribund (dying) animals are found, consideration should be given to humanely euthanize some of these individuals for necropsy and sample collection (see Section 8.6 in Pessier & Mendelson, 2017). This provides very fresh samples that are ideal for most laboratory methods used for disease investigation.

1. BASIC TISSUE SAMPLE COLLECTION PROTOCOL FOR AMPHIBIAN MORTALITY EVENTS (Wildlife Health Expert Not Available, or Field Situation with Limited Equipment)

- For half of the dead animals, make an incision into the coelomic cavity and expose the internal organs.
  - For very small animals or if a knife is not available, just fix the carcasses intact.
  - Place the opened carcass into a fixative solution such as 10% neutral buffered formalin (preferred) or 70% ethanol. The ideal ratio is 1 part animal carcass to 9 parts fixative solution.
- For the other half of the dead animals, freeze the carcasses whole or keep them cool (such as in a portable ice-chest) until they can be transported to a location where freezing is possible.
  - It is always better to save both fixed (formalin or ethanol) and frozen samples. If this is not possible, preference should be given to saving tissues fixed in formalin or ethanol.
  - Saving only frozen samples should be a last resort (but is better than no samples at all).
    - If freezing of samples is not possible, fixation in ethanol may allow for both histopathology as well as some molecular diagnostic tests (e.g., PCR)

2. **SHIPMENT OF SAMPLES.** For shipment of tissues that have been preserved in a fixative solution. Once carcasses or tissues have been in formalin or other fixative solution for a minimum of 48 hours, they are removed from fixative, wrapped in paper towels or gauze moistened with fixative, packed into sealed plastic bags and shipped to a pathologist. This minimizes the potential for leakage during shipment and reduces package weight (and shipment costs).
   - Materials should be shipped in a manner that follows International Air Transport Association (IATA) regulations for Dangerous/Hazardous Materials (see also https://www.gpo.gov/fdsys/pkg/FR-2011-07-20/pdf/2011-17687.pdf). Some general guidelines include:
     - Samples should be enclosed in a primary receptacle that is leak-proof.
     - The primary receptacle is then placed within a leak-proof secondary receptacle.
     - An absorbent material (e.g., paper towels) should be placed between the primary and secondary receptacles. The volume of material should be sufficient to absorb all of the fluid within the primary receptacle.
   - Major shipping companies have guidelines available to help with proper shipping of biological samples. More information available here: http://images.fedex.com/downloads/shared/packagingtips/pointers

3. **Disinfection and Biosecurity in the Field.** Concerns about the possibility of moving amphibian pathogens to new locations as the result of field research conducted on wild amphibians have led to a number of protocols for reduction of this risk (e.g., http://northeastparc.org/disinfection-protocol/). There are variations and sometimes contradictions between the different protocols, however, the basic principles of biosecurity for biologists working on wild amphibian populations are similar. Peer-reviewed publications including the addition of risk calculators to

assist the biologist in making good biosecurity decisions have recently become available (St-Hilaire et al. 2009; Phillott et al. 2010). A summary of recommended field practices includes:

- **Definition of the field site.** The first precaution against the possible spread of disease among amphibian populations is careful definition of the field site or sites. Researchers should use natural and man-made boundaries to help define the sites. Whenever possible, plans should be made ahead of time to work in only one site per outing, or have different groups working at each individual site to avoid cross-contamination (and transmission of disease) between sites.
- **On-site hygiene and biosecurity of equipment.** The use of disposable equipment discarded after use at a single site or on a single individual amphibian reduces the risk of spreading disease. All reusable equipment, including footwear, should be disinfected between sites, or dedicated to a single site (e.g., a single pair of rubber boots is purchased for each field site and used ONLY at that site). Consult the table in Section 5.10 of Pessier & Mendelson (2017) for details on the use of specific disinfectants including recommended concentrations and contact times.
    - Footwear and other reusable equipment should be made of materials that are easy to clean and disinfect (e.g., rubber boots are better than leather hiking boots).
    - Thorough cleaning of equipment is essential for removal of dirt and organic material prior to disinfection in the field. As noted in other sections, organic material inactivates many disinfectants. Scrub brushes and other implements to remove dirt should be part of the field equipment. If disinfectant solutions become contaminated with organic material or dirt they should be changed.
    - The quaternary ammonium compounds (see Section 5.2 in Pessier & Mendelson, 2017) have been recommended for field situations because they are concentrated and easy to transport into field situations (Johnson et al. 2003; Webb et al. 2007).
    - If disinfection is undertaken in the field, consideration should be given to the toxicity of chemicals to the environment. The quaternary ammonium compounds and Virkon® (see Section 5.2 of Pessier & Mendelson, 2017) are more environmentally friendly options compared to chlorine bleach (Johnson et al. 2003; Webb et al. 2007; Schmidt et al. 2009). If ranaviruses are a special concern Virkon® may have some advantages over the quaternary ammonium compounds (Bryan et al. 2009). Powdered bleach is another easily portable suggestion.
    - Vehicles are less likely to be a vector for the transmission of disease than footwear and field equipment, but still should be disinfected, especially if used to cross or enter a known contaminated site. The wheels and tires should be cleaned of all dirt and organic material and disinfected prior to leaving the site, using the same disinfectant that was used on footwear. Always

remember to disinfect footwear before getting into a vehicle to prevent pathogens from transferring to the floor or pedals.

- **Handling and collection of samples from amphibians:** When handling amphibians in the field, even within the same site, precautions should be taken to minimize the risk of transmitting pathogens between individual animals.
    - Non-powdered disposable gloves are the best choice when handling amphibians. Powdered gloves should be rinsed free of powder. A new pair of gloves should be used for each animal. If gloves are unavailable, it is slightly preferable to use bare hands, and wash hands between handling different animals (Mendez et al. 2008).
    - The greatest risk for spreading disease when handling amphibians occurs when animals are placed together in the same container or when containers are reused without being disinfected. Do not re-use collecting bags and utilize a new one for each animal.
    - Always handle animals as little as possible. Procedures that are quick, even if potentially painful, may cause less stress than longer procedures.
    - Animals should only be released at the site of capture and any sick or dead amphibians found should be preserved in 10% buffered formalin solution and submitted for disease diagnosis (see Chapter 9, Necropsy, in Pessier & Mendelson, 2017).
    - Instruments used for sample collection should be disinfected between use on different animals. For surgical instruments (e.g., scissors) and weighing equipment 70% ethanol is rapidly acting against the amphibian chytrid fungus (Johnson et al. 2003).
    - Although mentioned in some amphibian handling protocols the use of iodine-based compounds for sanitizing the skin prior to procedures such as toe-clipping or microchip implantation is not recommended because of toxicity concerns. Potential substitutes include 0.75% chlorhexidine or 2mg/L benzalkonium chloride (Wright, 2001).

B. SAMPLE COLLECTION FOR Bsal PCR. As of this version, some of the sample collection options for Bsal have not yet been documented; this information is provided based on techniques used for *Batrachochytium dendrobatidis* (Bd) and will be updated as new information becomes available. Based on what is known for Bd, the PCR procedure can be performed using a variety of different sampling methods including skin swabs, water bath, and tissue samples (e.g., toe clip; Hyatt et al. 2007).

- Skin swabs. The skin swab procedure is simple, minimally invasive and samples multiple areas of the skin that may be infected with Bsal (increasing the likelihood that infected areas will be sampled). Skin swabs generally are the preferred sampling method for Bsal PCR.
- Water bath. Samples using the water bath procedure require immediate centrifugation or micropore filtration *and are not practical in many settings*.
- Tissue samples. Toe clipping is an invasive procedure with associated ethical concerns and has the disadvantage of sampling only a small portion of potentially infected skin.

1. **Materials Needed.** The materials listed below are general guidelines needed to perform the skin swab procedure for Bsal PCR using realtime or quantitative PCR (qPCR) methods. There may be differences depending on the preferences of the laboratory processing the samples and the environmental conditions under which the swabs are obtained.
   - Powder-free latex or nitrile disposable gloves.
   - Sterile applicators ("swabs"); see "Swab Selection" in Pessier & Mendelson (2017).
   - 1.5 ml microcentrifuge tubes/cryovials.

   Storage of dry swabs at controlled room temperature/refrigeration or freezing is preferred, but 70% ethanol is an alternative especially if samples will be exposed to variable climate conditions, especially heat. Individual laboratories may have preferences about sample storage conditions; be sure to check in advance with the Participating Laboratory to which samples will be sent. For additional information see the section on "Storage of Skin Swab Samples" below.

2. **Swabbing Procedure 101.** Several videos demonstrating swabbing and associated biosecurity and prevention of contamination have been developed.
   i. Swabbing technique for qPCR: http://amphibiaweb.org/chytrid/index.html
   ii. Swabbing using wooden-stemmed swabs suitable for conventional PCR (see "How to Swab a Frog for Chytrid"): http://www.amphibianark.org/frog_gallery.html
   iii. General swabbing and associated biosecurity procedures: https://www.youtube.com/watch?v=a5CtPrGOK8c

3. **Avoiding Cross-contamination of Samples.** The PCR assays are very sensitive tests and can detect very small amounts of Bsal DNA. This is good for detecting animals that have very low-level infections with Bsal, but increases the risk that

samples from a non-Bsal infected animal can have false-positive results if they become contaminated with even small amounts of Bsal DNA from an infected animal. Therefore, it is very important to take precautions to avoid sample cross-contamination which include:

- A new pair of disposable latex or nitrile gloves should be used for each animal handled for testing (Mendez et al. 2008).
- Avoid contact of swabs (especially swab tips) with surfaces or substrates other than the skin of the animal to be tested.
- If instruments are used to cut the tip of the swab into cryovials, a freshly disinfected instrument must be used for each sample.
    - To disinfect instruments for this purpose, dip in 70% ethanol followed by flaming under an alcohol lamp.
    - Avoid using bleach solutions for disinfection because this can degrade Bsal DNA in swab samples (resulting in false-negative tests; Cashins et al. 2008).

4. **Avoiding PCR Inhibitors in Samples.** Foreign material such as dirt or plant matter can contain materials that inhibit the PCR reaction. This can result in a false-negative test result (animal is infected with Bsal, but it is not detected by the PCR test).
    - Prior to skin swabbing efforts should be made to manually remove heavy skin contamination. Animals may be gently rinsed with clean water prior to sampling, but vigorous washing should be avoided because of the potential to also rinse off Bsal infected skin cells or organisms.
    - If rinsing with water is used for cleaning, the water should not originate from the animal's enclosure or environment.
    - Laboratories that perform PCR for Bsal should always use exogenous internal positive controls to detect PCR inhibitors (Hyatt et al. 2007).

5. **Storage of Skin Swab Samples.** Storage of swabs after sample collection is an important consideration. Swabs can be stored air-dried or in 70% ethanol. Be sure to check in advance with the Participating Laboratory to which samples will be sent; individual laboratories may have preferences about sample storage conditions.

    For air-dried swabs, the major concern is high temperature extremes:
    - The DNA on air-dried skin swabs is remarkably stable, and experimentally, swabs have been stored for up to 18 months at room temperature (23°C) without a reduction in the sensitivity of the assay (Hyatt et al. 2007).
    - In contrast, exposure of swabs to very high temperatures (> 38°C) for 7 days can result in decreased recovery of pathogen DNA that could result in false-negative results for animals with low-level Bsal infections (Van Sluys et al. 2008).

    Therefore, it is recommended that air-dried skin swab samples be stored at as low a temperature as possible (Skerratt et al. 2008).
    - At 25°C (refrigerator) or lower.

- Samples should be frozen (–20°C or below) if sample analysis is not performed within six months of sample collection.
- See alternatives to low temperature storage (i.e., where refrigeration may not be possible) in Pessier & Mendelson (2017).

6. **Shipment of Swabs to the Laboratory.**
   - Ideally ship swabs by overnight or 2-day courier service (e.g., Federal Express; Canada Post Xpresspost, UPS, Purolator, etc.).
   - Consider using cold packs to guard against high temperature extremes.
   - Samples that have been previously frozen should be sent on dry ice to prevent freeze-thaw cycles.

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