

**A North American Strategic Plan to Control Invasions of
*Batrachochytrium salamandrivorans***

North American *Bsal* Task Force

2020

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A North American Strategic Plan to Control Invasions of *Batrachochytrium salamandrivorans*

North American *Bsal* Task Force

Summary

Chytridiomycosis is a fungal disease of amphibians that has led to widespread mortality and extinctions. It has been considered “the greatest threat to biodiversity of any known disease” (Wake and Vredenburg 2008). In 1999, the pathogen causing this disease was described: *Batrachochytrium dendrobatidis*, or *Bd* (Longcore et al. 1999). A second *Batrachochytrium* species that causes chytridiomycosis has been discovered more recently: *B. salamandrivorans*, or *Bsal* (Martel et al. 2013). *Bsal* has caused population extirpations 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; Wagner et al. 2019; Lötters et al. 2020). Several lines of evidence support an Asian origin of *Bsal* (Martel et al. 2013, 2020; Laking et al. 2017; Nguyen et al. 2017; Yuan et al. 2018). Susceptibility trials of salamander species native to North America revealed that some species are lethally affected, including all species evaluated in the newt family Salamandridae (Martel et al. 2014, Carter et al. 2020). Introduction of *Bsal* to North America, a hotspot of salamander diversity, could drastically reduce amphibian biodiversity and result in devastating ecosystem effects (Gray et al. 2015; Yap et al. 2015; Richgels et al. 2016, Basanta et al. 2019). To date, *Bsal* has not been detected in North America (defined as Canada, Mexico, and the United States; e.g., Govindarajulu et al. 2017; Klocke et al. 2017; Waddle et al. 2019, 2020). Since effective mitigation strategies have not been developed to combat *Bsal* in the field (but see Thomas et al. 2019 for review; Martel et al. 2020), the best threat-abatement strategy currently available is to prevent invasion and keep the pathogen from being introduced and becoming established in North America (Grant et al. 2017; Stegen et al. 2017). If *Bsal* is detected, a rapid response plan will be essential for managing and controlling its spread (Appendix 4).

In addition to these two main goals of **preventing invasion** and being able to **respond quickly** if *Bsal* were detected in North America, it is vital to develop a more comprehensive network of strategic actions. This “North American Strategic Plan to Control Invasions of *Batrachochytrium salamandrivorans*” (hereafter the “North American *Bsal* Strategic Plan”) outlines integrated, multi-pronged actions developed by the North American *Bsal* Task Force (or *Bsal* Task Force) for detecting and preventing the establishment of *Bsal* in North America. The North American *Bsal* Strategic Plan includes the following components: development of **response and control measures** to facilitate response and rapid mitigation actions; improved **diagnostic tools** to identify the pathogen with accuracy and efficiency, including a network of diagnostic laboratories able to analyze samples for *Bsal*; **research** to better understand *Bsal* effects on North American species and how to potentially safeguard susceptible species; application of **decision science** to frame and solve management problems, identify research needed to improve management, and guide data collection and use; a **surveillance and monitoring** program to maximize the potential for early detection of the fungus and

documentation of its distribution and spread if found; a **data management** system to track *Bsal* surveillance in the field and captivity and to aid in planning future studies; **outreach and communication** pathways for rapid dissemination of new developments; and a **clean trade program** to promote actions that reduce *Bsal* translocation in trade markets, such as the pet industry.

The *Bsal* Task Force is unique in that it is proactively designed, it is interactive among all components described above, and it includes decision science as a critical element in linking other components, especially surveillance, research, and management. The transdisciplinary approach to the North American *Bsal* Strategic Plan relies on the integration of the strategic planning of objectives and goals of the *Bsal* Task Force Technical Advisory Committee (TAC) and working groups that represent the key components outlined above. This approach has resulted in the identification of goals and actions for each component as well as an overarching Framework of Actions (below). Elements of the North American *Bsal* Strategic Plan, including the Rapid Response Plan (Appendix 4) and the Implementation Plan (Appendix 5), are intended to be updated as initial actions are implemented and new information, techniques, or priorities are identified.

Framework of Actions

1. Prevent invasion of *Bsal* into North America by encouraging stakeholders to work toward a clean trade program for amphibians that certifies individuals in trade are free of *Bsal* infection.
2. Develop and encourage use of the Rapid Response Plan (Appendix 4 in this Strategic Plan), which can be customized to meet local needs, to contain a *Bsal* outbreak.
3. Develop a network of diagnostic laboratories that can run validated tests to detect the presence of *Bsal* in animal or environmental samples in a timely manner.
4. Test for the occurrence of *Bsal* in Canada, Mexico, and the United States in the field and in captivity, 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 the understanding of the risk of *Bsal* introduction to North America and assess the invasion risk of this deadly pathogen to native North American amphibians through decision science analyses, research, and development of a common repository for aggregating and managing *Bsal* surveillance data.
6. Develop effective, scientifically justified prevention and mitigation strategies that prevent *Bsal*-associated infections and mortality.
7. As evidence-based *Bsal* response and management actions are developed, identify expedited pathways for permitting actions and facilitate regulatory processes to implement mitigation measures.
8. Work with partners to compile and disseminate surveillance and research results via social media, accessible web portal databases, and newsletter articles.
9. Build a network of partners that can communicate updates on *Bsal* developments and alert the public and scientific community if *Bsal* is detected in Canada, Mexico, or the United States.

Plan Estratégico de Norte América para Controlar la Invasión de *Batrachochytrium salamandrivorans*

Grupo de trabajo *Bsal* en Norte América

Resumen

La quitridiomycosis es una enfermedad fúngica que afecta a los anfibios 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 anfibios (Longcore et al. 1999). Más recientemente, se ha identificado una segunda especie de hongo patógeno que también causa quitridiomycosis: *B. salamandrivorans*, o *Bsal* (Martel et al. 2013). Varias evidencias apoyan la hipótesis de que el *Bsal* tiene un origen asiático (Martel et al. 2013, 2020; Laking et al. 2017; Nguyen et al. 2017; Yuan et al. 2018). *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; Wagner et al. 2019; Lötters et al. 2020). 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, Carter et al. 2020). La introducción de *Bsal* a Norte América podría reducir de manera dramática la biodiversidad de salamandras, así como causar efectos graves en el ecosistema, ya que esta región del planeta constituye 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 detectado en el continente americano. Debido a que no se han desarrollado medidas efectivas para mitigar la infección por *Bsal* en poblaciones naturales (pero ver revisión de Thomas et al. 2019; Martel et al. 2020), la mejor estrategia para disminuir los riesgos de esta enfermedad, es evitar que el patógeno se introduzca y 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 para controlar su propagación (Apéndice 4).

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. Este “Plan Estratégico de Norte América para Controlar la Invasión de *Batrachochytrium salamandrivorans*” (Plan Estratégico de *Bsal* en Norte América) propone acciones integradas y multifacéticas desarrolladas por el Grupo de Trabajo *Bsal* en Norte América para detectar y prevenir el establecimiento de *Bsal* en esta región del continente americano. El Plan Estratégico de *Bsal* en Norte América se compone de los siguientes elementos: desarrollar **medidas de manejo y respuesta** que faciliten una respuesta proactiva y el desarrollo de estrategias de mitigación rápida; **herramientas mejoradas 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; **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 científico para dar contexto y resolver problemas de manejo, identificar la investigación requerida para mejorar el manejo, así como guiar la colecta y uso de datos; un **programa de vigilancia y monitoreo** para maximizar las probabilidades de una detección temprana del hongo y documentar su distribución y propagación en caso de ser detectado; un **sistema de manejo de datos** para rastrear el monitoreo de *Bsal* en el campo y en cautiverio, y ayudar en la planeación de estudios futuros; **vías de comunicación y divulgación** para la diseminación rápida de nuevos avances o descubrimientos; y un **programa de comercio limpio** para promover acciones que reduzcan la translocación de *Bsal* en el comercio de especies, como es el caso de la industria de mascotas.

El Grupo de Trabajo *Bsal* se distingue por su naturaleza proactiva, y por un diseño interactivo de los elementos que lo componen. El Grupo se apoya en decisiones basadas en la ciencia como un elemento crítico que enlaza a todas sus actividades, en particular en lo que se refiere a vigilancia, investigación y manejo. El enfoque transdisciplinario del Plan Estratégico de *Bsal* en Norte América depende de la integración del Comité de Asesoría Técnica (TAC, por sus siglas en inglés), que facilita la comunicación entre los diferentes sub-grupos de trabajo que integran el Grupo de Trabajo *Bsal*. Esta integración se refleja en los elementos clave incluidos en este documento, incluyendo los objetivos y metas del TAC, los cuales pueden resumirse en el Plan de Acción (abajo). Algunos elementos del Plan Estratégico de *Bsal* en Norte América, incluyendo el Plan de Respuesta Rápida (Apéndice 4) y el Plan de Implementación (Apéndice 5), serán actualizados conforme se implementen sus recomendaciones y conforme surjan nuevas fuentes de información, técnicas o prioridades.

Plan de acción

1. Prevenir la invasión de *Bsal* a Norte América incentivando a los tomadores de decisiones para desarrollar un programa de comercio limpio para anfibios, el cual certifique que los organismos estén libres de la infección por *Bsal*.
2. Desarrollar e incentivar el uso de un plan de respuesta para contener un brote de *Bsal*, el cual está contenido en el Plan Estratégico y que puede adaptarse a las necesidades locales.
3. Desarrollar una red de laboratorios para diagnóstico que pueda realizar de manera eficiente pruebas estandarizadas para detectar la presencia de *Bsal* en muestras de anfibios o muestras ambientales.
4. Evaluar la ocurrencia de *Bsal* en Estados Unidos, Canadá y México en el campo y en cautiverio, reducir el riesgo de propagación de *Bsal* de organismos en cautiverio hacia organismos silvestres, y reducir la probabilidad de que los humanos acarreen *Bsal* de manera inadvertida a través de Norte América.
5. Avanzar en el entendimiento sobre los riesgos de la introducción de *Bsal* a Norte América y evaluar este riesgo mediante análisis de ciencia aplicada, investigación, y generación de un repositorio para incorporar y manejar datos de monitoreo de *Bsal*.
6. Desarrollar estrategias de mitigación y prevención que sean efectivas y sustentadas científicamente para prevenir la mortalidad y las infecciones asociadas a *Bsal* en anfibios.
7. Identificar vías expeditas para permitir acciones, facilitar procesos regulatorios e implementar estrategias de mitigación, mientras se desarrollan acciones de manejo y respuesta con base en evidencias científicas.

8. Trabajar con colaboradores y colegas para compilar y diseminar resultados de monitoreos e investigación sobre *Bsal* por medio de redes sociales, portales de bases de datos disponibles, y artículos o notas periodísticas.
9. Crear una red de colegas que puedan comunicar las actualizaciones en el tema de *Bsal* y alertar al público y a la comunidad científica en caso de que *Bsal* sea detectado en Estados Unidos, Canadá o México.

Stratégie nord-américaine de contrôle du *Batrachochytrium salamandrivorans* invasif

Le groupe de travail North American *Bsal* Task Force

Résumé

La chytridiomycose est une maladie fongique des amphibiens qui cause dans une large mesure la mortalité et l'extinction. Elle est considérée comme « la plus grande menace à la biodiversité de toutes les maladies connues » (Wake et Vredenburg, 2008) Le pathogène qui cause cette maladie a été identifié en 1999 : *Batrachochytrium dendrobatidis*, ou *Bd* (Longcore et coll., 1999). Une seconde espèce de *Batrachochytrium* causant la chytridiomycose a été découverte plus récemment : le *B. salamandrivorans*, ou *Bsal* (Martel et coll., 2013). *Bsal* cause la disparition de populations de salamandres tachetées *Salamandra salamandra* en Europe, où il semble avoir été récemment introduit et dont la distribution semble s'étendre (Spitzen-van der Sluijs et coll., 2016; Stegen et coll., 2017; Wagner et coll., 2019; Lötters et coll. 2020). Plusieurs éléments de preuve indiquent que *Bsal* serait d'origine asiatique (Martel et coll., 2013, 2020; Laking et coll., 2017; Nguyen et coll, 2017; Yuan et coll, 2018). Des essais de sensibilité des espèces de salamandres originaires d'Amérique du Nord ont révélé que certaines espèces sont mortellement affectées, et parmi elles toutes les espèces évaluées dans la famille de tritons salamandridés (Martel et coll., 2014, Carter et coll, 2020). Si *Bsal* était introduit en Amérique du Nord, haut lieu de la diversité des salamandres, cela pourrait réduire considérablement la biodiversité des amphibiens et avoir un effet dévastateur sur l'écosystème (Gray et coll., 2015; Yap et coll., 2015; Richgels et coll., 2016, Basanta et coll, 2019). À ce jour, *Bsal* n'a pas été détecté en Amérique du Nord (terme désignant le Canada, le Mexique et les États-Unis; Govindarajulu et coll. 2017; Klocke et coll., 2017; Waddle et coll, 2019, 2020). Puisque des stratégies d'atténuation efficaces n'ont pas été mises en place pour combattre *Bsal* sur le terrain (mais voir Thomas et coll., 2019 pour une revue de la littérature; Martel et coll, 2020), la meilleure stratégie de réduction de la menace présentement disponible serait de prévenir l'invasion et d'empêcher que ce pathogène ne soit introduit et ne s'établisse en Amérique du Nord (Grant et coll, 2017; Stegen et coll., 2017). S'il est détecté, un plan de réponse rapide sera essentiel pour la gestion et le contrôle de sa propagation (Annexe 4).

En plus de ces deux objectifs principaux de **prévention de l'invasion** et de capacité de **réponse rapide** si *Bsal* est détecté en Amérique du Nord, il est essentiel de mettre sur pied un réseau plus complet d'actions stratégiques. Le "North American Strategic Plan To Control Invasions of *Batrachochytrium salamandrivorans*" (North American *Bsal* Strategic Plan) détaille des actions intégrées sur plusieurs fronts et mises en marche par la North American *Bsal* Task Force pour détecter et prévenir l'établissement de *Bsal* en Amérique du Nord. Le North American *Bsal* Strategic Plan comprend les éléments suivants : la mise en œuvre de mesures de **réponse et contrôle** dynamiques déclenchant des actions d'atténuation rapides; de meilleurs **outils diagnostiques** permettant d'identifier le pathogène avec précision et efficacité, dont un réseau de laboratoires diagnostiques capables d'analyser des échantillons de *Bsal*; de la **recherche** pour mieux comprendre les effets de *Bsal* sur les espèces nord-américaines et peut-

être même protéger les espèces sensibles; l'application de la **science décisionnelle** pour définir et régler les problèmes de gestion, pour déterminer les besoins en recherche visant à améliorer la gestion et pour diriger la collecte et l'utilisation des données; un programme de **surveillance et de contrôle** pour maximiser le potentiel de détection précoce du champignon et pour documenter son éventuelle distribution et étendue; un système de **gestion des données** pour retracer les activités de surveillance de *Bsal* sur le terrain et en captivité, et aider à planifier les prochaines études; des voies de **communication et de sensibilisation** pour permettre de faire circuler les nouveaux éléments d'information; et un **programme de « commerce propre »** pour faire la promotion d'actions diminuant la translocation de pathogènes sur les marchés, comme le marché des animaux de compagnie. Le groupe *Bsal* Task Force est une initiative unique, car elle est dynamique, et tous les éléments décrits ci-dessus sont interactifs, avec la science décisionnelle comme point critique pour lier les autres éléments, dont la surveillance, la détection et la gestion. L'approche interdisciplinaire du North American *Bsal* Strategic Plan dépend de l'intégration de la planification stratégique des buts et objectifs du *Bsal* Task Force Technical Advisory Committee (TAC) and working groups, c.-à-d. les éléments clés décrits ci-dessus. Cette approche a mené à la détermination des buts et des actions pour chaque élément ainsi que pour le Cadre d'actions (voir ci-dessous). Les éléments du North American *Bsal* Strategic Plan, dont un plan de réponse rapide (Annexe 4) et un plan d'implantation (Annexe 5), devront être mis à jour quand les premières actions seront entreprises et que de nouvelles données, techniques ou priorités seront déterminées.

Cadre d'actions

1. Prévenir l'invasion de *Bsal* en Amérique du Nord en encourageant les parties prenantes à établir un programme de « commerce propre » pour les amphibiens qui certifie que les individus vendus n'ont pas d'infection au *Bsal*.
2. Mettre sur pied et promouvoir l'utilisation du plan de réponse décrit dans le plan stratégique, qui peut être adapté aux besoins locaux pour contenir une éclosion de *Bsal*.
3. Mettre sur pied un réseau de laboratoires diagnostiques capables d'exécuter des tests validés permettant de détecter à temps la présence de *Bsal* chez un animal ou dans un échantillon environnemental.
4. Dépister la survenue éventuelle de *Bsal* aux États-Unis, au Canada et au Mexique sur le terrain et en captivité, réduire le risque de débordement des amphibiens captifs vers les sauvages et réduire la possibilité que les humains jouent un rôle dans le transfert par inadvertance de *Bsal* en Amérique du Nord.
5. Améliorer la compréhension du risque lié à l'introduction de *Bsal* en Amérique du Nord et évaluer le risque d'invasion des amphibiens indigènes par ce pathogène mortel grâce à des analyses de science décisionnelle, à de la recherche et à la mise sur pied d'un référentiel commun pour colliger et gérer les données de surveillance de *Bsal*.
6. Grâce au soutien de la science, concevoir des stratégies de prévention et d'atténuation efficaces empêchant les infections et la mortalité liées au *Bsal*.
7. Pendant que des interventions et des cadres de gestion factuels de *Bsal* sont mis sur pied, en déterminer les voies d'accès privilégiées et aider au processus réglementaire menant à l'implémentation de mesures d'atténuation.

8. Travailler avec les partenaires pour colliger et distribuer les résultats de la recherche et de la surveillance à travers les réseaux sociaux, les bases de données accessibles en ligne et les articles des bulletins d'information.
9. Établir un réseau de partenaires capables de fournir des mises à jour de l'information concernant *Bsal* et d'alerter le public et la communauté scientifique si *Bsal* est détecté aux États-Unis, au Canada ou au Mexique.

Strategic Plan Overview: Vision, Purpose, and Components

The vision of the North American *Bsal* Strategic Plan is to help safeguard North American amphibians from a novel and emerging pathogen. Specifically, the risk is that the fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*) will spread to North America, where it is not currently known to occur, and will cause population losses of amphibians from the skin disease chytridiomycosis. The plan's purpose is to outline a comprehensive approach to reduce the likelihood of *Bsal* establishment in North America, develop and evaluate proactive management actions to reduce the risk of *Bsal* introduction and establishment, and develop actions to combat the disease if it were to be found. It is a multipronged strategy that engages a community of researchers, natural resource managers, and partners from the public sector in Canada, Mexico, and the United States.

The North American *Bsal* Strategic Plan includes background information on *Bsal* and the ecological significance of salamanders in ecosystems and a brief review of policy options aimed at preventing *Bsal* introduction to North America. The North American *Bsal* Task Force (or *Bsal* Task Force) is described, including the strategic goals of the oversight group (the Technical Advisory Committee [TAC]) and the following working groups: Response & Control; Diagnostics; Research; Decision Science; Surveillance & Monitoring; Data Management; Outreach & Communication; and Clean Trade. The Rapid Response Plan Template, which provides guidance for field or captive situations if *Bsal* were to be suspected in North America, is included as Appendix 4. The *Bsal* Implementation Plan, which outlines more specific goals and priorities of the TAC and working groups, is included as Appendix 5. Both Appendices 4 and 5 are expected to adapt over time to new information and updates will be posted on relevant websites (e.g., salamanderfungus.org).

At the time of this writing, *Bsal* is not known to occur in North America and strategies included herein are consistent with the high-alert condition of *Bsal* being yet undetected or rare in North America.

I. Background

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 amphibians, growing and reproducing in their skin. *Batrachochytrium dendrobatidis* (*Bd*) is known to infect frogs, salamanders, and caecilians, and has caused widespread population declines and extinctions, especially of frog species (Lips et al. 2006; Skerratt et al. 2007; Scheele et al. 2019). *Bd* and/or its metabolites are suspected of causing deleterious effects on crayfish, but definitive evidence of infection in invertebrates is lacking (McMahon et al. 2013). Amongst amphibians, some species can carry *Bd* infections without showing signs of the disease chytridiomycosis (e.g., Bullfrogs: Daszak et al. 2004), and some species are resistant to infection (Appendix 1). *Bsal* is a recently described, closely related

congener of *Bd* that has caused salamander population declines and extirpations in Europe (Stegen et al. 2017; Lötters et al. 2020; Martel et al. 2020).

Understanding the life history of the *Bsal* pathogen is important, as it relates to its modes of transmission and differs somewhat from that of *Bd*. In both *Bd* and *Bsal*, reproduction consists of the asexual production of spores (Robinson et al. 2020). A sexual stage has not been observed in *Bsal* or *Bd* but is common in other chytrids (Powell 2016). 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 predominantly types of zooplankton. That study also 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 (*Salamandra salamandra*) 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. Stegen et al. (2017) also reported that *Bsal* DNA could be detected from contaminated forest soils even after 200 days. The existence of both flagellated and encysted spores is likely to increase within-population transmission rates above what would occur with flagellated zoospores alone (Stegen et al. 2017).

Amphibian host species that are lethally infected and species that are tolerant of infection (carriers) can both 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 by *Bsal*, whereas both salamander and frog species can be carriers of *Bsal* (Martel et al. 2014; Nguyen et al. 2017, Stegen et al. 2017). 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). In the field, we lack a full understanding of *Bsal* transmission pathways, which are likely to have context-dependent dynamics. For example, transmission may vary with habitat heterogeneity, amphibian community composition, and *Bsal* physiological ecology.

Bsal evolved in Asia, where the lack of lethal infections suggests a long co-evolutionary history that has led to resistance or tolerance in native amphibian species (Laking et al. 2017). *Bsal* was recently discovered in Europe (Martel et al. 2013), and it appears to be spreading (e.g., Spitzen-van der Sluijs 2016; Lötters et al. 2020; Martel et al. 2020). Infections were first observed in the Netherlands, followed by the discovery of *Bsal* in Belgium and Germany. Most recent evidence suggests that Germany may be the site of the index case for introduction of *Bsal* into Europe (Lötters et al. 2020). The lethal effect of *Bsal* on some European amphibian species suggests a recently arrived pathogen that has encountered naïve hosts. The likely routes of within-continent spread are dispersal of infected amphibians among populations, movement of spores by other wildlife and humans, and release of infected individuals once held in captivity (Nguyen et al. 2017; Yuan et al. 2018; Martel et al. 2020). Spread of *Bsal* between continents is likely due to importation of infected species from locations where *Bsal* is 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* has been found on salamander species in China that are frequently imported. These findings 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 2). *Bsal* has infected over 20 amphibian species in the wild (Martel et al. 2013, 2014; Spitzen-van der Sluijs et al. 2016; Laking et al. 2017; Nguyen et al. 2017; Yuan et al. 2018) and at least nine species in captivity (Martel et al. 2014; Cunningham et al. 2015; Nguyen et al. 2017; Fitzpatrick et al. 2018; Sabino-Pinto et al. 2018; Yuan et al. 2018). In the wild, most infected species have been in the family Salamandridae (17 infected species). One frog species found to be infected in the wild was the Many-webbed Fire-bellied Toad (*Bombina microdeladigitora*) (Nguyen et al. 2017). This species is closely related to the Oriental Fire-bellied Toad (*Bombina orientalis*), which is widely imported into the United States in pet trade markets. An enormous number of Oriental Fire-bellied Toads ~~*B. orientalis*~~ (3.5 million individuals) were imported into the United States between 2001 and 2009. Nguyen et al. (2017) concluded that *Bsal* was vectored into the wild in Europe via the pet trade.

Susceptibility of amphibian species to *Bsal* infection

The *Bsal* case definition describes the presentation of the disease *Bsal* chytridiomycosis (White et al. 2016) resulting from *Bsal* infection. However, disease signs are not apparent in all infections. Four terms have been used to classify host response to *Bsal* (Martel et al. 2014; F. Pasmans, Ghent University, Belgium, pers. comm. to D. H. Olson, 23 June 2020): 1) lethal = infection that invariably (as far as we know) results in death after a single exposure to a low dose (e.g., 1000 zoospores); 2) susceptible = infection resulting in variable infection and disease dynamics, depending on dose of infection (and probably a large number of other determinants); 3) tolerant = infection never resulting in disease or mortality, given current knowledge; and 4) resistant = no natural infections known and no infection in laboratory trials. In the absence of knowledge of within-species variation in *Bsal* response, these terms have been used to classify species-level responses (Martel et al. 2014).

The effects of *Bsal* infection vary among 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 1). The Fire Salamander is highly susceptible to *Bsal*; indeed, once *Bsal* enters a population of Fire Salamanders, extirpation of the population can occur rapidly (Stegen et al. 2017). However, other salamander species, such as the Alpine Newt (*Ichthyosaura alpestris*), appear to be tolerant of infection, in the sense that they can carry an infection without experiencing morbidity. Frog species, such as the Common Mid-wide Toad, *Alytes obstetricans*, have also been reported to be either resistant to or tolerant of *Bsal* infections. Unpublished data on susceptibility trials in the laboratory also have indicated that some amphibian species are resistant to infection, some species carry infections but do not succumb, and some species are

lethally affected (Appendix 1). So far, nine species of salamanders are known to develop chytridiomycosis from *Bsal* and succumb, especially when infected at a high dose (10^6 zoospores). The finding, both 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 work of Martel et al. (2014).

Importantly, the Siberian Salamander (*Salamandrella keyserlingii*) has been found infected with *Bsal* in the wild (Martel et al. 2014). Given that *Salamandrella* is a very early-evolved genus within the order Caudata, this finding 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 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: the southeastern United States, the western United States, the south coast of British Columbia, and the highlands of central Mexico. The model developed by Richgels et al. (2016) used habitat suitability for *Bsal* and host richness in the United States and included risk of introduction from the pet trade. Their model predicted three zones of high risk: the Pacific coast, the southern Appalachian Mountains, and the mid-Atlantic region. 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 hotspot areas for surveillance. This model predicted areas of the Trans-Mexican Volcanic Belt, the Sierra Madre del Sur, the Sierra Madre Oriental, northern Oaxaca, the Mexican Gulf, and the Yucatán Peninsula as risk zones suitable for *Bsal* in Mexico, and it identified 13 hotspots with both high salamander diversity and suitability 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. They can be important in energy flow through these ecosystems and in suppression of leaf litter decomposition in terrestrial ecosystems, which functions to sequester carbon from the atmosphere. They can also be important as keystone species that affect ecosystem biodiversity. Dramatic declines and extinctions of salamander species in North America could, therefore, 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 consider the detection rate of a species indicate that surface populations can be <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*) were over $1/\text{m}^2$ in New Hampshire's Hubbard Brook Experimental Forest (Burton and Likens 1975a) and were up to $11/\text{m}^2$ in

Virginia's Shenandoah National Park (Jaeger 1979). The population density of a related species, the Southern Red-backed Salamander (*Plethodon serratus*), was estimated to be about 1/m² in Missouri (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 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/m². 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. Further, it has been suggested that predation is not a significant source of mortality for woodland salamanders in the genus *Plethodon* (Hairston 1987). If so, then a large fraction of primary production is entering the salamander community through a pathway that starts with leaf litter and proceeds to soil invertebrates and on to 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 could 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 CO₂ release into the atmosphere. An increase in leaf-shredding soil invertebrates and the resulting increase in leaf fragments could facilitate an increase in microbial decomposers, increasing total microbial respiration as a result. Three studies have demonstrated that total leaf litter decomposition is suppressed when woodland salamanders in control plots are 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 to 17%, thereby reducing soil carbon release to the atmosphere (i.e., by their predation of soil invertebrates, these salamanders affect carbon sequestration). Another study found a similar effect and estimated that, across its range in California, the species *Ensatina eschscholtzii* could prevent 72.3 metric tons of carbon from entering the atmosphere each year (Best and Welsh 2014). Although additional replication of these studies is required, they suggest that large declines of terrestrial salamanders could release large amounts of CO₂ into the atmosphere that otherwise would be stored in the soil ecosystem, and this release would have concomitant effects on climate change.

Furthermore, many amphibians have a key ecological functional role in the 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 in their body mass. These energetic subsidies are transported to terrestrial ecosystems upon metamorphosis for many species, including many 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 terrestrial ecosystem energetics.

Salamanders are keystone species in temporary pond ecosystems, meaning that their removal will have important impacts on the ecosystem. Studies from the 1980s in experimental ponds demonstrated that removal of the keystone species the Eastern Newt (*Notophthalmus 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*)—a dynamic that would change in the absence of newts. Based on recent *Bsal* susceptibility trials (Longo et al. 2019), the introduction of *Bsal* to North America could decimate newt populations across their range, which could have irreversible cascading effects on biotic communities, and associated changes in energy transport among ecosystems are a likely consequence.

II. Policy Review

Strategic planning to forestall *Bsal* emergence in North America intersects a variety of policy arenas. Regulatory processes may be intertwined with development of proactive measures to reduce the likelihood of *Bsal* transmission to North America (and within North America, should it be detected) and with use of reactive mitigations to control a known *Bsal* occurrence, should one be detected. Overall, a range of actions have been identified, each with policy implications: 1) restrictions on importation of *Bsal*-susceptible amphibian species; 2) protocols to ensure that any imported amphibians are free of the pathogen; 3) testing of amphibians already in captivity in North America and treatment of infected individuals and associated enclosures and fomites to remove *Bsal*; 4) a suite of mitigations for field use in response to *Bsal* detection, following lessons learned by Bosch et al. (2015), Martel et al. (2020), and research findings by Bernard and Grant 2020; and 5) field and laboratory biosecurity measures to reduce inadvertent human-mediated pathogen transmission.

Relative to the first action, concerns over *Bsal* have led to the implementation of partial to complete restrictions on amphibian imports in some nations, such as Canada, Switzerland, and the United States. Canada requires a permit for the importation of all salamanders whether live or dead (CBSA 2018). The United States has enacted an interim rule of the Lacey Act that banned importation of 201 species of salamanders (USFWS 2016). So far, Mexico has not banned any amphibian imports, and the country has trade programs with Asia and the United States but not with Europe. In addition, in 2015, the Pet Industry Joint Advisory Council (PIJAC) in the United States recommended a temporary and immediate voluntary trade moratorium of imports of Asian salamander species that were known to carry *Bsal* until such time as effective testing and treatment regimens could be developed and distributed (<http://www.petproductnews.com/News/PIJAC-Recommends-Immediate-Moratorium-on-Firebelly-and-Paddletail-Newt-Imports/>; accessed 2 June 2020). PIJAC of Canada also supported a temporary moratorium on importation of Asian-native Fire Belly and Paddle-tail Newts (*Cynops* spp and *Pachytriton labiatus*, respectively), which might transmit *Bsal* (<https://pijaccanada.blogspot.com/2016/05/environment-canada-helps-pet-stores.html>; accessed 17 June 2020).

The second action noted above, testing and treating *Bsal*-infected animals in international trade markets prior to import, has been addressed recently in Europe: the European Union (EU) enacted legislation to create a clean trade program for salamander species (EU 2018). This EU action can serve as a model for clean trade programs in other countries and can be a model for broader legislation that could apply to pathogens of all wildlife species. Also, the World Organization for Animal Health (OIE) has listed *Bsal* as an OIE-notifiable disease (OIE 2014; <https://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2020/>; accessed 2 June 2020). This status requires OIE member countries to provide animal health certificates to accompany imports of domesticated animals that are potential hosts of OIE-listed pathogens, but evidence of pathogen-free shipments is not required for wildlife in many countries, including the United States. In addition, OIE member countries are supposed to report OIE-listed pathogen detections, should they occur. Further development of these proactive measures, including establishment of a clean trade program and requiring pathogen-free certificates for import into North America, warrant additional consideration for broader implementation (see Clean Trade Working Group section below).

Relative to the third action, surveillance of captive animals has been conducted to a limited extent in North America, with no known *Bsal* detections to date (e.g., British Columbia, Canada: Govindarajulu et al. 2017; United States: Klocke et al. 2017). Additionally, the national Disease Task Team of Partners in Amphibian and Reptile Conservation (PARC) has implemented a “Herpetofaunal Disease Alert System” (HDAS) where anyone can send an alert email (herp_disease_alert@parcplace.org; Gray et al. 2018) if they observe an amphibian or reptile die-off related to apparent disease causes. This alert applies to both captive and field situations, and alerts have been received for both. For example, captive alerts have been received about apparently diseased amphibians from a pet store and a private breeding facility. With follow-up investigations, no *Bsal* detections have yet been confirmed. *Bsal* sampling of animals at field sites in North America also have not resulted in *Bsal* detections at this time, either by HDAS or by more formal research and monitoring studies (e.g., Govindarajulu et al. 2017, Waddle et al. 2019, 2020; see Surveillance & Monitoring Working Group section below).

Relative to the fourth action, field mitigations in response to *Bsal* detections have had limited testing at this time. Garner et al. (2016) considered strategies to mitigate die-offs of wild amphibians from chytridiomycosis to be “at best, nascent”, with some approaches having ethical and legal considerations. Hopkins et al. (2018) identified regulations that were likely to be policy hurdles for habitat (e.g., chemical applications and ground disturbing activities with federal funding or on federal lands) and host (e.g., species of concern) management in response to conceptual *Bsal* die-off scenarios at United States field sites. In two different isolated instances of actual amphibian die-offs in Europe, field trials using a variety of actions were conducted to treat outbreaks of chytridiomycosis caused by *Bd* (Bosch et al. 2015) and *Bsal* (Martel et al. 2020). These actions included site biosecurity and habitat management as well as host removal, treatment, and surveillance, and some involved local to regional authorities for management action approval. Regulations aside, Bernard and Grant (2020) conducted a workshop to evaluate expert opinion on the efficacy of a variety of actions for *Bsal* mitigation at United States field sites. The uncertainties identified by the workshop highlight research needed to establish method efficacy within different species, habitat, and geographic contexts (see also Stegen et al. 2017). At the time of this writing, there is a recognized priority to clarify, improve, and aid navigation

of North American policy regulations for *Bsal* management (see Management & Control Working Group section below) and to conduct research to advance understanding of the effectiveness of field mitigation actions (see Research Working Group section below).

Relevant to the fifth action, adding or heightening proactive biosecurity measures to forestall inadvertent human-mediated transmission of disease-causing pathogens is a topic that is gaining recognition and is highly relevant for *Bsal*. PARC's national and regional **Disease Task Teams** have been aiding this effort: 1) within- and between-site biosecurity measures for amphibian field work to forestall pathogen transmission were discussed by Gray et al. (2017); 2) disinfection alternatives for large equipment used in or near amphibian habitats were summarized by Julian et al. (2020); and, finally, ~~Haman et al. (in prep.)~~ 3) field-site contexts that could trigger use of enhanced biosecurity approaches rather than standard measures are currently being summarized. There is limited policy at the legislative level to enforce such biosecurity measures. Nevertheless, policies and practices are developed and observed among amphibian researchers at the institutional and agency levels. Specifically: 1) amphibian research permits through university or federal agency research programs' Institutional Animal Care and Use Committees (IACUCs) are increasingly addressing within- or between-field site biosecurity; 2) IACUC approvals require laboratory studies with *Bsal* to operate with heightened biosecurity levels, and standardized procedures for between-laboratory movement of *Bsal* cultures or samples have been established (see Research and Diagnostics Working Groups sections below); 3) some state and provincial scientific collection and use permits require language addressing disinfection protocols to safeguard amphibian health and welfare from human-mediated disease transmission; and 4) some research-reporting journals also require statements of biosecurity measures taken to forestall human-mediated spread of amphibian diseases, including *Bsal*. In addition, the World Organization for Wildlife Health (OIE 2020) has discussed potential *Bsal* transmission by human-mediated movements of contaminated water and fomites. To date, no broad policies for clean water or fomite movements have been established. However, water treatment with chemicals for invasive species, including disease-causing pathogens such as *Bsal*, has been conducted during some water draws for regional wildfire management in the western United States (Olson et al. 2013; Olson and Ronnenberg 2014), and national guidance for such clean water transport has been established in the United States (National Wildfire Coordinating Group 2017, 2020; US Forest Service 2017).

Despite this five-pronged approach to forestall *Bsal* emergence, which include various policies and standardized procedures, no single strategy or mix of strategies is failure-proof. Therefore, it remains imperative to continue developing and implementing proactive management to mitigate the risk of *Bsal* invasion while also pragmatically planning for the arrival of *Bsal* in North America. This approach is the foundational rationale for development of the North America *Bsal* Strategic Plan.

III. History and structure of the North American *Bsal* Task Force

The *Bsal* Strategic Plan is a product of the North American *Bsal* Task Force. Its purpose is to outline steps to reduce the likelihood of *Bsal* transmission to North America and what is necessary for a successful response to the detection of *Bsal* in North America. A brief history of

the *Bsal* Task Force and descriptions of the activities of the Technical Advisory Committee (TAC) and of each of the eight working groups, follows.

History of the North American *Bsal* Task Force

At a 2015 workshop in Colorado, hosted by the US Geological Survey (USGS) Amphibian Research and Monitoring Initiative (ARMI) and the USGS Powell Center, researchers and managers discussed approaches to understanding the epidemiology of *Bsal* and its potential consequences on biodiversity losses in the Americas (Grant et al. 2015). The *Bsal* Task Force was conceived at this workshop (Figure 1), and the Framework of Actions (Box 1) was developed to guide strategic planning along several themes, which are intended to be addressed simultaneously. Initially, seven interactive working groups (Figure 1) were formed to address different goals that are integrated with the *Bsal* Task Force’s Framework of Actions: 1) Response; 2) Diagnostics; 3) Research; 4) Decision Science; 5) Surveillance & Monitoring; 6) Data Management; and 7) Outreach & Communication. Recently, the Response Working Group began focusing on mitigation actions in the event that *Bsal* is detected in North America, and this group has been renamed the Response & Control Working Group (Figure 1). A Clean Trade Working Group was initiated in 2020 as the eighth working group. Progress of the *Bsal* Task Force and its component groups can be tracked by updates to the goals and priorities listed in the *Bsal* Implementation Plan (Appendix 5) and by activities listed in annual reports (available at salamanderfungus.org).

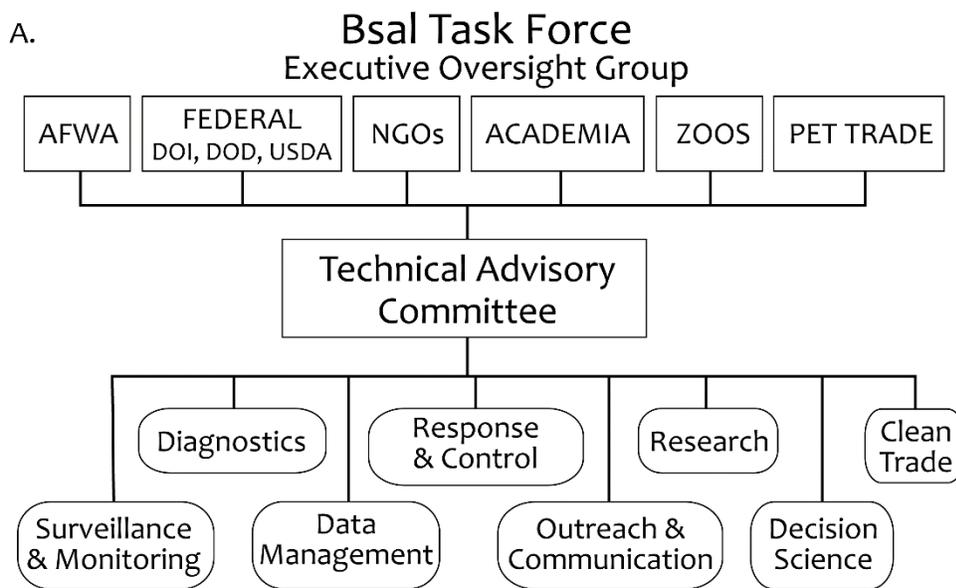


Figure 1. Structure of the North American *Bsal* Task Force.

Since their formation, working groups have met via periodic conference calls to outline new tasks and discuss progress on existing efforts. Group membership is open and inclusive, but the groups were initially founded with persons involved with disease research, natural resource management in state or provincial/territorial agencies and other government agencies,

environmental or conservation groups, non-governmental organizations (NGOs), and the pet industry. Each group has one to three leads, who help to coordinate personnel, manage the workload, and serve as members of the TAC (Figure 1).

Box 1. Framework of Actions

Upon organization of the *Bsal* Task Force in 2015, nine guiding principles for strategic planning along several themes were developed to forestall *Bsal* emergence in North America. This Framework of Actions led to the development of eight working groups and associated collaborations among researchers, managers, industry, and other partners across North America.

1. Prevent invasion of *Bsal* into North America by encouraging stakeholders to work toward a clean trade program for amphibians that certifies individuals in trade are free of *Bsal* infection.
2. Develop and encourage use of the Rapid Response Plan (Appendix 4 in this Strategic Plan), which can be customized to meet local needs, to contain a *Bsal* outbreak.
3. Develop a network of diagnostic laboratories that can run validated tests to detect the presence of *Bsal* in animal or environmental samples in a timely manner.
4. Test for the occurrence of *Bsal* in Canada, Mexico, and the United States in the field and in captivity, 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 the understanding of the risk of *Bsal* introduction to North America and assess the invasion risk of this deadly pathogen to native North American amphibians through decision science analyses, research, and development of a common repository for aggregating and managing *Bsal* surveillance data.
6. Develop effective, scientifically justified prevention and mitigation strategies that prevent *Bsal*-associated infections and mortality.
7. As evidence-based *Bsal* response and management actions are developed, identify expedited pathways for permitting actions and facilitate regulatory processes to implement mitigation measures.
8. Work with partners to compile and disseminate surveillance and research results via social media, accessible web portal databases, and newsletter articles.
9. Build a network of partners that can communicate updates on *Bsal* developments and alert the public and scientific community if *Bsal* is detected in Canada, Mexico, or the United States.

IV. Technical Advisory Committee (TAC)

The Technical Advisory Committee (TAC) is populated by the working group leads; representatives from Canada, Mexico, and the United States; and representatives from partner groups, including different government agencies, the IUCN Amphibian Survival Alliance (ASA), the Pet Industry Joint Advisory Council (PIJAC), and Partners in Amphibian and Reptile

Conservation (PARC). The roles of TAC members vary considerably, with strategic surveillance, research, and proactive planning being tri-lateral priorities across the three countries and advances in biosecurity procedures being contributed by PARC's national and regional Disease Task Teams. The TAC meets monthly by conference call to discuss ongoing work and allow for: 1) development or discussion of new information or tasks; 2) exploration of opportunities to collaborate on the development of products and grant proposals; and 3) discussion of communication, outreach and networking needs.

Objective: To serve as an integrated leadership body of the North American *Bsal* Task Force in collaboration with key partners, framing the scope and intent of the activities undertaken by the *Bsal* Task Force as a whole and acting as an advisory panel for working groups and ad-hoc subgroups.

Goals:

Goal 1. Provide leadership of the *Bsal* Task Force and ensure progress toward the Framework of Actions.

Goal 2. Develop and strengthen lines of communication between the *Bsal* TAC, as representative of the entire *Bsal* Task Force, and national leaders to address risk and response to *Bsal* emergence in North America.

The *Bsal* Implementation Plan (Appendix 5) provides more specific priorities under each of these two goals. Goals and priorities may adapt over time. TAC accomplishments and activities, including adaptive management of goals and priorities, are provided in annual reports and periodic updates to the *Bsal* Implementation Plan (available at salamanderfungus.org).

The first goal of the TAC is designed to adapt over time to new knowledge discovery and activities of working groups and *Bsal* Task Force members and partners. The second goal of the TAC is intended to work toward development of a more formal network of relevant partners among higher-level national leaders with jurisdiction over the natural heritage of species and biodiversity in Canada, Mexico, the United States, and the Indigenous nations of North America to serve as an Executive Oversight Group (Figure 1 depicts this concept, but such a group is not formalized at this time). This oversight group was originally envisioned to be a mechanism to inform managers or leaders of new *Bsal* information or emerging *Bsal* topics at higher organizational levels, such as US federal agencies, the Association of Fish and Wildlife Agencies (AFWA), the Canadian Wildlife Service (CWS), the Mexican Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), and PIJAC.

The initiation of a *Bsal* Task Force Executive Oversight Group was proposed to national leaders at the North American Wildlife and Natural Resources Conference in March 2016. Discussion there identified the need for such an oversight body to extend beyond *Bsal* and include other non-agricultural wildlife diseases with existing 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. Such oversight for non-agricultural wildlife diseases is the topic of

continued discussion and relates to a recognized gap in laws for wildlife health in the Canada, Mexico, and the United States. Although 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 toward agricultural animal health, so the Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act (WAPPRITA) is used to control the spread of *Bsal* via controlling salamander imports instead. These issues are examples of what the Executive Oversight Group could address.

V. Working Groups

The eight working groups of the North American *Bsal* Task Force, and their goals are introduced in this section. Additionally, ongoing working group tasks are described as priorities under each goal in the *Bsal* Implementation Plan (Appendix 5). The working groups are: Response & Control, Diagnostics, Research, Decision Science, Surveillance & Monitoring, Data Management, Outreach & Communication, and Clean Trade.

1. Response & Control Working Group

Objective: To provide guidance for the rapid response (including, but not limited to, eradication, containment, or other management and control responses) should *Bsal* be detected in North America.

Background: Natural resource 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, species with conservation status of concern, and differing state or provincial/territorial and federal jurisdictions, regulations, policy, and permitting. The *Bsal* Response & Control Working Group aims to bridge the gap between identifying and implementing scientifically-sound mitigation actions in response to a confirmed *Bsal* detection in North America 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, the purpose of the group is to facilitate efficient and rapid response to *Bsal* invasion.

Rapid Response Plan: In 2015, an initial Response Working Group focused on development of a *Bsal* Rapid Response Plan Template, addressing Framework Action 2 (Box 1). After comprehensive review by federal, state, provincial, and other potential users of the Rapid Response Plan, this task has been completed, and the final product is included as Appendix 4 (see also salamanderfungus.org). The contents of the Rapid Response Plan are meant to be customized by any agency or institution with management jurisdiction over wild or captive salamanders, so that the plan may serve as a template for actions required in case of suspected or confirmed *Bsal* detection.

The Rapid Response Plan Template 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 involve different levels of diagnostic information for sick or dead animals. In other words, all recommended actions occur after the laboratory has

made its determinations based on the *Bsal* case definition (White et al. 2016). Proactive actions to forestall *Bsal* emergence are not considered in this Rapid Response Plan Template but are being considered by the Decision Science Working Group, which is developing guidelines for implementing management actions before an introduction or outbreak is suspected or confirmed. The Rapid Response Plan Template provides 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 a captive assurance colony). Rapid containment and response measures may prevent broad impacts of the infection. 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 the ARMI Decision Science Lead, Dr. Evan Grant (ehgrant@usgs.gov), for assistance. The Rapid Response Plan is considered a living document that will be updated as more information becomes available. Updates will be posted on relevant websites (e.g., salamanderfungus.org).

After completion of the Rapid Response Plan Template (Appendix 4) and development of scenario-building exercises (Hopkins et al. 2018), it became apparent that several aspects of responses to *Bsal* detection in North America warranted further development. These are tasks included within current goals (below) of the expanded Response & Control Working Group. Tasks and activities of the working group related to each goal are described further in the *Bsal* Implementation Plan (Appendix 5; salamanderfungus.org).

Response & Control Working Group: In 2018, the newly reconfigured Response & Control Working Group was formed to continue and expand the work described above. Specifically, the Response & Control Working Group aims to facilitate an efficient and effective response, by development of *Bsal* control mitigations, if *Bsal* were to be detected at a field or captive site in North America. This effort is part of Framework Actions 6 and 7 (Box 1), which also intersect the aims of other working groups, such as the Research and Decision Science Working Groups.

Mitigation strategies can target the host or environment (see also the Research Working Group section below). 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 new, innovative ideas that 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; La Patra 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. 2014, 2015), environmental probiotics (Muletz et al. 2012), habitat alterations (e.g., fencing to reduce animal movements and human encroachment, water diversions to preempt *Bsal* transmission with water flow), and removal of infected hosts. These strategies have potential for mitigating *Bsal*'s impact on North American salamander biodiversity.

To implement *Bsal* management actions on the ground, government agencies may be required to follow national and/or state/provincial/territorial policies related to the potential environmental impacts resulting from such actions. For example, during scenario exercises of potential *Bsal* outbreaks in the United States (e.g., Hopkins et al. 2018), rapid responses were

identified to be potentially slowed by lack of permits for United States federal or state authorities to assist in field responses including ground-disturbing activities, disturbance to species with conservation status of concern, and use of chemical applications to address the *Bsal* fungus viability. The Response & Control Working Group activities currently focus on understanding the permits and approvals that may be needed for various *Bsal* actions in field or captive settings and expediting rapid permit processes. An initial analysis is underway to gain a better understanding of the complex network of permits and procedures in the United States and the processes that may expedite those approvals. Similar analyses for Canada and Mexico are anticipated.

US Federal Lands: United States 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 a variety of actions, including, but not limited to, permanent or temporary construction projects; limiting public access to public lands; using chemical or biological treatments; funding, assisting, conducting, or approving projects and permitting of private actions.

In the United States, 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 that can be difficult to navigate if you are not familiar with it and can take years to accomplish, but the process 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), which issues permits for chemical and biologic use based on the Clean Water Act and Clean Air Act.

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

1. Environmental Assessment (EA), which determines the significance of the action’s effects and finds alternative measures.
2. Environmental Impact Statement (EIS), which must be prepared if an action significantly affects the quality of the human environment.

If an action may occur more than once, or it occurs 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, but 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:

1. If the agency needs to take an action in response to an emergency, and the action would typically require preparation of an EIS, but 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 CFR § 1506.11) and proceed immediately to mitigate harm to life, property, or important resources.
2. 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 CFR § 1507.3(c))

US State Lands: There are currently 16 states with Environmental Quality Acts that require state and local agencies to perform EISs or at least Environmental Reviews (ER) before performing actions and applying for permits. Please see Appendix 3 for a list of states with Environmental Quality Acts, the specific act to which they are bound, and the governing body of the act.

Response & Control Working Group Goals:

Goal 1. Review and update the Rapid Response Plan Template as new information becomes available.

Goal 2. Facilitate and improve a natural resource agency's ability to take proactive and reactive actions to prevent occurrence and transmission of *Bsal* in North America.

Goal 3. Provide information and build understanding of *Bsal*, the *Bsal* Task Force, the Strategic Action Plan, and available management/mitigation options for federal agencies at the national and regional levels.

Goal 4. Brief and offer training to natural resource agencies (local to regional) about the North American *Bsal* Task Force and available management/mitigation options.

Goals and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5), and annual reports summarize the working group's activities (salamanderfungus.org).

2. Diagnostics Working Group

Objective: To assist with the promotion of consistent standards among the wildlife health community for diagnosing and reporting *Bsal*; to serve as a forum for exchanging ideas, working out challenges, and providing consultation relative to *Bsal* detection and diagnosis; and to provide expert advice concerning *Bsal* detection and diagnosis.

Background: Determination of effective, standardized diagnostic procedures for detection of novel disease-causing pathogens, such as *Bsal*, relies both on gross and microscopic examination of lesions in affected animals and on the detection and correct identification of the pathogen

through histochemical and molecular methods. Molecular detection of pathogens is a rapidly developing discipline largely reliant on technological advances in analyzing organismal DNA and RNA. Mortality due to *Bsal* was first described by Martel et al. (2013), who also provided a morphological description of the pathogen and the microscopic lesions it caused. Martel et al. conducted molecular characterization and diagnostic polymerase chain reaction (PCR) analyses of the 5.8S rRNA gene of *Bsal* from skin samples. *Bsal* is closely related to *Batrachochytrium dendrobatidis* (*Bd*), and Blooi et al. (2013) aimed to develop a duplex real-time PCR assay to detect both pathogens, as *Bsal* was not detected during application of *Bd*-specific PCR methods described by Annis et al. (2004) and Boyle et al. (2004). Cross-reactivity of testing procedures for disease-causing microbiota, with each other or environmental contaminants, has raised broad concerns for PCR-testing sensitivity and specificity (e.g., Iwanowicz et al. 2017); hence, a priority aim for additional disease diagnostic research is to develop effective testing tools for standardized use.

The *Bsal* Diagnostics Working Group was convened as a partnership among diagnosticians and researchers interested in further development of effective diagnostics tools for *Bsal*, addressing Framework Action 3 (Box 1). The Diagnostics Working Group is composed of professionals with expertise in the application and interpretation of an array of diagnostic tools, with members working in academia, diagnostic laboratories, and government agencies throughout North America and involved in detection and reporting of amphibian diseases, including *Bsal*.

Since the working group's assembly in 2015, collaborations between members of the group and others have achieved several initial goals. First, the *Bsal* case definition (White et al. 2016) was published to establish criteria to promote standardized communication of diagnostic results for diagnosis of *Bsal*-caused disease, i.e., *Bsal* chytridiomycosis. The case definition describes clinical and histopathological presentation of *Bsal* chytridiomycosis and references Martel et al. (2013) and Blooi et al. (2013) for molecular analyses by PCR. Since then, additional recommendations on diagnosing *Bsal* chytridiomycosis have been produced (see Thomas et al. 2018). Further, advancing the development of reliable standardized diagnostic procedures for *Bsal* is an overarching goal for the working group. Because of similarities between *Bd* and *Bsal*, working group aims have included addressing diagnostic procedures that accurately differentiate these pathogens, for example, when both are present at variable infection loads. Thus, a second achievement was the completion of a pilot multiple-laboratory round-robin proficiency test for *Bsal* detection by PCR in 2016 (Forzán et al., manuscript in progress). Third, an in situ hybridization protocol to detect *Bd* and *Bsal* cells in formalin-fixed paraffin-embedded tissues was developed (Ossiboff et al. 2019)—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. Ongoing work by the group is capitalizing upon the value of collaborations among diagnosticians and researchers with different expertise. Goals and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5), and annual reports summarize the working group's activities (salamanderfungus.org).

Diagnostic Working Group Goals:

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*.

Goal 2. Develop standardized and reproducible methods that will allow comparison across studies, with reliable detection of *Bd* and *Bsal* and reliable estimation of infection load once either or both pathogens are detected.

3. Research Working Group

Objective: To facilitate communication and collaboration among scientists studying *Bsal* in North America and to ensure high-quality research on *Bsal* is produced rapidly.

Background: The breadth of *Bsal* research needs span basic, applied, and theoretical science disciplines; advances in all three arenas are needed to build foundational knowledge essential for responding to *Bsal* emergence. Scientific studies across these arenas broadly address Framework Actions 4 through 6 (Box 1). To achieve a broad scope of work, the *Bsal* Research Working Group is a collaboration of scientists with a diversity of expertise from multiple disciplines, including molecular and cellular biology, immunology, ecology, mathematics, pathology, and social sciences. The group strives for inclusion and productive collaboration and to maintain or expand its participants to effectively address open research questions critical to our ability to respond to and manage *Bsal*. The working group is currently composed of >30 members, representing >20 organizations.

The Research Working Group maintains a list of research needs based on recent publications, our current state of knowledge, and key knowledge gaps. The working group ranks research needs on the list as Urgent, High, or Medium priority. Within each of these categories, research needs are considered to have equal importance. Each year, the list is updated as more information becomes available and ranks change. Ranks are associated with the thematic goals for the Research Working Group, listed below. The research goals identified below represent a comprehensive approach to advancing the understanding of *Bsal*'s potential impact on amphibian host communities and effective response and management approaches should *Bsal* be introduced into North America. The *Bsal* Implementation Plan (Appendix 5) further breaks down these goals into priority research studies (i.e., action items); achievements are listed in annual reports (salamanderfungus.org).

Research Working Group Goals:

Goal 1: Understand the role of human behavior and the pet trade in the spread and spillover of *Bsal*.

Goal 2: Identify critical transmission pathways and conditions under which *Bsal* is likely to emerge in amphibian host populations in North America (e.g., compartmental disease models).

Goal 3: Produce more informed *Bsal* risk models for North America through improved, objective classification of species susceptibility and tolerance to *Bsal* infection (e.g., integral projection models).

Goal 4: Identify effective methods for managing *Bsal*-induced disease and clearing *Bsal* infections in captive and field settings.

Goal 5: Quantify innate and adaptive immune responses to *Bsal* across species and environmental conditions.

Goal 6: Identify the mechanisms of *Bsal* pathogenesis.

Goal 7: Establish effective methods for detecting *Bsal* infections.

Goal 8: Estimate the interactive effects of *Bsal* with natural and anthropogenic stressors.

4. Decision Science Working Group

Objective: 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 evaluation of trade-offs and risks to overcome impediments to optimal decision-making.

Background: The Decision Science Working Group applies the theory, tools, and techniques from decision analysis to the complex decision-making process for mitigating the threat of *Bsal*, managing risk to native amphibian communities, and responding to *Bsal* detections in North America (Framework Actions 2 and 5; see Box 1). Application of decision science provides an effective interface between rapidly changing conditions in societal, policy, and science information, uncertainty, and operational planning, which together provide substantial benefits that facilitate effective and rapid decision-making in response to *Bsal* detection. The Decision Science Working Group is currently composed of members representing academic institutions and federal agencies (US Geological Survey, US Fish and Wildlife Service). Collectively, the group has decades of experience in decision science, amphibian and pathogen ecology, research, mathematical modeling, and direct work 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 the most effective means of controlling emerging infectious diseases, prevention is not fail-safe. Resource managers must consider multiple social, economic, and ecological objectives, and there are consequently difficult trade-offs for any given disease management strategy (e.g., an optimal action for managing a wildlife disease may result in declines in recreational or economic values). The complexity that arises in balancing numerous, competing demands on land managers 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 (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 provides 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.

Planning for the eventual arrival of *Bsal* in North America illustrates several decision-making impediments common in the world of wildlife disease. First, despite calls for improved responses to emerging infectious diseases in wildlife, management is seldom considered until a disease has been detected in a population. Lack of resources (i.e., time, money, and personnel) are often cited as reasons for not taking pre-emptive actions, but reactive approaches often limit the potential for control and increase the total cost of a response. Second, while preventing the arrival of a pathogen is the most effective means of controlling emerging infectious disease, it is not fail-safe. Once present in a new area or population, emerging diseases have the potential to impact competing social, economic, and ecological interests of North American resource managers, and there are consequently difficult trade-offs for any given disease prevention or management strategy (e.g., optimal actions for managing a wildlife disease may result in declines in recreational or economic values). Finally, acting under high levels of uncertainty is a hallmark of wildlife disease management. Choosing an untested management action can be difficult for managers to justify to the public, and acting in the face of uncertainty depends a great deal on an individual manager's tolerance for risk.

The application of decision science is increasing among natural resource agencies, as it provides rational and transparent frameworks for managing disease. Decision science tools, such as cost-benefit analysis or portfolio decision theory, can help managers better understand the opportunity costs of proactive action versus inaction. Multi-criteria decision analysis can be used to help examine trade-offs among competing social, political, economic, and ecological objectives. Finally, decision trees, expected value of information, and Bayesian belief networks are useful tools for understanding risk tolerance and examining the trade-offs between managing despite uncertainty and delaying action to gain additional information. By using the tools from decision science and behavioral psychology to facilitate conversations between researchers and wildlife managers and identify optimal management strategies, the Decision Science Working Group 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.

Since 2015, the Decision Science Working Group has made considerable progress on its initial goals. The inaugural meeting that led to the development of the North American *Bsal* Task Force was originally planned as a *Bsal* decision science workshop sponsored by the US Geological Survey. That workshop led to a report that was one of the *Bsal* Task Force's initial accomplishments (Grant et al. 2015) and helped springboard the first product of the Decision Science Working Group (Grant et al. 2017), which addressed proactive measures to forestall *Bsal* emergence in North America. Simultaneously with the formation of the *Bsal* Task Force, initial efforts of an independent subgroup that became the heart of the Decision Science Working

Group came to fruition as their United States risk assessment for *Bsal* emergence was published (Richgels et al. 2016). The Decision Science Working Group's current goals are designed to help researchers and managers identify and address the kinds of decision-making impediments outlined above and to advance the overall Framework Actions of the *Bsal* Task Force (Box 1). Goals and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5), and annual reports summarize the working group's activities (salamanderfungus.org).

Decision Science Working Group Goals:

Goal 1: Identify critical research that has the highest value of information, which will lead to an improved ability to manage *Bsal*.

Goal 2: Identify approaches to improve proactive management of *Bsal* when risk or competing objectives are impediments to action.

Goal 3: Conduct and update risk assessments.

Goal 4: Frame *Bsal* management problems at regional and resource manager levels.

Goal 5: Identify whether management should consider proactive, reactive, or a combination of management strategies, dependent on the presumed presence and spatial distribution of *Bsal*.

5. Surveillance & Monitoring Working Group

Objective: To facilitate and coordinate the surveillance of *Bsal* in North America.

Background: A coordinated surveillance effort is aimed at detecting the initial introduction of *Bsal* into North America, thereby allowing for a more effective rapid response to forestall further transmission and helping to safeguard the native fauna of the continent (Framework Action 4; see Box 1). Management and conservation actions cannot proceed effectively without the fundamental information about when and where *Bsal* is introduced to North America. While opportunistic *Bsal* sampling improves the odds of detecting *Bsal* compared to lack of sampling (e.g., Muths et al. 2009), this haphazard approach is unlikely to detect *Bsal* at the onset of its invasion. Instead, the Surveillance & Monitoring Working Group aim is the early detection of *Bsal* to allow for an effective and rapid response, with the ultimate vision of conserving amphibian biodiversity.

Achieving a broad and robust surveillance network is difficult and expensive because of the labor involved. No single organizational entity has been identified that has this capacity. Instead, the emphasis has been on coordinating and encouraging sampling for *Bsal* by diverse partners such that something close to a reasonable level of surveillance is achieved. Initial efforts toward this goal are described below. However, because of the limitations of initial approaches, current efforts of the Surveillance & Monitoring Working Group are aimed at building an

integrated network of partners in surveillance. This network will increase *Bsal* awareness, engage volunteer personnel and citizen scientists, utilize dispersed in-kind resources, and increase the amount of *Bsal* sampling.

Past Surveillance Efforts:

United States: In the United States, some sampling has been conducted by federal agencies. As part of the Surveillance & Monitoring Working Group's primary objective, a one-time major sampling effort was conducted by the US Geological Survey Amphibian Research and Monitoring Initiative (ARMI) in 2014 to 2017 (Waddle et al. 2019, 2020). This effort included sample sites across the United States, with resources allocated according to the estimated risk of *Bsal* occurrence as per the risk assessment model results developed by Richgels et al. (2016). Over 10,000 amphibians (mostly salamanders) were sampled. *Bsal* was not detected (Waddle et al. 2019; Waddle et al. 2020; data archived at amphibiandisease.org by the Data Management Working Group, see below). ARMI continues to sample at a very low level in select areas where resources allow. In addition, the US Fish and Wildlife Service 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 (Richgels et al. 2016).

In the United States and elsewhere, independent science investigations into *Bsal* detections from skin swabs of selected species in specific geographies are ongoing. For example, four reports of joint *Bsal* and *Bd* sampling in North America were published in Herpetological Review in 2017 (Olson 2019).

In addition to sampling, in the United States, an iNaturalist website 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 (PARC) national Disease Task Team set up a Herpetofaunal Disease Alert System (HDAS; email reports to herp_disease_alert@parcplace.org; include photograph, species affected, location, other relevant episode information, observer's name; Gray et al. 2018) that provides another way to gather observations of sick or dead amphibians that might not otherwise be reported. Communication also occurs between iNaturalist and HDAS, as oversight of iNaturalist reports occurs and possible disease events are identified and communicated. In both cases, observations are typically forwarded to the relevant state biologist, but in some cases, when deemed necessary, members of the HDAS or state authorities can use their personal networks to help facilitate further investigation.

Canada: In Canada, the provincial governments are the lead jurisdiction for amphibian disease surveillance. The provinces of British Columbia (BC) and Ontario have conducted the most intensive *Bd* and *Bsal* monitoring programs to date. In Ontario, over 900 amphibians were sampled opportunistically along a latitudinal gradient over a four-year period (2014–2017). All samples were tested for *Bsal*, and all tests were negative (Christina Davy, unpubl. data). In 2016, provincial biologists in BC 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 quantitative

polymerase chain reaction (qPCR) analyses in any swabs from the 82 wild newts and 15 captive salamanders sampled (Govindarajulu et al 2017). In many provinces, including 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. In addition, amphibians seized from the illegal pet trade will be tested for *Bsal* in BC.

The Canadian public can submit reports of sick or dead amphibians to the Canadian Wildlife Health Cooperative (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. Disease reports from Canadian locations that are received by the HDAS email address are forwarded to provincial authorities in Canada, similar to state reporting of HDAS reports received in the United States.

Mexico: In Mexico, surveys aimed at identifying *Bsal* in natural populations have been conducted by members of Dr. Gabriela Parra-Olea's research laboratory. 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-Olea, unpubl. data). In 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, they are starting efforts to provide certification to both of these research laboratories so that legal amphibian imports in Mexico can be screened for *Bsal*.

Surveillance & Monitoring Working Group goals are listed below. Goals and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5), and annual reports summarize the working group's activities (salamanderfungus.org).

IMAGE HERE (IMAGE 1, FROM MARK'S EMAIL)

Surveillance & Monitoring Working Group Goals:

Goal 1: Facilitate and support a wide-reaching, ongoing, coordinated, and sustainable *Bsal* surveillance program in Canada, Mexico, and the United States.

Goal 2: Identify *Bsal* sampling efforts that are occurring outside of efforts coordinated by the *Bsal* Surveillance & Monitoring Working Group.

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

Goal 4: Develop initial plans for post-detection monitoring, if *Bsal* were to be detected at a field or captive site in North America.

6. Data Management Working Group

Objective: Develop an online *Bsal* and *Bd* data management portal to accelerate sharing of planned or completed surveillance projects and scientific studies to accelerate the pace of learning about these pathogens tied to emerging infectious diseases and foster integrated efforts in research and monitoring across North America and among global communities.

Background: The Data Management Working Group is a multi-partner collaboration to retain a *Bsal* and *Bd* database of world amphibian-chytrid occurrences and projects for co-production of knowledge to improve the science and management of these pathogens. In particular, this working group works closely with the *Bsal* Task Force, the University of California at Berkeley (UC Berkeley) and its online collaborative resource AmphibiaWeb.org, and the US Forest Service, manager of the world *Bd* database through 2019, in previous collaboration with Imperial College, London (Olson et al. 2013; Olson and Ronnenberg 2014). Underscoring all sources of *Bsal* and *Bd* data are the independent investigators who have been vital to compiling and disseminating *Bsal* and *Bd* surveillance results. These data are now available via development of an accessible web portal database with data import and export, analysis, and multi-database linking functions (Framework Action 5 and 8; see Box 1). A web-portal reporting system for *Bsal* and *Bd* data provides larger-scale data management capabilities and can address novel multi-scale questions and metadata analyses across individual studies. This data-sharing capability can contribute to analyses that advance understanding of the risk of *Bsal* or *Bd* introduction to North America and assess disease risk of these potentially deadly pathogens to native amphibians in North America and elsewhere through aggregating and managing past and current disease sampling data in a common repository.

The Data Management Working Group has met its original goal by developing the Amphibian Disease Portal (amphibiandisease.org) to comprehensively manage data on the amphibian chytrid fungi *Bd* and *Bsal*. This standards-compliant, online portal is hosted as the Disease Portal webpage on AmphibiaWeb.org, managed by UC Berkeley. The portal, which includes public and private datasets, is aimed at accelerating information sharing among global scientists, natural resource managers, and the public regarding planned and ongoing surveillance projects and scientific studies as well as the results of completed studies. Data within the portal are especially important for aiding in rapid responses and decision science for allocation of limited resources available for research and management of these amphibian emerging infectious diseases and their affected hosts. In particular, comprehensive management of *Bd* and *Bsal* detection and no-detection data by location and project is useful for development of new scientific research studies, surveillance, and effective monitoring programs and for understanding disease dynamics of chytridiomycosis. Through its website, in particular the Data Dashboard, the Amphibian Disease Portal could be an effective outreach and technical interface for the research community. Further, web services to other scientific portals, such as AmphibiaWeb, help extend the portal's reach to other audiences in education and conservation.

The chytrid data management system aims to expand in scope and capacity. In addition to increasing registered portal users and *Bsal* and *Bd* data imports, the Amphibian Disease Portal is partnering with the global bio-sampling database Geome (<https://geome-db.org>) to deliver improved validation services, enhanced security, improved data accessibility such as through R

Statistics to enhance research and forecasting abilities. These developing functional capacity priorities of the web portal are described in the *Bsal* Implementation Plan (Appendix 5). Goals and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5), and annual reports summarize the working group's activities (salamanderfungus.org).

Data Management Working Group Goals:

Goal 1. Maintain comprehensive data management of *Bd* and *Bsal* samples for archived, aggregated monitoring and analytic modeling in the Amphibian Disease Portal (amphibiandisease.org).

Goal 2: Advance the functional capacity of the Amphibian Disease Portal (amphibiandisease.org).

7. Outreach & Communication Working Group

Objective: To facilitate *Bsal* communication and outreach in North America.

Background: The Outreach & Communication Working Group produces a variety of *Bsal*-related outreach communication materials, including a web presence, fact sheets, press releases, and briefing papers. 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 social media posts (Facebook and Twitter). In particular, the Outreach & Communication Working Group works with the Technical Advisory Committee (TAC) and its partners and other working groups within the *Bsal* Task Force to disseminate new *Bsal* information and products developed by the group and others. To increase the efficacy of dissemination, the Outreach & Communication Working Group continues to build an online network via social media, increasing followers and directing them to the salamanderfungus.org website, which serves as a hub and repository for published developments relating to issues, detections, and research regarding *Bsal*.

Although the Outreach & Communication Working Group is not tasked with conducting or publishing *Bsal* research, members help synthesize findings and communications from other groups within the *Bsal* Task Force for the purposes of producing lay articles meant to educate the public and highlighting key messages in social media posts. The scope of outreach to date has focused on national coverage in the United States, as there are salamanders at risk throughout the United States. Outreach may also be relevant for Canada and Mexico, but focused efforts outside the United States have not yet been initiated by the working group.

Finally, the Outreach & Communication Working Group organizes, designs, and publishes the annual report for the *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 salamanderfungus.org and is available to both the general public and scientific community. Goals of the working group are listed below, and priority actions per goal are described further in the *Bsal* Implementation Plan (Appendix 5; salamanderfungus.org).

Outreach & Communication Working Group Goals

Goal 1. Work with partners to disseminate syntheses, research, and other products or activities developed by the *Bsal* Task Force via social media and newsletter articles.

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

Goal 3. Independently, or with partners, produce public service announcements on the presence and implications of *Bsal*.

Goal 4. Update the *Bsal* Task Force website.

8. Clean Trade Working Group

Objective: To expand efforts to forestall potential human-mediated transmission of *Bsal* into North America via trade markets.

Background: Although *Bsal* was first described from infections of Fire Salamanders in Europe, an Asian origin of the pathogen was implicated from the outset (Martel et al. 2013). Additional support for the hypothesis that *Bsal* evolved in Asia has been forthcoming. No lethal infections have been found there, suggesting that a long co-evolutionary history has led to resistance or tolerance by amphibian species in Asia (Laking et al. 2017). In Europe, infections were first observed in the Netherlands (Spitzen-van der Sluijs 2016), followed by the discovery of *Bsal* in Belgium and Germany (Stegen et al. 2017, Lötters et al. 2020) and most recently in Spain (Martel et al. 2020). The lethal effect of *Bsal* on some European amphibian species suggests a recently arrived pathogen that has encountered naïve hosts. The likely routes of within-continent spread are dispersal of infected amphibians among populations, possibly movement of spores by waterbirds, and spillover of infected individuals once held in captivity (Nguyen et al. 2017; Yuan et al. 2018). Spread of *Bsal* between continents, including the emergence of *Bsal* in Europe, is likely due to importation of infected species from locations where *Bsal* is endemic (Nguyen et al. 2017). 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* has been found on salamander species in China that are frequently imported. These findings 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. Clean trade measures, such as development of *Bsal* testing and certification procedures that help ensure traded animals and fomites in imports to North America are *Bsal*-free, are likely the most effective proactive measures for forestalling *Bsal* emergence in North America (Grant et al. 2017). Such measures align with Framework Action 1 (Box 1).

The Clean Trade Working Group was established in early 2020 in partnership with the Pet Industry Joint Advisory Council (PIJAC), and its implementation goals and priorities are under development at this time. The objective of the stakeholder-led Clean Trade Working Group is for experts in the pet trade to organize and collaborate with subject matter experts,

regulators, and academics to determine what the potential components of a workable, comprehensive clean trade program for North America may include and to report these initial findings back to the *Bsal* Task Force Technical Advisory Committee (TAC). Currently, this working group has established a network of experts in amphibian and reptile care, husbandry, and pathogen and disease research, including industry experts with knowledge of the volume and dynamics of the amphibian trade. This group has been collaborating on a regular basis to identify potential components for a comprehensive North American clean trade program to help prevent the invasion of *Bsal* to North America while allowing for the legal and responsible importation of animals for the pet trade. For example, components under discussion at this time include a description of the current status of amphibian imports; a characterization of the United States and Canada amphibian trade network; and identification of factors or processes that could reduce potential pathogen transmission, amplification, and spillover.

Appendices

Appendix 2. Records of *Batrachochytrium salamandrivorans* (*Bsal*) in nature and in captivity.

Species Common Name	Species Scientific Name	Family	Location	Disposition (Wild/Captive)	Citation
Chinese Giant Salamander	<i>Andrias davidanus</i>	Cryptobranchidae	China	Captive	Yuan et al. 2018
Many-webbed Fire-bellied Toad	<i>Bombina microdeladigitora</i>	Bombinatoridae	Germany	Captive	Nguyen et al. 2017
Bosca's Newt [Iberian Newt]	<i>Lissotriton boscai</i>	Salamandridae	UK	Captive	Fitzpatrick et al. 2018
Anatolia Newt [Strauch's Spotted Newt]	<i>Neureergus strauchii</i>	Salamandridae	Netherlands	Captive	Fitzpatrick et al. 2018
Eastern Newt	<i>Notophthalmus viridescens</i>	Salamandridae	Netherlands	Captive	Fitzpatrick et al. 2018
Macedonian Crested Newt	<i>Triturus macedonicus</i>	Salamandridae	Netherlands	Captive	Fitzpatrick et al. 2018
North African Fire Salamander	<i>Salamandra algira</i>	Salamandridae	Germany	Captive	Sabino-Pinto et al. 2015
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Germany	Captive	Sabino-Pinto et al. 2015
Vietnamese Crocodile Newt	<i>Tylototriton vietnamensis</i>	Salamandridae	Vietnam	Captive	Martel et al. 2014
Many-webbed Fire-bellied Toad	<i>Bombina microdeladigitora</i>	Bombinatoridae	Vietnam	Wild	Nguyen et al. 2017
Chuxiong Fire-bellied Newt	<i>Cynops cyanurus</i>	Salamandridae	China	Wild	Yuan et al. 2018
Chinese Fire-bellied Newt	<i>Cynops orientalis</i>	Salamandridae	China	Wild	Yuan et al. 2018
Dayang Newt	<i>Cynops orphicus</i>	Salamandridae	China	Wild	Yuan et al. 2018
Clouded Salamander	<i>Hynobius nebulosus</i>	Hynobiidae	Japan	Wild	Martel et al. 2014
Alpine Newt	<i>Ichthyosaura alpestris</i>	Salamandridae	Netherlands	Wild	Spitzen-van der Sluijs et al. 2016
Alpine Newt	<i>Ichthyosaura alpestris</i>	Salamandridae	Belgium	Wild	Spitzen-van der Sluijs et al. 2016
Alpine Newt	<i>Ichthyosaura alpestris</i>	Salamandridae	Netherlands	Wild	Martel et al. 2014
Smooth Newt	<i>Lissotriton vulgaris</i>	Salamandridae	Netherlands	Wild	Spitzen-van der Sluijs et al. 2016
Japanese Clawed Salamander	<i>Onychodactylus japonicas</i>	Hynobiidae	Japan	Wild	Martel et al. 2014
--	<i>Pachytriton wuguanfui</i>	Salamandridae	China	Wild	Yuan et al. 2018
Asian Warty Newt sp.	<i>Paramesotriton aurantius</i>	Salamandridae	China	Wild	Yuan et al. 2018

Hong Kong Warty Newt	<i>Paramesotriton hongkongensis</i>	Salamandridae	China	Wild	Yuan et al. 2018
Tam Dao Salamander	<i>Paramesotriton deloustali</i>	Salamandridae	Vietnam	Wild	Martel et al. 2014
Tam Dao Salamander	<i>Paramesotriton deloustali</i>	Salamandridae	Vietnam	Wild	Laking et al. 2017
--	<i>Paramesotriton sp.</i>	Salamandridae	Vietnam	Wild	Laking et al. 2017
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Netherlands	Wild	Spitzen-van der Sluijs et al. 2016
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Belgium	Wild	Spitzen-van der Sluijs et al. 2016
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Germany	Wild	Spitzen-van der Sluijs et al. 2016
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Belgium	Wild	Martel et al. 2013
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Netherlands	Wild	Martel et al. 2014
Fire Salamander	<i>Salamandra salamandra</i>	Salamandridae	Belgium	Wild	Martel et al. 2014
Siberian Salamander	<i>Salamandrella keyserlingii</i>	Hynobiidae	Japan	Wild	Martel et al. 2014
Black Knobby Newt	<i>Tylototriton asperrimus</i>	Salamandridae	China	Wild	Laking et al. 2017
Black Knobby Newt	<i>Tylototriton asperrimus</i>	Salamandridae	Vietnam	Wild	Yuan et al. 2018
Chiang Mai Crocodile Newt	<i>Tylototriton uyenoii</i>	Salamandridae	Thailand	Wild	Martel et al. 2014
Himalayan Newt	<i>Tylototriton verrucosus</i>	Salamandridae	China	Wild	Yuan et al. 2018
Vietnamese Crocodile Newt	<i>Tylototriton vietnamensis</i>	Salamandridae	Vietnam	Wild	Laking et al. 2017
Ziegler's Crocodile Newt	<i>Tylototriton zieglerei</i>	Salamandridae	Vietnam	Wild	Martel et al. 2014
Ziegler's Crocodile Newt	<i>Tylototriton zieglerei</i>	Salamandridae	Vietnam	Wild	Laking et al. 2017
Sword-tailed Newt	<i>Cynops ensicauda</i>	Salamandridae	--	Wild*	Martel et al. 2014

Appendix 1. Results of *Bsal* Susceptibility Trials (Martel et al. 2014; unpubl. data: M. Gray [University of Tennessee], D. Woodhams [University of Massachusetts], J. Piovia-Scott [Washington State University]).

**Species that developed *Bsal* chytridiomycosis
(i.e., lethal and susceptible species)**

<i>Desmognathus auriculatus</i>	Plethodontidae
<i>Pseudotriton ruber</i>	Plethodontidae
<i>Eurycea wilderae</i>	Plethodontidae
<i>Aquiloerycea cephalica</i>	Plethodontidae
<i>Chiropterotriton spp.</i>	Plethodontidae
<i>Notophthalmus viridescens</i>	Salamandridae
<i>N. perstriatus</i>	Salamandridae
<i>N. meridionalis</i>	Salamandridae
<i>Taricha granulosa</i>	Salamandridae

**Species that are *Bsal* carriers
(i.e., tolerant species)**

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

Species that are resistant

<i>Desmognathus conanti</i>	Plethodontidae
<i>D. monticola</i>	Plethodontidae
<i>Plethodon shermani x teyahalee</i>	Plethodontidae
<i>Hemidactylium scutatum</i>	Plethodontidae
<i>Eurycea lucifuga</i>	Plethodontidae

Necturus maculosus
Lithobates sylvaticus

Cryptobranchidae
Ranidae

Appendix 3. List of US states with Environmental Quality Acts and the 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	Montana 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 be 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

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

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British Columbia

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 Ministry of Environment
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 Victoria BC V8W 9M1

Telephone: (250) 387-9755

Email: purnima.govindarajulu@gov.bc.ca

Helen Schwantje

Wildlife Veterinarian

Fish, Wildlife, and Habitat Branch
Ministry of Forests, Lands and Natural Resource Operations
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Kemptville ON K0GV1J0

Telephone: (613) 258-8320
Email: sbnymanh@uoguelph.ca

Manitoba

Bill Watkins

Biodiversity Conservation Zoologist
Wildlife and Fisheries Branch
Manitoba Conservation and Water Stewardship
Box 24, 200 Saulteaux Crescent
Winnipeg MB R3J 3W3

Telephone: (204) 945-8481
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Newfoundland and Labrador

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EHJV Stewardship Biologist
Wildlife Division
Dept of Environment and Conservation
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New Brunswick

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Fish & Wildlife Branch
Department of Natural Resources
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Northwest Territories

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Nova Scotia

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Nunavut

Melanie Wilson

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Department of Environment
Government of Nunavut
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Igloolik NU X0A 0L0

Telephone: (867) 934-2176

Email: [-MWilson@gov.nu.ca](mailto:MWilson@gov.nu.ca)

Ontario

Chris Heydon

Wildlife Health – Human-Wildlife Conflict Policy Advisor

Species Conservation Policy Branch

Ontario Ministry of Natural Resources and Forestry

300 Water Street, 5N

Peterborough ON K9J 8M5

Telephone: (705) 755-5378

Email: chris.heydon@ontario.ca

Parks Canada Agency

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Protected Areas and Conservation Directorate
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Prince Edward Island

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Telephone: (902) 569-7595
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Québec

Catherine DOucet

Ministère des Forêts, de la Faune et des Parcs
880, Chemin Ste-Foy, 2e étage
Québec (Québec) G1S 4X4

Téléphone: (418) 627-8694 x/p 7454
Courriel:
Catherine.Doucet@mffp.gouv.qc.ca

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Veterinarian
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Yukon

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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 Science 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. (2015a).
- 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

- A. **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).*
- B. **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.*
 1. **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.
 2. **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 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.

C. Points of Contact (PoCs): *Entities customizing this template should populate with preferred PoCs.*

1. 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.
2. Include Permit coordination contacts (state, federal, ESA, etc.)

D. 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:

1. The Diagnostic Laboratory Network established via the Bsal Task Force's Diagnostics Working Group (see www.salamanderfungus.org).
2. 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)
3. Wildlife Epidemiologists or Wildlife Disease Ecologists

E. **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)

1. 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.*
2. Rescue colonies. *Entities customizing this template should identify facilities to house rescued animals or those collected for the purpose of captive breeding and reintroduction.*
3. Museums or other storage facilities. *Entities customizing this template should identify facilities for voucher 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?*

F. **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:

- i. Biosecurity protocols for field, lab, use of live cultures, etc.
- ii. Swabbing and storage (and transportation) protocols
- iii. **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 fail-safe eradication, we recommend humane culling or euthanasia, and either:
 - 1. Preservation of infected individuals, per **Appendix 2(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 (Bloom et al. 2015a; Bloom 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 Bloom 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
- B. 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.

- C. 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.*

IMAGE HERE (2, FROM MARK'S EMAIL)

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):

- Results communicated by Participating Laboratory to:
 - Reporting Individual(s), who in turn informs:
 - Detection site landowner/manager
 - 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.
- a. 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 fail-safe 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 (Bloom et al. 2015a; Bloom 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:
 1. Treat per guidance in Bloom 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.*
 2. Swab treated animals post-treatment (see Appendix I, Section B) and submit samples to a Participating Laboratory to confirm Bsal eradication.
 3. 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](#).

- **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 CryoTubes™ or Vangard Cryos™ (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.

- **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 one part animal carcass to nine 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)
 - **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).
- 1) 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:
 - a. Samples should be enclosed in a primary receptacle that is leak-proof.
 - b. The primary receptacle is then placed within a leak-proof secondary receptacle.
 - c. 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.
 - 2) 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>
- **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:

- d. **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.
- e. **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.
 - a. 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).
 - b. 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.
 - c. 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).
 - d. 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.
 - e. 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.
- f. **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.

- a. 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).
- b. 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.
- c. Always handle animals as little as possible. Procedures that are quick, even if potentially painful, may cause less stress than longer procedures.
- d. 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).
- e. 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).
- f. 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).

- **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 *Batrachochytrium 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.
- **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.
 - a. Powder-free latex or nitrile disposable gloves.
 - b. Sterile applicators (“swabs”); see “Swab Selection” in Pessier & Mendelson (2017).
 - c. 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.

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

- d. A new pair of disposable latex or nitrile gloves should be used for each animal handled for testing (Mendez et al. 2008).
- e. Avoid contact of swabs (especially swab tips) with surfaces or substrates other than the skin of the animal to be tested.
- f. If instruments are used to cut the tip of the swab into cryovials, a freshly disinfected instrument must be used for each sample.
 - a. To disinfect instruments for this purpose, dip in 70% ethanol followed by flaming under an alcohol lamp.
 - b. Avoid using bleach solutions for disinfection because this can degrade Bsal DNA in swab samples (resulting in false-negative tests; Cashins et al. 2008).
- o **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).
- g. 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.
- h. If rinsing with water is used for cleaning, the water should not originate from the animal's enclosure or environment.
- i. Laboratories that perform PCR for Bsal should always use exogenous internal positive controls to detect PCR inhibitors (Hyatt et al. 2007).
- o **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:
- j. 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).
- k. In contrast, exposure of swabs to very high temperatures (> 38°C) for seven 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).

- l. At 25°C (refrigerator) or lower.

- m. Samples should be frozen (-20°C or below) if sample analysis is not performed within six months of sample collection.
- n. See alternatives to low temperature storage (i.e., where refrigeration may not be possible) in Pessier & Mendelson (2017).
 - **Shipment of Swabs to the Laboratory.**
 - o. Ideally ship swabs by overnight or 2-day courier service (e.g., Federal Express; Canada Post Xpresspost, UPS, Purolator, etc.).
 - p. Consider using cold packs to guard against high temperature extremes.
 - q. Samples that have been previously frozen should be sent on dry ice to prevent freeze-thaw cycles.

IMAGE HERE (IMAGE 3, FROM MARK'S EMAIL)

APPENDIX 5. North American Bsal Implementation Plan

Update: 13 October 2020

Summary

A North American Strategic Plan to Control Invasions of Batrachochytrium salamandrivorans (Bsal; Bsal Strategic Plan, available at salamanderfungus.org) is the result of ongoing collaborations of members of the North American Bsal Task Force and partners. The overarching objective of the Bsal Strategic Plan is to provide interdisciplinary scientific and managerial guidance to forestall emergence of Bsal in North America. This is a complex objective that has led to the development of a Technical Advisory Committee (TAC) and multiple Bsal Working Groups. The TAC serves an oversight function for Working Groups and provides communication with key partners, but also takes on overarching activities such as collaborative projects among Task Force members and partners. Working Groups are convened to advance knowledge relative to eight disciplinary themes and their integration: Response & Control; Diagnostics; Research; Decision Science; Surveillance & Monitoring; Data Management; Outreach & Communication; and Clean Trade. Actions of the TAC and Working Groups are developed in a hierarchical fashion outlined here in the North American Bsal Implementation Plan. First, the Framework of Actions summarizes the initial breadth of Task Force aims as nine general intention statements. Second, Goals of the TAC and Working Groups are statements that frame thematic activities specific to each group. Each of the nine statements in the Framework of Actions tiers to one or more Goals among groups. Third, Priorities of the TAC and Working Groups are specific tasks or activities undertaken under the theme of a Goal. Priorities are the iterative steps that have been identified to advance achievement of the Goal and may represent separate studies undertaken by Working Group members or subgroups, or stages in the development of programmatic operations to advance Bsal response activities. As the North American Bsal Task Force has been in operation since 2015, some initial goals' priority tasks have been completed. These are briefly summarized below, as well as the list of priorities-in-development at this time. The North American Bsal Implementation Plan is a living document to track current Goals, Priorities, and their separate tasks across working groups, as well as for the TAC and Bsal Task Force as an overarching leadership cadre. This Implementation Plan is written as Appendix 5 of the Bsal Strategic Plan, with updates to be posted on partner web pages (e.g., salamanderfungus.org; amphibiandisease.org; Partners in Amphibian and Reptile Conservation, Disease Task Team web page: <https://parcplace.org/resources/parc-disease-task-team/>). Periodic updates are expected at annual or longer time scales and are noted by the date on the first page.

Overview

The North American Bsal Task Force was initiated in June 2015 to address the complex facets of potential *Batrachochytrium salamandrivorans* (Bsal) emergence in North America and the projected dire outcomes that could ensue for native amphibian fauna due to this pathogen (Martel et al. 2014; Gray et al. 2015; Yap et al. 2015; Richgels et al. 2016). The North American Bsal Strategic Plan (Bsal Strategic Plan; salamanderfungus.org) summarizes interdisciplinary scientific and managerial guidance for a successful response to the detection of Bsal in North America. The Bsal Strategic Plan provides additional detail on: the amphibian chytrid fungus Bsal – the pathogen that can cause the disease Bsal chytridiomycosis in some amphibians; emergence of the disease Bsal chytridiomycosis from this skin microparasite in Europe, and current knowledge of the Asian origin of Bsal; and the origin, structure (Figure 1), and aims of the Task Force.

Herein, the Implementation Plan of the Bsal Strategic Plan is described in a 3-step hierarchical fashion. First, the *Framework of Actions* (1–9, below) provide intention statements to capture the overarching breadth of the initial objectives of the Task Force. Second, *Goals* describe the broad thematic aims undertaken by the: 1) Bsal Technical Advisory Committee (TAC), the leadership body of the Task Force and its key partners; and 2) the Working Groups, assembled to address separate disciplinary or operational topics in depth (Figure 1A). Goals of the TAC and Working Groups may tier to statements in the Framework of Actions. Currently, eight Working Groups address: Response & Control; Diagnostics; Research; Decision Science; Surveillance & Monitoring; Data Management; Outreach & Communication; and Clean Trade. Each Working Group has an Objectives statement to define its scope, under which its Goals define thematic activities. Third, to achieve the broad Goals of the different groups, more specific *Priorities* are developed. Priorities are independent task statements or activities that aid in a stepwise advancement of Goals. Priorities may align with specific studies or operational procedures to advance the efficacy of the Bsal Task Force.

The North American Bsal Implementation Plan describes the Objectives, Goals, and Priorities of TAC activities with its partners and each Working Group. Since 2015, several tasks have been addressed to date, and new Goals, Priorities and tasks have been developed over time. Per group, a summary is provided of completed tasks and current Goals and Priorities.

The North American Bsal Task Force is a non-affiliated partner organization. To implement the goals, priorities, and tasks of the Bsal Strategic Plan and Implementation Plan, in-kind resources of the myriad partners in North America (e.g., employers of Task Force members) are used. These include salary of personnel time to advance the various Plan activities, and in some cases operating and facility costs of projects undertaken. The North American Bsal Task Force is grateful for this support, as well as for volunteer hours donated to this effort. However, implementation of some work described below is heavily reliant upon obtaining external grants and agreements. As this is an integral part of selected Goal and Priority implementation, the financial planning that accompanies those aims is briefly included in this document. In addition, as implementation decisions are weighed with consideration of limited available resources, the relative ranking of the importance of goals and priorities may be highly relevant. General ranks as Urgent, High, and Medium are provided for this purpose.

The North American Bsal Implementation Plan is intended to adapt over time as tasks are completed, Priorities are met, new information is forthcoming, and new Goals and Priorities are identified by the TAC and the Working Groups. The Implementation Plan will be updated on associated web portals (e.g., salamanderfungus.org; amphibiadisease.org; Partners in Amphibian and Reptile Conservation Disease Task Team: <https://parcplace.org/resources/parc-disease-task-team/>), and dated accordingly. Periodic updates are expected at annual or longer time scales.

Framework of Actions

Upon organization of the Bsal Task Force, nine guiding principles for strategic planning along several themes were developed to forestall Bsal emergence in North America. This Framework of Actions led to the development of eight Working Groups and associated collaborations among researchers, managers, industry, and other partners across North America.

10. Prevent invasion of Bsal to North America by encouraging stakeholders to work toward a clean-trade program for amphibians that certifies individuals in trade are free of Bsal infection.
11. Develop and encourage use of the response plan contained in this Strategic Plan, which can be customized to meet local needs, to contain a Bsal outbreak.
12. Develop a network of diagnostic laboratories that can run validated tests to detect the presence of Bsal in animal or environmental samples in a timely manner.
13. Test for the occurrence of Bsal in the US, Canada and Mexico in the field and in captivity, 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.
14. Advance the understanding of the risk of Bsal introduction to North America and assess invasion risk of this deadly pathogen to native North American amphibians through decision science analyses, research, and development of a common repository for aggregating and managing Bsal surveillance data.
15. Develop effective, scientifically justified prevention and mitigation strategies that prevent Bsal-associated infections and mortality.
16. As evidence-based Bsal response-and-management actions are developed, identify expedited pathways for permitting actions, and facilitate regulatory processes to implement mitigation measures.
17. Work with partners to compile and disseminate surveillance and research results via social media, accessible web portal databases, and newsletter articles.
18. Build a network of partners that can communicate updates on Bsal developments and alert the public and scientific community if Bsal is detected in the US, Canada, or Mexico.

A.

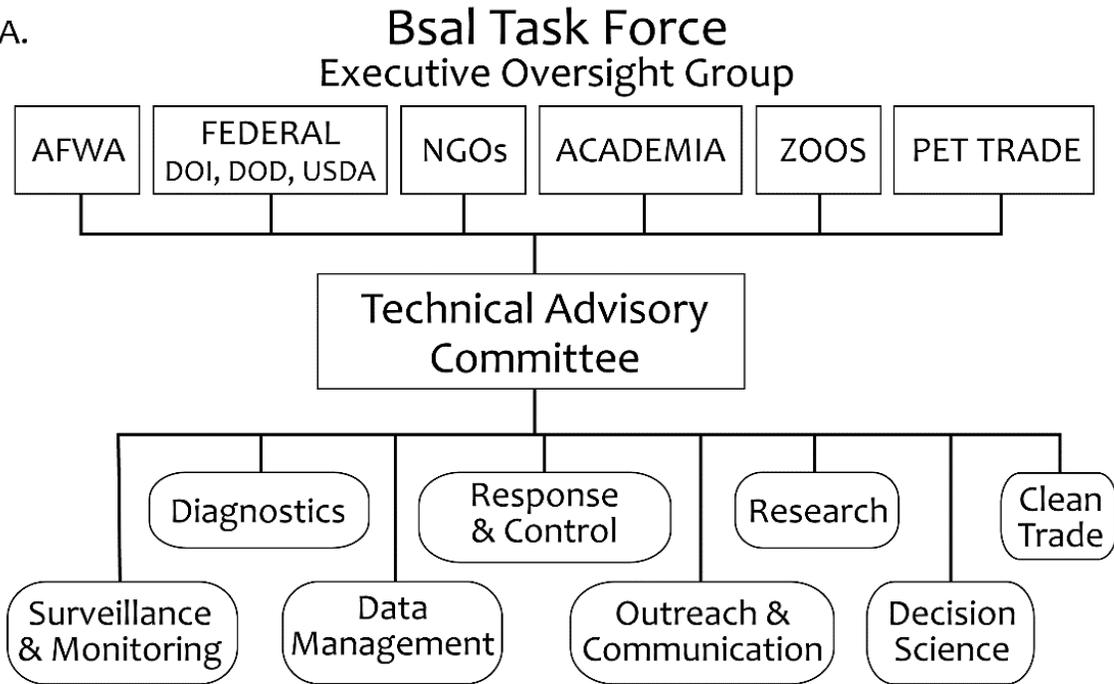


Figure 1. Structure of the Bsal Task Force.

1. Bsal Technical Advisory Committee (TAC)

OBJECTIVE: To serve as an integrated leadership body of the North American Bsal Task Force in collaboration with key partners, framing the scope and intent of the activities undertaken by the Task Force as a whole and as an advisory panel for working groups and ad-hoc subgroups.

Leads: Matt Gray, University of Tennessee, Knoxville, TN, USA, and Maria Forzan, Long Island University, Brookville, NY, USA

Past Leads: Dede Olson, US Forest Service, Corvallis, OR, USA; Jennifer Ballard, formerly of US Fish and Wildlife Service, Conway, AR, USA; Mike Adams, US Geological Survey, Corvallis, OR, USA; Reid Harris, formerly of James Madison University and Amphibian Survival Alliance, Harrisonburg, VA, USA; and Jake Kerby, University of South Dakota, Vermillion, SD, USA

Canada Lead: Cynthia Pekarik, Environment and Climate Change Canada, Gatineau, QC, Canada

Past Canada Leads: Jennifer Provencher, Science and Technology Branch, Ottawa, ON, Canada; Sam Iverson, Canadian Wildlife Service, Gatineau, QC, Canada

Mexico Leads: Gabriella Parra-Olea and Eria Rebollar, Universidad Nacional Autonoma de México, Federal District of Mexico City, Mexico

The structure of the North American Bsal Task Force centers upon the Technical Advisory Committee (TAC) and the eight Working Groups (Figure 1A). The TAC is populated by the Working Group leads, representatives from Canada, the USA, and Mexico, and partner groups including different government agencies, the IUCN Amphibian Survival Alliance (ASA), the Pet Industry Joint Advisory Council (PIJAC), and Partners in Amphibian and Reptile Conservation (PARC). The roles of TAC members vary considerably, with strategic surveillance, research, and proactive planning being tri-lateral priorities across the three countries, and advances in biosecurity procedures being contributed by PARC national and regional Disease Task Teams.

The TAC meets by conference call monthly, with a focus on new tasks of the TAC, progress reports on ongoing tasks, and round-robin reporting by Working Group leads and partners. New items have included actions or projects to be assigned or delegated to a subset of members, opportunities for products and grant proposals, subgroup activities with partners, and communication-outreach and networking needs. A lead for the TAC is rotated each year, adding broader perspectives to Task Force activities and ensuring shared leadership of developing products. The incoming and past leads help to transition work and knowledge. Decisions of the TAC are made by consensus. In particular, the TAC identifies novel implementation actions or priorities from new Bsal information forthcoming from the international Bsal research and management community, Working Groups, and partners.

Goal A.1. Leadership of the Bsal Task Force and ensuring progress toward the Framework Actions.

Rank: Urgent

Rationale: Coordinated oversight of the Bsal Task Force by Working Group leads and partners will aid inter-group communication and identification of strategic gaps in development of a program to forestall Bsal transmission to or potential translocation within North America.

Management Relevance: The Framework of Actions (1–9, above) of the Bsal Task Force were identified as the initial scope of interdisciplinary work needed to understand and forestall potential Bsal emergence in North America and protect our natural heritage biodiversity into the future. To address these actions and additional concerns that have been raised in a coordinated fashion, the TAC and its partners and ad-hoc subgroups have taken on a variety of specific tasks that have helped the Bsal Task Force to be better defined and developed (nine current priorities listed below). The Urgent Rank of this Goal has expedited development of numerous strategic actions, 2015 to present, raising awareness among natural resource managers and expediting response processes among both management and science communities.

Financial Needs: In-kind support from member agencies and institutions for time and limited travel; grants for publication costs, symposia, and workshops.

Priority A.1.1: Expanding North American collaborations for Bsal science and management with broader academic, governmental, non-governmental, and conservation interest groups.

Priority A.1.2: Foster inclusivity and diversity in the North American Bsal Task Force structure, participants, and leadership, including: representation across Canada, the US, Mexico, and the First Nations therein; geographic or cultural diversity within nations; disciplinary expertise including epidemiology, wildlife biology, wildlife health and veterinary sciences, ecology, trade, zoos and aquariums; employment sectors including academic and research institutions, government agencies, non-governmental or non-profit organizations, and business and industry organizations; and additional human dimensions such as gender, age, and ethnicity.

Priority A.1.3: Organization of international Bsal symposia with European partners to focus on communication of science and management directions in North America and Europe, two continents with heightened Bsal emergence concerns.

Priority A.1.4: Organization of focused Workshops in conjunction with international symposia or other relevant meetings, to leverage assembly of interested experts for advancing knowledge across the spectrum of the framework of actions in two continents with heightened Bsal emergence concerns, North America and Europe.

Priority A.1.5: Formally publish the North American Bsal Strategic Plan with online access for facilitate communication and referencing capability.

Priority A.1.6: Distinguish the North American Bsal Implementation Plan from the Bsal Strategic Plan and Bsal annual reports which iterate annual achievements and new activities.

Priority A.1.7: Update the Bsal Task Force website, salamanderfungus.org, to provide easier access to information and expand web pages per Working Group.

Priority A.1.8: Leverage expansion of Bsal communication and outreach efforts with other scheduled events and opportunities including Amphibian Week, 1–7 June 2020, being planned by Partners in Amphibian and Reptile Conservation.

Priority A.1.9: Provide support to all Working Groups as needed, but especially for the newest Clean Trade Working Group as it navigates assembly and task identification across the United States and Canada.

Background: Examples of past TAC implementation priorities under Goal A.1 that have already been completed and have advanced the Task Force development and mission have included: 1) an establishment report of the Bsal Task Force (Grant et al. 2015); 2) an overarching decision science framework for addressing Bsal emergence in North America, a product led by the Decision Science Working Group with broad collaboration by the TAC and partners (Grant et al. 2017; described further below); 3) completion of the Bsal Rapid Response Plan (Appendix 4, Bsal Strategic Plan), a product led by the Response & Control Working Group under advisement of the TAC and consultation with partners (Appendix 4 of the Bsal Strategic Plan; described further below); 4) a scenario-building exercise of responses if Bsal were to be detected in North America, led by US Geological Survey scientists with a subgroup of TAC members and partners (Hopkins et al. 2018); 5) a position paper describing the benefits of early release of Bsal occurrence information in novel areas without adverse implications for later scientific publication opportunities of principal investigators involved in the discovery (Adams et al. 2018); 6) the North American Bsal Strategic Plan, the overarching document within which this Implementation Plan is Appendix 5, which describes Bsal and its discovery, the Task Force initiation and structure, and the scope and interests of Working Groups; 7) Bsal Task Force annual reports of achievements, developed with the Outreach & Communication Working Group (2016 to present: salamanderfungus.org); and 8) development of early drafts of this Bsal Implementation Plan.

Bsal TAC members in association with key partners have acted or developed products that contribute to TAC and Working Group Goals. In particular, the Disease Task Team of Partners in Amphibian and Reptile Conservation includes Bsal TAC members and has taken on an important additional communication and outreach function for the North American Bsal Task Force. For example, they have developed: 1) a call to action for Bsal in North America (Gray et al. 2015); 2) a one-page briefing paper on Bsal (<https://parcplace.org/wp-content/uploads/2017/08/BsalBrief.pdf>); 3) guidance for herpetological pathogen surveillance, with Bsal applications specified (Gray et al. 2017); 4) a Herp Disease Alert System (Gray et al. 2018; <https://parcplace.org/resources/parc-disease-task-team/>) to help with a rapid response to disease-related die-offs in North America, including potential Bsal-related die-offs; and 6) biosecurity guidance to forestall transmission of pathogens including Bsal (Julian et al. 2020). In

addition, in 2019, Bsal TAC members and partners organized the first North American Symposium on *Batrachochytrium dendrobatidis*, held at the joint annual meetings of The Wildlife Society and the American Fisheries Society, in Reno, Nevada (Pereira et al. 2020; videos of invited talks: <https://itunes.apple.com/itunes-u/ut-forestry-wildlife-fisheries/id494866284>; accessed 13 October 2020).

The TAC also has helped in an advisory capacity with decisions to transition priorities of Bsal Working Groups as initial tasks were completed since 2015. For example, after the Rapid Response Plan (Appendix 4, Bsal Strategic Plan) was completed, the former Response Working Group moved to a new focus of developing mitigations in response to Bsal detection and expediting policies for permitting actions that may disturb wild environments if Bsal were to be detected, such as ground-disturbing activities that could occur with fencing of areas or water containment, and chemical applications at field sites with Bsal detection. This need was identified in part as the result of a scenario-building exercise led by TAC members from the US Geological Survey (Hopkins et al. 2018), when it was recognized that ground-disturbing activities to forestall Bsal transmission at known sites of first-emergence in North America could be federally funded and may require NEPA (National Environmental Protection Act) policy to be followed, and use of chemical applications at field sites may require Environmental Protection Agency Section-16 approval. Hence, a new focus of the former Response Working Group was influenced by TAC members involved in the scenario exercise, which included an interaction of multiple partners and Working Groups, such as members of the Surveillance & Monitoring and Response groups. The name of the Response Working Groups was changed to reflect the new goals and priorities it was assuming: Response & Control Working Group. Additionally, the TAC concurred with the decision by the Surveillance & Monitoring Working Group to move to a new phase of United States national Bsal field-site surveillance and monitoring with partners at colleges and universities once their initial Goal, led by US Geological Survey partners, had been completed to analyze ~10,000 animals for Bsal across the US. Lastly, a new Clean Trade Working Group was recently initiated after TAC members were invited to speak at an annual meeting of the herpetofaunal trade industry sponsored by the Pet Industry Joint Advisory Council, which focused new attention on framework Action 1, above. This Working Group is hoped to expand efforts to address potential human-mediated transmission of Bsal into North America via trade markets.

Goal A.2: Develop and strengthen lines of communication between the Bsal TAC and national leaders to address risk and response to Bsal emergence in North America.

Rank: Medium

Rationale: Development of a network of relevant partners to address concerns for Bsal in North America includes establishment of communication networks to higher-level national leaders with jurisdiction over the natural heritage of species and biodiversity in Canada, the US, Mexico, and First Nations therein.

Management Relevance: An Executive Oversight Group (Figure 1) was envisioned to be created at a higher level than the TAC as a mechanism to more formally communicate with diverse natural resource managers or wildlife health leaders working at higher organizational levels of the government, especially as new Bsal information became evident with high relevancy for nations. These higher-level managers or leaders potentially include national-scale

personnel such as US Department staff in the Departments of Interior and Agriculture, the Association of Fish and Wildlife Agencies (AFWA) which includes federal, provincial and state leaders, the Canadian Wildlife Service (CWS), and the Pet Industry Joint Advisory Council (PIJAC). The Medium ranking of this Goal reflects the complexity of authorities that need to align to facilitate agreement to convene an Executive Oversight Group.

Financial Needs: In-kind support of members' time, limited travel.

Priority A.2.1: Work toward development of an Executive Oversight Group for communication to higher levels of natural resource management in North America.

Priority A.2.2.: Maintain regular communication with national liaisons about Bsal Task Force or information developments.

Priority A.2.3: In particular, communicate with herpetofaunal, wildlife, epidemiological, or natural resource specialists in Mexico and nations of the Caribbean to inform them of Bsal Task Force or information developments that may be relevant to management of their natural heritage of amphibian species.

Background: The initiation of the Bsal Task Force Executive Oversight Group (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. Although an EOG has not yet been formally convened to address multiple wildlife diseases, an EOG for non-agricultural wildlife diseases is the topic of continued discussion and communications across groups and with leaders across jurisdictions and authorities has increased since 2016.

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 toward 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 issues that the EOG could address. This aim of the Bsal Task Force TAC to engage an effective oversight group among relevant government agencies is an ongoing task. Currently, key national liaisons in different government sectors have been identified for communication, if relevant topics arise. The resulting structure of the Bsal Task Force (Figure 1A), envisioned to be a hierarchical structure with overarching counsel from the EOG, is informally occurring as wildlife health guidance continues to develop with key partners interfacing with the actions of the TAC and Working Groups. Priorities under this Goal reflect ongoing activities in this arena.

2. Working Groups

Eight Bsal Working Groups have overarching goals per disciplinary area or thematic topic, which address one or more statements of the overarching Framework of Actions (above), with

more detailed priorities of focused activities. The following sections detail the implementation plans of the objectives, goals, and priorities for the Response & Control, Diagnostics, Research, Decision Science, Surveillance & Management, Data Management, Outreach & Communication, and Clean Trade Working Groups.

Working Group membership was planned to be open and inclusive but was initially founded with persons involved with disease research, natural resource management in state, provincial or territorial and other government agencies, environmental or conservation groups, non-governmental organizations, and the pet industry. Each Working Group has 1–3 leads, who help to coordinate personnel, manage the workload, and participate in the Bsal Technical Advisory Committee.

B.1. Response & Control Working Group

OBJECTIVE: To provide guidance for the rapid response (including, but not limited to eradication, containment, or other management and control responses) should Bsal be detected in North America.

Working Group Lead: Laura Sprague, US Fish and Wildlife Service, Idaho, USA

Past Working Group Lead: Priya Nanjappa, formerly of Association of Fish and Wildlife Agencies, Partners in Amphibian and Reptile Conservations, Washington, D.C.

Background: Natural resource 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, species with conservation status of concern, differing state or provincial/territorial, and federal jurisdictions, regulations, policy and permitting. The Bsal Response & Control Working Group was formed to bridge the gap between identifying and implementing scientifically-sound mitigation actions in response to a confirmed Bsal detection in North America 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, the purpose of the Group is to *facilitate efficient and rapid response to Bsal invasion*.

The Response & Control Working Group initially focused on development of a Bsal Rapid Response Plan Template, addressing implementation framework Action 2, above. After comprehensive review by federal, state, provincial and other potential users of the Response Plan, this task has been completed and is included as Appendix 4 of the Bsal Strategic Plan (salamanderfungus.org). The Rapid Response Plan is intended to be 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 included in Appendix 4 are based on what an entity using this plan might do after receiving information regarding a potential Bsal detection and then detection confirmation from a laboratory with expertise in Bsal diagnostics. In other words, the initial response may be from a suspected Bsal outbreak, and all subsequent recommended actions occur after diagnostic laboratories have made determinations based on the Case Definition of Bsal (White et al. 2016; a product of the Diagnostics Working Group). The

Bsal Rapid Response Plan and its guidance 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 outbreak may be warranted. The Bsal Rapid Response Plan provides 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. 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. The Response Plan is considered a living document that will be updated as more information becomes available.

After completion of the Rapid Response Plan Template (Appendix 4) and development of scenario-building exercises (Hopkins et al. 2018; Canessa et al. 2020), several aspects of responses to Bsal detection in North America warranted further development. These are tasks included in current Priorities listed below.

Bsal Mitigation: The Response & Control Working Group currently focusses on mitigation strategies that may forestall Bsal occurrence and transmission, if Bsal were to be detected at a field or captive site in North America. Development of Bsal mitigations is part of framework Actions 6, and 7, above, which also intersect the Research and Decision Science Working Groups.

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; La Patra 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. 2014, 2015), environmental probiotics (Muletz et al. 2012), habitat alterations such as fencing to reduce animal movements and human encroachment and water diversions to forestall Bsal transmission with water flow, 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 state/provincial policies related to their potential environmental impacts. For example, in particular, during scenario-response exercises of potential Bsal outbreaks in North America (e.g., Hopkins et al. 2018), rapid responses were identified to be potentially slowed by lack of permits for federal or state authorities to assist in field responses including ground-disturbing activities, disturbance to species with conservation status of concern, and use of chemical applications to address the Bsal fungus viability. The Response & Control Working Group activities currently focus on understanding the US permits and approvals that may be needed for various Bsal actions in field or captive settings and expediting rapid permit processes. It is anticipated that similar assessments will be conducted for Canada and Mexico.

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.

In the USA, 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 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) which 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:

1. 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.
2. 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.

The Response & Control Working Group works in close association with the TAC and other Bsal Working Groups and partners. These include the Decision Science, Surveillance & Monitoring, and the Clean Trade Working Group. In addition, there is continued dialogue with the Research Working Group, Diagnostics Working Group, Outreach & Communication Working Group, Partners in Amphibian and Reptile Conservation, and the US Geological Survey Research and Monitoring Initiative. For example, the Decision Science Working Group helps biologists decide upon a course of action given likelihood of success, the Research Working Group focuses on testing possible disease management options, and the Response & Control working group assists biologists with implementing management strategies.

Goal B.1.1: Review and update the Rapid Response Plan as new information becomes available.

Rank: High

Rationale: As knowledge accrues and response plans are trialed in workshop exercises or in real field or captive situations abroad or in North America, new information or lessons-learned may be applied to adapt and improve the Rapid Response Plan.

Management Relevance: A variety of agencies, institutions, and authorities including the public domain may be stakeholders in a Bsal response. An efficient proactively created Response Plan can aid rapid action if Bsal were to be detected. Adapting current plans as new information becomes available elevates plan efficacy and can streamline processes and reduce uncertainty among management partners.

Financial Needs: In-kind support from member agencies and institutions for time and limited travel.

Priority B.1.1.1: Stay current with new information that may be used during response actions if Bsal were to be detected in North America, including decision science systems, new research on Bsal mitigations, results of Bsal responses in Europe, biosecurity advances, and surveillance results.

Priority B.1.1.2: Interact with other relevant Bsal Working Groups to stay abreast of developing scientific or management breakthroughs that may affect Bsal response guidance.

Priority B.1.2.3: In the event of a Bsal detection in North America, offer assistance for the context-specific customization of the Rapid Response Plan using the template developed by the Response & Control Working group.

Goal B.1.2: Facilitate and improve a natural resource agency’s ability to take proactive and reactive actions to prevent occurrence and transmission of Bsal in North America.

Rank: 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.

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.

Financial needs: In-kind support from member agencies and institutions for time and limited travel.

Priority B.1.2.1: 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

Priority B.1.2.2: Define and outline justifications for management actions, including effectiveness and possible (or lack of) environmental impacts for applying for Cat Ex approval.

Priority B.1.2.3: Identify permits for each jurisdiction in North America that may be needed for effective Bsal mitigation during in response to Bsal detection at a field or captive site.

Priority B.1.2.4: Work with relevant government agencies that have oversight for procedures to do activities with potential environmental impacts at sites under different site contexts in order to develop an expedited plan for a rapid mitigation of Bsal occurrence and transmission.

Priority B.1.2.5: Work with relevant government agencies that have oversight for procedures to work with species of conservation concern at sites with Bsal detections, including their capture, Bsal testing, quarantine in captive settings, treatment, relocation, or euthanization.

Priority B.1.2.6: Develop “blanket documents” for exemption or permitting requests, as feasible per jurisdiction and context.

Priority B.1.2.7: Identify a list of contacts for rapid submission to relevant permitting agencies; and develop a communication chain for expediting processes.

Priority B.1.2.8: Explore mechanisms to set up an emergency response fund and how to disperse such funds.

Goal B.1.3: Provide information and build understanding of Bsal, the Bsal Task Force, the Strategic Action Plan, and available Management/Mitigation options for Federal Agencies at the national and regional levels.

Rank: High

Rationale: Local and regional 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.

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

Financial needs: In-kind support from member agencies and institutions for time and limited travel; external funding resources may be needed for hard-copy brochure production, if necessary.

Priority B.1.3.1: Develop briefing materials about Bsal, the Bsal Task Force and Strategic Plan, and available Bsal management tools (in collaboration with TAC and the Outreach & Communications Working Group).

Priority B.1.3.2: Develop a preliminary list of key national (in collaboration with the TAC) and regional (in collaboration with the Disease Task Team of Partners in Amphibian and Reptile Conservation, as they have this list for their Herp Disease Alert System) wildlife health and natural resource contacts for briefing material distribution.

Priority B.1.3.3: Devise a plan for briefing material distribution at national and regional scales.

Priority B.1.3.4: 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.

Goal B.1.4: Brief and offer training to local-to-regional natural resource agencies about the North American Bsal Task Force and available Management/Mitigation options.

Rank: High

Rationale: Local and regional 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.

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

Financial needs: In-kind support from member agencies and institutions for time, travel, and printing of training materials; grants may be needed to cover costs of in-person, on-site standardized training across North America.

Priority B.1.4.1: Develop training materials for Bsal mitigation actions (in collaboration with the Outreach & Communication Working Group).

Priority B.1.4.2: Conduct a mock training session to assess efficacy of pilot approach developed for roll-out of Management/Mitigation options. Revise procedures as warranted.

Priority B.1.4.3: Provide training workshops which could be done in-person or remotely targeting local, regional, and national natural resource management groups (may vary by region, state/province, or country).

B.2. Diagnostics Working Group

OBJECTIVE: To assist with the promotion of consistent standards for diagnosis and reporting Bsal among the wildlife health community, and to serve as a forum to exchange ideas, work out challenges, and provide consultation relative to Bsal detection and diagnosis, and to provide expert advice concerning Bsal detection and diagnosis.

Working Group Lead: Maria Forzan, College of Veterinary Medicine, Long Island University, NY, USA

Past Working Group Lead: Jacob Kerby, University of South Dakota, Vermillion, SD, USA

Background: Determination of effective standardized diagnostics procedures for detection of novel disease-causing pathogens such as Bsal relies both on gross and microscopic examination of lesions in affected animals and on the detection and correct identification of the pathogen through histochemical and molecular methods. Molecular detection of pathogens is a rapidly developing discipline largely reliant on technological advances analyzing organismal DNA and RNA. Mortality due to Bsal was first described by Martel et al. (2013), along with a morphological description of the pathogen and the microscopic lesions it caused. Martel et al. conducted molecular characterization and diagnostic PCR (polymerase chain reaction) analyses of the 5.8S rRNA gene of Bsal from skin samples. Bsal is closely related to *Batrachochytrium dendrobatidis* (*Bd*), and Blooi et al. (2013) aimed to develop a duplex real-time PCR assay to detect both pathogens, as Bsal was not detected during application of *Bd*-specific PCR methods described by Annis et al. (2004) and Boyle et al. (2004). Cross-reactivity of testing procedures for disease-causing microbiota with each other or environmental contaminants has raised broad concerns for PCR-testing sensitivity and specificity (e.g., Iwanowicz et al. 2017), and hence a priority aim for additional disease diagnostic research is to develop effective testing tools for standardized use.

The Bsal Diagnostics Working Group was convened as a partnership among diagnosticians and researchers interested in further development of effective diagnostics tools for Bsal, addressing implementation framework Action 3, above. The Diagnostics Working Group is composed of professionals with expertise in the application and interpretation of an array of diagnostic tools, with members working in academia, diagnostic laboratories and government agencies throughout North America and involved in detection and reporting of amphibian diseases, including Bsal.

Since the Working Group's assembly in 2015, collaborations between members of the group and others have achieved several initial Goals. First, the Bsal case definition (White et al. 2016) was published to establish criteria to promote standardized communication of diagnostic results for diagnosis of Bsal-caused disease. The case definition provides clinical and histopathological presentation of Bsal disease, and references Martel et al. (2013) and Blooi et al. (2013) for molecular analyses by PCR. Second, advancing the development of reliable standardized diagnostic procedures for Bsal has been an overarching goal for the Working Group. In 2016, a pilot multiple-laboratory round-robin proficiency test for Bsal detection by PCR was conducted (Forzán et al., manuscript in progress). Third, an *in situ* hybridization protocol to detect *Bd* and Bsal cells in formalin-fixed paraffin-embedded tissues was developed (Ossiboff et al. 2019) - 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. Ongoing work by the group is capitalizing upon the value of collaborations among diagnosticians and researchers with different expertise.

The Diagnostics Working Group has close interactions with the Research Working and Surveillance & Monitoring Working Group, and has intermittent interactions with the Response & Control, Data Management, and Outreach & Communications Working Groups.

Goal B.2.1: Establish a long-term program for inter-laboratory quality control and evaluation of protocols for the detection of wildlife pathogens, particularly *Bd* and Bsal.

Rank: 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 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.

Management Relevance: Development of efficient diagnostic procedures expedites sample processing and delivery of results with known accuracy levels; this reduces uncertainty for rapid response actions to be implemented by natural resource managers, should Bsal be detected in North America.

Financial Needs: Cost Estimate: 60,000 USD; partial funding provided by Environment and Climate Change Canada.

Priority B.2.1.1: Determine a laboratory that can produce bi-annual sets of samples containing pre-determined concentration of inactivated *Bd* and *Bsal* zoospores in solution.

Priority B.2.1.2: 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.

Priority B.2.1.3: 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.

Priority B.2.1.4: Provide a set of blind samples that include blanks, and one or both amphibian chytrid fungi (*Bsal* and *Batrachochytrium dendrobatidis*).

Priority B.2.1.5: 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.

Goal B.2.2: Develop standardized and reproducible methods that will allow comparison across studies, with reliable detection of *Bd* and *Bsal*, and reliable estimation of infection load once either or both pathogens are detected.

Rank: 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.

Management Relevance: Development of efficient and standardized diagnostic procedures allows comparison of results among contexts such as sites; this provides a foundation for geographic or taxonomic comparisons of *Bsal* detection and reduces uncertainty for rapid response actions to be implemented by natural resource managers, should *Bsal* be detected in North America.

Financial Needs: 135,000 USD; unfunded at present.

Priority B.2.2.1: Establish a short list of protocols that are most likely to be used across agencies and institutions.

B.2.2.2: 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.

B.2.2.3: Provide detailed instructions on the protocols to test to participating laboratories.

B.2.2.4: Define common metrics that laboratories should report to determine detection, quantification and variability of chytrid detection using molecular tools.

B.2.2.5: Provide a set of blind samples that include blanks, and one or both amphibian chytrid fungi (*Bsal* and *Batrachochytrium dendrobatidis*).

B.2.2.6: Collate reports from participating laboratories and establish the protocol(s) that yielded most consistent results and determine variability.

B.2.2.7: Establish a mechanism to provide laboratories with the standard(s) deemed most appropriate based on the round robin results.

B.3. Research Working Group

OBJECTIVE: To facilitate communication and collaboration among scientists studying *Bsal* in North America and to ensure that high-quality research on *Bsal* is produced rapidly.

Working Group Lead: Doug Woodhams & Molly Bletz, University of Massachusetts, Boston, USA

Past Working Group Leads: Matt Gray, University of Tennessee, Knoxville, TN, USA; Reid Harris, formerly of James Madison University and Amphibian Survival Alliance, Harrisonburg, VA, USA

Background: The breadth of *Bsal* research needs span basic, applied, and theoretical science disciplines; advances in all three arenas are needed to build foundational knowledge essential for responding to *Bsal* emergence. Scientific studies across these arenas broadly address Framework Actions 4-6, above. To achieve a broad scope of work, the *Bsal* Research Working Group is a collaboration of scientists with a diversity of expertise from multiple disciplines, including molecular and cellular biology, immunology, ecology, mathematics, pathology, and social sciences. The group strives for inclusion and productive collaboration, and to maintain or expand its participants to effectively address open research questions critical to our ability to respond to and manage *Bsal*. The Working Group is currently composed of >30 members, representing >20 organizations.

The Research Working Group maintains a list of research needs based on recent publications, our current state of knowledge, and key knowledge gaps. The Working Group ranks research needs on the list as Urgent, High, or Medium priority. Within each of these categories, research needs are considered as having equal importance. Each year the list is updated as more information becomes available and ranks change. Ranks are associated with the thematic Goals for the Research Working Group, listed below. The research Goals identified below represent a comprehensive approach to advancing the understanding of *Bsal*'s potential impact on amphibian host communities and effective response and management approaches should it be introduced into North America. The Goals are broken down into priority research studies (i.e., action items); achievements are listed in annual reports (salamanderfungus.org).

The Research Working Group interacts with several other Working Groups (Figure 1B), including key ties to the Decision Science, Data Management, and Diagnostics Working Groups and additional interactions on specific topics with the Response & Control, Communication & Outreach, and Clean Trade Working Groups. In particular, research can inform management decisions and can evaluate effectiveness of intervention strategies collectively identified in collaboration with the Decision Science Working Group and the Response & Control Working Group. The Research Working group endeavors to reduce uncertainties that impede proactive and responsive strategies. Decision Science 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 4, 7) can be evaluated in a decision analysis framework to identify the optimal strategy, given species-specific susceptibility (Goal 3), and calculate the importance of reducing remaining uncertainties to improve decisions. Hence, it is essential that the Research Working Group interacts with the other working groups to produce research with applied implications.

Goal B.3.1: Understand the role of human behavior and the pet trade in the spread and spillover of *Bsal*.

Rank: Urgent.

Rationale: 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. 2012). 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, and specifically relative to *Bsal* transmission routes in trade markets.

One of the most likely routes 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 US Fish and Wildlife Service rule banning the trade of some salamander genera may have reduced the likelihood of infected animals entering the US 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 US in the past 10 years, and their estimates did not include frogs, which constitute 94% of imported amphibians. Indeed, *Bsal* has been documented in trade in Europe (Nguyen et al. 2017; Fitzpatrick et al. 2018; Sabino-Pinto et al. 2018), 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 et al. (2017) performed preliminary surveillance for *Bsal* in the US pet trade and did not detect it. However, their small sample size prevented detection of the pathogen at low prevalence (Yuan et al. 2018). Limited *Bsal* surveillance studies in the pet trade have been published for Canada or Mexico (Govindarajulu et al. 2017). 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 human dimensions. For example, understanding 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, understanding carrier capacity of highly trade amphibians, and human dimension surveys to characterize public awareness, perceptions and behaviors associated with *Bsal*.

Management Relevance: The North American Model of Wildlife Management relies on evidence-based decision making. For an effective *Bsal* management response, science-based support for context-specific pathogen occurrence, host disease susceptibility, and efficacy of management or treatment alternatives is imperative to reduce uncertainty in manager or policy-maker decision making. 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.

Financial Needs: 30,000 USD per state/province/port of entry; 15,000 USD per species; partial funding received for species research. Klocke et al. (2017) study addressed Priority 1.

Priority B.3.1.1: 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.

Priority B.3.1.2: Estimate the susceptibility of potential *Bsal* hosts (salamanders and frogs) in the pet trade (e.g., species commonly imported from Southeast Asia).

Priority B.3.1.3: 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*.

Goal B.3.2: Identify critical transmission pathways and conditions under which *Bsal* is likely to emerge in amphibian host populations in North America (e.g., compartmental disease models).

Rank: Urgent.

Rationale: Identifying the importance of transmission pathways under varying conditions is fundamental to characterizing the epidemiology of host-pathogen systems and developing effective disease intervention strategies (Tien and Earn 2010; Langwig et al. 2012).

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; Stegen et al. 2017). 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).

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.

Financial Needs: 2,100,000 USD; partial funding by National Science Foundation for Priorities 1–5. Longo et al. (2019) study addressed Priority 6.

Priority B.3.2.1: Estimate latency period of infection and recovery rate for pre- and post-metamorphic amphibian hosts at biologically relevant temperatures.

Priority B.3.2.2: 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.

Priority B.3.2.3: Estimate daily contact rates of amphibian hosts at relevant temperatures and densities when exposed to different complexities of habitat structure.

Priority B.3.2.4: Estimate probability of *Bsal* transmission between infected and uninfected amphibian hosts (within and between species) at different post-exposure durations and temperatures

Priority B.3.2.5: Estimate the duration of zoospore persistence in water and soil given differences in various environmental conditions (e.g., temperature, micropredators, soil moisture, water chemistry, and bacterial presence).

Priority B.3.2.6: Estimate the influence of co-infection with other pathogens (e.g., *Bd*, ranavirus) on the likelihood of *Bsal* transmission and development of chytridiomycosis.

Priority B.3.2.7: Determine the probability of transmission or translocation of *Bsal* by non-amphibian hosts (e.g., crayfish, waterfowl, humans) and understand their role as potential biological reservoirs.

Goal B.3.3: Produce more informed *Bsal* risk models for North America through improved, objective classification of species tolerance to *Bsal* infection (e.g., integral projection models).

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. Evidence for species susceptibility is being rapidly expanded (Martel et al. 2014; Carter et al. 2020; Barnhart et al. 2020; unpubl. data). Since autumn 2015, the susceptibility of a number of North American amphibian species to *Bsal* has been estimated among several US laboratories (Appendix 1). Integral projection models (IPMs) 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. IPMs 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.

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.

Financial Needs: 15,000 USD per species, Priority 1, partially funded by BAND Foundation; 300,000 USD each, Priority 2 (partially funded by Smith Conservation Fellowship and National Science Foundation) and Priority 3 (partially funded by National Science Foundation); 200,000 USD, Priority 4, funded by US Fish and Wildlife Service.

Priority B.3.3.1: 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).

Priority B.3.3.2: Estimate the impact of habitat characteristics (temperature, pH, salinity, zooplankton abundance, etc.) on *Bsal* infection risk.

Priority B.3.3.3: 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).

Priority B.3.3.4: 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.

Goal B.3.4: Identify effective methods to manage *Bsal*-induced disease and clear *Bsal* infections in captive and field settings.

Rank: 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 possible conservation frontiers for field-based mitigation (Bletz et al. 2013; Garner et al. 2016; Thomas et al. 2019). 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. A recent review by Thomas et al. (2019) possible strategies to mitigate *Bsal*, critical gaps and future directions.

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 (Bloom 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).

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.

Financial Needs: 800,000 USD total, Priorities 1–6; 150,000 USD per strategy, partially funded by David H Smith Fellowship, Foundation for the Conservation of Salamanders, US Fish and Wildlife Service; Priority 7, partially funded by National Science Foundation; 400,000 USD, Priority 8, partially funded by US Fish and Wildlife Service.

Priority B.3.4.1: 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.

Priority B.3.4.2: Identify *Bsal*-consuming aquatic micropredators from natural habitats and test micropredator augmentation strategies.

Priority B.3.4.3: 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, nanoparticle technology).

Priority B.3.4.4: Explore the use of *Bsal* removal methods (e.g., attractants or traps).

Priority B.3.4.5: Explore the genetic correlates of disease resistance and the possibility of selective breeding hosts for *Bsal* resistance.

Priority B.3.4.6: 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).

Priority B.3.4.7: 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.

Priority B.3.4.8: Determine the effectiveness of reducing host density or altering relative abundance of host species with different infection tolerances on invasion potential of *Bsal*.

Priority B.3.4.9: Identify volatile organic compound (VOC)-producing, *Bsal*-inhibitory microbes and their inhibitory compounds.

Priority B.3.4.10: Test alternative antifungal compounds for use on a broad host taxonomic range and across life-history stages.

Priority B.3.4.11: Test the use microbes and/or compounds to clear existing infections and minimize side effects.

Goal 5: Quantify innate and adaptive immune responses to *Bsal* across species and environmental conditions.

Rank: 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 includes 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).

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.

Financial Needs: 1.3 million USD, partial funding from National Science Foundation, *Notophthalmus viridescens* focus.

Priority B.3.5.1: 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.

Priority B.3.5.2: 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.

Priority B.3.5.3: Determine whether amphibians or symbionts produce antifungal small molecule compounds.

Priority B.3.5.4: Determine how the skin microbiome (bacteria, fungi, viruses) interacts with immune responses and influences disease susceptibility. Also, determine if the skin microbiome be manipulated, and whether it varies with environmental conditions and host genetics.

Priority B.3.5.5: Establish hematological reference values and determine how these parameters reflect immunity to *Bsal* infection in amphibian hosts.

Priority B.3.5.6: Determine whether there is protective immunity that 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).

Goal B.3.6: Identify the mechanisms of *Bsal* pathogenesis.

Rank: 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 Ca²⁺), 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.

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.

Financial Needs: 1.2 million USD; partially funded by US National Science Foundation.

Priority B.3.6.1: Quantify the changes in plasma electrolyte concentrations in Bsal-infected salamanders.

Priority B.3.6.2: Identify whether bacterial invasion from the skin via Bsal lesions and sepsis are contributing factors to pathogenesis.

Priority B.3.6.3: Identify tissue tropism for Bsal-infected amphibians.

Priority B.3.6.4: Explore mechanisms of attraction to (chemotaxis) and physical binding of zoospores to hosts.

Priority B.3.6.5: Determine whether Bsal releases lymphotoxic or cytotoxic molecules.

Goal B.3.7: Establish effective methods for detecting Bsal infections.

Rank: Urgent

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 and therefore rapid and accurate detection of the pathogen is crucial. This objective intersects directly with the Diagnostic Working Group. Recent histological advances have provided a novel tool for identifying coinfections using RNAScope (Ossiboff et al. 2019).

Management Relevance: Detecting and tracking existing *Bsal* infections from captive-housed amphibians is critical 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.

Financial Needs: 350,000 USD, unfunded.

Priority B.3.7.1: Develop new diagnostic tools and improve existing tools.

Goal B.3.8: Estimate the interactive effects of Bsal with natural and anthropogenic stressors.

Rank: 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.

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.

Financial Needs: 150,000 USD per stressor, Priority 1; 300,000 USD Priority 2.

Priority B.3.8.1: Conduct susceptibility trials that include common natural and anthropogenic stressors (e.g., hydration, salinity, pesticides) to determine if outcomes following *Bsal* exposure are altered.

Priority B.3.8.2: Conduct susceptibility trials in complex settings that include community features such as predation and trophic interactions and changing habitat quality

B.4. Decision Science Working Group

OBJECTIVE: 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 trade-offs and risk to overcome impediments to optimal decision-making.

Working Group Lead: Evan Grant, US Geological Survey Patuxent Wildlife Research Center, Turner Falls, MA, USA

Current Working Group Members: Katrina Alger (USGS National Wildlife Health Center), Riley Bernard (University of Wyoming), Stefano Canessa (University of Ghent), Brittany Mosher (University of Vermont), Katherine Richgels (USGS National Wildlife Health Center), Robin Russell (USGS National Wildlife Health Center), and Alex Wright (Michigan State University).

Background: The Decision Science Working Group applies decision science to the potentially complex decision-making process of *Bsal* response and management (implementation framework Action 2, above). The Decision Science Working Group is currently composed of members representing multiple Federal and academic organizations. Collectively, the group has decades of experience in decision science, amphibian and pathogen ecology, research, mathematical modeling, and development of collaborative relationships with wildlife 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 fail-safe. 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 the 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 (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.

Since 2015, the Decision Science Working Group has made considerable progress on initial Goals. The inaugural meeting sponsored by the US Geological Survey that led to the development of the North American Bsal Task Force was originally planned as a Bsal decision science working group. That workshop led to a report that was one of the initial Task Force accomplishments (Grant et al. 2015) and helped springboard the first product of the Decision Science Working Group (Grant et al. 2017), the first steps in identifying proactive measures to forestall Bsal emergence in North America. Simultaneously with the forming of the Task Force, initial efforts of an independent subgroup that became the heart of the Decision Science Working Group came to fruition as their US risk assessment for Bsal emergence was published (Richgels et al. 2016). These efforts are tied to subsequent Goals and Priorities of the Decision Science Working Group, below.

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 Decision Science Working Group Goals below are designed to respond to these challenges, and many are interactive with other Working Groups, especially Response & Control, Research, Data Management, and Outreach & Communication.

Goal B.4.1: Identify critical research that has the highest value of information which will lead to an improved ability to manage Bsal.

Rank: High

Rationale: Identification of critical research needs that impede decision-making is of paramount importance for responsive and proactive management of Bsal emergence in North America. The collaborative development of research priorities between land managers and researchers is an integral component for creating and evaluating effective and efficient management solutions.

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 Decision Science Working Group 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.

Financial Needs: 500,000 USD

Priority B.4.1.1: 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.

Goal B.4.2: Identify approaches to improve proactive management for Bsal when competing objectives or risk are impediments to action.

Rank: High

Rationale: Proactive management to forestall Bsal emergence reduces the need for crisis-management approaches that can be less effective, less cost-efficient, and potentially higher risk for values of concern such as threatened species or personal property.

Management Relevance: Involvement of the natural resource management community in proactive measures to forestall Bsal transmission in North America has several strategic advantages: it raises awareness of the potential Bsal threat to native species; it increases the likelihood Bsal is detected before it gains a significant foothold on the continent; as biosecurity measures are enacted, there are likely ‘spillover’ benefits to forestall transmission of other pathogens, microparasites, or invasive species that similarly affect endemic hosts; it improves management success; and it is cost-effective relative to reactive measures that may include long-term rescue of local endemic hosts at the risk they may become conservation reliant for their persistence.

Financial Needs: 500,000 USD

Priority B.4.2.1: Use simulation, modeling, and optimization techniques to identify optimal actions given various impediments (i.e., uncertainty or competing objectives).

Priority B.4.2.2: Using these models, evaluate possible trade-offs of action versus inaction, and estimate costs of delaying action. Evaluate individual and agency risk tolerance and effect on optimal actions under different levels and sources of uncertainty.

Goal B.4.3: Conduct and update Bsal risk assessments.

Rank: 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 2014) 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.

Management Relevance: Knowledge advances can be used to inform adaptive management processes, improving management efficacy. This cycle can be applied to decision science and risk assessments, where knowledge advances can be used to update parameters in risk models and to alter management and policy decisions. In the Bsal context, use of multiple Bsal prevention strategies, rather than reliance on a single measure, can be a more effective strategy to forestall Bsal emergence on this continent. In addition to an import restriction on salamanders, addition of alternative measures warrant consideration.

Estimated Cost: 200,000 USD

Priority B.4.3.1: 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 US, Canada, and Mexico

Priority B.4.3.2: 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.

Goal B.4.4: Frame Bsal management problems at regional and resource manager levels.

Rank: 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 Decision Science Working Group 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.

Management Relevance: Having natural resource managers work together with decision science specialists can expedite development of multiple effective management options for Bsal response, that take diverse management contexts into consideration.

Financial Needs: 680,000 USD

Priority B.4.4.1: 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 trade-offs among habitats, amphibian populations, and pathogen occurrence and prevalence.

Priority B.4.4.2: Hold a series of structured decision-making workshops with managers (i.e., USFWS refuge biologists, regional land managers, Canadian Fish and Wildlife biologists, etc.) that have expressed interest in identifying where proactive management can be implemented and what the barriers to implementing proactive management are across regions and management entities.

Priority B.4.4.2: 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.

Goal B.4.5: Identify whether management should consider proactive, reactive, or a combination of management strategies, dependent on the presumed presence and spatial distribution of Bsal.

Rank: 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 & Monitoring and Research Working Groups.

Management Relevance: Bsal surveillance increases the likelihood of detection early in Bsal invasion of North America. Improved surveillance designs with application of decision science tools can help maximize early-detection likelihoods. This increases the likelihood of a positive management response and is much more cost-effective.

Needs: 360,000 USD

Priority B.4.5.1: 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.

Priority B.4.5.2: 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.

B.5. Surveillance & Monitoring

OBJECTIVE: To facilitate and coordinate surveillance of Bsal in North America.

Working Group Leads: Mike Adams, US Geological Survey, Corvallis, OR, USA; Jenifer Walke, Eastern Washington University, Cheney, WA, USA; Olya Milenkaya, Warren Wilson College, Swannanoa, NC, USA

Past Working Group Lead: Hardin Waddle, US Geological Survey, Gainesville, FL, USA

Background: A coordinated surveillance effort is aimed to detect the initial introduction of Bsal in North America, thereby allowing for a more effective rapid response to forestall further transmission, helping to safeguard the native fauna of the continent (framework Action 4). Management and conservation actions cannot proceed effectively 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 (e.g., Muths et al. 2009), this haphazard approach is unlikely to detect Bsal at the onset of its invasion. Instead, the vision of the Surveillance & Monitoring Working Group is to facilitate early detection of Bsal to allow for an effective and rapid response, with the ultimate goal of conserving biodiversity.

Achieving a broad and robust surveillance network is difficult and expensive due to the labor involved. No single organizational entity has been identified that has this capacity. Instead, the emphasis has been on coordinating and encouraging sampling for Bsal by diverse partners such that something closer to a reasonable level of surveillance is achieved. Initial efforts toward this goal are described below. However, due to limitations of initial approaches, current efforts of the Surveillance & Monitoring Working Group are aimed to build a network of undergraduate teaching institutions to engage students in surveillance. This will increase Bsal awareness, engage volunteer personnel and citizen scientists, utilize dispersed in-kind educational institution resources, and increase the amount Bsal sampling.

Past Surveillance Efforts:

USA: In the US, a one-time major sampling effort was conducted by the US Geological Survey Amphibian Research and Monitoring Initiative (ARMI), as part of this Working Group's mission. This effort included sample sites across the US with site resources allocated according to the estimated risk of Bsal occurrence as per the risk assessment model results developed by Richgels et al. (2016). Over 10,000 amphibians (mostly salamanders) were sampled. Bsal was not detected (Waddle et al. 2019, 2020). ARMI continues to sample at a very low level in select areas where resources allow. In addition, the US Fish and Wildlife Service 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 website 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: email

reports to herp_disease_alert@parcplace.org; include photograph, species affected, location, other relevant episode information, observer's name) that provides another way to gather observations of sick or dead amphibians that might not otherwise be reported. Communication also occurs between iNaturalist and HDAS, as oversight of iNaturalist reports occurs and possible disease events are identified and communicated. In both cases, observations are typically forwarded to the relevant state biologist but in some cases, when deemed necessary, members of the HDAS or state authorities can use their personal networks to help facilitate further investigation. Lastly, in the US and elsewhere, independent science investigations into Bsal detections from skin swabs of selected species in specific geographies are ongoing, for example multiple reports of joint Bsal and Bd (*B. dendrobatidis*) sampling in North America were published in *Herpetological Review* in 2017 (Olson 2019).

Canada: 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 (Christina 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. In addition, 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. Disease reports to the HDAS email address are forwarded to provincial authorities in Canada, similar to state reporting of HDAS reports received in the US.

Mexico: In Mexico, surveys directed to identify Bsal in natural populations have been conducted by members of Dr. Gabriela Parra-Olea's research laboratory. 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-Olea, unpubl. data). In 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 provide certification of both of these research laboratories so that legal amphibian imports in Mexico can be screened for Bsal.

The Surveillance & Monitoring Working Group has strong ties to the Data Management Working Group, which helps compile Bsal surveillance reports and scientific studies from outside the efforts of the North American Bsal Task Force (Goal 2). They also coordinate with

the Diagnostics Working Groups which conducts Bsal diagnostics research, and the Response Working Group to help formulate monitoring responses if Bsal were to be detected. As needed, they work with the Clean Trade Working Group for Goal 3, and the Decision Science Working Group, as their risk assessments have guided initial priority sampling efforts.

Goal B.5.1: Facilitate and support a wide-reaching, ongoing, coordinated and sustainable Bsal surveillance program in the USA, Canada, and Mexico.

Rank: High

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.

Management Relevance: Early detection of Bsal in North America improves management efficacy to forestall Bsal spread, increasing population and species persistence likelihoods at lower financial burdens. It is a win-win strategy for retaining North American amphibian biodiversity and reducing stressors to the larger ecosystems within which they are embedded.

Financial Needs: In-kind support from member agencies and institutions (especially US Geological Survey's Amphibian Research and Monitoring Program) for time, some laboratory analyses, and travel; grant proposals planned for partner sampling efforts, travel, and laboratory analytical costs. 15,000 USD per year for SNAPS portion of Priority 1; 100,000 USD per year in 2021–2023 for initial efforts with broader US, Mexico, and Canada surveillance; 200,00 USD per year for scaled-up efforts in 2023-2025.

Priority B.5.1.1: Establish a Bsal surveillance program in cooperation with US colleges and universities, the Student Network for Amphibian Pathogen Surveillance (SNAPS) (e.g., this broad priority includes development of partnership roles, a student training curriculum, sampling protocols, safety and biosecurity procedures, necessary permits and permissions for working with wildlife and working with students within course constraints or as part of extracurricular activities, data management and archival, data reporting procedures, and attribution of efforts).

Priority B.5.1.2: Facilitate and support ongoing Bsal surveillance in Mexico and Canada.

Goal B.5.2: Identify Bsal sampling efforts that are occurring outside of efforts coordinated by the Bsal Surveillance & Monitoring Working Group.

Rank: High

Rationale: Researchers and managers are conducting their own Bsal surveillance across North America outside of efforts spearheaded by the Bsal Task Force, 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.

Management Relevance: Multipronged, coordinated surveillance has a higher likelihood of detecting Bsal early upon its emergence in North America, reducing long-term management costs of Bsal management and protection of rare species. Coordination of efforts can reduce survey redundancies, provide better inference to the surveyed landscape and host taxa, and be

more cost-efficient. Strategic coordination may influence independent sampling in much needed areas where gaps in effort have been revealed.

Financial Needs: In-kind support from member agencies and institutions for time, some laboratory analyses, and limited travel; grant proposals planned for partner sampling efforts, travel, and laboratory analytical costs. No cost estimate at this time as workplan is in development.

Priority B.5.2.1: Coordinate with the Bsal Research Working Group and other amphibian disease researchers across North America who are conducting Bsal surveillance as part of their broader research programs.

Priority B.5.2.2: Establish additional pathways of communication with a broader sector of amphibian biologists, permitting agencies and institutions, and diagnostic laboratories to develop a means of identifying personnel conducting Bsal surveillance without compromising confidential research plans.

Priority B.5.2.3: Encourage Bsal and *Bd* surveyors to input their planned, ongoing, or past efforts into the chytrid data management portal: amphibiandisease.org.

Goal B.5.3: Support and facilitate sampling of amphibians in the pet trade.

Rank: High

Rationale: Bsal is likely to be introduced to North America through amphibian trade. Therefore, surveillance among captive amphibians is a logical priority for the early detection of Bsal. Furthermore, detection of Bsal in captive animals 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.

Management Relevance: Forestalling Bsal introduction to the wild in North America is of premier importance for retaining the integrity of North American wildlife and ecosystems, a primary goal of natural resource managers. Early Bsal detection in North American trade markets and captive populations (e.g., amphibians used as pets, food, and traditional medicines, and populations retained in zoos, aquariums, museums, and biomedical research) can improve Bsal management efficacy and reduce costs of Bsal mitigation actions in North America.

Financial Needs: In-kind support from member agencies and institutions for time, some laboratory analyses, and limited travel; grant proposals planned for partner sampling efforts, travel, and laboratory analytical costs. No cost estimate at this time as workplan is in development.

Priority B.5.3.1: Coordinate with the newly established Clean Trade Working Group to support and facilitate Bsal sampling of amphibians in the pet trade.

Priority B.5.3.2: Develop a study plan for animal sampling or subsampling and eDNA water sampling for Bsal at ports of entry.

Priority B.5.3.3: Develop study plan for animal subsampling and eDNA water sampling of batches in pet stores in cooperation with state and provincial agencies.

Goal B.5.4: Develop initial plans for post-detection monitoring, if Bsal were to be detected at a field or captive site in North America.

Rank: Urgent

Financial Needs: In-kind support from member agencies and institutions for time.

Priority B.5.4.1: In cooperation with the Decision Science Working Group, partners, and existing scenario-development exercises of Bsal detection in different site geographic and taxonomic contexts, develop example sampling designs to detect Bsal extent at a novel detection site before mitigation actions are fully implemented.

Priority B.5.4.2: In cooperation with the Decision Science Working Group, partners, and existing scenario-development exercises of Bsal detection in different site geographic and taxonomic contexts, develop example sampling designs to detect Bsal extent at a detection site after different types of mitigation actions are fully implemented.

Priority B.5.4.3: To fine-tune monitoring designs at known or suspected Bsal detection sites, develop study plans to compare efficacy of alternative approaches; develop *a priori* IACUC and state or federal permission forms, and determine if expedited approvals could be gained.

B.6. Data Management Working Group

OBJECTIVE: Develop an online Bsal and *Bd* data management portal to accelerate sharing of planned or completed surveillance projects and scientific studies to accelerate the pace of learning about these pathogens tied to emerging infectious diseases, and to foster integrated efforts in research and monitoring across North America and among global communities.

Working Group Leads:

Michelle Koo, Director, AmphibiaWeb, University of California, Berkeley, CA, USA

Deanna H. Olson, USDA Forest Service, Corvallis, OR, USA

Background: The Data-Management Working Group works with the North American *Bsal* Task Force, its partners, and independent investigators to compile and disseminate *Bsal* and *Bd* surveillance results via development of an accessible web portal with data import, export, analysis, and multi-database linking functions (Framework Action 8). A web-portal reporting system for *Bsal* and *Bd* data provides larger-scale data management capabilities and can address novel multi-scale questions across individual studies. This data sharing capability can contribute to analyses that serve to advance understanding of the risk of *Bsal* or *Bd* introduction to North America and assess disease risk of these potentially deadly pathogens to native amphibians in North America and elsewhere through aggregating and managing past and current disease sampling data in a common repository (Framework Action 5). Lastly the data portal may serve as a data repository to meet journal requirements for data access thus providing a community service to research.

The Data Management Working Group has met its original Goal by developing the Amphibian Disease portal (<https://amphibiandisease.org>) to comprehensively manage data on the amphibian chytrid fungi *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*). This online portal is aimed to accelerate information sharing among global scientists, natural resource managers, and the public regarding planned and ongoing surveillance projects and scientific studies, as well as the results of completed studies. Data within the portal is especially important to aid in rapid responses and decision science for allocation of limited resources available for research and management of these amphibian emerging infectious diseases and their affected hosts. In particular, comprehensive data management of *Bd* and *Bsal* detection and no-detection data by location and project are useful for development of new scientific research studies, surveillance and effective monitoring programs, and for understanding disease dynamics of chytridiomycosis. The Amphibian Disease portal (<https://amphibiandisease.org>) meets these aims 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 by displaying aggregated data summaries. Further, web services to other scientific portals such as AmphibiaWeb help extend its reach to the other audiences in education and conservation.

The Data Management Working Group is allied with the Surveillance & Monitoring, Research, Decision Science, and Outreach and Communication Working Groups, with anticipated potential interaction with the Clean Trade Working Group.

Goal B.6.1. Maintain comprehensive data management of *Bd* and *Bsal* samples for archived, aggregated monitoring and analytic modeling in the Amphibian Disease Portal, amphibiandisease.org.

Rank: Urgent

Rationale: The Data Management Working Group aims to expand the scope and capacity of the new web-based chytrid data management system (amphibiandisease.org). In addition to increasing registered portal users and *Bsal* and *Bd* data imports, the AmphibianDisease portal is partnering with the global bio-sampling database Geome (<https://geome-db.org>) to deliver improved validation services, enhanced security, improved data accessibility such as through R Statistics to enhance research and forecasting abilities.

Management Relevance: The portal serves as a data management system for chytrid survey information and a communication mechanism among surveillance teams to show where efforts are planned, ongoing, or completed. This maximizes early detection of *Bsal* in North America and is cost effective so that redundant efforts are not launched. Early detection reduces future potential costs of natural resource managers to sustain or restore their natural systems.

Financial Needs: In-kind support from member agencies and institutions for time and limited travel; grant proposals planned for temporary hiring of web programmers to advance web user interfaces and database management procedures. 30,000 USD per year for web developer to create SNAPS website for use by colleges and universities across the USA, and other working group developments. 10,000 USD per year *Bd* and *Bsal* data updates from the literature;

Priority B.6.1.1: Import Bsal surveillance projects and associated data as projects are planned (e.g., SNAPS, discussed by Surveillance & Monitoring Working Group above) and completed and becomes available from Bsal Task Force partners, and independent researchers.

Priority B.6.1.2: Develop systems and networks to identify data from Bsal surveillance and science reports.

Priority B.6.1.3: Update and upload the world *Bd* database from *Bd*-maps.net and the Global *Bd* mapping project managed by the US Forest Service Pacific Northwest Research Station; this larger database serves as a model for Bsal, and lessons learned from *Bd* data management can improve Bsal data management efficiencies.

Priority B.6.1.4: Improve collaborations with Bsal Task Force Working Groups including Surveillance & Monitoring, Decision Science, Research, and Outreach & Communication for planning of sampling efforts, and to coordinate with their outreach for improved surveillance efficiencies across North America.

Goal B.6.2: Advance the functional capacity of the Amphibian Disease web portal amphibiandisease.org.

Rank: High

Rationale: Web Portal management and improvements are dynamic as security systems adapt, and technology advances enabling broader analysis applications and weaving of multiple datasets across websites.

Management Rationale: Improving portal functional capacities attract more users to the site, improving data inclusivity and expanding analytical capabilities. The result is enhanced understanding of Bsal current and future distribution and host taxonomic patterns of infection and disease. Such knowledge can guide management actions for Bsal mitigation or host species biosecurity.

Financial Needs: In-kind support from member agencies and institutions for time; grant proposals planned for temporary hiring of web programmers to advance web user interfaces and database management procedures. 10,000–25,000 USD per year for web-programmer upgrades; 70,000 USD for data scientist programmer for one yr.

Priority B.6.2.1: Facilitate data import and export procedures, add new analytical applications for access.

Priority B.6.2.2: Expand web programming to enhance analytical capacities, e.g., incorporate interactive web displays of data and correlation charts or modeling.

Priority B.6.2.3: Expand integration capacity among databases, for example to better integrate with the AmphibiaWeb portal with reciprocal links and access to geographic, habitat, or climate databases, and other enhancements such as to update data with current species taxonomic information.

2.7. Outreach & Communication Working Group

OBJECTIVE: To facilitate Bsal communication and outreach in North America.

Working Group Leads: Mark Mandica, Amphibian Foundation, USA; Alex Shepack, Florida International University, Miami, FL, USA

Past Working Group Leads: Jillian Farkas, University of South Dakota, Vermillion, SD, USA

Background: The Outreach & Communications Working Group produces a variety of Bsal-related outreach communication materials that include a website, fact sheets, press releases, and briefing papers. 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). In particular, the Working Group works with the TAC and its partners and other Working Groups within the North American Bsal Task Force to disseminate new Bsal information and products developed by the group and others.

In order to increase the efficacy of dissemination, the Outreach & Communication Working Group 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.

Whereas the Working Group is not tasked with conducting or publishing Bsal research, Working Group members do help to synthesize findings and communications within the Bsal Task Force for the purposes of producing lay articles meant to educate the public, and highlighting key messages in social media posts. The scope of outreach to date has focused on US national coverage, as there are salamanders at risk throughout the United States. Outreach may also be relevant for Canada and Mexico, but focused efforts outside the US have not yet been initiated by the Working Group.

Finally, the Outreach & Communications Working Group organizes, designs and publishes the Annual Report for the Bsal Task Force. This annual report summarizes activities, achievements and priorities for the TAC, its partners, and all Working Groups, and provides the current known status of the Bsal fungus. This report is published on the Salamander Fungus website and is widely available to both the general public and scientific-management community. The Outreach & Communications Working Group interacts with the TAC, partners, and all other Working Groups.

Goal B.7.1. Work with partners to disseminate syntheses, research, and other products or activities developed by the Bsal Task Force via social media and newsletter articles.

Rank: High

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*.

Management Relevance: This communication group is a dominant resource for management agency information about *Bsal*. Forging these networks of communication before *Bsal* is detected in North America will facilitate communication during a potential crisis such as a die-off, for a more measured and effective response.

Financial Needs: In-kind support from member agencies and institutions for time and limited travel; some grant proposals are anticipated, no estimates available at this time.

Priority B.7.1.1: Echo *Bsal* information and key findings of new scientific research publications in new publication outlets.

Priority B.7.1.2: Develop publication partner relations.

Priority B.7.1.3: Provide outreach for new programs and operations of the *Bsal* Task Force, such as development of SNAPS with colleges and universities (see Surveillance & Monitoring, above).

Priority B.7.1.4: Provide media releases of *Bsal* related information for relevant US national or North American events.

Goal B.7.2: Continue to build a network on social media to communicate developments within the *Bsal* Task Force.

Rank: Urgent

Rationale: Social media is a powerful communication tool that can reach different sectors of the community. Building a network of social media partners can expand the *Bsal* Task Force purpose and aims.

Management Relevance: Outreach via social media can connect broader citizen scientists and the public to *Bsal* efforts, enhancing awareness, and improving partnerships with natural resource specialists in institutions and agencies.

Financial Needs: In-kind support from member agencies and institutions for time and limited travel.

Priority B.7.2.1: Explore utility of additional media applications for outreach and communication.

Priority B.7.2.2: Build social media presence.

Priority B.7.2.3: Summarize new information for newsletters, blogs, and social media posts.

Goal B.7.3: Independently or with partners, produce Public Service Announcements on the presence and implications of *Bsal*.

Rank: Medium

Rationale: Multimedia communication can reach broader audiences.

Management Relevance: Multimedia communication can reach agency managers, and as partners with common goals, be used by them for their own messaging.

Financial Needs: In-kind support from member agencies and institutions for time.

Priority B.7.3.1: Work toward development of film or video outreach materials; identify videographers, develop material with TAC, produce video.

Priority B.7.3.2: Work toward development of additional flyers or briefing papers on Bsal; identify printer, develop materials and content with TAC, produce flyer, ship to partners.

Goal 2.7.4: Update the Bsal Task Force Website.

Rank: High

Rationale: The website is a go-to resource for North American Bsal information, including updated recent news.

Management Relevance: Natural resource managers access the website for rapid information used in their decision making.

Financial Needs: In-kind support from member agencies and institutions for time.

Priority B.7.4.1: Work with TAC members or partners including the Amphibian Survival Alliance to restructure and repopulate a more navigable website structure.

Priority B.7.4.2: Work with TAC members or partners including the Amphibian Survival Alliance to develop Working Group web pages and content.

Priority B.7.4.3: Work with TAC members or partners including the Amphibian Survival Alliance to develop a better Bsal publications list for the website and maintain updates to this list.

B.8. Clean Trade Working Group

OBJECTIVE: To expand efforts to forestall potential human-mediated transmission of Bsal into North America via trade markets.

Working Group Lead: Josh Jones, Pet Industry Joint Advisory Council, Alexandria, VA, USA

Background: Although Bsal was first described from infections of Fire Salamanders in Europe, from the outset an Asian origin of the pathogen was implicated (Martel et al. 2013). Additional support has been forthcoming that 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). In Europe, infections were first observed in the Netherlands (Spitzen-van der Sluijs 2016), followed by its discovery in Belgium and Germany (Stegen et al. 2017, Lötters et al. 2020), and most recently in Spain (Martel et al. 2020). 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 waterbirds, and by spillover of infected individuals once held in captivity (Nguyen et al. 2017; Yuan et al. 2018). Spread of Bsal between continents including the emergence of Bsal in Europe is likely due to importation of infected species from locations where Bsal is endemic (Nguyen et al. 2017). 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. Clean Trade measures such as development of Bsal testing and certification procedures that traded animals and fomites in imports to North America are Bsal-free are likely the most effective proactive measure to forestall Bsal emergence in North America (Grant et al. 2017). This is Framework Action 1, above.

The Clean Trade Working Group was established in early 2020 in partnership with the Pet Industry Joint Advisory Council, and its implementation goals and priorities are under development at this time. The mission of the stakeholder-led clean trade workgroup is for experts in the pet trade to organize and collaborate with subject matter experts, regulators, and academia to determine what the potential components of a workable, comprehensive clean trade program for North America may include, and to report these initial findings back to the Bsal Task Force Technical Advisory Committee. Currently, this working group has established a group of experts in the care, husbandry, and research of pathogens and diseases in reptiles and amphibians, including industry experts with knowledge of the volume and dynamics of the amphibian trade. This group has been collaborating on a regular basis to identify potential components for a comprehensive North American clean trade program to help prevent the invasion of Bsal to North America while allowing for the legal and responsible importation of animals for the pet trade. For example, components under discussion at this time include: a description of the current status of amphibian imports; a characterization of the US and Canada amphibian trade network; and identification of factors or processes that could reduce potential pathogen transmission, amplification, and spillover.

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