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Credit: Steven Gnam

Credit: Colleen Olfenbuttel

Credit: Bruce Lucas

Erratum

In Volume 14.1, we reported that the Donald H. Rusch Memorial Game Bird Research Scholarship was being continued through the generosity of Dr. Charles Meslow and was renamed to recognize his contribution. However, the name of the award won't be changed to include Dr. Meslow's name until after his death. We apologize for the error.

The Next Threat

HOW DO WE STOP FUNGAL DISEASE FROM DEVASTATING NORTH AMERICAN SALAMANDERS?

By Kenzie E. Pereira, Matthew J. Gray, Jacob L. Kerby, Evan H. Campbell Grant and Jamie Voyles

This article is based on discussions at the first North American Symposium on Batrachochytrium salamandrivorans*, which took place Sept. 30, 2019, at the joint conference of The Wildlife Society and the American Fisheries Society in Reno, Nevada.*

The rise of fungal diseases has taken a huge toll on wildlife populations across the globe, and it continues to challenge conservation efforts to protect endangered wildlife taxa, such as amphibians.

In the last 20 years, amphibians have experienced never-before-seen declines with nearly half of all species threatened by extinction. Although many factors are to blame, the fungal skin disease chytridiomycosis has helped drive the decline of over 500 amphibian species worldwide.

Chytridiomycosis is caused by at least two types of chytrid fungi, *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*). Since its discovery in the late 1990s, *Bd* has been found all over the world, with frogs taking the greatest hit. It was not until 2010, that a sudden population crash of European fire salamanders led scientists to discover a second chytridiomycosis-causing fungus, *Bsal*. In contrast to *Bd, Bsal* is deadliest to salamanders. Given the notoriety of *Bd* and the large-scale impact it's had on the world's amphibians, scientists quickly acknowledged that *Bsal* could be the next great threat to North America's biodiversity.

The arrival of *Bsal* in Europe is presumed to have resulted from the globalized trade of infected amphibians. Though captive and wild salamander

Credit: Bruce Lucas

If *Bsal* appears in North America, biologists fear the eastern newt may play a role in spreading it.

populations in Europe have suffered from disease outbreaks caused by *Bsal*, no such outbreaks have yet been reported in North America. In 2016, in an effort to slow *Bsal's* spread into the U.S., the U.S. Fish and Wildlife Service listed 201 salamander species as injurious wildlife due to their potential to become infected with *Bsal*, which resulted in banning their importation and trade. Nevertheless, given that approximately 99% of amphibians still imported into the U.S. originate from *Bsal* endemic regions, such as Asia, and there are currently no regulations mandating the clean trade of pathogen-free amphibians imported into the U.S., the risk of *Bsal* to the North American continent is a serious concern.

Credit: Patrice Clemenza

▲ **Duquesne University researcher Kenzie Pereira, a member of the North American *Bsal* Task Force, uses skin swabs to test for *Bsal* and *Bd* in wild populations of eastern newt.**

The entry and establishment of *Bsal* in North America could have substantial impacts on amphibian biodiversity — especially salamanders. North America is home to approximately 350 different salamander species, making the continent a global hotspot for salamander biodiversity. Though *Bsal* can infect both salamanders and frogs, salamanders suffer the greatest rates of disease and death when exposed to the fungus. Newts (salamanders belonging to the family Salamandridae) are widespread throughout the U.S. and are especially at risk of dying from *Bsal*.

Preparing for battle

The presumed absence of *Bsal* in North America gives wildlife professionals a unique opportunity to prepare for *Bsal*'s arrival before it has a chance to cause population declines and disrupt native ecosystems. In 2015, scientists, wildlife managers and veterinarians joined forces during the first international working group at the U.S. Geological Survey Powell Center to form the North American *Bsal* Task Force with the goal of developing and organizing strategies to combat a North American *Bsal* invasion.

Currently, the *Bsal* Task Force has more than 100 members, divided among seven working groups. Each group is focused on a key element to understand, prevent and respond to the introduction and spread of *Bsal* in the U.S. The pet industry also has representation on the *Bsal* Task Force and is currently working with members to devise strategies for clean trade. In addition, groups like the Decision Science Working Group strive to bridge the gaps between scientists and wildlife agencies to improve the ability to respond to the *Bsal* threat.

While the North American *Bsal* Task Force website (salamanderfungus.org) provides several resources (including a *Bsal* Rapid Response Template) to help wildlife professionals quickly respond to suspected cases of *Bsal* in the wild, the development and implementation of specific strategies that can be used across a variety of outbreak scenarios requires the joint efforts of government agencies, conservation and wildlife health organizations, academia and professionals in the pet industry.

To promote such collaborations, the North American *Bsal* Task Force, in conjunction with TWS and the Wildlife Disease Association, organized a symposium on *Bsal* which took place during the 2019 Joint Conference of TWS and AFS. The symposium included 13 presentations on the current research of North American scientists, a panel discussion and a workshop hosted by USGS. Key outcomes from the symposium are summarized below, highlighting what is currently known about *Bsal,* the current gaps in knowledge and possible strategies for managing *Bsal* disease outbreaks.

What is *Bsal*?

Bsal is a fungus within the phylum *Chytridiomycota* that invades and feeds off of the skin of amphibians. Currently, there is only one known

Bsal strain — AMFP — that is highly deadly in salamander hosts. Frogs can also become infected by the AMFP strain but typically do not develop the disease, chytridiomycosis.

The life cycle of *Bsal* is divided into infective (free-living zoospores) and reproductive (stationary vase-like structures known as 'zoosporangia') stages, similar to *Bd*. However, *Bsal* is unique in that it produces two zoospore types: swimming zoospores (which lack a cell wall) and environmental zoospores (which have a thick cell wall). Where swimming zoospores can actively move through the environment over a limited distance, environmental zoospores float freely if they become dislodged from the host.

While *Bsal* seems to grow best at cooler temperatures (below 25°C), the discovery of *Bsal* infections in Vietnamese salamanders at 26 degrees Celsius suggests that additional studies are needed to fully understand the fungus's thermal thresholds.

Where did *Bsal* come from and where is it now?

Bsal is believed to have originated in Asia, where it exists among native amphibians without causing apparent harm. The fungus is also abundant among commonly traded Asian amphibians, including the fire-bellied toad (*Bombina spp*.), which makes up a large proportion of imported amphibians in the U.S.

To date, *Bsal* has been detected in six European countries including the Netherlands (where it was first discovered), Belgium, the United Kingdom, Germany, Sweden and Spain. Die-offs of wild salamanders are known in the Netherlands, Belgium, Germany and Spain.

Though monitoring efforts have been limited, *Bsal* has not yet been detected in captive or wild salamanders in the U.S. Research presented by Delia Basanta, of the Universidad Nacional Autónoma de México, also suggests that *Bsal* is currently absent from numerous sites in Mexico.

How is *Bsal* detected and diagnosed?

Scientists routinely swab amphibians and use a technique known as qualitative polymerase chain reaction (qPCR) to simultaneously screen amphibians for *Bsal* and *Bd* and estimate the severity of infections. A definitive diagnosis, however, typically requires a combination of qPCR and microscopic examination of the skin.

Just recently, scientists developed a microscopic method — *in-situ* hybridization — that allows researchers to distinguish *Bsal* from *Bd* in the skin of co-infected amphibians. While these methods are reliable and appropriate for small-scale diagnostic testing, more time- and cost-effective methods are needed to screen the millions of amphibians entering the U.S. each year.

Jesse Brunner, of Washington State University, described an alternative method of using environmental DNA to detect fungal DNA from the water used to hold amphibians and demonstrated

Credit: Todd Amacker

▲ **Focal lesions appear on the snout of a rough-skinned newt (*Taricha granulosa*) infected with *Bsal*.**

Credit: Todd Pierson

▼ **The Blue Ridge two-lined salamander (*Eurycea wilderae*) is one of several plethodontid salamander species susceptible to *Bsal*.**

that this method can be efficient and effective in detecting *Bsal*. Though the details of this method are in development, eDNA testing could facilitate the rapid screening of entire shipments of imported amphibians with minimal samples, thus making the implementation of clean trade programs more feasible.

What are the signs of chytridiomycosis?

Bsal chytridiomycosis has been called "death by a thousand holes." Heavy infection loads can result in focal necrotic skin lesions across the entire body. These lesions are often accompanied by abnormal behaviors that can include anorexia, convulsions, lethargy and loss of the righting reflex. Debra Miller, of the University of Tennessee, explained that clinical and anatomic responses of rough-skinned newts (*Taricha granulosa*) to chytridiomycosis include altered white blood cell ratios, protein levels and some electrolyte concentrations.

Credit: Kathi Bletz

University of Massachusetts Boston researcher Molly Bletz collects salamander skin secretions to investigate proactive strategies for combating *Bsal*.

Miller's findings imply that *Bsal* might kill hosts through a combination of impaired skin function and systemic processes, which may make infected amphibians fair game for opportunistic pathogens such as secondary bacterial infections.

Are all amphibians at risk?

Experimental studies show that not all amphibian species become infected or develop chytridiomycosis when exposed to the *Bsal* fungus. Such differences in susceptibility have been broadly categorized as: resistant (do not become infected), carrier (develop subclinical infection), vulnerable (develop clinical infection with little to no mortality) and highly vulnerable (clinical infection and high mortality).

Jonah Piovia-Scott, at Washington State University, and colleagues at the University of Tennessee and University of Massachusetts Boston tested the susceptibilities of 31 North American salamander species. While over half of the species tested became infected by *Bsal,* only those in the families *Plethodontidae* (lungless salamanders) and *Salamandridae* (newts) were deemed 'highly vulnerable.'

It is believed that the eastern newt (*Notophthalmus viridescens*) and several species of the genus *Taricha* (Pacific newts) may play a major role in the spread of *Bsal* if it arrives in North America. The ability of *Bsal* to infect many amphibian species will most certainly make it difficult to control the fungus in North America, as has been the case in Europe.

What do we know about Bsal susceptibility?

Amphibian susceptibility to *Bsal* chytridiomycosis is not fully understood but is likely influenced by numerous host, environmental and pathogen-specific factors. Previous studies show that amphibians with low susceptibility to *Bd* infection tend to produce skin secretions that are better at killing the fungus *in-vitro* — when tested in a test tube — compared to more susceptible species.

Following this principle, Molly Bletz, of the University of Massachusetts Boston, showed that the antifungal properties of skin secretions collected from wild salamanders (genera *Notophthalmus* and *Eurycea*) varied between and within species and could potentially be used as a tool for identifying high-risk species and populations. Although amphibian skin secretions contain mixtures of small peptides and large proteins, bacteria and numerous other compounds, scientists have predicted amphibian susceptibility to *Bd* based on the antifungal properties of the peptide component alone.

Using a similar methodology, Kenzie Pereira, of Duquesne University, found little evidence that the ability of peptides to kill *Bsal in-vitro* was related to *Bsal* susceptibility in four salamander species (genera *Ambystoma, Cryptobranchus, Desmognathus,* and *Plethodon*). Pereira also showed that the peptides often had different effects on the growth of *Bd* compared to *Bsal*. Collectively, these studies suggest that while there may be some overlap between the immune response of hosts to *Bd* and *Bsal*, it should not be assumed that results from *Bd* studies are translatable to the *Bsal* system. Further, Ana Longo, of the University of Florida, showed that despite mounting an immune response to *Bsal* infection, the eastern newt (*N. viridescens*) does not typically survive *Bsal* encounters, further suggesting the high risk of this common species to *Bsal*.

On a related topic, other presenters, including Davis Carter, of the University of Tennessee; Doug Woodhams, of the University of Massachusetts; and Louise Rollins-Smith, of Vanderbilt University, showed that environmental temperature can influence the development of *Bsal* chytridiomycosis in eastern newts (*N. viridescens*), the production and antifungal properties of peptides and proteins (within skin secretions) and microbial communities on the skin. These results suggest that *Bsal* epidemics in North America might follow seasonal, latitudinal and elevational trends.

Lastly, Ana Longo, of the University of Florida, reported that co-infection of *Bsal* with *Bd* could increase mortality more than exposure to either fungus alone. Because the presence of *Bd* is widespread in North American amphibians, including populations of eastern newt (*N. viridescens*), the introduction of *Bsal* in *Bd* endemic areas may worsen adverse impacts and declines.

How is *Bsal* transmitted?

Bsal transmission can occur via direct (host-to-host) and indirect (environment-to-host) routes and is influenced by host density. Angela Peace, of Texas Tech University, combined knowledge on *Bsal* and *Bd* epidemiology within a mathematical framework showing the importance of various pathways and host densities on the transmission of *Bsal*.

Credit: Riley Bernard

▲ **Scientists evaluate and discuss potential management actions for *Bsal* during a U.S. Geological Survey workshop.**

Specifically, Peace found that while host-to-host contact may be the main transmission pathway in small populations (low host density), environmental transmission routes might dominate in larger populations (greater host density).

Matt Gray, of the University of Tennessee, also presented evidence that transmission of *Bsal* is density dependent. Gray found that increasing habitat complexity may reduce the number of times that hosts come into contact with one another and ultimately, *Bsal* transmission. Together, these studies suggest that disease intervention strategies aimed at disrupting *Bsal* transmission might be effective but require further evaluation.

Difficult decisions

Decisions on selecting and putting disease management actions into use are complicated and often made in the face of uncertainty. This is partly because such decisions require the cooperation of multiple management authorities and jurisdictions and compliance with laws and mandates. These decisions also involve the consideration of the overall objectives of wildlife management agencies, which can include minimizing financial costs as well as the ecological consequences if management actions are not correctly executed.

While research helps to reduce some of the uncertainties inherent to emerging diseases, the complexity of ecological systems makes it impossible to get rid of uncertainty entirely. To further complicate the decision process, potential nontarget impacts of disease management actions must also be carefully considered. The decision to simply delay management actions until additional information is received may, in itself, increase the risk for biodiversity loss. To minimize these risks, decisions should be made using a science-based management framework.

Despite the challenges and uncertainties associated with the emergence of *Bsal*, Evan Grant, a USGS researcher and principal investigator of the Northeast Amphibian Research and Monitoring Initiative, and Riley Bernard, a postdoctoral research associate with Pennsylvania State University, have been working with several state and federal natural resource managers to help make decisions for reducing the risk of *Bsal,* should it be introduced, to the populations they manage.

What do we do now?

To help bridge the gap between resource managers and scientists, Grant and Bernard organized a workshop on behalf of the USGS to evaluate some of the proposed management actions in the following potential *Bsal* outbreak scenarios: 1) Newts in a vernal pond in the Northeast (genus *Notophthalmus*); 2) Pacific Northwest (genus *Taricha*); and 3) Stream salamanders (family *Plethodontidae*) in the Great Smoky Mountains National Park.

During the workshop, participating scientists were asked to give their opinions on expected impacts of proposed management actions on outcomes such as pathogen and host survival, nontarget ecological impacts and public acceptance of the method by stating the direction — increasing or decreasing — and magnitude of each action. Their responses were summarized and presented by Grant, highlighting a subset of possible preferred management actions to *Bsal* invasion.

Though we are far from overcoming the challenges and uncertainties associated with selecting possible actions to manage a *Bsal* disease outbreak, at the close of the workshop, it was clear that diverse outbreak scenarios will require different management actions. Further, the identification and implementation of effective management actions for preventing biodiversity loss while minimizing nontarget ecological impacts will require the continued collaborations among scientists, wildlife professionals and other stakeholders.

Never say die

The threat of *Bsal* is upon us. Under current regulations, *Bsal's* entry in the U.S. is inevitable — if it is not already here and has not been detected. To prepare for *Bsal* disease outbreaks, scientists need to continue working to understand the possible impacts of *Bsal* on North American ecosystems, identify high-risk regions and amphibian populations and develop disease management strategies that quickly contain and permanently remove *Bsal* from the environment or help salamander populations coexist with the fungus.

From Aug. 30 to Sept. 5, wildlife professionals will be gathering in Cuenca, Spain, at the International Conference of the WDA for the First Global Symposium and Workshops on *Bsal*. This meeting will continue the progress made during the symposium in Reno and will include the insightful experience of Europeans currently in the thick of the *Bsal* battle. (For information, go to www.cuenca2020.com.)

Through collaborations across countries and disciplines, we hope to limit the effects of *Bsal* on global biodiversity and ensure the survival and persistence of salamanders for many generations to come.

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Evan H. Campbell Grant, PhD, is a research biologist for the U.S. Geological Survey and principal investigator for the USGS' northeast region of the Amphibian Research and Monitoring Initiative.

Jamie Voyles, PhD, is an associate professor of biology at the University of Nevada.