

A European early warning system for a deadly salamander pathogen

Tariq Stark, An Martel, Frank Pasmans, Valarie Thomas, Maarten Gilbert, Annemarieke Spitzen

Introduction

The chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) was described in 2013 after the rapid decline of a Fire Salamander (*Salamandra salamandra*) population in the south of the Netherlands (Spitzen et al., 2013; Martel et al., 2013). This population experienced a 99.9% decline over a seven-year period with no signs of recovery. Soon after the description of *Bsal* more outbreak sites with massive population crashes were discovered in Belgium, Germany and in additional sites in the Netherlands (Spitzen et al., 2016; Dalbeck et al., 2018). In most cases Fire Salamander populations were affected but Alpine Newts (*Ichthyosaura alpestris*) and Smooth Newts (*Lissotriton vulgaris*) also suffered mortalities (Spitzen et al., 2016; Stegen et al., 2017). In 2017 *Bsal* was also detected on Great Crested Newts (*Triturus cristatus*) and Palmate Newts (*Lissotriton helveticus*) in Germany (Dalbeck et al., 2018) but without obvious clinical signs. *Bsal* is deadly to nearly all European urodelans (newts and salamanders) and poses a massive threat to European urodelan diversity (Martel et al., 2014).

Bsal originated in Asia and likely arrived in Europe via the trade in Asian salamanders and certain Asian anurans (frogs and toads) (Martel et al 2014; Laking et al., 2017; Ngyuen et al., 2017; Yuan et al., 2018). From these vectors it is believed *Bsal* spilled over to European salamanders and newts, which are naive to the pathogen, and most die soon after being infected. Recently Stegen et al (2017) found that Alpine Newts with low *Bsal* infection intensity

may persist with the fungus, and even clear the infection. This means that these newts can vector the fungus, as can some anuran species. *Bsal* has also been detected in populations of captive urodelans in Germany, the Netherlands, Spain and the United Kingdom (Cunningham et al., 2015; Sabino-Pinto et al., 2015; Fitzpatrick et al., 2016; Actieplan – België, 2017).

Legislation has anticipated halting the spread of the fungus via trade. The USA banned the interstate transport and import of salamanders and newts in 2016, Switzerland has banned the import, as has Hungary, and the European Union has recently announced animal health protection measures for intra-Union trade in salamanders in order to prevent further introduction of *Bsal* (<http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018D0320&from=NL>).

The current knowledge of the distribution of *Bsal* outside of its native range is incomplete. It might be present in more European countries than currently known. Early detection of *Bsal* induced population declines is therefore very important. Urodelans with small ranges and/or small population sizes are especially at risk. In order to stem the tide of amphibian population declines and prevent mass extinctions, the European Commission issued the tender ENV.B.3/SER/2016/0028, Mitigating a new infectious disease in salamanders to counteract the loss of European biodiversity.

General objectives

Bsal has devastating effects on European urodelans and measures to protect them from these are of the utmost importance. The general objectives of this project can be summarized as followed:

1. Delineate the current range of *Bsal* in Europe
2. Create an Early Warning System which allows the rapid detection of novel *Bsal* outbreaks
3. Development of an emergency action plan (short term)
4. Provide proof of concepts for sustainable long-term mitigation measures.

This article focusses mostly on the first and second objective. More on the third (www.bsaleurope.com/emergency-action-plans/) and fourth (www.bsaleurope.com/mitigation-and-reporting/) objectives can be found on www.BsalEurope.com.

Early Warning System Website

We created an online platform www.BsalEurope.com (Figure 1) in order to educate the European public about *Bsal*, with the aim of detecting novel *Bsal* outbreaks throughout Europe. This website replaces the previous website (<https://bsalinfoeurope.wixsite.com/eubsal-mitigation2017>). The

The screenshot shows the homepage of BsalEurope.com. At the top, there's a navigation bar with links to Home, What is Bsal?, Report cases, Bsal Diagnostic Centres, Public Awareness Material, Early Warning System, Publications, and Contact. Below the navigation is a large image of a yellow salamander. To the left of the image is a box titled "Salamanders and newts at risk" containing text about the threat of Bsal to European salamanders and newts, and a "Read more" button. To the right of the image is a sidebar with a logo for "GOUTTER REUKEMA" and a section titled "General objectives". Further down, there are sections for "Tweets" by @BsalEurope, "COMMISSION DECISION 2013/402/EU OF 26 JUNE 2013 concerning veterinary and zootechnical checks applicable to intra-Community trade in certain live animals and products made from the meat of such animals and amending Council Directive 91/621/EEC as regards the application of the principles governing the recognition of third countries for the purposes of the veterinary and zootechnical controls and authorising Member States to take certain measures in respect thereof" dated Mar 7, 2018, and another "Tweets" section by @BsalEurope.

Figure 1. Home page of the BsalEurope web site (www.BsalEurope.com).

BsalEurope web site provides general information on *Bsal* (www.bsaleurope.com/b-salamandriverans/), from pathogen characteristics, clinical signs and hosts, to the European distribution of *Bsal*, options for treatment and prevention. The map of the current known distribution of *Bsal* in Europe will be kept up to date. In addition, several public awareness materials (www.bsaleurope.com/public-awareness-material/) were created which aid in the early detection of *Bsal* outbreaks. Reporting dead and moribund animals is very important, therefore regional hotlines (www.bsaleurope.com/report-cases/) and *Bsa*/ diagnostic centres (www.bsaleurope.com/laboratories/) are listed on BsalEurope. Comprehensive lists of scientific and popular scientific papers can also be found on the website (www.bsaleurope.com/sample-page/) in addition to more helpful resources. BsalEurope will be updated on a regular basis, so be sure to bookmark it!

Public awareness materials

On BsalEurope several public awareness videos and documents can be found that were specifically created for this project: three videos and several informative leaflets. The first of the animated videos (www.youtube.com/watch?v=kss8B7V_zAA&t=1s) shows where *Bsal* originates from, outlines its effect on European urodelans and where you can report dead and moribund animals. The second animation (www.youtube.com/watch?v=-WgYZME-GA9Y) outlines best practices for captive populations: biosecurity measures, quarantining of newly acquired specimens and a call to report dead and moribund animals (figure 2). The third video (www.youtube.com/watch?v=i-WJtmPdkng) stresses the importance of adherence to a field hygiene protocol in order to reduce the risk of human-mediated pathogen dispersal. Both animated clips have subtitles in sixteen

European languages in order to reach a large (European) audience. The clips can be found on the Reptile, Amphibian and Fish Conservation Netherlands YouTube channel (www.youtube.com/channel/UCdzWX2q4OhU0KGx_kRcixig). The other public awareness materials are three easy-to-use leaflets on recognition of *Bsal* in urodelans (www.bsaleurope.com/recognize-sick-animals/), including FAQ's for fieldwork and captive collections), disinfection protocols for fieldwork (www.sossalamander.nl/LinkClick.aspx?fileticket=wilFeby6by8%3d&portalid=17&language=nl-NL) and heavy machinery (www.sossalamander.nl/LinkClick.aspx?fileticket=FAncbfR0tck%3d&portalid=17&language=nl-NL, figure 3).

Report Cases

In order to detect *Bsal* outbreaks and hence delineate the current range of the pathogen in Europe an Early Warning System is of the essence. If you have found a salamander or newt that is not the obvious victim of traffic or predation, then please report it to your nearest regional hotline (www.bsaleurope.com/report-cases/). Eight hotlines have now been established in eight European Union countries. The network of diagnostics centres (www.bsaleurope.com/laboratories/) in the European Union currently consists of fourteen laboratories in eleven countries. Ghent University centralizes all data on *Bsal* outbreaks and monitoring. Additionally, a European network of stakeholders that will report urodelan population declines is being developed.

If you notice suspicious deaths or sick animals, please take the following steps:

- Do not handle sick or dead amphibians with your bare hands
- Use your mobile phone or other device to take multiple photos of the animal (from all sides)
- Be sure to include photos of any obvious lesions which you notice on its body
- Make a note of the location, date, time, and the number of animals which are sick or dead
- In Europe it is prohibited to collect wildlife (alive or dead) from the environment, therefore please contact the relevant hotline as soon as possible for further action.



Figure 2. Screenshot of one of the animated videos.

Monitoring

The detection of disease-induced population declines relies heavily on long-term monitoring schemes. Several European NGO's developed programs where individual amphibian species, populations and communities are followed in long-term monitoring schemes. Long-term studies are extremely valuable to assess population trends and can act as an Early Warning System when populations are declining (especially rapid declines). Sharp declines can be a sign of the involvement of a pathogen, like *Bsal*, and follow-up research can quickly be deployed.

Are you interested in participating in such a program? Please contact one of the regional hotlines.

Contact and follow us

An Early Warning System only works when many organisations, professionals and volunteers work together. Questions about this project can be directed to Prof. Dr. An Martel of Ghent University via email (An.Martel@ugent.be) or via the contact form on the web site (www.bsaleurope.com/contact/). The project also has its own Facebook Page (@Bsalmandriverans) and Twitter account (@BsalEurope). **Be sure to follow us!**

Partners

This project is a collaboration of Ghent University (Belgium: Flanders), Natagora (Belgium Walloon), The Spanish National Research Council (Spain), Reptile, Amphibian and Fish Conservation Netherlands (the Netherlands), Centre d'Ecologie Fonctionnelle et Evolutive (France), Genoa University (Italy), Trier University (Germany), and Zoological Society of London (United Kingdom). This tender (ENV.B.3/SER/2016/0028 Mitigating a new infectious disease in salamanders to counteract the loss of European biodiversity) was issued by the European Commission.

Figure 3: Example of one of the leaflets on hygiene protocols.

Disinfection protocol fieldwork

Click on Tools, Sign, and Comment to access additional features.

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Background

This document provides simple but effective measures that can help limit the spread of fungi and viruses pathogenic to amphibians in disease free areas. The advice listed below only encompasses "standard" field research methods. In case of reintroductions, translocation of animals, etc. stricter hygienic requirements are in order.

Many emerging infectious diseases, among which the chytrid fungi *Batrachochytrium salamandrinorum* and *B. dendrobatidis*, but also ranavirus, currently pose a significant threat to amphibians in Europe. Anthropogenic spread of pathogens has been identified as a considerable threat to amphibian health. We encourage all biologists, researchers and volunteers to disinfect their field material.

This way, we can reduce the spread and 'buy' time while both field- and laboratory trials are run in order to counter/mitigate the effects of these disease agents.

Advice

- Only handle amphibians when absolutely necessary. There are no limitations in the field as long as precautionary measures are taken in account.
- Also take precautionary measures in account when you work with freshwater fish, aquatic invertebrates or aquatic plants.
- Always return amphibians to the exact location¹ where they were caught.
- When handling amphibians one needs to wear disposable (powderless) gloves. Nitril gloves are recommended. Non-perfumed hand sanitizer (which contains ethanol) is also effective for disinfecting your hands afterwards.
- All materials used on a location¹ need to be disinfected before using them at another site.
- Boots and wading suits that have been in direct contact with water or muddy soil need to be disinfected thoroughly.
- Park your vehicle preferably on paved road and not in soft, muddy soil or vegetation.
- Dead and sick amphibians can pose a high ecological risk. Only handle them with disposable gloves, report them to the proper authorities and if possible – and legally allowed to – take them with you (dead animals). Transport dead animals in two plastic bags in order to prevent leakage. Report dead and sick salamanders directly to your research institute.

Disinfect your field material

Handle dead amphibians with disposable gloves.

Help to prevent mass mortality.

¹ A location is defined as a unique pond or stream system that is not directly connected to other waters in the area.