Report: Tender ENV.B.3/SER/2016/0028

Mitigating a new infectious disease in salamanders to counteract the loss of European biodiversity

Ghent University, Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Avian Diseases (Prof. dr. A. Martel and Prof. dr. F. Pasmans)

Repile, Amphibian and Fish Conservation the Netherlands (RAVON) (dr. A.M. Spitzen-van der Sluijs, dr. M.J. Gilbert)

Agencia Estatal Consejo Superior De Investigaciones Cientifica (CSIC) (dr. J. Bosch)

Centre d'Ecologie Fonctionelle et Evolutive ((UMR 5175 CEFE and PSL-EPHE) (Prof. dr. C. Miaud)

University of Genova (UNIGE) (Prof. dr. S. Salvidio, Prof. dr. E. Grasselli)

Trier University (Prof. dr. S. Lötters, Prof. dr. M. Veith)

Zoological Society of London (ZSL) (Prof. dr. A. Cunningham)

Natagora (A. Laudelout, T. Kinet)

European Commission

Report: Tender ENV.B.3/SER/2016/0028

Mitigating a new infectious disease in salamanders to counteract the loss of European biodiversity

Ghent University, Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Avian Diseases (Prof. dr. A. Martel and Prof. dr. F. Pasmans)

Repile, Amphibian and Fish Conservation the Netherlands (RAVON) (dr. A.M. Spitzen-van der Sluijs, dr. M.J. Gilbert)

Agencia Estatal Consejo Superior De Investigaciones Cientifica (CSIC) (dr. J. Bosch)

Centre d'Ecologie Fonctionelle et Evolutive ((UMR 5175 CEFE and PSL-EPHE) (Prof. dr. C. Miaud)

University of Genova (UNIGE) (Prof. dr. S. Salvidio, Prof. dr. E. Grasselli)

Trier University (Prof. dr. S. Lötters, Prof. dr. M. Veith)

Zoological Society of London (ZSL) (Prof. dr. A. Cunningham)

Natagora (A. Laudelout, T. Kinet)

European Commission

Table of Content

Executive summary

The recent incursion of the chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) in Europe poses an unprecedented threat to urodele (salamanders and newts) diversity across Europe, with the possibility to drive species to extinction. Legal frameworks (Habitats Directive, Bern Convention on the Conservation of European Wildlife and Natural Habitats, Convention on Biological Diversity) oblige the European Union to preserve its biodiversity from such threats.

By funding this project, the European Union has taken significant steps in protecting its threatened amphibians from this disease. A pan-European Early Warning System, consisting of a network of one central lab, regional hotlines and 16 diagnostic labs in 12 EU countries, was developed to raise broad-scale awareness in a European network of stakeholders, centralize and coordinate efforts. The combination of active and passive surveillance resulted in the detection of 28 Bsal outbreaks in the Netherlands, Belgium, Germany and Spain, affecting at least six urodele species including species listed in annex IV of the Habitats Directive (*Triturus marmoratus* and *Triturus cristatus*). Detection of *Bsal* in nature is invariably associated with mortality events and the infection has been demonstrated to persist for at least ten years in an affected population.

The regional hotlines have proven to be essential in the detection of new *Bsal* outbreaks, after which active surveillance efforts were set up in order to delineate the extent of the outbreaks by surveillance of nearby urodele populations.

Emergence of *Bsal* in Spain at over 1000 km of the index outbreak site threatens the survival of Europe's most threatened newt (*Calotriton arnoldi*) and demonstrates the ability of the pathogen to cross large distances quickly. The clear link of this outbreak with released pet animals and the high prevalence of *Bsal* in private urodele collections stresses the risk of pathogen spillover and supports measures to ensure a "clean trade" (absence of pathogens) in amphibians.

A combined effort of authorities, management and scientists delivered proof that drastic and continued actions in the field may contain and even eradicate *Bsal* in natural systems through a combination of fencing, disinfection, host removal and active surveillance of a perimeter. Initiating mitigation measures quickly is key, stressing the importance of a functional early warning system and the ability to rapidly impose a response.

Development of a *Bsal* Action Plan enables the EU and EU member states to implement measures to prevent *Bsal* driven loss of urodele diversity. The plan provides prioritization of European urodele taxa, identifying 14 urodele taxa at high risk of extinction within 10 years after *Bsal* incursion, 13 of which are included in annex IV of the Habitats Directive. Both general and taxon specific actions are proposed that should minimize the risk of *Bsal* driven loss of urodele diversity in the EU.

Preventing *Bsal* introduction and fast elimination after incursion in natural ecosystems through maintaining the Early Warning System, supporting clean trade of amphibians throughout the chain and implementing the action plan in the EU member states is strongly recommended to the EU to meet its obligations to protect its biodiversity.

Given the limited number of *Bsal* outbreaks known and the availability of mitigation tools, eradication of *Bsal* from Europe should be envisaged.

Background

Amphibians are the most threatened vertebrate group globally (as assessed by the International Union for Conservation of Nature, IUCN) with more than 40% of the species being at risk of extinction (Catenazzi, 2015). In Europe, 59% of amphibian species are in decline and 23% threatened (Temple and Cox, 2009).

Fungal diseases are known to exert a massive impact on populations of certain animal species in the wild. One of the best known examples is the amphibian skin disease chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). Being linked to the decline of at least 500 amphibian species, including 90 species extinctions, this fungus has caused the greatest biodiversity loss ever recorded due to a disease agent (Scheele et al., 2019). The most pronounced negative impact of *Bd* is noticed in specific regions (mostly mountainous) in the Americas, Australia and the Iberian Peninsula. Although *Bd* is widespread across Europe, *Bd* outbreaks seem patchily distributed and mainly confined to a limited number of species in the Iberian Peninsula and the Pyrenees (Olson et al., 2013; Lips, 2016).

In 2013, the novel fungus *Batrachochytrium salamandrivorans* (*Bsal*) was described, following the discovery of a massive crash of a fire salamander population in the Netherlands (Spitzen-van der Sluijs et al., 2013a; Martel et al., 2013). *Bsal* is believed to have recently spilled over from Asian urodeles in the pet trade to European salamanders (Martel et al., 2014; Laking et al., 2017). This fungus has been shown to be highly pathogenic to most urodelan taxa naturally occurring in Europe and currently causes mortality in wild salamander and newt populations in four EU member States: the Netherlands (first detected in 2013), Belgium (first detected in 2013), Germany (first detected in 2015) and recently dectected (2018) in Spain (Martel et al., 2013; 2014; 2020; Spitzen-van der Sluijs et al., 2016). Prior to the start of this project, *Bsal* was shown to be present in captive urodeles in the United Kingdom and Germany. In the Netherlands, where *Bsal* outbreaks were first described, the loss of individuals in a population of the fire salamander (*Salamandra salamandra*) over a 7-year period was estimated at 99,9%, with no signs of recovery so far (Spitzen-van der Sluijs et al., 2016). While the fire salamander is still common in other parts of the EU, similar population crashes in other urodelan species with small ranges and small population sizes are likely to significantly increase the extinction risk of these species of conservation importance through stochastic events.

Given the continued risk of human mediated *Bsal* introduction in naïve regions, it is likely that *Bsal* will soon approach some of the urodelan species-rich regions in Europe, with a number of narrow-ranged, endemic species of conservation importance (all of which are covered by Annex IV of the EU Habitats Directive 5). With the anticipated further spread and risk of focal introductions of *Bsal*, Europe is currently facing a new threat for these species of conservation importance which require urgent mitigation measures to be put in place. Preventive and sustainable mitigation measures should be designed to counteract the impact of the epidemic, both in the short and the long-term. If no prompt actions are undertaken, a rapid loss of biodiversity and even species extinctions can be expected all over Europe (Garner et al., 2016).

Although *Bsal* infections can be successfully treated in captive animals, measures to effectively control the infection in wild populations are not currently available (Canessa et al., 2018). Hence the importance of reducing the risk of importing novel *Bsal* strains into Europe, for example through trade bans or a regulated trade. However, this would not solve the pressing problem of the ongoing outbreaks in Europe and further intra-European dispersal. The development of mitigation scenarios for the regions with *Bsal* outbreaks is challenging and should aim at minimizing the negative effects of the pathogen on wildlife population health and to maximize population persistence. This could be achieved by both a reduction (ideally to zero) in the prevalence of *Bsal* in the population and a reduction of the *Bsal* load in infected animals. Applying measures that reduce infection load and prevalence could steer the current epidemic scenario, characterized by mass host die offs, towards host-pathogen co-existence (and, ideally, pathogen elimination) with host population survival. Mitigation measures should be directed towards influencing one or several of the corner stones of the disease triangle, defined as the interplay between host, pathogen and environment factors that determine the outcome of an infection. Their focus could thus be on reducing pathogen virulence, increasing host resistance and/or reducing environmental suitability for *Bsal*.

General objectives

Protecting European urodelan species against the devastating effects of *Bsal* requires urgent measures to be put in place. Besides preventing a further entry of this amphibian pathogen into Europe, these measures should aim at minimizing the impact of *Bsal* on European urodelan species. Any *Bsal* abatement plan requires a clear overview of the current extent of the *Bsal* distribution in Europe. Therefore, the first and second objectives of this contract are to <u>delineate the current *Bsal* range in Europe</u> and to <u>establish an Early Warning System (EWS)</u> that should allow rapid detection of novel *Bsal* outbreaks. Efficient mitigation requires apt measures both in the short and the longer term. Therefore, the third and fourth objectives of this study are to <u>develop a *Bsal* Action Plan</u> for the short term and to <u>provide proof of concepts for sustainable long-term mitigation measures</u>. The objectives, measures and results of the contract are presented on a dedicated website.

This contract is done in a multi-Member State collaborative effort with efficient centralisation and exchange of information and expertise, involving the most relevant stakeholders in each of the Member States concerned.

Specific tasks

Task 1: Delineating the current range of Bsal in Europe

At the beginning of the project, outbreaks of *Bsal* were known from the Netherlands, Germany and Belgium (Spitzen-van der Sluijs et al., 2016) (Figure 1 and Table 1). Key to any attempt to mitigate the impact of *Bsal* is to acquire detailed information on its distribution in Europe. Here, we determined the actual current range of *Bsal* in Europe. Using prior knowledge of the known outbreak sites, the neighbouring regions were investigated for the presence of *Bsal* in the local salamander populations.

This task involved the following steps:

a) Collection of skin samples from fire salamanders to determine the extent of the current *Bsal* outbreak in Europe in all regions of Europe that are neighbouring the known outbreak localities

b) Quantifying *Bsal* DNA in the skin samples using established techniques

c) Examining dead specimens to establish the causative role of *Bsal* in observed mortality in those regions that are neighbouring the known outbreak localities

Methods

To delineate the *Bsal* range, we assessed its presence in skin swabs collected from fire salamanders (*S. salamandra*). This species is used as sentinel species given its high susceptibility to the disease (Martel et al., 2014) and the expected prevalence of *Bsal* in an infected population is relatively high (approx. 50%, Stegen et al., 2017).

The current distribution of *Bsal* was determined by collecting skin swabs from *S. salamandra* populations in Germany (51 populations), Belgium (30 populations) and France (30 populations). The envisaged number of 30 samples per population allows reliable (95% confidence) detection of 1 *Bsal* positive at a prevalence of 10%. Collecting 30 samples was not feasible in all populations examined due to low population density. Sampling sites were determined based on the up to date knowledge of *Bsal* outbreak sites at the start of the project (Figure 1 and Table 1). Sample collection was done in compliance with all relevant EU and national regulations. Proper hygienic measures were taken to ensure biosecurity of the sampling: all materials and clothing were decontaminated after a population had been sampled. Hygienic protocols were developed by the project partners (RAVON, Ghent University (UGent)) and a uniform hygiene protocol was provided to all partners and made publicly available (see www.bsaleurope.com and disinfection and hygiene protocols in Informative leaflets).

Identical protocols for the collection of skin swabs (Spitzen- van der Sluijs et al., 2013b) and the analysis of skin swabs (Blooi et al., 2013) were performed in all three countries by partners of the consortium (UGent, Belgium; Trier University, Germany; CEFE, France). In brief, duplicate skin swabs were taken from the skin of five fire salamanders per population. These samples were analysed using the above mentioned qPCR protocol in the country of origin and in the central laboratory (UGent), to ensure interlaboratory congruence.

When dead animals were found in the sampled regions, a qPCR on a skin sample and a gross necropsy followed by histopathology were performed to establish the causative role of *Bsal* in those mortalities.
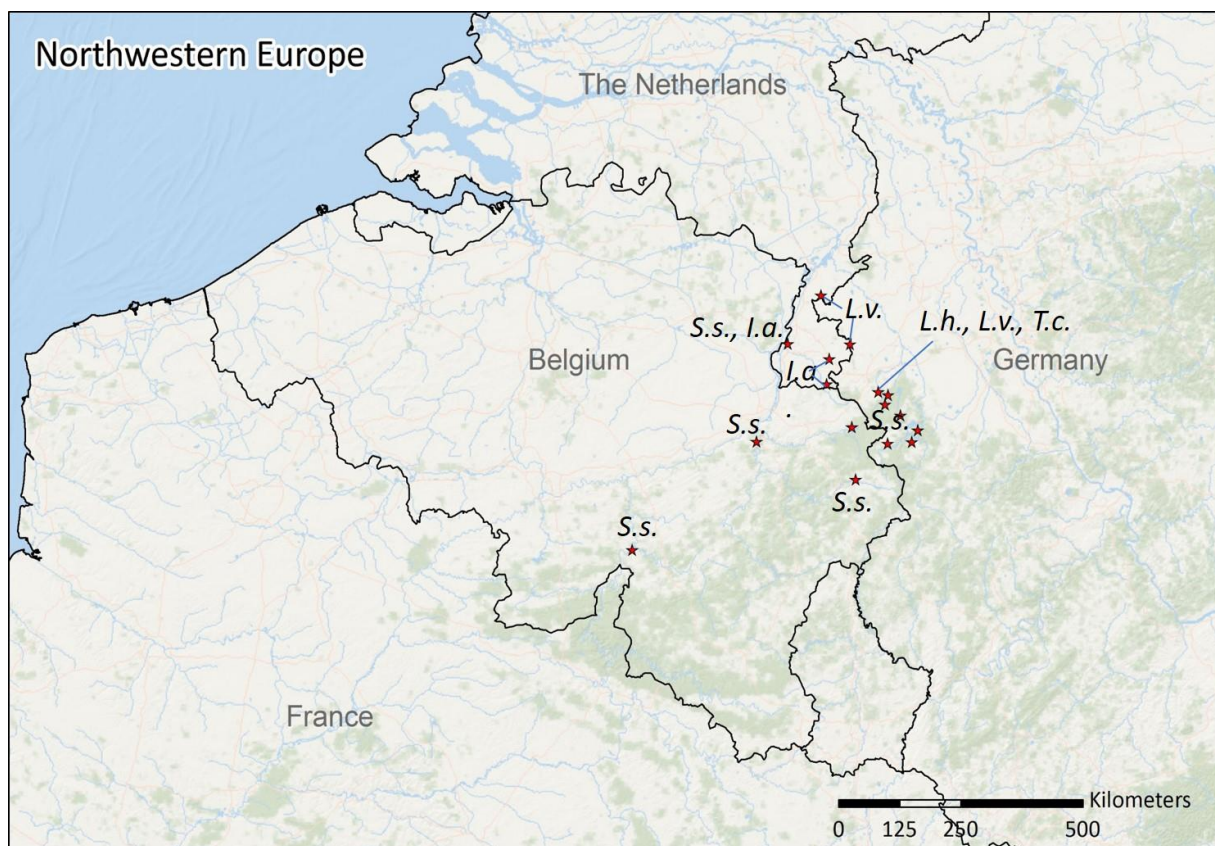
Figure 1. Sites in Europe where *Bsal* was detected prior to the commencement of the tender project. *I.a.* (*Ichthyosaurus alpestris*), *L.h.* (*Lissotriton helveticus*), *L.v.* (*Lissotriton vulgaris*), *S.s.* (*Salamandra salamandra*), *T.c.* (*Triturus cristatus*).

Table 1. Detailed information regarding outbreak sites prior to tender commencement. For each outbreak, the year of detection within a urodelan species, the outbreak location and coordinates (Latitude, Longitude) are presented.

Country	Year	Outbreak location	Latitude	Longitude	Species
Belgium	2013	Eupen	50.627727	6.08914079	*Salamandra salamandra*
	2014	Robertville	50.453241	6.109822	*Salamandra salamandra*
	2014	Liège (Sart-Tilman)	50.580567	5.570157	*Salamandra salamandra*
	2016	Dinant	50.216981	4.89063	*Salamandra salamandra*
Germany	2015	Belgenbach	50.573024	6.28390029	*Salamandra salamandra*
	2015	Fischbach-Vicht	50.735354	6.286172	*Salamandra salamandra*
	2015	Solchbach	50.703659	6.270637	*Salamandra salamandra*
	2016	Simonskall, Kalltall	50.667126	6.354061	*Salamandra salamandra*
	2016	Brockenberg	50.745226	6.234003	*Lissotriton helveticus, Lissotriton vulgaris, Triturus cristatus*
	2017	Haftenbach	50.6180	6.4489	*Salamandra salamandra*
	2017	Sauerbach	50.5791	6.4168	*Salamandra salamandra*
The Netherlands	2010	Bunderbos	50.90554	5.73955208	*Salamandra salamandra*
	2013	Bunderbos	50.90554	5.73955208	*Ichthyosaurus alpestris*
	2014	Pepinusbeekdal	51.066104	5.920909	*Lissotriton vulgaris*
	2014	Putberg	50.854796	5.96689543	*Ichthyosaurus alpestris*
	2015	Vijlenerbosch	50.77145	5.95063222	*Ichthyosaurus alpestris*
	2015	Wormdal	50.903916	6.07849514	*Lissotriton vulgaris*

Results

The sampling sites of the active surveillance in Belgium, France and Germany are presented in Figures 2, 3 and 4. More detailed information per country can be found in Annex 1.

In Belgium, France and Germany 702, 844 and 1189 amphibians were sampled in the context of the active surveillance respectively. In Belgium and France all tests yielded *Bsal* negative results. Apart from two *Bsal* positive populations in the Ruhrhöhen region of Germany, which were included in the active surveillance program after initial *Bsal* detection through passive surveillance, all populations tested negative for *Bsal* in Germany. Control swabs showed interlaboratory congruence between the central lab (UGent, Belgium) and the labs in Germany and France. The Belgian sampling revealed an incoherent pattern of *Bsal* distribution, which cannot be explained by population connectivity (for example: lack of evidence for direct spread to neighbouring populations, lack of evidence for dispersal through waterways). The latter was also observed in the Netherlands where a healthy fire salamander population was detected adjacent and connected to the initial outbreak site in Bunderbos (Spitzen-van der Sluijs et al., 2018a).

Figure 2. Active surveillance sampling sites of *S. salamandra* populations in Belgium.

Detailed information about the sampling location and sample size can be found in Annex 1_Belgium.

Figure 3. Active surveillance sampling sites of *S. salamandra* populations in France.

Detailed information about the sampling location and sample size can be found in Annex 1_France.
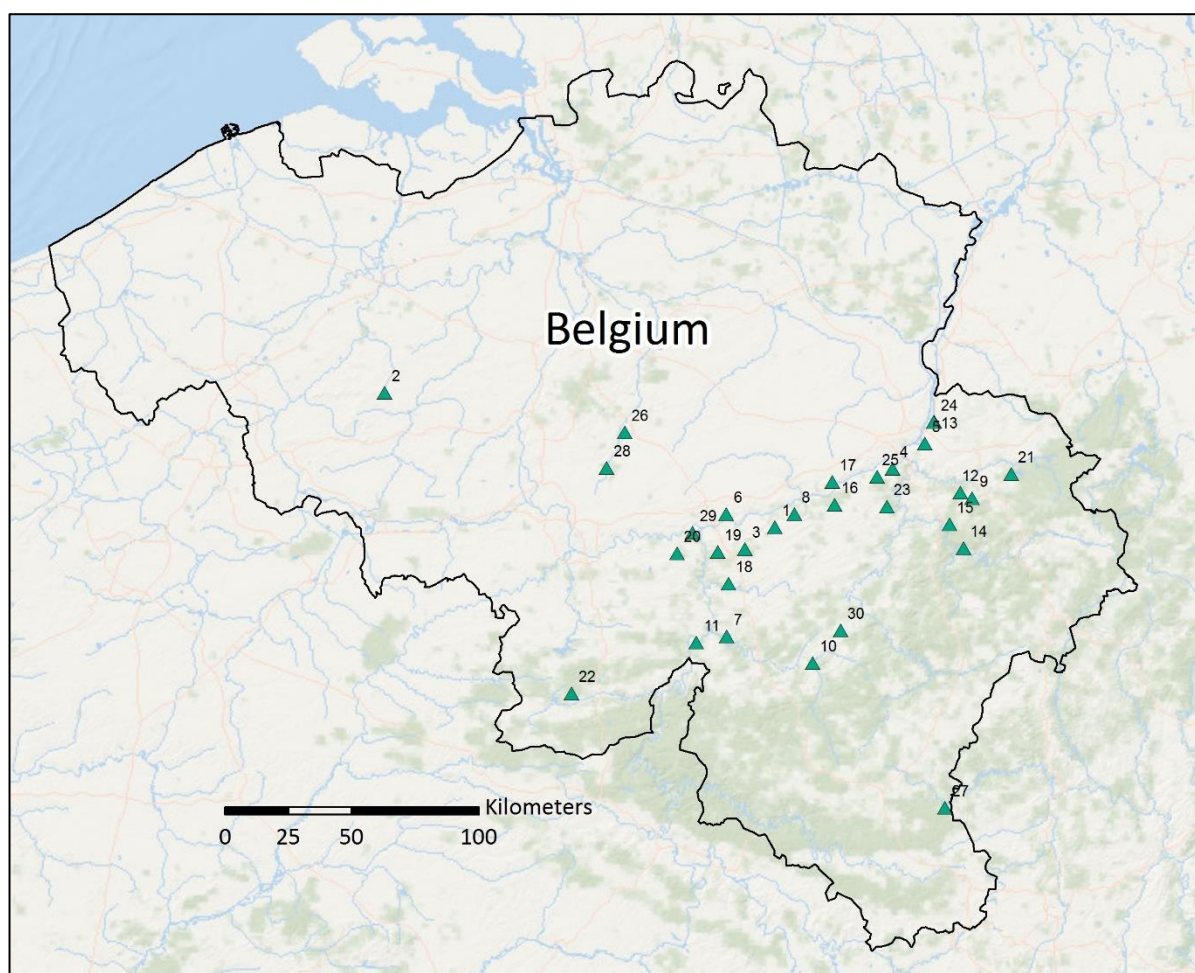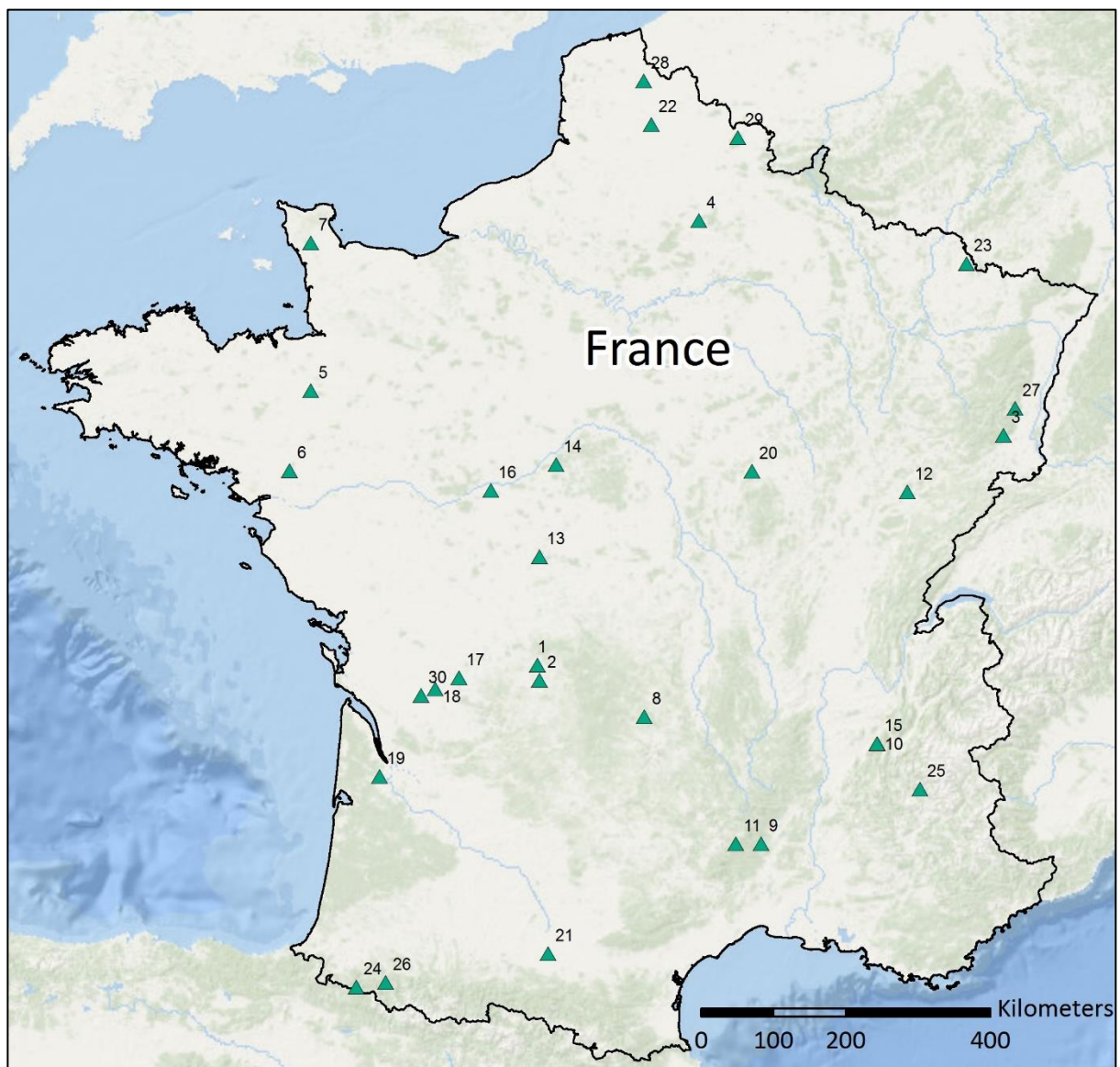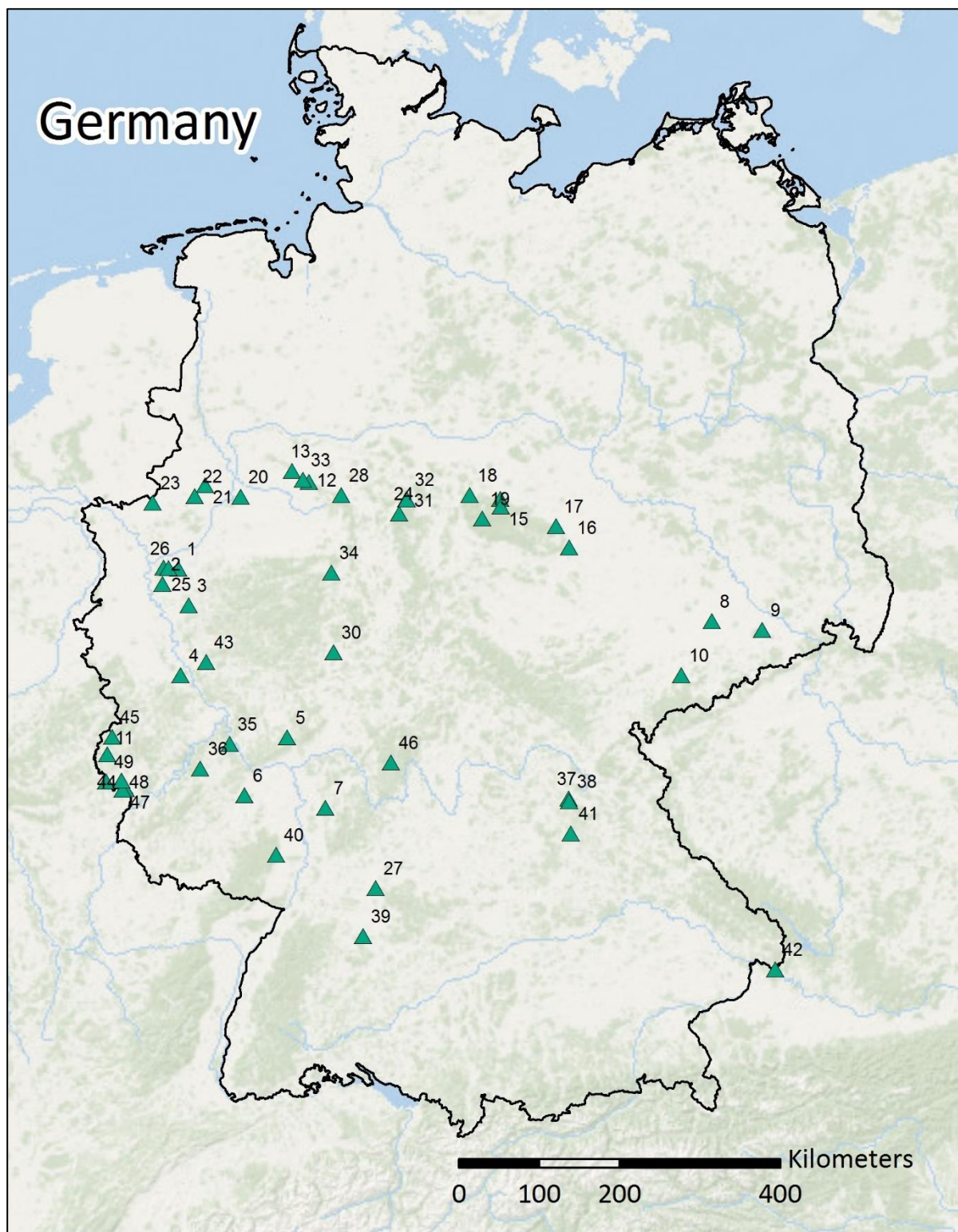
Figure 4. Active surveillance sampling sites of *S. salamandra* populations in Germany.

Detailed information about the sampling location and sample size can be found in Annex 1 Germany.

In subsequent years (not foreseen in the project proposal), different project partners have continued to collect active surveillance data through specific research projects financed through external funding (Figures 5 and 6, Annex 1_Continued). In **Belgium**, between the end of 2017 and mid 2019, 131 fire salamanders, coming from (nearby) locations with confirmed previous outbreaks, were tested for the presence of *Bsal*. Fire salamanders from one previously known outbreak site tested positive for *Bsal*. **Germany** focused on two confirmed outbreak sites, detected through the passive surveillance program, and seven areas close to these confirmed outbreak sites and sampled 230 fire salamanders. All populations tested positive for *Bsal*, in total 23 individuals being *Bsal* positive. **The Netherlands** continued to follow up the fire salamander population in Bunderbos, which remained positive in 2018, approximately a decade after the initial detection. In total 1322 salamander and newt species were sampled, 18 of which were positive for *Bsal,* belonging to different species (*Ichthyosaura alpestris, Lissotriton vulgaris, Salamandra salamandra).* **French partners** included different species in their active surveillance effort, sampling 243 urodeles belonging to different species (*Calotriton asper, Euproctus montanus, Salamandra corsica, Salamandra lanzai, Speleomantes strinatii)* from 13 different sites. All tested negative for *Bsal*. **Italy** and **Spain** focused on a variety of (sub)species (Italy: *Ichthyosaura alpestris, Lissotriton italicus, Lissotriton vulgaris, Salamandra atra aurorae, Salamandra lanzai, Salamandrina terdigitata, Triturus carnifex;* Spain: *Bufo spinosus, Calotriton arnoldi, Chioglossa lusitanica, Lissotriton boscai, Pleurodeles waltl, Rana temporaria, Salamandra salamandra, Triturus marmoratus*), some of which listed as endemic (sub)species on the Habitat Directives II or critically endangered on the IUCN Red list, coming from various populations spread over the countries. In total respectively 84 and 289 animals were tested for *Bsal,* all of them being negative.
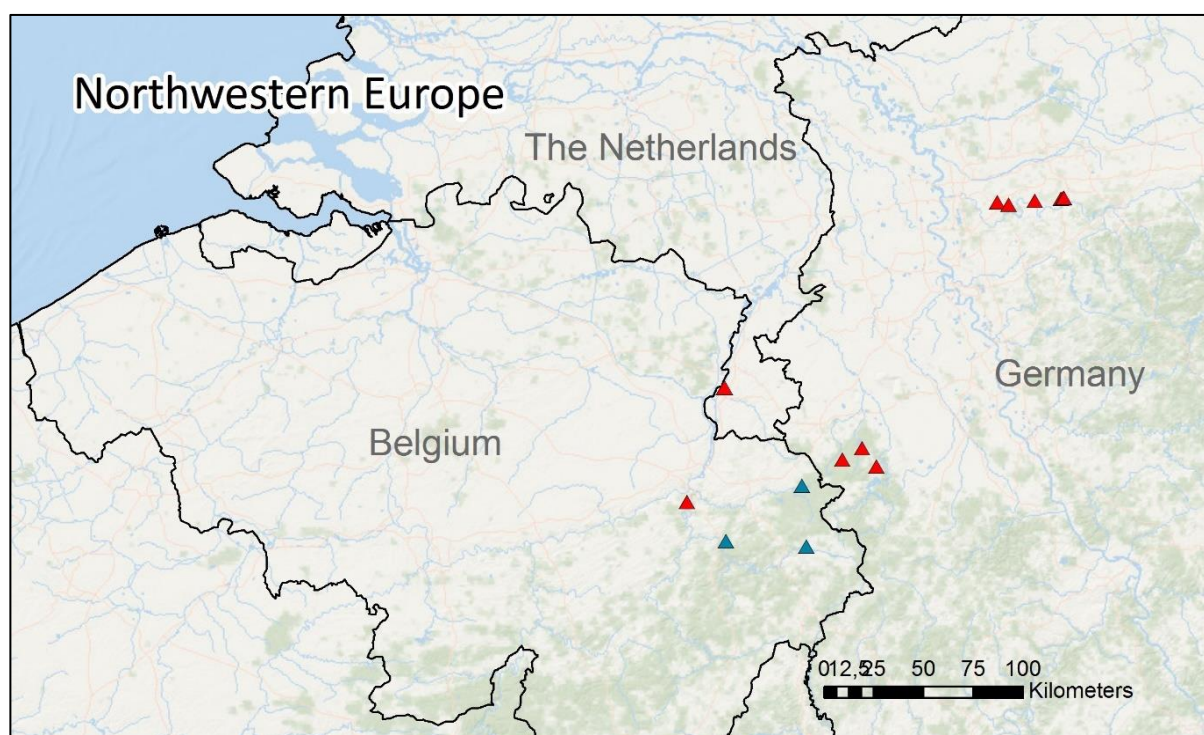
Figure 5. Continued active surveillance as a follow up of previously known outbreak sites and sites in the close proximity of these outbreak sites (Belgium, Germany, the Netherlands). *Bsal* positive (red triangles) and *Bsal* negative (green triangles) during continued active surveillance.
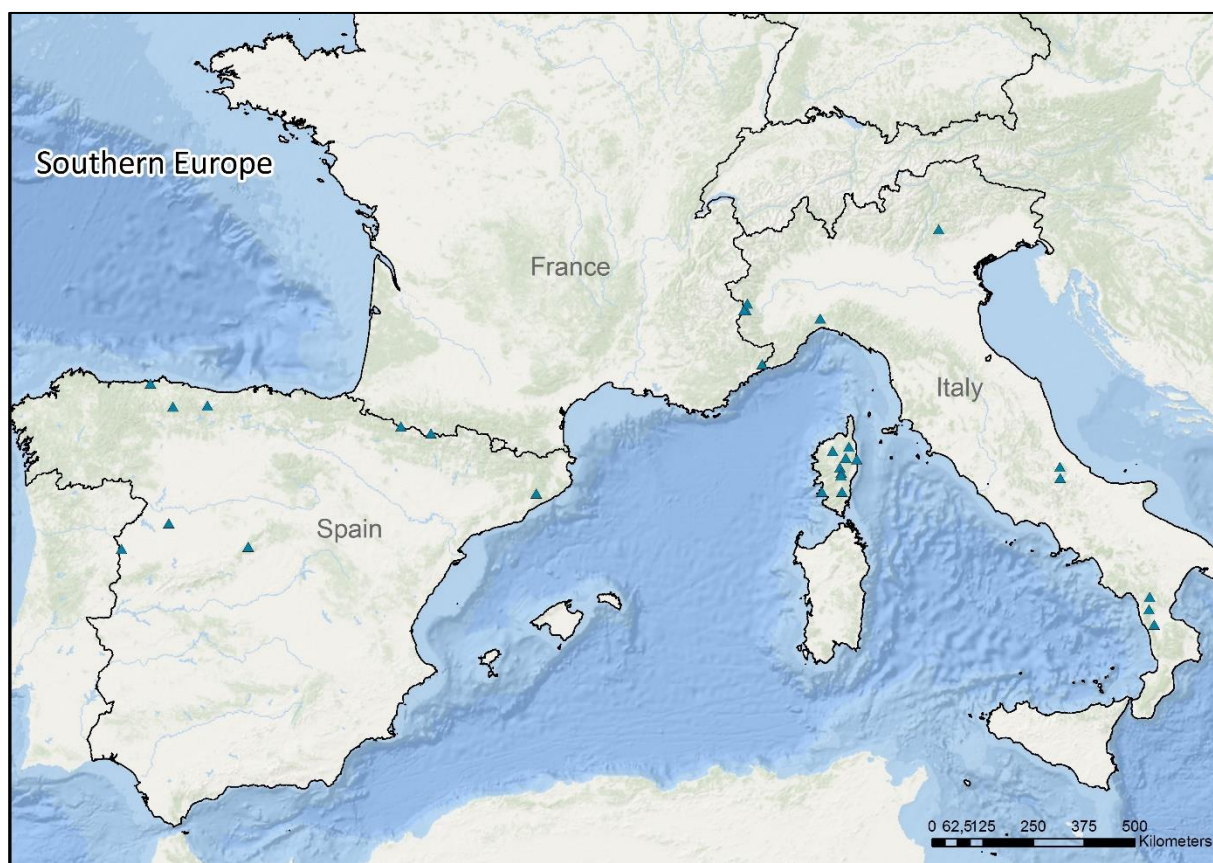
Figure 6. Continued active surveillance (France, Italy, Spain) with inclusion of other urodelan species. *Bsal* negative (green triangles) during continued active surveillance.

In conclusion: during the active surveillance performed specifically for the tender project, no new outbreak sites of *Bsal* were detected. However, during the continued active surveillance (performed in addition to the tender initiative) seven new *Bsal* outbreak sites were discovered in Germany, all located nearby previously known outbreak sites, detected by the passive surveillance program of the Early Warning System. All other new *Bsal* outbreaks were detected via the Early Warning System (see task 2) of the project.

Detailed information regarding outbreaks detected during the Tender project by active and passive surveillance is provided in Table 2 and Figure 7.

An overview of all sites in Europe where *Bsal* has been detected up to the present is provided in Figure 8.
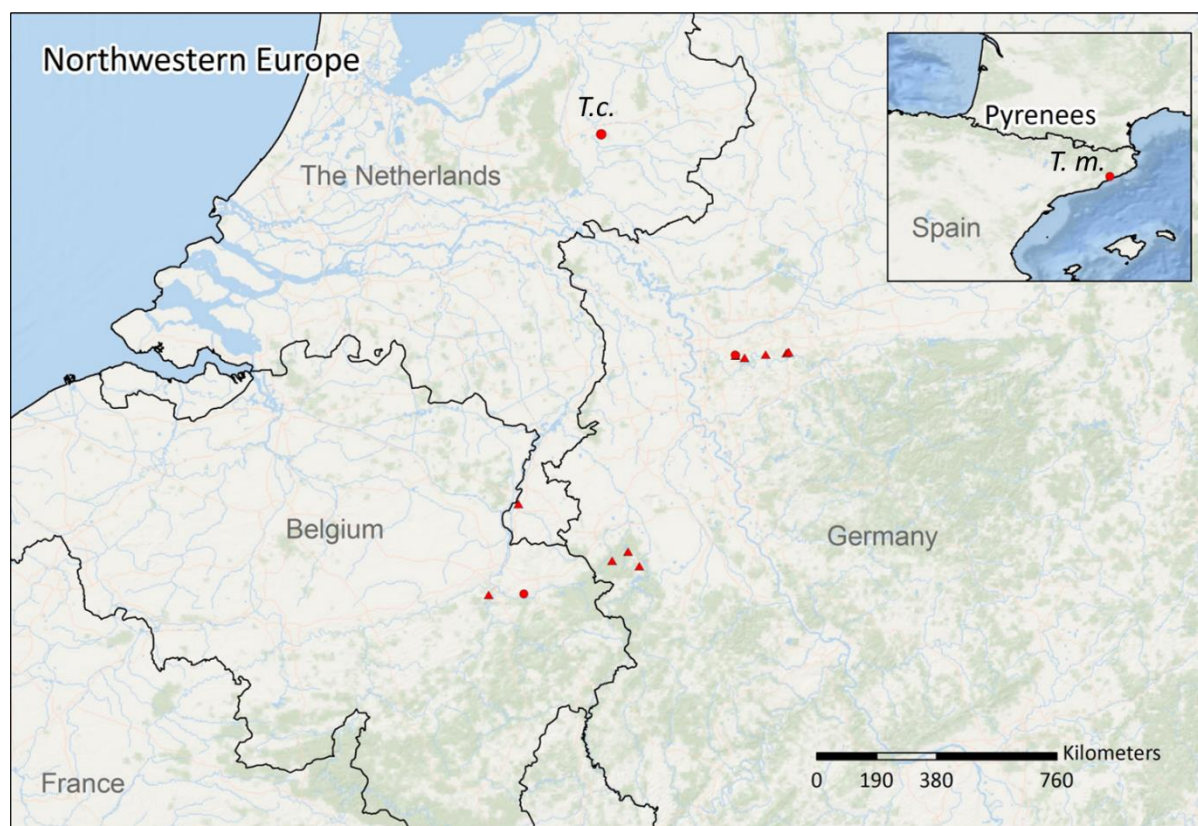
Figure 7. Sites in Europe (Belgium, Germany, the Netherlands and Spain) where *Bsal* was detected after commencement of the tender based on the results of active surveillance (indicated with triangles) (task 1) and passive surveillance (indicated with circles) by the regional hotlines (see task 2). *T.c* (*Triturus cristatus*), *T.m.* (*Triturus marmoratus*).

Table 2. Detailed information regarding outbreaks detected during the Tender project. For each outbreak, the year of detection within a urodelan species, the outbreak site and coordinates (Latitude, Longitude) are presented. Active (A) /Passive (P) surveillance

| Country	Year	Site	Latitude	Longitude	Species	A/P
Belgium	2017	Liège (Sart-Tilman)	50.580567	5.570157	*Salamandra salamandra*	A
	2020	Olne	50.582561	5.76903697	*Salamandra salamandra*	P
Germany	2017	Essen Fulerum	51.432957	6.965708	*Salamandra salamandra*	P+A
	2017	Essen Stadtwaldt	51.431025	6.968945	*Salamandra salamandra*	P+A
	2018	Zweifallshammer	50.682388	6.423373	*Salamandra salamandra*	A
	2018	Bochum, Botanischer Garten	51.442199	7.266148	*Salamandra salamandra*	A
	2018	Bochum Dahlhausen	51.435073	7.139507	*Salamandra salamandra*	A
	2018	Bochum, Klosterbusch	51.442880	7.270582	*Salamandra salamandra*	A
	2018	Bochum Lottental	51.440284	7.256134	*Salamandra salamandra*	A
	2018	Essen, Eschenstraße	51.423689	7.019866	*Salamandra salamandra*	A
	2018	Essen, Stadtwald	51.431025	6.968945	*Salamandra salamandra*	A
	2018	Nordeifel Solchbachtal	50.701780	6.270098	*Salamandra salamandra*	A
	2018	Weberbach	50.735089	6.359651	*Salamandra salamandra*	A
The Netherlands	2018	Bunderbos	50.9055	5.7396	*Salamandra salamandra*	A
	2019	Gorssel	52.203303	6.207423	*Triturus cristatus*	P
Spain	2018	Catalonia	41.615678	2.470966	*Triturus marmoratus*	P

15
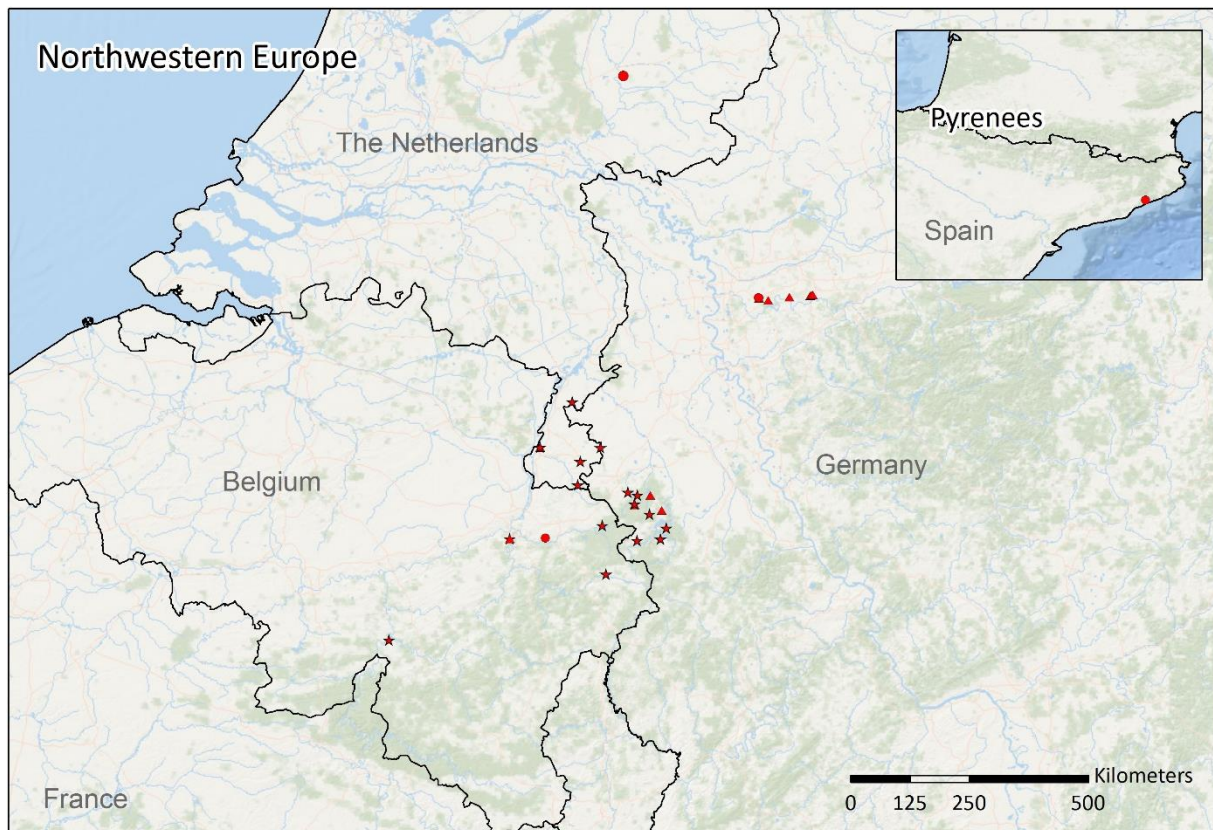
Figure 8. Sites in Europe (Belgium, Germany, the Netherlands, Spain) where *Bsal* has been detected up to the present. Prior to tender (indicated with stars), active surveillance (indicated with triangles), passive surveillance (indicated with circles).

Bsal in pet and invasive urodeles

Understanding the introduction routes of an emerging pathogen in wild living amphibian populations in Europe is essential in order to set up prevention and mitigation measures. *Bsal* has been shown to be endemically present in wild living urodele populations in east Asian countries (Martel et al., 2014; Laking et al., 2017; Beukema et al., 2018; Yuan et al., 2018). Since some of the urodele species are heavily traded internationally, introduction of *Bsal* most likely occurred through the international pet trade. Project partners discovered the presence of *Bsal,* in collections of pet urodeles in the Netherlands (2 collections), Belgium (1), Germany (10), the United Kingdom (4) and Spain (1) (Fitzpatrick et al., 2018; Sabino-Pinto et al., 2018) and a clear epidemiological link, associated with trade events, was established between the *Bsal* positive collections (Fitzpatrick et al., 2018). In Italy and Spain the presence of *Bsal* in captive collections was also investigated in respectively 68 and 70 individuals (covering a variety of amphibians species). All animals tested negative for *Bsal.*

In Spain, intentionally released pet newts that became invasive (*Triturus anatolicus*) were shown to be the likely source of infection for native newts (*Triturus marmoratus*, Martel et al., 2020).

Conclusions

The combination of active and passive surveillance resulted in accurate delineation of the current range of *Bsal* in Europe. Up to the present, the presence of *Bsal* has been identified in 28 natural populations of urodeles the Netherlands, Belgium, Germany and Spain.

Occurrence of *Bsal* in nature was invariably associated with field mortality in urodeles.

We demonstrated widespread occurrence of *Bsal* in collections of urodeles in captivity in the United Kingdom, Belgium, the Netherlands, Germany and Spain.

Recent incursion in Spain at over 1000 km from the index outbreak site in the Netherlands was associated with mass mortality of indigenous newts, threatening the nearby, critically endangered Monseny brook newt (*Calotriton arnoldi*). This outbreak is linked to intentional release of invasive pet newts (Martel et al., 2020).

Mortality in wild urodeles, caused by Bsal, was confirmed in *Salamandra salamandra* and *Triturus marmoratus* (Listed in annex IV of the Habitats Directive).

Mortality and population declines, caused by *Bsal*, are likely to occur in *Triturus cristatus* (Listed in annex IV of the Habitats Directive), given high *Bsal* loads in deceased specimens in the Netherlands, extensive mortality of closely related species in captivity (see Fitzpatrick et al., 2018) and the wild (*T. marmoratus*).

The contractual obligation for task 1 is fulfilled.

Task 2: Setting up a European Early Warning System (EWS)

This task involved establishing a European EWS according to the following steps:

a) setting up an EU central laboratory for *Bsal*. This laboratory centralised and reported data from *Bsal* outbreaks in Europe and, if necessary, confirmed the diagnosis of suspected cases.

b) establishing a European network of diagnostic centers. Each of which has the capacity for fast and accurate diagnosis of *Bsal* infections performing qPCR.

c) establishing a European network of research institutions, administrations, NGOs, and other relevant bodies that engage in population monitoring and sanitary surveillance of wild (and, if relevant, captive bred) urodelan populations.

Set-up of an EU Bsal central diagnostic lab

The EU *Bsal* central diagnostic lab was established at UGent. This lab houses all necessary infrastructure and expertise in pathology and microbiology and has the necessary competencies to build capacity for proper *Bsal* diagnosis in Europe. The responsibilities of the central lab were:

a) Confirming positive *Bsal* cases from project partners using necropsy, histopathology, qPCR and, where appropriate, sequencing (see below, regional hotlines). *Bsal* outbreaks in 4 countries (Belgium, Germany, the Netherlands, Spain) were confirmed in two host species (*Salamandra salamandra* and *Triturus marmoratus*). Causal role of *Bsal* in mortality of great crested newts (*Triturus cristatus*) could not be confirmed using histopathology due to post mortem decay.

b) Confirming qPCR screening results from project partners by testing a subset of samples (task 1). The lab assessed congruency between results in 350 samples, collected for task 1.

c) Providing the *Bsal* standards and a ring test for *Bsal* qPCR to diagnostic labs requesting *Bsal* diagnostic quality control testing. In total, 16 labs in 12 countries were involved and succceeded (see Annex 2).

d) Developing detailed sampling and hygiene protocols for *Bsal* fieldwork and amphibian husbandry. Hygiene protocols for *Bsal* fieldwork and amphibian husbandry have been developed. English, Dutch, French, Spanish and Italian versions can be found at http://bsaleurope.com/hygiene-protocols/ (Annex 6). Educational and instructional videos and Standard Operating Procedures (SOPs) on etiology, epidemiology, pathogenesis, pathology, sampling, diagnosis, treatment and prevention of *Bsal* were developed during the project and can be found on http://bsaleurope.com/videos/ (Annex 6).

e) Constructing and maintaining the EU *Bsal* project website (www.bsaleurope.com) (see task 5)

f) Centralising data about *Bsal* outbreaks and present and publish them on the *Bsal* project website (see task 5).

Set-up of an EU network of diagnostic centers capable of diagnosing the disease using qPCR diagnostics

We built capacity by developing an EU wide network of diagnostic labs with the capability to diagnose *Bsal* infections in as many European countries as possible. Therefore, UGent (*Bsal* central diagnostic lab) provided information on the set up of the diagnostic test in a lab, provided *Bsal* standards and set up a qPCR-ring test for quality control of diagnostic labs. For this qPCR-ring test, UGent provided the participating labs with several blinded samples, each containing a known number of *Bsal* genomic equivalents. After the test was carried out by the respective lab, results were returned to UGent to verify the performance of the lab. Performance was deemed acceptable if no false positive or false negative results were obtained and if positive samples were within a tenfold range of the known concentration.

Currently, there are 16 diagnostic labs in 12 European countries: Austria (1), Belgium (1), Czech Republic (1), Croatia (1), France (2), Germany (4), Italy (1), Slovenia (1), Spain (1), Sweden (1), UK (1), Poland (1) (Figure 9) which have successfully participated in the *Bsal* ring test. The list of the labs is presented in Annex 2 and has been made publicly available on the website http://bsaleurope.com/laboratories/.

Belgium:
1. Faculty of Veterinary Medicine, Ghent University
United Kingdom:
2. Zoological Society of London, Institute of Zoology
Germany:
3. Faculty of Geography/Geosciences, Trier University
4. Landesbetrieb Hessisches Landeslabor (LHL)
5. Laboklin
6. Zoological Institute, Technische Universität Braunschweig
Sweden:
7. National Veterinary Institute Uppsala
Poland:
8. Institute of Zoology and Biomedical Research, Jagiellonian University
Czech Republic:
9. Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno
Austria:
10. Veterinärmedizinische Universitat Wien
Slovenia:
11. Biotechnical faculty, University of Ljubljana
Croatia:
12. Faculty of Veterinary Medicine, University of Zagreb
Italy:
13. DISTAV – University of Genoa
France:
14. PSL Research University, CEFE
15. L'Ecole Nationale Supérieure Agronomique de Toulouse
Spain:
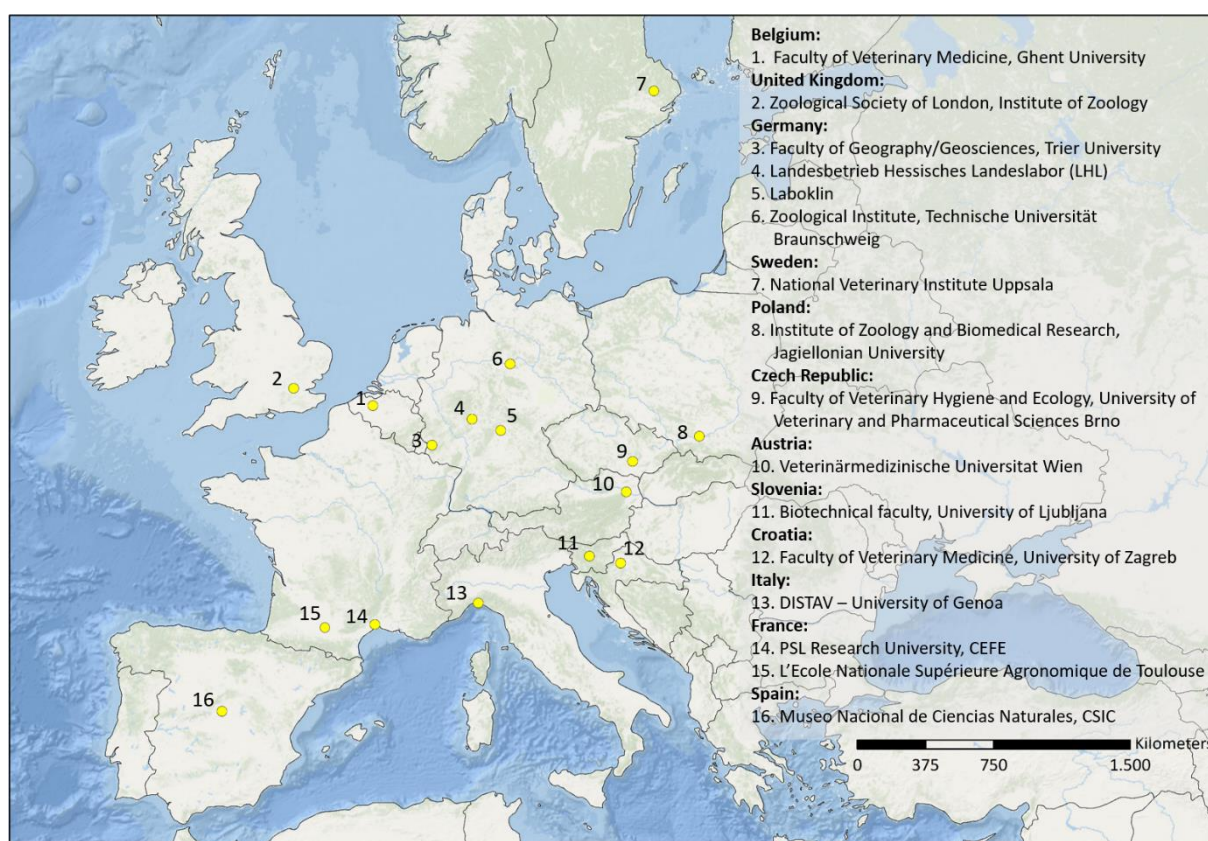16. Museo Nacional de Ciencias Naturales, CSIC

Figure 9. The current EU *Bsal* diagnostic network of labs that succesfully participated in the qPCR-ring test.

Establishment of a network of regional hotlines

The creation of a network of regional hotlines is crucial to establish an early warning system. The hotlines set up a passive surveillance system by spreading information about *Bsal* to regional stakeholders (research institutions, administrations, NGOs, relevant scientific societies, association of animal breeders, pet animal shops and herpetology enthousiasts) and collecting suspect cases.

Hotlines (contact information in Annex 3) have been established in seven European countries: Belgium, France, Germany, Italy, The Netherlands, Spain and the UK. Awareness of the hotlines was raised through various methods including presentations, animations, videos, flyers, project partners' websites and the *Bsal* project website (www.bsaleurope.com). Details on public awareness measures for the individual hotlines can be found in Annex 6.

The regional hotlines have been demonstrated to be crucial in detecting new outbreaks. A total of 539 dead amphibians were reported. (Detailed information can be found in Annex 4).

In **Belgium**, the hotline was contacted 26 times to report a total of 147 animals. One report coming from a mortality event in Spain. One location in Olne, where many fire salamanders were found dead, tested positive for *Bsal* and was localized close to previous outbreak sites in Belgium in the province of Liège.

In **France**, 77 amphibians from 32 locations were reported via the hotline, all resulting in negative *Bsal* tests.

In **the Netherlands**, the hotline was contacted 30 times to report a total of 72 dead amphibians. Two crested newts (*Triturus cristatus*), coming from one site, tested positive for *Bsal* by qPCR. Post mortem decay of tissues precluded further histopathological analyses.

In **Spain**, the regional hotline was contacted 19 times to report 49 animals. One of the amphibians was a captive-bred animal while the others were detected in the wild. All animals tested negative for *Bsal.*

In **Germany**, two mortality events in Essen reported through the regional hotline, with 19 fire salamanders being infected with *Bsal*. In addition, concerned citizens also used the hotline to solicit more information on *Bsal*.

In **Italy**, there were no dead amphibians reported via the hotline however two amphibian breeders after receiving some information on the disease, used the hotline to find more information on *Bsal* and initiated *Bsal* testing of their amphibian collections.

In the **UK**, 150 amphibians coming from 107 locations were reported via the hotline. All animals tested negative for *Bsal.*

In some cases, a hotline of a different country was contacted to report a disease or mortality event. The hotline of the Netherlands was contacted twice for Belgian cases. The hotline of Spain was contacted two times, once for a case in Morocco and once for a case in Austria. The hotline of Belgium was contacted once for a mortality event in Spain, resulting in the detection of 6 *Bsal* positive *Triturus marmoratus* individuals (Martel et al., 2020).

Set-up of a European network of stakeholders in the most relevant EU Member States, involved in the monitoring of salamander populations

RAVON created a network of stakeholders in 18 member states. Individuals and institutions in 23 countries were contacted and up to date, we have received responses from 18 European countries: Austria, Belgium Croatia, Estonia, France, Germany, Hungary, Italy, the Netherlands, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, UK and Ukraine. The list of responsive stakeholders is provided in Annex 5.

Disinfection protocols for field material and large equipment, as well as informative leaflets regarding *Bsal*-recognition leaflet, disinfection and hygiene protocols have been developed (Annex 6). These protocols have been well received and several stakeholders have made their own versions to reach their volunteers. For instance the French organisation LPO Franche-Comté provides information on amphibian diseases on their website (http://franche-comte.lpo.fr/index.php?m_id=20178) and they provide a disinfection protocol adapted from the one we have provided (Annex 6). Two animations (https://www.youtube.com/watch?v=kss8B7V_zAA and https://www.youtube.com/watch?v=-WgYZMEGA9Y), which have been translated in 17 languages (Bulgarian, Dutch, English, French, German Hungarian, Italian, Portuguese, Spanish, Serbian (cirillic and latinic), Slovenian, Swedish, Polish, Russian and Macedonian, Czech and Slowak) and an animation regarding the hygiene protocol used for fieldwork (https://www.youtube.com/watch?time_continue=3&v=i-WJtmPdkng&feature=emb_logo) (Annex 6 Educational videos) have been developed. The animations have been widely shared on the internet and have reached a large audience.

The Italian project partner constructed a Wikipedia page in Italian on *Bsal*: https://it.wikipedia.org/wiki/Batrachochytrium_salamandrivorans. Providing information in multiple languages strongly increases the outreach and herewith the impact of the information, and therefore an article on this *Bsal* project was published both in English and Spanish (Stark et al., 2018; Annex 6).

The contractual obligation for task 2 is fulfilled.

Task 3: Development of a *Bsal* action plan

This task involved the following steps:

a) Prioritisation of species, based on known or expected susceptibility to *Bsal*, inclusion in the Annexes of the Habitats Directive, range and population size (if available), etc.

b) Development of general and species specific protocols, potentially covering both for *in situ* and ex *situ* measures and standard operating procedures for prioritized species.

This task delivered one general Action Plan (covering issues horizontally applicable to all species) and species specific measures. The species-specific action plans cover all European urodele taxa.

Action plan

The *Bsal* Action Plan consists of three main parts: a conservation prioritization list for all European urodelan species based on a qualitative *Bsal* risk assessment for all Eurodelan species; a general *Bsal* mitigation action plan for all European urodeles, describing all actions needed prior to, during and after a *Bsal* incursion into a new region; and a species specific *Bsal* mitigation action plan for each European urodelan species, providing details about *Bsal* susceptibility, the risk *Bsal* poses to the persistence of the particular species and *Bsal* risk mitigation, as well as a proposal for the delineation of conservation units for each European urodelan species.

The *Bsal* Action plan is attached in Annex 7.

Prioritisation of species

To define conservation priorities a qualitative risk assessment was performed based on available knowledge and expert judgement for all European urodelan species and subspecies in order to assess the likely impact of *Bsal* on the persistence of these taxa.

Based on published and non-published evidence of Bsal susceptibility, the risk that *Bsal* poses to a particular urodelan taxon at the population level could be assessed with a certain degree of confidence, dependent on the availability of information. To assess the degree of risk (low, medium, high) at species and subspecies level, the species/subspecies distribution range size was combined with the population level risk at two time frames (10 years and 100 years post-incursion of *Bsal,* reflecting the immediately required short-term actions and the long-term risk for urodelan biodiversity when restraining from actions). The resulting risk on species and subspecies level increases with decreasing range sizes for Bsal susceptible taxa. Outcomes were assessed by expert judgment, explaining slightly deviant risk categories for some taxa.

Of the 40 European urodelan species, 30 (75.0%) are considered to be at high risk, five (12.5%) are considered to be at medium risk and five (12.5%) are considered to be at low risk at the population level. At the species level over a 10-year time frame, ten (25.0%) are considered to be at high risk of extinction, six (15.0%) are considered to be at medium risk and 24 (60.0%) are considered to be at low extinction risk. Over a time frame of 100 years, 16 (40.0%) species are considered to be at high risk of extinction, 16 (40.0%) are considered to be at medium risk and eight (20.0%) are considered to be at low extinction risk. For many of the assessed subspecies, the *Bsal* risk category is identical to, or higher than, the species-level risk category.

Development of general and species specific protocols

General protocol

The distribution of both urodelan species and Bsal transcends country borders, therefore coordinated actions between countries are needed to safeguard urodelan biodiversity. Each individual country, and the EU as a whole, has the responsibility to maintain a favourable conservation status for all urodelan species occurring within their territories. The overriding objective is the preservation of urodele biodiversity in Europe. In most cases, three broad objectives can be expected, (1) minimise the risk of *Bsal* introduction, (2) contain/eradicate the pathogen and (3) preserve the affected population.

Within this Action Plan, urodelan (sub)species are assessed based on the risk *Bsal* poses to their sustainable persistence. As scientific knowledge of *Bsal* advances, estimated risks may change. Individual countries and the EU should react as fast and flexibly as possible to these changes when needed, possibly with the support of a European *Bsal* Working Group.

When pathogens invade new species or geographic areas, several phases of the invasion process can be discerned (Langwig et al., 2015). This enables invasion phase-specific measures to be devised (Spitzen-van der Sluijs, 2018a). Ideally, the ability to enact these measures should be put in place in advance of *Bsal* incursion and decisions to implement them should be made when there still is an opportunity to act (Martin et al., 2012). The invasion of the European continent by *Bsal* is still at an early stage, so there is still time to adopt adequate pre-emptive actions and to develop plans to prevent the future spread of the pathogen, or to mitigate its impacts should spread occur. However, disease eradication should be envisaged in all cases, which requires a clear and long-term commitment of the EU and its member states.

Invasion phase-specific measures are key in the response to *Bsal* and these are listed below (Figure 10). Here, three invasion phases are considered: 1) pre-invasion phase (the fungus has not yet invaded the considered country or urodelan population), 2) invasion (epidemic) phase (the fungus has entered the country or population and causes either no added mortality (no or low susceptibility hosts) or the fungus causes mass mortality (high susceptible hosts)) and 3) established (endemic) phase (the fungus remains present albeit possibly at a low prevalence, however continues to cause mortality in susceptible hosts, threatening species conservation).

Obtaining as much relevant, quality data as possible is required in order to reduce uncertainties about the actions required and with regard to the best and most efficient allocation of resources. Bearing in mind the destructive global impact of *Bd* (the fungus closely related to *Bsal* that also causes catastrophic declines due to chytridiomycosis), we cannot afford to wait for post-hoc crisis management (Grant et al., 2017) with regard to *Bsal* if amphibian biodiversity is to be protected. This means we need to translate available scientific knowledge into practical management as pragmatically as possible. The control of infectious diseases often demands rapid decision-making in the face of scarce knowledge, limited time for learning, and challenges turning the available scientific knowledge into actions (Canessa et al., 2020; Grant et al., 2017). Yet, complexity and uncertainty are not excuses for inaction (Lindgren et al., 2012).
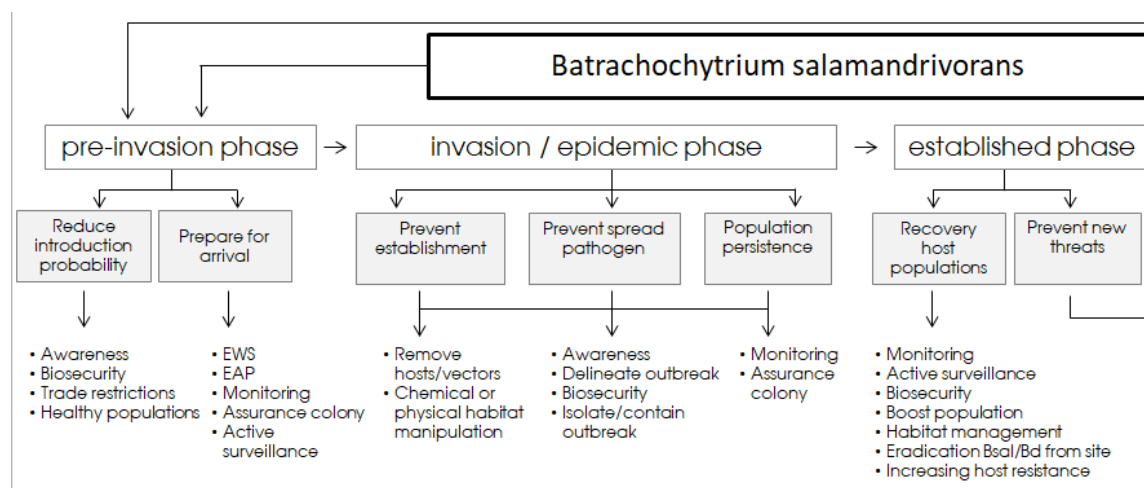

Figure 10. Illustration of the three invasive phases: pre-invasion (the fungus has not yet invaded the considered population), invasion or epidemic phase and the third phase, in which a population might go extinct due to *Bsal*, or the situation could become endemic in which the pathogen is present, at low prevalence, but continues to cause mortality (from: Spitzen-van der Sluijs, 2018b).

Here, the general actions for each *Bsal* risk category are summarised, while in the species-specific protocols, additional species or lineage-specific actions are listed, if applicable. In all cases, upon definitive diagnosis of a *Bsal* outbreak, disease eradication must be envisaged.

High risk
- Implement biosecurity measures to prevent the human-facilitated *Bsal* incursion.
- Ensure proper habitat management.
- Set up long-term population monitoring.
- Set up active and passive Bsal surveillance.
- Prepare and initiate *ex situ* measures.

Medium risk
- Implement biosecurity measures to prevent the human-facilitated *Bsal* incursion.
- Ensure proper habitat management.
- Set up passive *Bsal* surveillance.
- Set up long-term population monitoring, at least at locations with high likeliness of exposure to *Bsal*.
- Prepare *ex situ* measures.

Low risk
- Implement biosecurity measures to prevent the human-facilitated *Bsal* incursion.
- Ensure proper habitat management.
- Set up passive *Bsal* surveillance, at least at locations with high likeliness of exposure to *Bsal*.

Species specific protocols

This section covers *Bsal*-related conservation measures for all currently recognized European urodelan species. For each species, the major intraspecific lineages, often defined as subspecies, are described. Each intraspecific lineages should preferably be used as a conservation unit.

According to the *Bsal* risk status of a given conservation unit, different general actions are needed. These actions can be on the scale of population, intraspecific lineage, subspecies or species, depending on the conservation priorities.

The contractual obligation for task 3 is fulfilled.

Task 4: Proof of concept for long term and sustainable mitigation

This task involved the following steps:

a) providing an overview of potential sustainable mitigation measures

b) selection of the best candidate sustainable measure

c) development of a protocol for the application of the selected measure

d) providing proof of concept that the application of this measure protects a salamander community in an experimental setup.

a) Using an extensive literature survey, an overview of potential sustainable mitigation measures, their advantages and limitations, has been developed and can be found in Annex 8.

Below, a summary of these potential mitigation methods, which can be divided into pre- and post-exposure measures, is provided.

Pre-exposure measures:

Taking actions to prevent the introduction and spread of *Bsal* into naïve regions is currently considered as the most efficient control method available. This could be effectuated by:
- imposing trade restrictions/bans on amphibian trade and performing pre-import screening for *Bsal* in the live animal trade,
- screening captive amphibian collections and treating of *Bsal* positive collections in order to eliminate the *Bsal* reservoir in captive collections (striving for a clean trade),
- setting up and implementing monitoring, surveillance and early-warning systems to detect *Bsal* incursion into the wild as well as the expansion of its range following its introduction,
- implementing strict biosecurity measures to avoid anthropogenic spread of *Bsal* in between/into amphibian habitats,
- increasing host resistance through vaccination, bioaugmentation or selective breeding.

Post-exposure measures:

Once there has been a *Bsal* incursion to a novel site, potential mitigation methods focus on:

- decreasing the impact of *Bsal* by
  1) reducing the fungal load in the environment, through biological (e.g. micropredators), physical (e.g. pond drying) or chemical (e.g. decontamination treatments) manipulation of the environment;
  2) reducing the fungal load on the host species (e.g. *in situ* treatment of the amphibian host);
  3) safeguarding amphibian populations through bioaugmentation or vaccination
- preventing the further spread of *Bsal* by reducing the fungal load (e.g. removal of amphibian community),
- creating barriers to halt the spread of *Bsal,*
- setting up conservation strategies to prevent population extirpation,
- collecting information, vital for setting up conservation programs: setting up monitoring, (active and passive) surveillance systems to follow up on amphibian populations in *Bsal* outbreak sites and the expansion of the *Bsal* range.

In summary, the main objective of mitigating *Bsal* induced chytridiomycosis should be to preserve susceptible amphibian species and populations, and protect biodiversity. Each above mentioned approach has its benefits and limitations, however any single method is unlikely to accomplish the desired conservation outcome. A combination of methods may have the best chance of success.

In this respect, long-term, context-dependent, multi-faceted approaches are needed to successfully mitigate adverse effects of *Bsal*. These approaches should be initiated pre-arrival of the pathogen. While *ex situ* conservation and preventive measures aimed at improving biosecurity by banning or restricting amphibian trade may be implemented quickly, the establishment of *ex situ* assurance colonies, for species threatened with extinction, should be considered as soon as possible.

All information has been summarized and published in following article Thomas et al. (2019) see also Annex 8.

Thomas, V., Wang, Y., Van Rooij, P., Verbrugghe, E., Baláz, V., Bosch, J., Cunningham, A.A., Fischer, M.C., Garner, T.W.J., Gilbert, M.J., Grasselli, E., Kinet, T., Laudelout, A., Lötters, S., Loyau, A., Miaud, C., Salvidio, S., Schmeller, D.S., Schmidt, B.R., Spitzen-van der Sluijs, A., Steinfartz, S., Veith, M., Vences, M., Wagner, N., Canessa, S., Martel, A., Pasmans, F. (2019) **Mitigating *Batrachochytrium salamandrivorans* in Europe**. Amphibia-Reptilia. DOI:10.1163/15685381-20191157

b) Based on the literature overview and previous results, strategies that were not further examined due to lack of either estimated feasibility and/or efficacy include vaccination and *in situ* treatment.

c and d) The first option that has been explored is bioaugmentation ([Annex 9](#), Bletz et al., 2018). The principle of bioaugmentation is that microbial communities may confer protection against pathogen infection. We first examined skin microbiota of healthy, wild fire salamanders, isolated bacteria that inhibited *Bsal* growth, and studied whether addition of these bacteria would alter the course of a *Bsal* infection in fire salamanders.

> Wild, healthy fire salamanders were shown to maintain a complex skin microbiota, be it at very low densities. Some of the bacteria within this skin microbiota produced anti-fungal components. Through daily addition of bacteria inhibiting or killing *Bsal* on the skin of fire salamanders, we were able to increase the bacterial densities of these specific bacteria and slowing down the disease progression and mortality rate in fire salamanders. However, cessation of administration quickly resulted in very low numbers of skin bacteria. Thus, although healthy fire salamanders in the wild do maintain bacteria inhibiting or killing *Bsal*, the salamander skin maintains bacterial communities at such low levels that they are not capable of protecting the salamanders against *Bsal* infection.

Overall, we conclude that bioaugmentation does not show promise for effective mitigation and is unlikely to be of use for *in situ* treatment of fire salamanders in the wild. These results were published in Bletz et al. (2018), see also [Annex 9](#).

> Bletz M.C., Kelly M., Sabino-Pinto J., Bales E., Van Praet S., Bert W., Boyen F., Vences M., Steinfartz S., Pasmans F., Martel A. (2018) **Disruption of skin microbiota contributes to salamander disease**. Proceedings of the Royal Society B. 285:20180758. http://dx.doi.org/10.1098/rspb.2018.0758

For the second proof of concept study, we used the natural disease outbreak in Spain to test a combined, rigorous mitigation approach. The **creation of barriers** holds some promise, given the persistence of a healthy fire salamander population at less than 1 km from the index outbreak in the Netherlands (Spitzen-van der Sluijs et al., 2018a) and the poor dispersal capacity of *Bsal*. This was combined with **host removal (focusing on the entire amphibian community)**, **habitat management** and **disinfection** and **biosecurity** measures during the *Bsal*-outbreak in the field in 2018 in Spain ([Annex 10](#)).

> *Bsal* emerged in the Montnegre i el Corredor Natural Park in Catalonia (NE Spain), causing mass mortality in indigenous marbled newts (*Triturus marmoratus*) and posing an acute threat to the survival of nearby populations of the critically endangered montseny brook newt (*Calotriton arnoldi*). Disease management was initiated shortly after *Bsal* detection in a close collaboration between policy (Catalan Government, the Barcelona Provincial Council (managers of the Montnegre i el Corredor Natural Park), Forestal Catalana S.A., Grup de Recerca de l'Escola de la Natura de Parets del Vallès (GRENP),

Centre de Recuperació d'Amfibis i Reptils de Catalunya (CRARC), and the Institute of Evolutionary Biology (CSIC-UPF)) and science.

Disease control included biosecurity, habitat management and disinfection, host removal and intensive disease surveillance throughout the park. The control strategy was based on a combination of a mitigation action previously used to combat *B. dendrobatidis* in Mallorcan midwife toads (Bosch et al., 2015) and by epidemiological models suggesting that removal of the host community is currently the only possible response to eliminate a *Bsal* outbreak (Canessa et al., 2018; 2019).

In summary the protocol used to mitigate *Bsal:*

1) Implementation of a strict biosecurity protocol to avoid the spread of emergent diseases within and outside the Park (Usage of a new pair of nitrile gloves for each individual amphibian. After each field visit, disinfection of all equipment that came into contact with the infected environment with a 1% Virkon® solution for at least 5 minutes).
2) Installation of a fence around the perimeter of the aquatic reservoir to stop people and mammals entering the infected area.
3) Installation of pit-fall traps with a drift fence around the nearly drained water point to capture amphibians moving into the water.
4) Installation of a hanging bird net around the water point to prevent birds from accessing the water and spreading the disease.
5) Disinfection of the environment of the nearly drained water point of the infected site with Chlorine.
6) Surveying the infected point to test and remove all amphibian specimens found.
7) Periodical control of the area performing sanitary controls in the surroundings of the infected area.

This approach resulted in containment but not eradication of *Bsal* in a two-year time frame. Continued efforts will be necessary.

The inability to eradicate disease in this case, even following detection and coordinated response using best practice, demonstrates the necessity of intercepting wildlife diseases at an early stage, before the invasion of natural systems. Despite the inability to eradicate the disease, we were able to temporarily contain the disease within the infected area. This case clearly demonstrates the necessity of early warning systems and the implementation of coordinated actions following emergency action plans that can be used immediately upon pathogen detection. Although the mitigation measures used in this case were already drastic, even more drastic measures, such as remediation of the terrestrial reservoir, are recommended.

Given the likelihood of spillover of infection from a captive source, we propose an integral chain management of trade-associated wildlife diseases, aimed at minimizing the probability of disease introduction.

Current evidence points to the role of the captive *Bsal* reservoir combined with amphibian movements as likely vehicle for further *Bsal* introductions in naïve regions. Extending the regulations for commercial trade (EU2018/1882) to include the private sector, now exempted from the legislation, is highly advised. Also the implementation of strict biosecurity protocols for any activities planned in amphibian habitats is encouraged.

Results were published in Martel et al. (2020), see also Annex 10.

Martel A., Vila-Escale M., Fernández-Giberteau D., Martinez-Silvestre A., Canessa S., Van Praet S., Pannon P., Chiers K., Ferran A., Kelly M., Picart M, Piulats D., Li Z., Pagone V., Pérez-Sorribes L., Molina C., Tarragó-Guarro A., Velarde-Nieto R., Carbonell F., Obon E., Martínez-Martínez D., Guinart D., Casanovas R., Carranza S., Pasmans F. (2020). **Integral chain management of wildlife diseases**. Conservation Letters. *e*12707. DOI:10.1111/conl.12707

The contractual obligation for task 4 is fulfilled.

Task 5: Presentation of the project results

Project website

The European *Bsal* project website https://Bsalinfoeurope.wixsite.com/eu*Bsal*mitigation2017 was created in 2017 and is on its second iteration (**www.bsaleurope.com**).
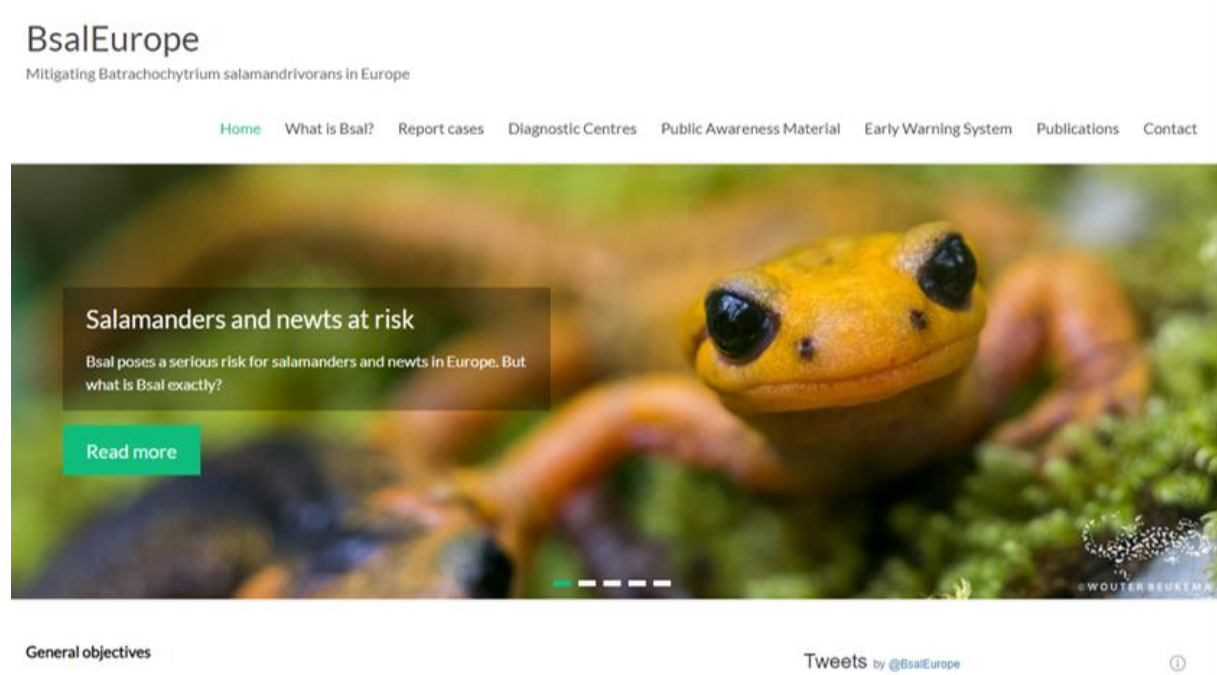
The website presents the contract objectives and measures, as well as the contract results, findings and publications related to these objectives and measures.

This dynamic website has evolved and continues to evolve as new information is discovered or published:

- General information about *Bsal* (characteristics, clinical signs, host range, treatment and prevention and discovery of *Bsal* in novel locations): http://bsaleurope.com/b-salamandrivorans/
- Diagnostic laboratories joining the *Bsal* diagnostic network: http://bsaleurope.com/laboratories/
- Public awareness material (informative and educational videos/cartoons, informative leaflets and flyers): http://bsaleurope.com/public-awereness-material/
- scientific and non-scientific publications: http://bsaleurope.com/sample-page/
- Information about the early warning system and where to report suspicious cases (hotline information): http://bsaleurope.com/early-warning-system/ and http://bsaleurope.com/report-cases/

Scientific information about *Bsal* and the tender are also available on project partner websites such as:

- https://www.gardenwildlifehealth.org/
- https://www.ravon.nl
- https://www.mncn.csic.es

and on stakeholder websites such as:

- https://www.natuurenbos.be/ (governemental agency: Nature and Forestsry Agency, Belgium)
- https://www.natuurpunt.be/ (NGO, Belgium)
- https://www.salamanders.nl/ (NGO, the Netherlands)
- https://www.arc-trust.org/ (NGO, UK)

Furthermore, scientific *Bsal* information is shared on various facebooksites (@ravonNL, @salamandrivorans, @wildlifehealth, @wildlifehealthghent).

Besides the BsalEurope website, the project also has a Facebook page and Twitter account, updating the followers on the project's progress and postings. The EU *Bsal* project's Facebook page has 747 (18th of February 2020) Facebook followers and its Twitter account has 298 (18th of February 2020) followers who keep informed of the project's progress and postings.

During conferences (SEH conferences, Dead or Alive – Towards a sustainable wildlife trade One World – One Health recommendations) results of the project were presented and the website www.bsaleurope.com was promoted.

Project meetings

Three project meetings were organised during the Tender Project:

20/09/2017 (12 – 13.30): SEH-conference in Salzbourgh

Attendance list

Frank Pasmans, Elena Grasselli, Sebastiano Salvidio, Stefan Lötters, Michael Veith, Valarie Thomas, Annemarieke Spitzen

Apologies for absence: Claude Miaud

External Specialist: Benedikt Schmidt

Summary of the meeting

- Presentation of a short overview of the current results of the Tender project.
- Division of the tasks between the different partners, based on the project objectives.
- Specifying sampling locations and efforts needed for the active surveillance program.
- Discussing the preparation of general documents (leaflets, video's,…).

02/09/2019 (13 – 17h): SEH-conference in Milan

Attendance list

Frank Pasmans, An Martel, Elena Grasselli, Sebastiano Salvidio, Stefan Lötters, Annemarieke Spitzen-Van der Sluijs, Maarten Gilbert, Andrew A. Cunningham, Lieze Rouffaer, Stefano Canessa
External Specialist: Benedikt Schmidt
Apologies for absence: Michael Veith, Arnaud Laudelout, Thierry Kinet, Claude Miaud

Summary of the meeting

- Presentation of a short overview of the current results of the Tender project
    - Active surveillance, diagnostic laboratory, regional hotlines, proof of concepts for sustainable mitigation measures, *Bsal*-website, instructional video's
    - Overview of tasks that still need to be done.
- A brief overview of the Action Plan was presented (Species prioritization, General Action Plan, Species Specific protocols)
    - All partners had the opportunity to provide their suggestions and comments on the currently available plan, which were discussed during the meeting.
    - The plan will be adjusted to the latest suggestions and comments and will be sent for revision to all partners before the final closure meeting.
- Setting a date for the closure meeting.

27/01/2020 (13 – 17h): University of Ghent

Attendance list

Frank Pasmans, An Martel, Elena Grasselli, Sebastiano Salvidio, Stefan Lötters, Annemarieke Spitzen-Van der Sluijs, Maarten Gilbert, Andrew A. Cunningham, Lieze Rouffaer, Arnaud Laudelout, Thierry Kinet
Skype-meeting: Claude Miaud
Apologies for absence: Michael Veith

Summary of the meeting

- Presentation of a short overview of the final results of the Tender project
    - All objectives of the Tender project have been met.
- A brief overview of the changes made in the Action Plan was presented.
    - All partners had the opportunity to provide their suggestions and comments on the currently available plan, which were discussed during the meeting. All partners gave their approval of the action plan (according to the final suggestions made during the meeting).
    - RAVON will take all suggestions into account for the final *Bsal* Action plan and will forward the plan to the external reviewer (Benedikt Schmidt).

The contractual obligation for task 5 is fulfilled.

Conclusion and recommendations

During this project, several key findings with regard to *Bsal* **epidemiology** in Europe were identified:

1) *Bsal* is widespread and has been identified in **28 amphibian populations in 4 countries** (Belgium, the Netherlands, Germany and Spain). *Bsal* presence was invariably associated with urodele mortality in the field.

2) There is **no evidence of of population recovery** after *Bsal* incursion, corroborating the risk of population extirpation or even species extinction events.

3) *Bsal* was demonstrated to be the causal agent of urodele mortality in **at least two urodele species**: fire salamanders (*Salamandra salamandra*) and marbled newts (*Triturus marmoratus*) and linked to mortality in at least great crested newts (*Triturus cristatus*). Both *Triturus* species are listed in annex IV of the Habitats Directive.

4) *Bsal* **is capable of persisting for at least ten years** in an infected ecosystem, as demonstrated by the detection of *Bsal* in mortality events in fire salamanders at the index outbreak in 2018. This finding renders spontaneous eradication of *Bsal* from the European continent unlikely.

5) *Bsal* **emerged in Spain in 2018** in a mortality event in wild newts (Martel et al., 2020). Emergence is strongly linked with invasive urodeles, released from captivity. This finding expands the *Bsal* range in Europe by approximately 1000 km and stresses the vulnerability of urodele rich regions that are either geographically isolated from the core *Bsal* outbreak region (e.g. the islands of Corsica, Sardinia, Karpathos) or currently deemed out of reach of natural spread of *Bsal* (e.g. the Alps, the Italian peninsula).

6) *Bsal* was shown to occur widely in **private collections of pet keepers** across Europe. Traffic in these animals poses a distinct risk of pathogen dispersal to naïve regions. Eliminating this captive reservoir is highly advisable and may be achieved by systematic screening and treatment and follow up of *Bsal* positive collections, complemented with trade restrictions. The European Union is strongly advised to work towards a "clean trade" (absence of pathogens) in amphibians.

7) The application of **rigorous mitigation measures** (fencing, disinfection, culling, surveillance) resulted in disease containment but not eradication. Eradication will likely require prolonged action and additional measures (in this case soil sanitation).

The early warning system with the regional hotlines has been demonstrated to be crucial in the early detection of new outbreaks, the prime example being the very recent detection of a *Bsal* outbreak in wild urodeles in Spain. Early disease detection in combination with emergency action plans ready to use and aimed at disease eradication minimizes the response time and increases the likelihood of pathogen eradication or containment. **Maintaining the early warning system will be key** in future attempts for disease mitigation.

All evidence combined suggests *Bsal* will not spontaneously disappear from European amphibian communities and further expansion and loss of urodele populations is likely. Even if costly, on the long term, preventive measures and installing radical mitigation measures shortly after detection of novel outbreaks is likely to be the most cost-effective option. Since we demonstrated that mitigation curbs *Bsal* disease, with the potential of disease eradication,

Europe has to meet its obligations to protect its threatened species against such incursions (as mentioned in Habitats Directive). It is therefore recommended that **each *Bsal* outbreak is met with a quick and drastic response, aiming at disease eradication**.

The tender consortium, complemented with European experts has developed a protocol describing the most appropriate **actions to be taken upon or before incursion of *Bsal***. This document provides a clear guideline that can be used as such by all relevant authorities.

The expansion of the *Bsal* range into the Iberian Peninsula, with a high level of endemic urodeles, stresses the importance of species prioritization for conservation and the development of species specific action plans. The European Association of Zoos and Aquaria has shown a distinct interest in participating in ex situ programs. Providing a clear overview of conservation priorities and protocols for the action plans has been developed and should allow fast implementation. We recommend **immediate implementation of the action plan** at least for the 14 taxa that were estimated to be acutely threatened within a 10 year timeframe after *Bsal* incursion (*Salamandra lanzai, Salamandra atra aurorae, Salamandra atra pasubiensis, Salamandra salamandra almanozris, Speleomantes ambrosii ambrosii, Speleomantes ambrosii bianchii, Speleomantes flavus, Speleomantes genei, Speleomantes supramontis, Speleomantes sarrabusensis, Lyciasalamandra helverseni, Lyciasalamandra luschani basoglui, Calotriton arnoldi, Triturus karelinii*).

Demonstrated persistence of *Bsal* in an affected ecosystem (in casu the index outbreak site) stresses the importance of developing **long term, sustainable mitigation**, in addition to preventive measures (biosecurity) and rapid response actions that target early eradication of *Bsal*. Developing such measures will be a challenge on the long term and requires an in depth understanding of the host-pathogen-environment interaction.

References

Beukema, W., Martel, A., Nguyen, T.T., Goka, K., Schmeller D.S., Yuan Z., Laking, A.E., Nguyen, T.Q., Lin, C.-F., Shelton, J., Loyau, A., Pasmans, F. (2018) Environmental contect and differences between native and invasive niches of *Batrachochytrium salamandrivorans* affect invasion risk assessments in the Western Palearctic. *Diversity and Distributions.* **24**, 1788-1801. DOI:10.1111/ddi.12795

Bletz, M.C., Kelly, M., Sabino-Pinto, J., Bales, E., Van Praet, S., Bert, W., Boyen, F., Vences, M., Steinfartz, S., Pasmans, F., Martel, A. (2018) Disruption of skin microbiota contributes to salamander disease. *Proceedings of the Royal Society B.* **285**(1885). DOI:10.1098/rspb.2018.0758

Blooi, M., Pasmans, F., Longcore, J. E., Spitzen-Van Der Sluijs, A., Vercammen, F., Martel, A. (2013) Duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in amphibian samples. *Journal of Clinical Microbiology.* **51**, 4173–4177. DOI:10.1128/jcm.02313-13

Bosch, J., Sanchez-Tomé, E., Fernández-Loras, A., Oliver, J.A., Fischer, M.C., Garner, T.W.J. (2015) Successful elimination of a lethal wildlife infectious disease in nature. *Biology Letters.* **11**(11). DOI:10.1098/rsbl.2015.0874

Canessa, S., Bozzuto, C., Grant, E.H.C., Cruickshank, S.S., Fisher M.C., Koella, J.C., Lötters, S., Martel, A., Pasmans, F., Scheele, B.C., Spitzen-van der Sluijs, A., Steinfartz, S., Schmidt, B.R. (2018) Decision-making for mitigating wildlife diseases: From theory to practice for an emerging fungal pathogen of amphibians. *Journal of Applied Ecology.* **55**:1987-1996. DOI:10.1111/1365-2664.13089

Canessa, S., Bozzuto, C., Pasmans, F., Martel, A. (2019) Quantifying the burden of managing wildlife diseases in multiple host species. *Conservation Biology.* **33**(5), 1131-1140. DOI:10.1111/cobi.13313

Canessa, S., Spitzen-van der Sluijs, A., Stark, T., Allen, B.E., Bishop, P.J., Bletz, M., Briggs, C.J., Daversa, D.R., Gray, M.J., Griffiths, R.A., Harris, R.N., Harrison, X.A., Hoverman, J.T., Jervis, P., Muths, E., Olson, D.H., Price, S.J., Richards-Zawacki, C.L., Robert, J., Rosa, G.M., Scheele, B.C., Schmidt, B.R., Garner, T.W.J. (2020) Conservation decisions under pressure: Lessons from an exercise in rapid response to wildlife disease. *Conservation Science and Practice.* 2:e141. DOI:10.1111/csp2.141

Catenazzi, A. (2015). State of the world's amphibians. *Annual review of environment and resources,* **40**, 91-119. DOI:10.1146/anurev-environ-102014-021358

Fitzpatrick, L.D., Pasmans, F., Martel, A., Cunningham, A.A. (2018) Epidemiological tracing of *Batrachochytrium salamandrivorans* identifies widespread infection and associated mortalities in private amphibian collections. *Scientific Reports.* **8**(13845). DOI:10.1038/s41598-018-31800-z

Garner, T.W.J., Schmidt, B.R., Martel, A., Pasmans, F., Muths, E., Cunningham, A.A., Weldon, C., Fisher, M.C., Bosch, J. (2016) Mitigating amphibian chytridiomycoses in nature. *Philosophical Transactions of the Royal Society B.* **371**(1709). DOI:10.1098/rstb.2016.0207

Grant, E.H.C., Muths, E., Katz, R.A., Canessa, S., Adams, M.J., Harris, M.C., Harris, R.N., Hossack, B., Huyvaert, K.P., Kolby, J., Lips, K.R., Lovich, R.E., McCallum, H.I., Mendelson III, J.R., Nanjappa, P., Olson, D.H., Powers, J.G., Richgels, K.L.D., Russel, R.E., Schmidt, B.R., Spitzen-van der Sluijs, A., Watry, M.K., Woodhams, D.C., White, C.L. (2017) Using decision analysis to support proactive management of emerging infectious wildlife diseases. *Frontiers in Ecology and the Environment.* **15**(4), 214-221. DOI:10.1002/fee.1481

Laking, A.E., Ngo, H.N., Pasmans, F., Martel, A., Nguyen, T.H. (2017) *Batrachochytrioum salamandrivorans* is the predominant chytrid fungus in Vietnamese salamanders. *Scientific Reports* **7**(44443). DOI:10.1038/srep44443

Langwig, K.E., Voyles, J., Wilber, M.Q., Fricj, W.F., Murray, K.A., Bolker, B.M., Collins, J.P., Cheng, T.L., Fisher, M.C., Hoyt, J.R., Lindner, D.L., McCallum, H.I., Puschedorg, R., Rosenblum, E.B., Toothman, M., Willis, C.K.R., Briggs, C.L., Kilpatrick, A.M. (2015) Context-dependent conservation responses to emerging wildlife diseases. *Frontiers in Ecology and the Environment*. **13**(4), 195-202. DOI:10.1890/140241

Lindgren, E., Andersson, Y., Suk, J.E., Sudre, B., Semenza, J.C. (2012) Monitoring EU emerging infectious disease risk due to climate change. *Science*. **336**(6080), 418-419. DOI:10.1126/science.1215735

Lips, K.R. (2016) Overview of chytrid emergence and impacts on amphibians. *Philosophical Transactions Royal Society B.* **371**. DOI:10.1098/rstb.2015.0465

Martel, A., Spitzen-van der Sluijs, A., Blooi, M., Bert, W., Ducatelle, R., Fisher, M. C., Woeltjes, A., Bosman, W., Chiers, K., Bossuyt, F., Pasmans, F. (2013) *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proceedings of the National Academy of Science of the United States.* **110**(38), 15325-15329. DOI:10.1073/pnas.1307356110

Martel, A., Blooi, M., Adriaensen, C., Van Rooij, P., Beukema, W., Fisher, M.C., Farrer, R.A., Schmidt, B.R., Tobler, U., Goka, K., Lips, K.R., Muletz, C., Zamudio, K.R., Bosch, J., Lötters, S., Wombwell, C., Garner, T.W.J., Cunningham, A.A., Spitzen-van der Sluijs, A., Salvidio, S., Ducatelle, R., Nishikawa, K., Nguyen, T.T., Kolby, J.E., Van Bocxlaer, I., Bossuyt, F., Pasmans, F. (2014) Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science*. **346**(6209), 630–631. DOI:10.1126/science.1258268

Martel, A., Vila-Escale, M., Fernández-Giberteau, D., Martinez-Silvestre, A., Canessa, S., Van Praet, S., Pannon, P., Chiers, K., Ferran, A., Kelly, M., Picart, M, Piulats, D., Li, Z., Pagone, V., Pérez-Sorribes, L., Molina, C., Tarragó-Guarro, A., Velarde-Nieto, R., Carbonell, F., Obon, E., Martínez-Martínez, D., Guinart, D., Casanovas, R., Carranza, S., Pasmans, F. (2020) Integral chain management of wildlife diseases. *Conservation Letters*. *e*12707. DOI:10.1111/conl.12707

Martin, T.G., Burgman, M.A., Fidler, F., Kuhnert, P.M., Low-Choy, S., McBride, M., Mengersen, K. (2012) Eliciting expert knowledge in conservation science. *Conservation Biology*. **26**(1), 29-38. DOI:10.1111/j.1523-1739.2011.01806.x

Olson, D.H., Aanensen, D.M., Ronnenberg, K.L., Powell, C.I., Walker, S.F., Bielby, J., Garner, T.W., Weaver, G., The *Bd* Mapping Group, Fisher, M.C. (2013) Mapping the global emergence of *Batrachochytrium dendrobatidis,* the amphibian chytrid fungus. *Plos One* **8**(2): e56802. DOI:10.1371/journal.pone.0056802

Sabino-Pinto, J., Veith, M., Vences, M., Steinfartz, S. (2018) Asymptomatic infection of the fungal pathogen *Batrachochytrium salamandrivorans* in captivity. *Scientific Reports.* **8**(11767). DOI:10.1038/s41598-018-30240-z

Scheele, B.C., Pasmans, F., Skeratt, L.FL., Berger, L., Martel, A., Beukema, W., Acevedo, A.A., Burrowes, P.A., Carvalho T., Catenazzi, A., De la Riva, I., Fisher, M.C., Flechas, S.V., Foster, C.N., Frías-Álvarez, P., Garner, T.W.J., Gratwicke, B., Guayasamin, J.M., Hirschfeld, M., Kolby, J.E., Kosch, T.A., La Marca, E., Lindenmayer, D.B., Lips, K.R., Longo, A.V., Maneyro, R., McDonald, C.A., Mendelson III, J., Palacios-Rodriguez, P., Parra-Olea, G., Richards-Zawacki, C.L., Rödel, M.-O., Rovito, S.M., Soto-Azat, C., Toledo, L.F., Voyles, J., Weldon, C., Whitfield, S.M., Wilkinson, M., Zamudio, K.R., Canessa S. (2019) Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*. **363**, 1459-1463. DOI:10.1126/science.aav0379

Spitzen-van der Sluijs, A., Spikmans, F., Bosman, W., de Zeeuw, M., van der Meij, T., Goverse E., Kik, M., Pasmans, F., Martel, A. (2013a) Rapid enigmatic decline drives the fire salamander (*Salamandra salamandra*) to the edge of extinction in the Netherlands. *Amphibia-Reptilia.* **34**: 233-239. DOI:10.1163/15685381-00002891

Spitzen-van der Sluijs, A., Martel, A., Hallmann, C., Bosman, W., Garner, T.W.J., Van Rooij, P., Jooris, R., Haesebrouck, F., Pasmans, F. (2013b) Environmental determinants promote recent endemism of *Batrachochytrium dendrobatidis* infections in amphibian assemblages in the absence of disease outbreaks. *Conservation Biology.* **28**(5), 1302-1311. DOI:10.1111/cobi.12281.

Spitzen-van der Sluijs, A., Martel, A., Asselberghs, J., Bales, E. K., Beukema, W., Bletz, M. C., Dalbeck, L., Goverse, E., Kerres, A., Kinet, T., Kirst, K., Laudelout, A., Marin da Fonte, L. F., Nollert A., Ohlhoff, D., Sabino-Pinto, J., Schmidt, B. R., Speyboreck, J., Spikman, F., Steinfartz, S., Veith, M., Vences, M., Wagner, N., Pasmans, F., Lotters, S. (2016) Expanding Distribution of Lethal Amphibian Fungus *Batrachochytrium salamandrivorans* in Europe. *Emerging Infectious Dis*eases. **22**(7), 1286-1288. DOI:10.3201/eid2207.160109

Spitzen-van der Sluijs, A., Stegen, G., Bogaerts, S., Canessa, S., Steinfartz, S., Jannsen, N., Bosman, W., Pasmans, F., Martel. A. (2018a) Post-epizootic salamander persistence in a disease-free refugium suggests poor dispersal ability of *Batrachochytrium salamandrivorans*. *Scientific Reports.* **8**(3800). DOI:10.1038/s41598-018-22225-9

Spitzen-van der Sluijs, A. (2018b). It takes three to tango. The impact of chytridiomycosis on native amphibians in the Netherlands. PhD thesis, Ghent University, Laboratory of Veterinary Bacteriology and Mycology, Faculty of Veterinary Medicine

Stark, T., Martel, A., Pasmans, F., Thomas, V., Gilbert, M., Spitzen-van der Sluijs, A. (2018) A European early warning system for a deadly salamander pathogen. *FrogLog*. 26(1):42-43.

Stark, T., Martel, A., Pasmans, F., Thomas, V., Gilbert, M., Spitzen-van der Sluijs, A. (2018) A European early warning system for a deadly salamander pathogen. *AArk Newsletter*. 42:12-14.

Stegen, G., Pasmans, F., Schmidt, B. R., Rouffaer, L. O., Van Praet, S., Schaub, M., Canessa, S., Laudelout, A., Kinet, T., Adriaensen, C., Haesebrouck, F., Bert, W., Bossuyt, F., Martel, A. (2017) Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. *Nature*, **544**(7650), 353–356. DOI:10.1038/nature22059

Temple, H.J., Cox, N.A. (2009) European Red List of Amphibians. Luxembourg: Office of Official Publications of the European Communities.

Thomas, V., Wang, Y., Van Rooij, P., Verbrugghe, E., Baláz, V., Bosch, J., Cunningham, A.A., Fisher, M.C., Garner, T.W.J., Gilbert, M.J., Grasselli, E., Kinet, T., Laudelout, A., Lötters, S., Loyau, A., Miaud, C., Salvidio, S., Schmeller, D.S., Schmidt, B.R., Spitzen-van der Sluijs, A., Steinfartz, S., Veith, M., Vences, M., Wagner, N., Canessa, S., Martel, A., Pasmans, F. (2019) Mitigating *Batrachochytrium salamandrivorans* in Europe. *Amphibia-Reptilia.* **40**:265-290. DOI:10.1163/15685381-20191157

Yuan, Z., Martel, A., Wu, J., Van Praet, S., Canessa, S., Pasmans, F. (2018) Widespread occurrence of an emerging fungal pathogen in heavily traded Chinese urodelan species. *Conservation Letters.* **11**:e12436. DOI:10.1111/conl.12436

Annexes

Annex 1: Localities sampled to delineate the current range of *Bsal* in Europe

For the tested fire salamander populations (Pop), the region, locality and coordinates (Latitude and Longitude) are provided, the number of swabs (n° swabs) taken per population on a certain date and the *Bsal* status are given.

Belgium

Pop	Date	Region	Locality	Latitude	Longitude	N° of swabs	*Bsal* status
1	20/02/2017	Namur	Coutisse	50.463226	5.126061	5	Negative
2	8/03/2017	Hainaut	Flobecq	50.765051	3.744878	11	Negative
3	18/03/2017	Namur	Gesves	50.413149	5.020961	28	Negative
4	2/05/2017	Liege	Seraing	50.594909	5.542909	30	Negative
5	19/05/2017	Liege	Liège (Nifiet)	50.651058	5.657861	21	Negative
6	2/06/2017_08/08/2017	Namur	Marche-Les-Dames	50.492301	4.955210	21	Negative
7	11/05/2017_1/07/2017	Namur	Furfooz	50.215800	4.956019	42	Negative
8	03/05/2017_8/09/2017	Liege	Huy	50.492792	5.195327	42	Negative
9	11/09/2017	Liege	Theux	50.528162	5.825166	31	Negative
10	13/09/2017	Namur	Jemelle, Piste cyclable	50.155227	5.261291	35	Negative
11	13/09/2017	Namur	Hastiere, Bois de Vesti	50.202162	4.849232	31	Negative
12	14/09/2017	Liege	Theux, Bois de Wiselez	50.541410	5.782921	30	Negative
13	29/09/2017	Liege	Bois de Julienne, Argenteau	50.700243	5.690341	15	Negative
14	7/10/2017	Liege	Bois de Sûreté, Targnon	50.415491	5.794612	22	Negative
15	30/08/2017_7/10/2017	Liege	Nonceveux/Sedoz	50.469042	5.745974	50	Negative
16	22/10/2017	Liege	Fagne Fargeron	50.514141	5.337480	33	Negative
17	22/10/2017	Liege	Grand Bois de Jehay	50.565628	5.330764	31	Negative
18	23/10/2017	Namur	Herbois, Durnal	50.335364	4.962908	30	Negative
19	24/10/2017	Namur	Bois de Dave	50.406823	4.923994	30	Negative
20	24/10/2017	Namur	Bois de la Haute Marlange	50.404263	4.780233	33	Negative
21	11/11/2017	Liege	Bois de la Borchêne	50.583064	5.963408	0	Negative
22	2017	Namur	Bois d'Aublain	50.087187	4.407116	21	Negative
23	3/05/2017	Liege	Esneux	50.50957	5.52347	15	Negative
24	2/05/2017	Liege	Argenteau	50.69921	5.68955	15	Negative
25	2/05/2017	Liege	Seraing	50.57705	5.48961	13	Negative
26	18/09/2017	Brabant Wallon	Ottignies-Louvain-La-Neuve	50.67638	4.59534	19	Negative
27	28/06/2017	Luxembourg	Martelange	49.825593	5.729159	15	Negative
28	28/06/2017	Brabant Wallon	Genappe	50.59676	4.53146	16	Negative
29	27/06/2017	Namur	Namur	50.45019	4.83552	7	Negative
30	8/08/2017	Luxembourg	Marche-en-Famenne	50.22945	5.35925	10	Negative
					Total	**702**	**Negative**

France

Pop	Date	Region	Latitude	Longitude	N° of swabs	*Bsal* status
1	20/03/17	Bois de la Bastide Limoges 87280 Limoges	45.862691	1.282455	11	Negative
2	31/03/17	Château Ligoure 87110 Ligoure	45.730381	1.303491	39	Negative
3	13/09/17	Route Forestière du Steinby, 68800 Thann	47.8104	7.0666	30	Negative
4	29/09/17	Forêt Domaniale de Coucy Basse, 02670 Folembray	49.571937	3.283728	30	Negative
5	29/09/17	Forêt de Rennes, 35340 Liffré	48.1825	-1.5347	30	Negative
6	29/09/17	Forêt du Gavre, 44130 Le Gavre	47.516823	-1.799117	30	Negative
7	07/10/17	Les Maresquiers, 50460 Cherbourg-en-Cotentin	49.3913	-1.5338	30	Negative
8	08/10/17	Champs-sur-Tarentaine-Marchal 15270	45.412024	2.603782	30	Negative
9	10/10/17	Petite route communale menant de Clamoux au hameau du Trental, commune Peyremale 30160	44.296526	4.056948	22	Negative
10	10/10/17	Canyon des Ecouges; SPN 38 isère	45.177168	5.503219	29	Negative
11	19/10/17	PN Cevennes	44.297672	3.743426	27	Negative
12	20/10/17	Sources des trois templiers proche 70150 Pin	47.3389	5.8722	30	Negative
13	24/10/17	Bellebouche, 36290 Mézières-en-Brenne	46.7891	1.3076	30	Negative
14	24/10/17	Forêt Domaniale de Boulogne, 41250 Neuvy	47.56597	1.511	30	Negative
15	01/11/17	Pélussin 42410 Parc Naturel Regional du Pilat	45.179702	5.497138	11	Negative
16	08/11/17	Parc de Granmont, 37200 Tours	47.350240	0.702156	30	Negative
17	10/11/17	Forêts de la Braconne et de Bois Blanc	45.7508	0.3058	30	Negative
18	24/11/17	Champmillon 16290	45.656026	0.011703	32	Negative
19	28/11/17	Forêt du Haillan 33185 Le Haillan	44.892528	-0.679664	35	Negative
20	10/03/18	Precy-le-sec/ Annay-la-côté/ Lucy-le-bois / source Vau de bouche à Sauvigny-le-Bois 89200	47.513863	3.940428	34	Negative
21	16/03/18	Forêt d'Esperce, 31190 Esperce	43.313613	1.410260	30	Negative
22	09/04/18	Bois départemental de Maroeuil 62144 Mont-Saint-Éloi	50.337959	2.695381	20	Negative
23	13/04/18	Ravin du Grossbach 57550 Hargarten-aux-Mines	49.224203	6.608125	24	Negative
24	24/05/2018	Forêt d'Iraty 64560 Larrau	43.012001	-0.966746	29	Negative
25	4/06/2018	L'Esparcelet, 05800 Saint-Firmin	44.781799	6.029976	13	Negative
26	05/06/18	Vallée d'Aspe, 64490 Sarrance	43.050783	-0.602211	30	Negative
27	21/10/18	Soultzbach-les-bains 68230	48.034148	7.21025	30	Negative
28	24/09/2019	Foret de Nieppe Drève Forestière	50.683333	2.600000	30	Negative
29	2/10/2019	Foret domaniale de Mormal	50.233333	3.766667	30	Negative
30	27/10/19	Bois de Mongin, Saint-Preuil	45.596764	-0.166251	38	Negative
				Total	**844**	**Negative**

Germany

Pop	Date	Region	Locality	Latitude	Longitude	N° of swabs	*Bsal* status?
1	03/2017	Ruhrhöhen	Essen Heisingen, Schellenberger Wald	51.412469	7.04811	12	Negative
2	03/2017	Ruhrhöhen	Ratinger Wald (zw. Essen und Ratingen)	51.304827	6.869358	22	Negative
3	03/2017	Bergisches Land	Remscheid (Tyrol und Eschbachtal)	51.154859	7.164698	30	Negative
4	21/04/2018	Ville	Kottenforst	50.66252	7.071624	24	Negative
5	04/2017	Taunus	Idstein	50.217173	8.265894	30	Negative
6	04/2017	Nahetal	Niederhausen, Stausee	49.803681	7.789785	30	Negative
7	04/2017	Odenwald	Felsenmeer, Lautertal	49.71231	8.692314	30	Negative
8	04/2017	Mittelsachsen	Kriebstein (Zschopau)	51.042831	13.011898	25	Negative
9	04/2017	Erzgebirge / Ergebirgsvorland	Dresden (Tharandt)	50.983195	13.576219	25	Negative
10	04/2017	Erzgebirge und Ergebirgsvorland	Hartenstein, Landkreis Zwickau	50.661939	12.671357	15	Negative
11	21/06/2017	Südeifel	Arzfeld	50.095483	6.251197	3	Negative
12	22-24/06/2017	Teutoburger Wald	Bot. Garten Bielefeld	52.014698	8.511353	22	Negative
13	25/06/2017 - 11/08/2017	Teutoburger Wald	Borgholzhausen Ravensberg	52.088711	8.317183	30	Negative
14	06-07/2017	Harz	Bad Harzburg (Eckertalstraße)	51.898946	10.646149	5	Negative
15	06-07/2017	Harz	Ilsetal	51.8451	10.6566	5	Negative
16	06-07/2017	Harz	Siebigerode	51.562173	11.418255	20	Negative
17	06-07/2017	Harz	Meisdorf	51.709798	11.269965	31	Negative
18	06-07/2017	Harz	Wolfshagen	51.923595	10.303128	26	Negative
19	25/07/2017	Harz	Morgenbrodstaler Graben (Clausthal-Zellerfeld)	51.762997	10.445509	39	Negative
20	8/08/2017	Münsterland	Wolbecker Tiergarten	51.910658	7.745656	14	Negative
21	08/2017	Münsterland	Baumberge, NSG Bombecker Aa	51.990031	7.350149	32	Negative
22	08/2017	Münsterland	Roruper Holz, Coesfeld	51.914888	7.231555	34	Negative
23	08/2017	Münsterland	Rhede, Rösing Busch	51.870503	6.761849	20	Negative
24	16/09/2017	Weserbergland	Solling, Mühlenberg	51.795175	9.514919	50	Negative
25	16/09/2017	Ruhrhöhen	Mülheim a.d.R. Wald	51.418568	6.884523	30	Negative
26	16/09/2017	Ruhrhöhen	Mülheim, Rumbachtal	51.416113	6.937303	7	Negative
27	15/10/2017	Kraichgau	Heilbronn	49.126126	9.255500	10	Negative
28	20-26/10/2017	Teutoburger Wald	Detmold, Freilichtmuseum	51.923554	8.871985	20	Negative
29	2017	Lahnberge	Marburg	50.826739	8.781213	17	Negative
30	09-10/2017	Thüringer Wald	NSG Waldecker Schlossgrund	50.915809	11.777532	15	Negative
31	10/2017	Weserbergland	Hooptalhütte Negenborn	51.894474	9.595518	30	Negative
32	10/2017	Weserbergland	Solling, Shilo Ranch	51.891693	9.604916	50	Negative
33	10/2017	Teutoburger Wald	Schwedenschanze, Kirchdornberg	52.029909	8.441465	25	Negative
34	10/2017	Sauerland, Rothaargebirge	Oberes Diemeltal, Hleminghausen	51.386061	8.759663	30	Negative
35	10/2017	Mittelrhein / Hunsrück	Rheinbay, Partelsbach	50.173356	7.62397	30	Negative
36	10/2017	Mittelrhein / Hunsrück	Altlayer Bachtal	49.993891	7.292991	29	Negative

37	10/2017	Fränkische Schweiz	Pottenstein 1	49.778186	11.413166	10	Negative
38	10/2017	Fränkische Schweiz	Pottenstein 2	49.758562	11.417500	30	Negative
39	10/2017	Raum Stuttgart	Stuttgart Stadtpopulation Stäffele	48.772791	9.115469	30	Negative
40	03/2018	Pfälzer Wald	Haardt	49.370101	8.144377	7	Negative
41	03/2018	Fränkische Alb	Hersbrucker Alb	49.525000	11.437374	30	Negative
42	03/2018	Bayerischer Wald	Donauleiten Obernzell	48.525530	13.722739	30	Negative
43	03/2018	Siebengebirge	Hennef	50.755387	7.357836	10	Negative
44	12/03/2018	Südeifel	Irrel	49.847058	6.456859	22	Negative
45	10/04/2018	Südeifel	Watzbach/Brandscheid	50.221331	6.309445	36	Negative
46	10-12/04/2018	Spessart	Wiesthal (Hochspessart)	50.037548	9.428539	10	Negative
47	11/04/2018	Südeifel	Ernzen	49.842228	6.423395	16	Negative
48	11/04/2018	Südeifel	Hauchenbach/Peffingen	49.908848	6.410098	24	Negative
49	12/04/2018	Südeifel	Gentingen	49.902447	6.243511	17	Negative
50	03/2017	Ruhrhöhen	Essener Stadswald	51.431025	6.968945	35	**4 Positive**
51	03/2017	Ruhrhöhen	Essen Fulerum	51.432957	6.965708	15	**15 Positive**
					Total	**1189**	

Extra active surveillance data per country partner

For the tested sites (Site), the region, locality and coordinates (Latitude and Longitude) are provided. The number of swabs (n° swabs) taken per population within a site on a certain date and the *Bsal* status are given.

Belgium

Follow up of fire salamander populations in known outbreak sites and sites located close to outbreak sites.

Site	Sampling date	Region	Locality	Latitude	Longitude	N° of Swabs	*Bsal* status
1	April-September 2018 (6 sampling efforts)	Liège	Robertville	50.453581	6.108975	13	Negative
	May-June 2019 (5 sampling efforts)	Liège	Robertville	50.453581	6.108975	5	Negative
2	May 2017 (2 sampling sessions)	Liège	Liege (Sart-Tilman)	50.580567	5.570157	29	**<u>Positive</u>**
	November-December 2017 (3 sampling sessions)	Liège	Liege (Sart-Tilman)	50.580567	5.570157	18	Negative
	May-December 2018 (6 sampling sessions)	Liège	Liege (Sart-Tilman)	50.580567	5.570157	9	Negative
	January-May 2019 (6 sampling sessions)	Liège	Liege (Sart-Tilman)	50.580567	5.570157	32	Negative
3	April-Novermber 2018 (3 sampling sessions)	Liège	Eupen	50.624383	6.088359	1	Negative
4	June 2018 (2 sampling sessions)	Liège	Nonceveux	50.46889	5.74459	12	Negative
	April 2019 (2 sampling sessions)	Liège	Nonceveux	50.46889	5.74459	12	Negative
					Total	**131**	

France

Sampling of other urodelan species.

Site	Date	Region/Locality	Latitude	Longitude	Species	N° of swabs	*Bsal* status
1	Sept/2017	PN Mercantour	43.85001	7.449293	*Speleomantes strinatii*	19	Negative
2	Apr/2018	Forêt Domaniale de Chiavari, 20138 Coti-Chiavari	41.772597	8.771765	*Salamandra corsica*	15	Negative
3	May/2018	Forêt de Vizzavone, 20219 Vivario	42.173277	9.169640	*Salamandra corsica*	30	Negative
4	Jun/2018	Forêt territoriale de Rospa Sorba, Caralba	42.090295	9.203405	*Euproctus montanus*	35	Negative
5	Jun/18	Fontana lungo strada D147	42.444000	9.013000	*Euproctus montanus*	2	Negative
6	Jun/18	Ruisseau du Prunello	42.309461	9.550903	*Euproctus montanus*	1	Negative
7	Jul/18	Ruisseau du Canneto	42.512345	9.373689	*Euproctus montanus*	6	Negative
	Jul/18	Ruisseau de Cippetto	42.519927	9.337097	*Euproctus montanus*	2	Negative
	Jul/18	Ruisseau de Vadoni	42.53392	9.296501	*Euproctus montanus*	7	Negative
8	Jul/18	Ruisseau de Muratellu	41.776594	9.219787	*Euproctus montanus*	1	Negative
	Jul/18	Ruisseau de Titinella	41.782100	9.212940	*Euproctus montanus*	3	Negative
	Jul/18	Ravin de Tijeta	41.784093	9.211819	*Euproctus montanus*	5	Negative
9	Jul/18	Ruisseau de Giugenti	42.329851	9.304396	*Euproctus montanus*	10	Negative
10	Jul/18	Ruisseau de Lattugona	42.042400	9.194900	*Euproctus montanus*	7	Negative
11	Jul/2018	PNR Queyras	44.704648	7.041028	*Salamandra lanzai*	30	Negative
12	Aug/2018	Gavarnie, Cirque de Troumouse 65120 Gèdre	42.733365	0.10503	*Calotriton asper*	35	Negative
13	Aug/2018	Vallon à l'ouest de Bérat-du-haut; PN Pyrénées Atlantiques	42.847606	-0.563016	*Calotriton asper*	35	Negative
					Total	**243**	**0**

Germany

Follow up of fire salamander populations in known outbreak sites and sites located close to outbreak sites.

Site	Date	Region	Latitude	Longitude	N° of swabs	*Bsal* status	Publication
1	2018	Nordeifel (Zweifallshammer)	50.682388	6.423373	25	**Positive (3)**	Lötters et al., 2018, Salamandra 54
2	2018	Ruhrhöhen (Bochum, Botanischer Garten)	51.442199	7.266148	23	**Positive (1)**	Lötters et al., 2018, Salamandra 54
3	2018	Ruhrhöhen (Bochum Dahlhausen)	51.435073	7.139507	1	**Positive (1)**	Lötters et al., 2018, Salamandra 54
4	2018	Ruhrhöhen (Bochum, Klosterbusch)	51.442880	7.270582	2	**Positive (1)**	Lötters et al., 2018, Salamandra 54
5	2018	Ruhrhöhen (Bochum Lottental)	51.440284	7.256134	4	**Positive (1)**	Lötters et al., 2018, Salamandra 54
6	2018	Ruhrhöhen (Essen, Eschenstraße)	51.423689	7.019866	98	**Positive (8)**	Lötters et al., 2018, Salamandra 54
7	2018	Ruhrhöhen (Essen, Stadtwald)	51.431025	6.968945	20	**Positive (1)**	Schulz et al., 2018, Natur in NRW 4
8	2018	Nordeifel (Solchbachtal)	50.70178	6.270098	25	**Positive (1)**	research project
9	2018	Nordeifel (Weberbach)	50.735089	6.359651	32	**Positive (6)**	research project
				Total	**230**	**23**	

Italy

Active surveillance of different (sub)species from various sites.

Site	Date	Region	Latitude	Longitude	(Sub)species	N° of swabs	*Bsal* status
1	Jul/2017	Abruzzo	42.187618	14.049244	*Lissotriton italicus*	8	Negative
	Jul/2017	Abruzzo	42.187618	14.049244	*Triturus carnifex*	6	Negative
	Jul/2017	Abruzzo	42.187618	14.049244	*Triturus carnifex*	2	Negative
2	Jul/2017	Abruzzo	42.002619	14.057026	*Triturus carnifex*	9	Negative
3	Mar/2018	Basilicata	39.541804	16.141861	*Lissotriton italicus*	6	Negative
4	Apr/2018	Calabria	40.015363	16.045129	*Salamandrina terdigitata*	14	Negative
	Apr/2018	Calabria	40.015363	16.045129	*Triturus carnifex*	9	Negative
5	Jun/2018	Basilicata	39.800782	16.030399	*Lissotriton italicus*	2	Negative
6	May/2019	Piemonte	44.574001	8.737722	*Ichtyosaura alpestris*	19	Negative
	May/2019	Piemonte	44.574001	8.737722	*Lissotriton vulgaris*	6	Negative
7	Jul/2019	Prov. Trento	45.961138	11.363402	*Salamandra atra aurorae*	2	Negative
	Sept/2019	Prov. Trento	45.961138	11.363402	*Salamandra atra aurorae*	1	Negative
					Total	**84**	Negative

Spain

Active surveillance of different species from various sites.

Site	Date	Region	Latitude	Longitude	Species	N° of swabs	Bsal status
1	Apr/2017	Montseny Natural Park, Barcelona	41.744	2.438	*Pleurodeles waltl*	1	Negative
	Apr/2017	Montseny Natural Park, Barcelona	41.744	2.438	*Triturus marmoratus*	2	Negative
	Apr-Jun/2017	Montseny Natural Park, Barcelona	41.744	2.438	*Calotriton arnoldi*	11	Negative
	Apr-Jun/2017	Montseny Natural Park, Barcelona	41.744	2.438	*Calotriton arnoldi*	32	Negative
	May-Jun/2017	Montseny Natural Park, Barcelona	41.744	2.438	*Rana temporaria*	2	Negative
	Jun/2017	Montseny Natural Park, Barcelona	41.744	2.438	*Bufo spinosus*	4	Negative
	Jun/2018	Montseny Natural Park, Barcelona	41.744	2.438	*Lissotriton boscai*	20	Negative
	Jun-Jul/2018	Montseny Natural Park, Barcelona,	41.744	2.438	*Calotriton arnoldi*	30	Negative
	May/2019	Montseny Natural Park, Barcelona	41.744	2.438	*Calotriton arnoldi*	35	Negative
	May/2019	Montseny Natural Park, Barcelona	41.744	2.438	*Bufo spinosus*	2	Negative
	May/2019	Montseny Natural Park, Barcelona	41.744	2.438	*Salamandra salamandra*	8	Negative
	Jul/2018	Montseny Natural Park, Barcelona	41.744	2.438	*Bufo spinosus*	1	Negative
2	Jun/2018	Picos de Europa National Park	43.183	-4.855	*Salamandra salamandra*	20	Negative
3	Jul/2018	Sierra de Guadarrama National Park, Madrid	40.858	-3.946	*Salamandra salamandra*	8	Negative
4	Jul/2018	Pian del Re, Crissolo, Italy	44.701	7.093	*Salamandra lanzai*	25	Negative
5	Oct/2018	Puerto Seguro, Salamanca	40.826	-6.761	*Lissotriton boscai*	2	Negative
	Oct/2018	Puerto Seguro, Salamanca	40.826	-6.761	*Pleurodeles waltl*	2	Negative
	Oct/2018	Puerto Seguro, Salamanca	40.826	-6.761	*Triturus marmoratus*	2	Negative
6	Oct/2018	Zamora	41.255	-5.711	*Pleurodeles waltl*	32	Negative
	Oct/2018	Zamora	41.255	-5.711	*Triturus marmoratus*	3	Negative
7	Jul/2019	Pellice, Torino, Italy	44.808	7.118	*Salamandra salamandra*	5	Negative
	Jul/2019	Pellice, Torino, Italy	44.808	7.118	*Salamandra lanzai*	20	Negative
8	Oct/2019	Somao, Asturias	43.537	-6.118	*Chioglossa lusitanica*	1	Negative
	Oct/2019	Somao, Asturias	43.537	-6.118	*Lissotriton boscai*	11	Negative
9	nov/2019	Soto de Aller, Asturias	43.162	-5.621	*Salamandra salamandra*	10	Negative
					Total	**289**	Negative

The Netherlands

Follow up of fire salamander populations in Bunderbos

Date	Locality	Latitude	Longitude	Species	N° of swabs	Bsal status
Apr/17	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	1	negative
May/17	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	6	negative
May/17	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	5	negative
Jun/17	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	45	negative

Jul/17	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	7	negative
Jul/17	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	1	negative
Aug/17	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	3	negative
Sep/17	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	2	negative
Sep/17	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	9	negative
Oct/17	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	21	negative
Oct/17	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	20	negative
Nov/17	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	15	negative
Dec/17	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	9	**Positive (1)**
Mar/18	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	7	negative
Mar/18	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	1	negative
Apr/18	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	332	**Positive (10)**
Apr/18	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	13	**Positive (1)**
Apr/18	Bunderbos	50,90553985	5,739552081	*Lissotriton vulgaris*	41	**Positive (5)**
May/18	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	153	negative
May/18	Bunderbos	50,90553985	5,739552081	*Lissotriton helveticus*	13	negative
May/18	Bunderbos	50,90553985	5,739552081	*Lissotriton vulgaris*	57	negative
May/18	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	2	negative
Jun/18	Bunderbos	50,90553985	5,739552081	*Lissotriton vulgaris*	13	negative
Aug/18	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	17	negative
Sep/18	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	18	negative
Sep/18	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	4	negative
Oct/18	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	11	negative
Oct/18	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	19	negative
Nov/18	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	27	negative
Nov/18	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	13	negative
Dec/18	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	9	negative
Dec/18	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	9	negative
Mar/19	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	65	negative
Mar/19	Bunderbos	50,90553985	5,739552081	*Lissotriton vulgaris*	50	negative
Mar/19	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	1	negative
Apr/19	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	78	negative
Apr/19	Bunderbos	50,90553985	5,739552081	*Lissotriton vulgaris*	26	negative
Apr/19	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	9	negative
May/19	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	49	negative
May/19	Bunderbos	50,90553985	5,739552081	*Lissotriton vulgaris*	43	**Positive (1)**
May/19	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	4	negative
Jun/19	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	2	negative
Jun/19	Bunderbos	50,90553985	5,739552081	*Lissotriton vulgaris*	46	negative
Sep/19	Bunderbos	50,90553985	5,739552081	*Ichthyosaura alpestris*	1	negative
Sep/19	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	22	negative
Oct/19	Bunderbos	50,90553985	5,739552081	*Salamandra salamandra*	23	negative
				Total	1322	

Annex 2: List of *Bsal* diagnostic labs recognized to perform *Bsal* qPCR in Europe

Austria

Department für Integrative Biologie and Evolution, Veterinärmedizinische Universitat Wien (Vetmeduni Vienna)

Contact person: Dr. Steve Smith (Steve.smith@vetmeduni.ac.at)

Address: Savoyenstraße 1, 1160 Vienna

Belgium

Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University

Contact persons: Prof. An Martel (an.martel@ugent.be), Prof. Frank Pasmans (frank.pasmans@ugent.be)

Address: Salisburylaan 133, 9820 Merelbeke

Czech Republic

Department of Ecology and Diseases of Game, Fish and Bees, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno

Contact person: Dr. Vojtech Baláž (balazv@vfu.cz)

Address: Palackého tř. 1946/1, PSČ 612 42 Brno

Croatia

Department of Poultry Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb

Contact person: Dr. Maja Lukac (mlukac@vef.hr)

Address: Heinzelova 55, 10000 Zagreb

France

PSL Research University, CEFE UMR 5175, CNRS, Université de Montpellier, Université Paul-Valéry Montpellier, EPHE, Biogéographie et Ecologie des Vertébrés

Contact person: Prof. Claude Miaud (claude.miaud@cefe.cnrs.fr)

Address: 1919 route de Mende, 34293 Montpellier

*ENSAT (*L'Ecole Nationale Supérieure Agronomique de Toulouse)

Contact person: Dr. Dirk Schmeller

Address: Avenue de l'Agrobiopole, Auzeville-Tolosane, 31316 Castanet-Tolosan Cedex

Germany

Biogeography Department, Faculty of Geography/Geosciences, Trier University

Contact person: Prof. (apl) Dr. Stefan Lötters (loetters@uni-trier.de)

Address: 54286 Trier

Laboklin

Contact: www.laboklin.de

Address: Steubenstr. 4, 97688 Bad Kissingen

Zoological Institute, Technische Universität Braunschweig

Contact person: Prof. Dr. Miguel Vences (m.vences@tu-braunschweig.de)

Address: Mendelssohnstr. 4, 38106 Braunschweig,

Landesbetrieb Hessisches Landeslabor (LHL)

Contact person: Dr.Tobias Eisenberg (Tobias.Eisenberg@lhl.hessen.de)

Address: Schubertstraße 60 - Haus 13, 35392 Gießen; Mailing address: Postfach 10 06 52, 35336 Gießen

Italy

DISTAV - Università degli Studi di Genova

Contact persons: Dr. Sebastiano Salvidio (salvidio@dipteris.unige.it), Dr. Elena Grasselli (elena.grasselli@gmail.com)

Address: Corso Europa 26, 16132 Genova

Slovenia

Department of Biology, Biotechnical faculty

Contact persons: Dr. Nina Gunde-Cimerman, Dr. Rok Kostanjšek (T: 386 1 320 33 73)

Address: Večna pot 111, SI-1000 Ljubljana

Spain

Museo Nacional de Ciencias Naturales, CSIC

Contact person: Dr. Jaime Bosch (bosch@mncn.csic.es)

c/ Jose Gutierrez Abascal 2, 28006 Madrid

Sweden

National Veterinary Institute, Department of Microbiology, Molecular Diagnostics

Contact person: Mr. Mats Isaksson (Mats.isaksson@sva.se)

Address: Travvägen 22, 75189 Uppsala

UK

Zoological Society of London, Institute of Zoology

Contact person: Prof. A. Cunningham (a.cunningham@ioz.ac.uk)

Address: Regent's Park, London NW1 4RY

Poland

Institute of Zoology and Biomedical Research Department of Comparative Anatomy, Jagiellonian University

Contact person: Maciej Pabijan (maciej.pabijan@uj.edu.pl)

Address: Gronostajowa 9, 30-387, Kraków

Annex 3: *Bsal* Hotline details

Belgium

Ghent University:
      Email: meldpuntziekeamfibieen@ugent.be

France

Centre d'Ecologie Fonctionnelle et Evolutive (CEFE):
      Website: http://www.alerte-amphibien.fr/

Germany

Trier University:
      Email: loetters@uni-trier.de
      Tel: +49 (0)651 201-4174

UK

Zoological Society of London:
      Website: www.gardenwildlifehealth.org
      Tel: 020 7449 6685

Italy

Genoa University (UNIGE):
      Email: salvidio@dipteris.unige.it
      Tel: +39-010 3538027

The Netherlands

Reptile, Amphibian and Fish Conservation the Netherlands (RAVON):
      Website: http://www.ravon.nl/Contact/tabid/1127/Default.aspx
      Email: ziektes@ravon.nl / a.spitzen@ravon.nl / m.gilbert@ravon.nl
      Tel: +31(0)24 – 74106000

Spain

Spanish National Research Council (CSIC):
      Email: bosch@mncn.csic.es
      Tel: +34677772402

Switzerland (as an external partner also has set up a regional hotline)

Karch:
      Email: benedikt.schmidt@ieu.uzh.ch
      Tel: +41 32 718 36 00 / +41 32 718 36 12

Annex 4: Detailed information of the passive surveillance results in the context of the early warning system

Reported cases to the *Bsal* hotlines (negative = negative for *Bsal*, positive = positive for *Bsal*)

Country	Date	Hotline	Location (province) [Latitude-Longitude]	Species	Number of Amphibians	*Bsal* status
Belgium	2017	Belgium	Ninglispo (Liège)	*Salamandra salamandra*	1	Negative
Belgium	2017	Belgium	Séraing (Liège)	*Salamandra salamandra*	2	Negative
Belgium	2017	Belgium	Lomprez Wellin (Luxembourg)	*Salamandra salamandra*	4	Negative
Belgium	April 2017	Belgium	Sint Lambrechts Woluwe (Brussels, Flemish Brabant)	*Ichthyosaura alpestris*	1	Negative
Belgium	2017	Belgium	Spa (Liège) [50.493600; 5.858190]	*Salamandra salamandra*	1	Negative
Belgium	May 2017	Belgium	Tielt (West Flanders)	*Lissotriton helveticus*	1	Negative
Belgium	June 2017	The Netherlands	Luxembourg	*Salamandra salamandra*	1	Negative
Belgium	February 2018	Belgium	Waasmunster (East Flanders)	*Ichthyosaura alpestris*	1	Negative
Belgium	August 2018	Belgium	Doeveren Forest, Zedelgem (West Flanders)	*Lissotriton helveticus*	2	Negative
				Lissotriton vulgaris	3	
Belgium	March 2018	Belgium	Mollendaalbos, Oud Heverlee (Flemish Brabant)	*Salamandra salamandra*	1	Negative
Belgium		Belgium	Antheit (Liège)	*Ichthyosaura alpestris*	10	Negative
Belgium	April 2018	Belgium	Tervuren Parc [50.822407; 4.531014] (Flemish Brabant)	*Bufo bufo*	40	Negative
Belgium	April 2018	Belgium	Neder-Over-Heembeek (Brussels, Flemish Brabant)	*Ichthyosaura alpestris*	1	Negative
Belgium	April 2018	Belgium	Sonian Forest (Brussels)	*Rana temporaria*	2	Negative
Belgium	May 2018	Belgium	Sint Blasius Boekel (East Flanders)	*Salamandra salamandra*	1	Negative
Belgium	May 2018	The Netherlands	Gemmenich	*Lissotriton vulgaris*	1	Negative
Belgium	September 2018	Belgium	Les bois de la Julienne, Visé – Argenteau (Liège)	*Salamandra salamandra*	1	Negative
Belgium	February 2019	Belgium	Tielt (West Flanders)	*Ichthyosaura alpestris*	1	Negative
Belgium	April 2019	Belgium	Paul Straubant street, Ukkel (Flemish Brabant, Brussels)	*Bufo bufo*	3	Negative
Belgium	April 2019	Belgium	Grimminge [50.782869; 3.935652] (East Flanders)	*Bufo bufo*	6	Negative
Belgium	April 2019	Belgium	Flossendelle [50.8129; 4.4736] (Flemish Brabant)	*Bufo bufo*	1	Negative

Belgium	April 2019	Belgium	Flossendelle [50.8129; 4.4736] (Flemish Brabant)	*Rana temporaria*	1	Negative
Belgium	April 2019	Belgium	Geraardsbergen (East Flanders)	*Ichthyosaura alpestris*	5	Negative
Belgium	April 2019	Belgium	Sint Lievens Houtem [50.924664; 3.909767] (East Flanders)	*Lissotriton helveticus*	1	Negative
Belgium	October 2019	Belgium	Mozet (Namen)	*Salamandra salamandra*	1	Negative
Belgium	January 2020	Belgium	Olne [50.582561; 5.769036971](Liège)	*Salamandra salamandra*	50	**<u>Positive</u>**

Country	Date	Hotline	Location (Province)	*Species*	Number of amphibians	*Bsal* status
The Netherlands	February 2017	The Netherlands	Ooij (Gelderland)	*Triturus cristatus*	1	Negative
The Netherlands	March 2017	The Netherlands	Geulle (Limburg)	*Salamandra salamandra*	1	Negative
The Netherlands	March 2017	The Netherlands	Amsterdam (Noord-Holland)	*Ichthyosaura alpestris*	1	Negative
The Netherlands	April 2017	The Netherlands	Slekkerhout, Haverland (Limburg)	*Lissotriton vulgaris*	1	Negative
The Netherlands	April 17	The Netherlands	Bunderbos (Limburg)	*Salamandra salamandra*	1	Negative
The Netherlands	April 2017	The Netherlands	Hoofddorp (Noord-Holland)	*Lissotriton vulgaris*	8	Negative
The Netherlands	April 2017	The Netherlands	Vijfhuizen (Noord-Holland)	*Lissotriton vulgaris*	1	Negative
The Netherlands	April 2017	The Netherlands	Warmond (Zuid-Holland)	*Lissotriton vulgaris*	1	Negative
The Netherlands	May 2017	The Netherlands	Druten (Gelderland)	*Triturus cristatus*	1	Negative
The Netherlands	May 2017	The Netherlands	Norg (Drenthe)	*Triturus cristatus*	1	Negative
The Netherlands	June 2017	The Netherlands	Laren (Noord-Holland)	*Lissotriton vulgaris*	1	Negative
The Netherlands	July 2017	The Netherlands	Liesbos, Breda (Noord-Brabant)	*Triturus cristatus*	1	Negative
The Netherlands	September 2017	The Netherlands	Schiedam (Zuid Holland)	*Lissotriton vulgaris*	8	Negative
The Netherlands	October 2017	The Netherlands	Bunderbos (Limburg)	*Salamandra salamandra*	1	Negative
The Netherlands	December 2017	The Netherlands	Assen (Drenthe)	*Ichthyosaura alpestris*	1	Negative
The Netherlands	March 2018	The Netherlands	Wageningen (Gelderland)	*Triturus cristatus*	1	Negative
The Netherlands	March 2018	The Netherlands	Kasteel Elsloo, Bunderbos (Limburg)	*Ichthyosaura alpestris*	1	Negative
The Netherlands	April 2018	The Netherlands	Schiedam (Zuid-Holland)	*Ichthyosaura alpestris*	1	Negative
The Netherlands	April 2018	The Netherlands	Gorssel (Gelderland)	*Triturus cristatus*	2	**<u>Positive</u>**
The Netherlands	April 2018	The Netherlands	Rhenen (Utrecht)	*Lissotriton vulgaris*	1	Negative

Country	Date	Hotline	Region	Species	Number of amphibians	*Bsal* status
The Netherlands	April 2018	The Netherlands	Wageningen (Gelderland)	*Lissotriton vulgaris*	1	Negative
The Netherlands	April 2018	The Netherlands	Woudrichem (Noord-Brabant)	*Lissotriton vulgaris*	1	Negative
The Netherlands	April 2018	The Netherlands	Gorssel (Gelderland)	*Lissotriton vulgaris*	4	Negative
The Netherlands	April 2018	The Netherlands	Gorssel (Gelderland)	*Lissotriton vulgaris*	4	Negative
The Netherlands	May 2018	The Netherlands	Houthem (Limburg)	*Lissotriton vulgaris*	4	Negative
The Netherlands	May 2018	The Netherlands	Wageningen (Gelderland)	*Lissotriton vulgaris*	19	Negative
The Netherlands	May 2018	The Netherlands	Maastricht (Limburg)	*Lissotriton vulgaris*	1	Negative
The Netherlands	June 2019	The Netherlands	Elsloerbos (Bunderbos-North)	*Ichthyosaura alpestris*	1	Negative

Country	Date	Hotline	Region	*Species*	Number of amphibians	*Bsal* status
Germany	March 2017	Germany	Essener Stadtwald [51.431025; 6.968945] (Ruhrhöhen)	*Salamandra salamandra*	35	**4 Positives**
Germany	March 2017	Germany	Essen Fulerum [51.432957; 6.965708] (Ruhrhöhen)	*Salamandra salamandra*	15	**15 Positives**

Country	Date	Hotline	Region [Latitude; Longitude] (Department)	Species	Number of Amphibians	*Bsal* status
France	March 2017	France	Azé [46.435077; 4.771861] (Loir-et-Cher)	*Salamandra salamandra*	1	Negative
France	March 2017	France	Bois de la Bastide Limoges, Limoges [45.862691; 1.282455] (Haute-Vienne)	*Salamandra salamandra*	1	Negative
France	March 2017	France	Bourg-Charente [45.673087; -0.221380] (Charente)	*Salamandra salamandra*	9	Negative
France	April 2017	France	Morbecque [50.676864; 2.568848] (Nord)	*Salamandra salamandra*	1	Negative
France	April 2017	France	Morbecque [50.676864; 2.568848] (Nord)	*Salamandra salamandra*	1	Negative
France	October 2017	France	Saint Laurent de Ceris [45.941017; 0.476551] (Charente)	*Salamandra salamandra*	1	Negative
France	October 2017	France	Trois-palis [45.638634; 0.054389] (Charente)	*Salamandra salamandra*	1	Negative
France	October 2017	France	Vindelle [45.718867; 0.104376] (Charente)	*Salamandra salamandra*	1	Negative
France	October 2017	France	Pressignac [45.807654; 0.738524] (Charente)	*Salamandra salamandra*	1	Negative

France	October 2017	France	Valbonnais, source de Gragnolet, Entraigues; PN Ecrins hautes Alpes [44.884506; 5.970695] (Isère)	*Salamandra salamandra*	1	Negative
France	October 2017	France	Saint-Amant-de-Nouère [45.745244; 0.002907] (Charente)	*Salamandra salamandra*	1	Negative
France	October 2017	France	vers Chalonne / Fontaine de pisse-menu [45.668525; 0.097830] (Charente)	*Salamandra salamandra*	1	Negative
France	October 2017	France	D453, entre le château de Portes et le hameau de Clamoux, commune de Portes, Cévennes [44.269096; 4.029414] (Gard)	*Salamandra salamandra*	3	Negative
France	October 2017	France	Bois de Confracourt, Confracourt [47.664814; 5.897501] (Haute-Saõne)	*Salamandra salamandra*	8	Negative
France	November 2017	France	Champmillon [45.656026; 0.011703] (Charente)	*Salamandra salamandra*	1	Negative
France	November 2017	France	Champmillon [45.656026; 0.011703] (Charente)	*Salamandra salamandra*	1	Negative
France	2017	France	Saint sauveur en puisage [47.6376; 3.1661] (Yonne)	*Ichthyosaura alpestris*	1	Negative
	2017			*Lissotriton helveticus*	7	
France	March 2018	France	La Chapelaude [46.422098; 2.502087] (Allier)	*Triturus cristatus*	2	Negative
France	March 2018	France	La Chapelaude [46.422098; 2.502087] (Allier)	*Lissotriton helveticus*	5	Negative
France	March 2018	France	Ravin de Dalem / Sennerloch, Dalem [49.244423; 6.619036] (Moselle)	*Salamandra salamandra*	6	Negative
France	March 2018	France	Esperce [43.313613; 1.410260] (Haute-Garonne)	*Salamandra salamandra*	5	Negative
France	May 2018	France	Le Désert en Valjouffray (pont de la Bonne) Parc national des écrins [44.867214; 6.091099] (Isère)	*Salamandra salamandra*	1	Negative
France	May 2018	France	Pont du prêtre Parc national des écrins [44.894371; 5.868386] (Isère)	*Salamandra salamandra*	1	Negative
France	May 2018	France	Lac de Soum - Arrens-Marsous [42.974570; -0.246457] (Hautes-Pyrénées)	*Lissotriton helveticus*	2	Negative
France	June 2018	France	Ancien canal, Parc national des écrins [44.874196; 6.102309] (Isère)	*Salamandra salamandra*	3	Negative

Country	Date	Hotline	Region [Latitude; Longitude] (Province)	Species	Number of amphibians	Bsal status
France	June 2018	France	Champchauzat, Parc national des écrins [44.921070; 5.958871] (Isère)	*Salamandra salamandra*	1	Negative
France	June 2018	France	Route de valsenestre, Parc national des écrins (05) [44.888520; 6.020854] (Isère)	*Salamandra salamandra*	4	Negative
France	June 2018	France	Ruisseau du Sue, Parc national des écrins [44.893268; 6.024014] (Isère)	*Salamandra salamandra*	1	Negative
France	June 2018	France	Sentier du Sue, Parc national des écrins [44.897765; 6.025105] (Isère)	*Salamandra salamandra*	1	Negative
France	June 2018	France	Route de Gioberney, La Chapelle-en-Valgaudémar [44.818406; 6.201138] (Hautes-Alpes)	*Salamandra salamandra*	3	Negative
France	October 2018	France	Meylan, Siphon piscine [45.228332; 5.774498] (Isère)	*Salamandra salamandra*	1	Negative

Country	Date	Hotline	Region [Latitude; Longitude] (Province)	*Species*	Number of amphibians	*Bsal* status
Spain			Captivity	*Cynops pyrrhogaster*	1	Negative
Spain	June 2017	Spain	Valsaín [40.880; -4.028] (Segovia)	*Salamandra salamandra*	1	Negative
Spain	June 2017	Spain	Valsaín [40.880; -4.028] (Segovia)	*Triturus marmoratus*	2	Negative
Spain	July 2017	Spain	Ibón de Perramo [42.655; 0.558] (Huesca)	*Calotriton asper*	1	Negative
Spain	July 2017	Spain	Ibón de Acherito [42.879; -0.711] (Huesca)	*Calotriton asper*	1	Negative
Austria	August 2017	Spain	Lake Turner [46.578; 14.577]	*Ichthyosaura alpestris*	13	Negative
Spain	September 2017	Spain	Teverga [43.161;-6.098] (Asturias)	*Salamandra salamandra*	1	Negative
Spain	September 2017	Spain	Tui [42.046; -8.644] (Pontevedra)	*Lissotriton helveticus*	4	Negative
				Lissotriton boscai	1	
Spain	September 2017	Spain	[37.520; -7.210] (Huelva)	*Salamandra salamandra*	1	Negative
Spain	October 2017	Spain	Soncillo [42.970; -3.786] (Burgos)	*Salamandra salamandra*	5	Negative
Spain	October 2017	Spain	Zamalvide [43.292; -1.905] (Basque Country)	*Lissotriton helveticus*	1	Negative
Morocco	November 2017	Spain	Targuist [34.940; -4.328]	*Salamandra algira*	1	Negative

Spain	March 2018	Belgium	Montnegre i el Corredor Natural Park in Catalonia (Barcelona)	*Triturus marmoratus*	6	**Positive**
Spain	March 2018	Spain	[36.669; -5.485] (Cádiz)	*Salamandra salamandra*	5	Negative
Spain	July 2018	Spain	Somiedo Natural Park [43.103; -6.252] (Asturias)	*Lissotriton helveticus*	2	Negative
Spain	July 2018	Spain	Tui [42.046; -8.644] (Pontevedra)	*Lissotriton helveticus*	2	Negative
Spain	August 2018	Spain	Valsaín [40.880; -4.028] (Segovia)	*Salamandra salamandra*	3	Negative
Spain	April 2019	Spain	La alhambra [37.177; -3.591] (Granada)	*Pleurodeles watl*	2	Negative
Spain	May 2019	Spain	Sierra de Alcaraz [38.670; -2.493] (Albacete)	*Salamandra salamandra*	2	Negative

Country	Report Date	Hotline	Region	Grid Ref.	Species	Number of amphibians	Bsal status
United Kingdom	10/04/2017	United Kingdom	London	TQ2985	*Lissotriton vulgaris*	1	Negative
United Kingdom	11/04/2017	United Kingdom	South East	SP8003	*Lissotriton vulgaris*	3	Negative
United Kingdom	20/05/2017	United Kingdom	East of England	TQ8487	*Rana temporaria*	1	Negative
United Kingdom	31/05/2017	United Kingdom	South East	TQ7565	*Bufo bufo*	1	Negative
United Kingdom	13/06/2017	United Kingdom	East Midlands	SK6241	*Rana temporaria*	1	Negative
United Kingdom	28/06/2017	United Kingdom	East of England	TL4657	*Alytes obstetricans*	1	Negative
United Kingdom	11/07/2017	United Kingdom	South West	SY9996	*Rana temporaria*	1	Negative
United Kingdom	17/07/2017	United Kingdom	South East	TR0033	*Rana temporaria*	1	Negative
United Kingdom	20/07/2017	United Kingdom	South West	ST6554	*Rana temporaria*	1	Negative
United Kingdom	31/07/2017	United Kingdom	East of England	TL2224	*Rana temporaria*	1	Negative
United Kingdom	08/08/2017	United Kingdom	East of England	TL1798	*Bufo bufo*	1	Negative
United Kingdom	17/08/2017	United Kingdom	East Midlands	SP86	*Bufo bufo*	2	Negative
United Kingdom	23/08/2017	United Kingdom	East of England	TL7003	*Rana temporaria*	1	Negative
United Kingdom	25/09/2017	United Kingdom	North West	SD3142	*Rana temporaria*	1	Negative
United Kingdom	27/11/2017	United Kingdom	North West	SD3142	*Rana temporaria*	1	Negative
United Kingdom	04/12/2017	United Kingdom	South East	TQ2706	*Rana temporaria*	1	Negative
United Kingdom	09/02/2018	United Kingdom	South West	SW8261	*Rana temporaria*	2	Negative

United Kingdom	22/02/2018	United Kingdom	London	TQ3462	*Rana temporaria*	1	Negative
United Kingdom	01/03/2018	United Kingdom	East of England	TL4658	*Rana temporaria*	2	Negative
United Kingdom	07/03/2018	United Kingdom	South East	TQ0644	*Rana temporaria*	2	Negative
United Kingdom	08/03/2018	United Kingdom	East of England	TL2232	*Rana temporaria*	3	Negative
United Kingdom	08/03/2018	United Kingdom	London	TQ3462	*Rana temporaria*	1	Negative
United Kingdom	13/03/2018	United Kingdom	South West	ST7565	*Rana temporaria*	1	Negative
United Kingdom	14/03/2018	United Kingdom	London	TQ2591	*Rana temporaria*	1	Negative
United Kingdom	15/03/2018	United Kingdom	South East	TQ1404	*Rana temporaria*	3	Negative
United Kingdom	16/03/2018	United Kingdom	South East	SU9960	*Rana temporaria*	1	Negative
United Kingdom	17/03/2018	United Kingdom	London	TQ3478	*Rana temporaria*	3	Negative
United Kingdom	20/03/2018	United Kingdom	East of England	TL4459	*Rana temporaria*	1	Negative
United Kingdom	27/03/2018	United Kingdom	Yorkshire and The Humber	SD9927	*Rana temporaria*	2	Negative
United Kingdom	31/03/2018	United Kingdom	South West	ST8499	*Rana temporaria*	2	Negative
United Kingdom	01/04/2018	United Kingdom	West Midlands	SJ6733	*Rana temporaria*	1	Negative
United Kingdom	02/04/2018	United Kingdom	South West	SO9619	*Rana temporaria*	1	Negative
United Kingdom	02/04/2018	United Kingdom	South West	SO8816	*Rana temporaria*	1	Negative
United Kingdom	02/04/2018	United Kingdom	East of England	TQ8190	*Rana temporaria*	1	Negative
United Kingdom	03/04/2018	United Kingdom	Yorkshire and The Humber	SK3081	*Rana temporaria*	1	Negative
United Kingdom	03/04/2018	United Kingdom	North West	SJ2893	*Bufo bufo*	1	Negative
United Kingdom	04/04/2018	United Kingdom	South West	SX4874	*Rana temporaria*	1	Negative
United Kingdom	05/04/2018	United Kingdom	South East	SU9504	*Rana temporaria*	1	Negative
United Kingdom	06/04/2018	United Kingdom	West Midlands	SJ6906	*Rana temporaria*	1	Negative
United Kingdom	07/04/2018	United Kingdom	North West	SJ4465	*Rana temporaria*	1	Negative
United Kingdom	07/04/2018	United Kingdom	South West	ST8650	*Rana temporaria*	1	Negative
United Kingdom	08/04/2018	United Kingdom	Wales	SN7437	*Rana temporaria*	1	Negative
United Kingdom	08/04/2018	United Kingdom	North West	NY0637	*Rana temporaria*	1	Negative
United Kingdom	08/04/2018	United Kingdom	South East	SU9993	*Pelophylax lessonae*	1	Negative
United Kingdom	09/04/2018	United Kingdom	Wales	ST0584	*Rana temporaria*	1	Negative
United Kingdom	11/04/2018	United Kingdom	South West	ST7898	*Rana temporaria*	3	Negative

United Kingdom	11/04/2018	United Kingdom	South West	SX2461	*Bufo bufo*	2	Negative
United Kingdom	12/04/2018	United Kingdom	South West	SO8816	*Rana temporaria*	1	Negative
United Kingdom	15/04/2018	United Kingdom	West Midlands	SO2956	*Rana temporaria*	1	Negative
United Kingdom	16/04/2018	United Kingdom	Yorkshire and The Humber	SD9743	*Rana temporaria*	1	Negative
United Kingdom	16/04/2018	United Kingdom	Yorkshire and The Humber	SE5400	*Rana temporaria*	2	Negative
United Kingdom	17/04/2018	United Kingdom	South Scotland	NS7997	*Rana temporaria*	1	Negative
United Kingdom	17/04/2018	United Kingdom	South East	SU8287	*Bufo bufo*	2	Negative
United Kingdom	18/04/2018	United Kingdom	East of England	TL1799	*Rana temporaria*	1	Negative
United Kingdom	18/04/2018	United Kingdom	London	TQ2883	*Lissotriton vulgaris*	1	Negative
United Kingdom	19/04/2018	United Kingdom	South East	SU6811	*Rana temporaria*	1	Negative
United Kingdom	19/04/2018	United Kingdom	East of England	TL1799	*Rana temporaria*	1	Negative
United Kingdom	20/04/2018	United Kingdom	Yorkshire and The Humber	SE3239	*n/a*	1	Negative
United Kingdom	20/04/2018	United Kingdom	North West	SJ4465	*Lissotriton vulgaris*	2	Negative
United Kingdom	06/05/2018	United Kingdom	West Midlands	SJ9600	*Rana temporaria*	1	Negative
United Kingdom	26/05/2018	United Kingdom	South West	ST8026	*Lissotriton vulgaris*	4	Negative
United Kingdom	29/06/2018	United Kingdom	South East	TQ2106	*Rana temporaria*	1	Negative
United Kingdom	01/07/2018	United Kingdom	Yorkshire and The Humber	SE0320	*Rana temporaria*	2	Negative
United Kingdom	10/07/2018	United Kingdom	South East	TQ7735	*Lissotriton vulgaris*	1	Negative
United Kingdom	15/07/2018	United Kingdom	East of England	TQ4498	*n/a*	1	Negative
United Kingdom	30/07/2018	United Kingdom	South West	SY9792	*Rana temporaria*	1	Negative
United Kingdom	30/07/2018	United Kingdom	East of England	TM5291	*Rana temporaria*	2	Negative
United Kingdom	30/07/2018	United Kingdom	East of England	TQ8486	*n/a*	2	Negative
United Kingdom	01/08/2018	United Kingdom	South East	SU1504	*Rana temporaria*	1	Negative
United Kingdom	03/08/2018	United Kingdom	East of England	TL2224	*Rana temporaria*	1	Negative
United Kingdom	07/08/2018	United Kingdom	East of England	TL2224	*Rana temporaria*	1	Negative
United Kingdom	07/08/2018	United Kingdom	East of England	TQ8486	*n/a*	4	Negative
United Kingdom	14/08/2018	United Kingdom	West Midlands	SP0858	*Rana temporaria*	1	Negative
United Kingdom	02/09/2018	United Kingdom	Wales	SO2811	*n/a*	1	Negative
United Kingdom	12/09/2018	United Kingdom	London	TQ2986	*Rana temporaria*	1	Negative

United Kingdom	03/10/2018	United Kingdom	South East	TR1166	*Bufo bufo*	1	Negative
United Kingdom	14/10/2018	United Kingdom	London	TQ2591	*Rana temporaria*	1	Negative
United Kingdom	05/01/2019	United Kingdom	South Scotland	NS5355	*Rana temporaria*	2	Negative
United Kingdom	03/03/2019	United Kingdom	South West	ST5376	*Rana temporaria*	1	Negative
United Kingdom	05/03/2019	United Kingdom	North West	NX9720	*Bufo bufo*	2	Negative
United Kingdom	08/03/2019	United Kingdom	London	TQ3889	*Rana temporaria*	2	Negative
United Kingdom	17/03/2019	United Kingdom	North West	NX9720	*Bufo bufo*	2	Negative
United Kingdom	17/03/2019	United Kingdom	South East	SP3505	*Bufo bufo*	2	Negative
United Kingdom	18/03/2019	United Kingdom	South West	SY6989	*Rana temporaria*	1	Negative
United Kingdom	25/03/2019	United Kingdom	South West	ST6478	*Rana temporaria*	1	Negative
United Kingdom	30/03/2019	United Kingdom	South East	SU8287	*Bufo bufo*	1	Negative
United Kingdom	23/04/2019	United Kingdom	South East	SU1418	*Bufo bufo*	2	Negative
United Kingdom	04/05/2019	United Kingdom	South West	SX8178	*Lissotriton helveticus*	2	Negative
United Kingdom	22/05/2019	United Kingdom	East Midlands	SK3833	*Lissotriton vulgaris*	2	Negative
United Kingdom	23/05/2019	United Kingdom	South West	SO9326	*Lissotriton vulgaris*	2	Negative
United Kingdom	27/05/2019	United Kingdom	South East	TQ3004	*Rana temporaria*	2	Negative
United Kingdom	03/06/2019	United Kingdom	South West	ST8026	*Lissotriton vulgaris*	1	Negative
United Kingdom	12/06/2019	United Kingdom	South East	TQ2704	*Rana temporaria*	1	Negative
United Kingdom	26/06/2019	United Kingdom	South East	TQ7207	*Lissotriton vulgaris*	1	Negative
United Kingdom	01/07/2019	United Kingdom	East of England	TL4310	*Rana temporaria*	4	Negative
United Kingdom	02/07/2019	United Kingdom	West Midlands	SP2154	*Rana temporaria*	1	Negative
United Kingdom	09/07/2019	United Kingdom	North West England	SJ3581	*Rana temporaria*	1	Negative
United Kingdom	15/07/2019	United Kingdom	North West England	SD2957	*Epidalea calamita*	1	Negative
United Kingdom	17/07/2019	United Kingdom	North West England	SJ8292	*Rana temporaria*	1	Negative
United Kingdom	28/07/2019	United Kingdom	East of England	TQ8194	*Rana temporaria*	2	Negative
United Kingdom	04/08/2019	United Kingdom	North West	SJ8292	*Rana temporaria*	1	Negative
United Kingdom	04/08/2019	United Kingdom	North West	SJ3581	*Rana temporaria*	1	Negative
United Kingdom	05/08/2019	United Kingdom	South West	SP1601	*Rana temporaria*	1	Negative
United Kingdom	06/08/2019	United Kingdom	North West	SJ3581	*Rana temporaria*	1	Negative

United Kingdom	13/08/2019	United Kingdom	North Scotland	NM6588	*Bufo bufo*	1	Negative
United Kingdom	27/08/2019	United Kingdom	East of England	TL2622	*Rana temporaria*	1	Negative
United Kingdom	01/09/2019	United Kingdom	East of England	TL8115	*Rana temporaria*	1	Negative

Annex 5: List of responsive stakeholders

Country	Institution	Contact Person	Contact Details
Austria	Haus der Natur, Salzburg	Peter Kaufmann	peter.kaufmann@hausdernatur.at
	Universität Salzburg /Österreichische Gesellschaft für Herpetologie (ÖGH)	Andreas Maletzky	andreas.maletzky@sbg.ac.at
	Technisches Büro für Biologie	Florian Glaser	florian.glaser@aon.at
		Gerda Ludwig	gerda.ludwig@gmx.at
	Schönbrunner Tiergarten GmbH, Wien	Doris Preininger	D.Preininger@zoovienna.at
Belgium	Agentschap Natuur en Bos	Muriel Vervaeke	muriel.vervaeke@lne.vlaanderen.be
	INBO	Jeroen Speybroeck	jeroen.speybroeck@inbo.be
	Natuurpunt	Anke Geeraerts	anke.geeraerts@natuurpunt.be
		Dominique Verbelen	dominique.verbelen@natuurpunt.be
	Natagora		salamandre@natagora.be
	Aves	Thierry Kinet	thierry.kinet@aves.be
Croatia	Kroatische Natuurhistorisch Museum	Eduard Kletecki	info@hpm.hr
	Faculty of Veterinary medicine, University Zagreb	Maja Lukac	mlukac@vef.hr
Estonia	Ministry of the Environment,	Merike Linnamagi	merike.linnamagi@envir.ee
	University of Tartu	Riinu Rannap	riinu.rannap@ut.ee

France	Association BUFO	Jean-Paul Vacher	association@bufo-alsace.org
	LPO Franche-Comté	Alix Michon	franche-comte@lpo.fr alix.michon@lpo.fr
	Groupe ornithologique et naturaliste du Nord-Pas-de-Calais (GON)	Robin Quevillart	contact@gon.fr robin.quevillart@gon.fr
	CEFE, CNRS, Universite de Montpellier	Claude Miaud	Claude.MIAUD@cefe.cnrs.fr
Germany	DGHT	Paul Bachhausen	salamanderseiten@gmx.de
	Trier Uni	Michael Veith	veith@uni-trier.de
	Braunschweig Uni	Miguel Vences	m.vences@tu-braunschweig.de
	Trier University	Lötters, Stefan	loetters@uni-trier.de
	Biostationen	Lutz Dalbeck	lutz.dalbeck@biostation-dueren.de
		Dagmar Ohlhoff	dagmar.ohlhoff@biostation-dueren.de
	Leipzig University	Sebastian Steinfartz	steinfartz@uni-leipzig.de
Hungary	Hungarian Natural History Museum	Judit Vörös	jvoros@nhmus.hu
	project manager at MME BirdLife Hungary	Balint Halpern	balint.halpern@mme.hu
Italy	University Florence	Claudia Corti	claudia.corti@unifi.it
	University of Genoa (Unige)	Sebastiano Salvidio	salvidio@dipteris.unige.it
		Elena Grasselli	elena.grasselli@gmail.com
	private	Vincenzo Ferri	drvincenzoferri@gmail.com

The Netherlands	European Association of Zoos and Aquaria	Allan Muir	allan.muir@eaza.net
	Salamandervereniging		https://www.salamanders.nl/contact/
Portugal	USA, UK, Faculdade de Ciências Universidade de Lisboa	Gonçalo M. Rosa	goncalo.m.rosa@gmail.com
	CIBIO-InBIO, Porto	Guillermo Velo-Antón	guillermo.velo@gmail.com
Romania	Ovidius University Constanta	Dan Cogalniceanu	dan_cogalniceanu@yahoo.com
Serbia	Faculty of Sciences and Mathematics, University of Niš	Jelka Crnobrnja Isailović	jelka.c.i@gmail.com
Slovakia	State Nature Conservancy	Viliam Vongrej	viliam.vongrej@sopsr.sk
		Daniel Jablonski	jablonski.dan@gmail.com
Slovenia	Center za kartografijo favne in flore	Katja Poboljsaj	katja.poboljsaj@ckff.si
	University	Rok Kostanjsek	rok.kostanjsek@bf.uni-lj.si
Spain	Museo Nacional de Ciencias Naturales, CSIC	Jaime Bosch	mcnbp3d@mncn.csic.es
Sweden	Länsstyrelsen Skåne, Malmö	Christer Persson	Christer.Persson@lansstyrelsen.se
	Uppsala University	Simon Karvemo	simon.karvemo@ebc.uu.se
UK	ZSL	Andrew Cunningham	A.Cunningham@ioz.ac.uk
	Amphibian and Reptile Conservation Trust	John Wilkinson	John.Wilkinson@arc-trust.org
		Jim Foster	Jim.Foster@arc-trust.org
Ukraine	Oles Honchar Dnipropetrovsk National University, Dnipro	Viktor Gasso	viktor.gasso@gmail.com
	Ornamental Fish	Dominic Whitmee	dominic@ornamentalfish.org

Annex 6: Public Awareness Activities from each project partner

Belgium

- The European *Bsal* project website:

  created in 2017: https://Bsalinfoeurope.wixsite.com/eu*Bsal*mitigation2017

  Information transferred to the current website: www.bsaleurope.com

- Scientific communication addressed to any audience, including governmental authorities and NGO

Joint Conference: 69th Wildlife Disease Association (WDA) and 14th European Wildlife Disease Association (EWDA): 'Managing Wildlife Diseases for Sustainable Ecosystems': "First global symposium and workshops on *Batrachochytrium salamandrivorans* (*Bsal*)",
- Symposium organised by Pasmans F., Gray M., Martel A., Bletz M., Cunningham AA., Miller D. (31st of August 2020)
- Workshops organised by Pasmans F., Martel A., Pessier A., Grant E., Canessa S., Briggs C., Wilber M. (31st of August 2020)

Evenet Symposium 2020 (10th two-day symposiumon Eco-Evolutionaty Dynamics), Kortrijk 8 and 9 January 2020. Oral presentations (Erens J. "Long-term persistence of European salamander populations following disease invasion"; Lammens L. "One strain is not the other-Susceptibility to antifungals varies between strains of a highly virulent chytrid fungus"; Kelly M. "Thriving after host extinction: intraspecific variation and isolate-specific metabolite capacities of *Batrachochytrium salamandrivorans*"; Van Leeuwenberg R. "Agricultural fungicides: hope to save amphibians from chytridiomycosis?")

Creation of educational video posted on the www.bsaleurope.com website ('Prevention: for pet keepers', 'Prevention for naturalists'. December 2019. Announced and link distributed through Facebook (Wildlife Health Ghent, B. salamandrivorans) and Twitter (@BsalEurope)

Belgium Biodiversity Platform: *Dead or alive: towards a sustainable wildlife trade: One world One Health Recommendations*, Brussels. 3-4th of December 2019. Oral presentation (Pasmans F. "Reptile and amphibian pets: Health benefits and threats"), Poster presentation (Project Poster) and bsaleurope-website (www.bsaleurope.com) demonstration.

3rd symposium on Research in Zoo/Wildlife and Tropical Medicine. Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, 2/12/2019. Oral presentation: Pasmans F. "Infectious threats to biodiversity loss"

Societas Europaea Herpetologica (SEH) Conference, Milan. 2-6th of September 2019. Poster presentation (Mitigating a new infectious disease in salamanders to counteract the loss of European biodiversity) and Oral presentations (Pasmans F. and Martel A. "Setting the scene: the *Bsal* threat to European urodeles"; Beukema W. "Beneficial amphibian thermal behaviour

remains constrained by the environment in the face of pathogenic invasion"; Canessa S. "Developing an early response plan to invasion by *Batrachochytrium salamandrivorans"*)

Creation of educational video's posted on the www.BsalEurope.com website ('Etiology and epidemiology of *Bsal*', 'Clinical signs, pathology and pathogenesis', 'Diagnosis', 'Treatment'. August 2019. Announced and link distributed through Facebook (Wildlife Health Ghent, B. salamandrivorans) and Twitter (@BsalEurope)

Media news: VRT NWS Karrewiet, April 2019. Education of children through their news-channel.
> https://www.facebook.com/vrtnws/posts/10158368990354622?comment_id=1015837 9172864622&comment_tracking=%7B%22tn%22%3A%22R%22%7D

Addition of *Batrachochytrium salamandrivorans* to list of notifiable diseases in the Belgium National law: Belgisch Staatsblad (26.06.2019): 3 juni 2019 Koninklijk besluit tot wijziging van het koninklijk besluit van 3 februari 2014 tot aanwijzing van de dierziekten die vallen onder de toepassing van hoofdstuk III van de dierengezondheidswet van 24 maart 1987 en tot regeling van aangifteplicht.

ZSL Symposium. Mitigating single pathogen and co-infection that threaten amphibian biodiversity. London, UK. 23th-26th of April 2019. Oral presentations (Martel A. and Pasmans F. "*Bsal*: translating basic science in mitigation actions"; Canessa S. "Decision making for mitigating amphibian diseases"), Poster presentations (Laking et al. "The importance of fire salamanders (*Salamandra Salamandra terrestris*) within Belgian forest ecosystems"; Verbrugghe et al. "Mimicking early host-chytrid interactions using an *in vitro*-infection model")

Fungal Genetics Conference, Pacific Grove, California, USA. 12th-17th of March 2019. Oral presentation (Martel A. "*Batrachochytrium salamandrivorans* infections in amphibians").

NCRS meeting, Holderness, USA. 2018. "*Batrachochytrium salamandrivorans*: interactions with its host"

ISHAM meeting, Amsterdam, Nederland. 2018. "*Batrachochytrium salamandrivorans* in amphibians"

Development of national Bsal-Action Plan in Belgium: Chytridiomycose *Batrachochytrium salamandrivorans* (*Bsal*), Actieplan – België, Brussel, 2017. https://www.natuurenbos.be/sites/default/files/inserted-files/actieplan_bsal_be_finale_versie_20170321.pdf

ACRS, Cambridge, UK. 24-25/6/2017. *Batrachochytrium salamandrivorans*: imminent threat to European urodelan diversity.

EWDA student chapter, Liège, Belgium. 2017 From basic science to action: the case of chytrid infections.

9th meeting of the Bern Convention Group of Experts on Amphibians and Reptiles, Trondheim, Norway. 2017 Infectious diseases in European amphibians.

SEVC Barcelona, Spain 9-11/11/2017 What we know about amphibian diseases.

SEH meeting Salzburg, Austria, 2017. Round table with presentation of tender to the European herpetological community.

Bsal working group, Belgium. Brussels, Belgium. 2016-2017: several updates on the state of the art of Bsal and its mitigation.

A Metro newspapaper article "Un champignon "dévoreur" de salamandres"

Bsal flyer created in collaboration with Claude Miaud: http://rainne.natagora.be/fileadmin/Rainne/salamandre/Bsal_Flyer_versionWall_br.pdf

A Natagora magazine article "Le dévoreur de salamandres sous la loupe"

Bsal information on Natagora website: http://rainne.natagora.be/index.php?id=salamandre

Dissemination of public awareness material to Natuurpunt en ANB (Agency for Nature and Forests)

- Talks addressed to stakeholders of the general public (pet keepers, nature conservation societies etc)

AG Urodela (DGHT), largest society of salamander hobbyists in the world, Gersfeld, Germany, 18-20/10/2019. Oral presentation: Pasmans F., Martel A. (2019) "*Bsal* and *Bd*: what's new".

Symposium for the general public, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, 27/4/2019. Oral presentation (Pasmans F. "Safe Kermit").

Presentation to volunteers, on 11/2/2018 in Marche-en-Famenne "Le champignon pathogène *Batrachochytrium salamandrivorans* en Wallonie et mise en place d'un suivi des populations de la Salamandre terrestre (Arnaud Laudelout & Thierry Kinet / Natagora)"

TERRA (Belgium terrarium keepers), Antwerp, Belgium 2017 the future of keeping reptiles and amphibians as pets.

11de studiedag Hyla (working group for amphibians and reptiles, NGO Natuurpunt). 16th of December 2017. Martel A. "Chytridiomycosis in België"

Dutch Salamander Society, Tilburg, the Netherlands. 2016 Chytrid infections in European amphibians: multiple actors in a globalized world.

Internationale Fachtagung zum Feuersalamander – Lurch des Jahres 19-20th of November 2016 DGHT Field Herpetology, Basel, Switzerland. Oral Presentation (Pasmans F. "Chytrid infections in European urodelans: a complex tale").

DGHT AG Urodela (largest society of urodele keepers), Gersfeld, Germany, 2016 Neues uber *Bsal*.

10de studiedag Hyla (working group for amphibians and reptiles, NGO Natuurpunt). 8th of November 2016. Martel A. "*Batrachochytrium salamandrivorans (Bs):* bedreiging voor Belgische amfibieën"

France

- Scientific communication addressed to any audience, including governmental authorities and NGO

Oral communication and poster (Repto Terra Expo, and Festival International du Film de Ménigoute Ornithologique de de Ménigoute, Rencontres herpétologiques du Est, presentation to national parks foundation)

Dissemination of public awareness material to various institutions in the herpetological network for further distribution:
- Société Herpétologique de France (SHF),
- Réserve Naturelles de France (RNF),
- Office National des Forêts (ONF),
- Centre Permanent d'Initiatives pour l'Environnement (CPIE),
- Agence Française pour la Biodiversité (AFB)

Articles in regional newspaper and online "Midi Libre" in the South of France: https://www.midilibre.fr/

Bsal flyer created in collaboration with Claude Miaud: http://rainne.natagora.be/fileadmin/Rainne/salamandre/Bsal_Flyer_versionWall_br.pdf

Website of Groupe Ornithologique du Nord-Pas-de-Calais:

- https://gon.fr/gon/urgent-un-champignon-mortel-pour-les-urodeles-aux-portes-du-departement-du-nord/

- https://www.facebook.com/1386212491636370/photos/a.1388255981432021.1073741 828.1386212491636370/1740036679587281/?type=3&comment_id=1740074392916 843&comment_tracking=%7B%22tn%22%3A%22R%22%7D

Website "Les zones humides": Information regarding *Bsal* and the early warning system in place in France: http://www.zones-humides.org/actualit%C3%A9-734

Bsal information on EU *Bsal* project partner's own website 'Centre d'Ecologie Fonctionelle et Evolutive': https://www.cefe.cnrs.fr/fr/

Bsal information on http://www.alerte-amphibien.fr/maladies-amphibiens.html; http://www.alerte-amphibien.fr/chytridiomycose.html; http://www.alerte-amphibien.fr/signaler-mortalite.php

Germany

- Scientific communication addressed to any audience, including governmental authorities and NGO

Societas Europaea Herpetologica (SEH) Conference, Milan. 2-6 September 2019. Oral presentation (Lötters S. "Germany, the hotspot of Bsal emergence")

Lötters, S., N. Wagner & M. Veith: Die Salamanderpest. Bedrohung durch *Bsal,* ein neues invasive Pathogen. Informationsaustausch SGD-Nord – Fach Biogeographie/Universität Trier, March 2018.

Lötters, S.: Zur aktuellen Verbreitung des invasiven Amphibien-Pathogens *Batrachochytrium salamandrivorans* in Europa. — Jahrestagung, Österreichische Gesellschaft für Herpetologie (ÖGH), Wien (Austria), January 2018.

Lötters, S.: Aktueller Kenntnisstand zur Verbreitung und Gefährdung durch *Bsal.* — Naturschutzseminar LBV und LARS, Amberg (Germany), October 2017.

Lötters, S., L. Dalbeck, H. Düssel-Siebert, K. Kirst, A. Martel, D. Ohlhoff, F. Pasmans, S. Steinfartz, M. Vences, N. Wagner, J. Wegge & M. Veith: The status of *Batrachochytrium salamandrivorans* in its potential center of dispersal in the Eifel, Germany. — European Congress of Herpetology by SEH, Salzburg (Austria), September 2017.

Lötters, S., L. Dalbeck, H. Düssel-Siebert, K. Kirst, A. Martel, D. Ohlhoff, F. Pasmans, S. Steinfartz, M. Vences, N. Wagner, J. Wegge & M. Veith: The status of *Batrachochytrium salamandrivorans* in its potential center of dispersal in the Eifel, Germany. — 53. Jahrestagung, Deutsche Gesellschaft für Herpetologie und Terrarienkunde (DGHT), Ulm (Germany), September 2017.

Lötters, S.: Zum Status vom "Salamanderfresserpilz" (*Batrachochytrium salamandrivorans*) in Deutschland. — Symposium des Landesfachausschuss Feldherpetologie des NABU, Bingen (Germany), March 2017.

Internationale Fachtagung zum Feuersalamander – Lurch des Jahres 19-20th of November 2016 DGHT Field Herpetology, Basel, Switzerland. Oral Presentation (Lötters S. "Der Salamanderpilz in Deutschland").

Citizen science / passive fire salamander surveillance campaign (addressed to any reader, especially private people frequently visiting salamander habitats). In cooperation with *Stiftung Natur und Umwelt Rheinland Pfalz (SNU),* a flyer was launched this month stimulating people

to report via a webpage records of fire salamanders in the state of Rhineland-Palatinate. https://artenfinder.rlp.de/node/3

Active information to professional and amateur field herpetologists / semi-active fire salamander surveillance **(**addressed to people with a scientific and/or conservation background actively doing research or conservation in the field) via personal contacts they have actively sensitized 22 people in all states of Germany where fire salamanders occur about *Bsal.* They do not systematically monitor fire salamander populations but do from time to time actively visit populations they know to record their status and to report about unusual mortality (which then will be studied)

Spread information to DGHT Arbeitsgruppe Urodela, the largest society of salamander hobbyists in the world http://www.ag-urodela.de/en/bsal/

- Scientific publications addressed to readers with scientific background such as governmental authorities and NGO people

Dalbeck, L., Düssel-Siebert, H., Kerres, A., Kirst, K., Koch, A., Lötters, S., Ohlhoff, D., Sabino-Pinto, J., Preißler, K., Schulte, U., Schulz, V., Steinfartz, S., Veith, M., Vences, M., Wagner, N. & Wegge, J. (2018): Die Salamanderpest und ihr Erreger *Batrachochytrium salamandrivorans* (*Bsal*): aktueller Stand in Deutschland. — Zeitschrift für Feldherpetologie, 25: 1-22

Wagner, N., Lötters, S., Bauer, S.D., Beninde, J., Ewen, J., Fichera, G., Feiler, L., Feldmeier, S., Göb, D., Gussone, L., Harms, W., Harzheim, M., Hassenjürgen, L., Kolwelter, C., Krone, S., Lambing, A., Marin da Fonte, L.F., Martens, A., Peters, J., Pfeifer, L., Pfrommer, J., Reinhardt, N., Sandvoß, M., Schafft, M., Schulte, U., Tull, F., Unterberg, K., & Veith, M. (2017): Zur Verbreitung des Gebänderten Feuersalamanders (*Salamandra salamandra terrestris* Lacépède, 1788) im Naturpark Südeifel sowie im rheinland-pfälzischen Teil des Naturparks Nordeifel (Amphibia Urodela Salamandridae). — Dendrocopos, 44: 7-20.

Italy

- Scientific communication addressed to any audience, including governmental authorities and NGO (ISPRA, Societas Herpetologica Italica)

Created *Bsal* Wiki page (in Italian) https://it.wikipedia.org/wiki/Batrachochytrium_salamandrivorans

Bsal Facebook group created "Salamandrivorans" https://www.facebook.com/groups/134273207141409/?ref=br_rs

Reached out to Italian herpetological mailing list (erpetologia @yahoogroups.com)

Reached out to Italian vertebrate mailing list (vertebrati@liste.cilea.it)

Information (also flyer created) provided to private keepers and breeders (Verona Reptiles Expo)

Created *Bsal* logo for Italian population

Article in UNIGE Newsletter http://scienza.unige.it/node/92

- <u>Talks addressed to stakeholders of the general public (pet keepers, nature conservation societies etc)</u>

Stefano Canessa, conference: "A un passo dal baratro (e ritorno)" (One step away from the abyss (and back). University of Genova, 16 December 2019.

Associazione Didattica Museale Genova, educational laboratory. La sesta estinzione. Anfibi in pericolo (The sixth extinction. Amphibians in danger. For school students, during the Festival della Scienza 2018, 27 October - 4 November, Genova)

Elena Grasselli and Sebastiano Salvidio, conference: Cambiamenti pericolosi- Il declino degli anfibi e l'importazione di specie alloctone (Dangerous changes - Amphibian decline and the trade of alien species. Open conference during the Festival della Scienza 2018, 27 October Genova)

Emmanuel Biggi led conference on amphibian conservation at UNIGE

Spain

- <u>Scientific communication addressed to any audience, including governmental authorities and NGO</u>

ZSL Symposium. Mitigating single pathogen and co-infection that threaten amphibian biodiversity. April 2019. Poster presentations (Salvidio et al. *Batrachochytrium salamandrivorans* in Italy: First data from wild populations and captive collections; Grasselli et al. Wound healing as a possible mechanism contributing to resistance to chytridiomycosis)

Project website information included on project partner's own website: http://www.mncn.csic.es

New website being developed which provides reccomendations to amphibian pet keepers on amphibian pet shops which meet legal requirements and practice good biosecurity measures. This website also provides amphibian pet shop owners with advice on how to maintain healthy collections: amphibianpetadvisor.com

Flyer for amphibian husbandry

The Netherlands

- <u>Scientific communication addressed to any audience, including governmental authorities and NGO</u>

Societas Europaea Herpetologica (SEH) Conference, Milan. 2-6 September 2019. Poster presentation and oral presentation (Spitzen A. and Gilbert M. *Batrachochytrium salamandrivorans* in the Netherlands: past, present and future perspectives)

ZSL Symposium. Mitigating single pathogen and co-infection that threaten amphibian biodiversity. April 2019. Oral presentation (Spitzen A. "What makes a small country big: the ubiquitousness of amphibian pathogens in the Netherlands")

Created animations:
- Created animations on public participation in Bsal Early Warning System [launched in Febr. 2020]. Available on YouTube (RAVON Channel) and www.bsaleurope.com, subtitles will be provided.
- general *Bsal* information*: https://www.youtube.com/watch?v=kss8B7V_zAA
- amphibian husbandry*: https://www.youtube.com/watch?v=-WgYZMEGA9Y
- *Bsal* fieldwork hygiene protocol: https://www.youtube.com/watch?v=i-WJtmPdkng&t=53s

*available with subtitles in Bulgarian, Czech, Dutch, English, French, German, Hungarian, Italian, Macedonian, Polish, Portuguese, Russian, Serbian, Slovak, Slovenian, Spanish and Swedish

Created informative leaflets:
- for veterinarians: http://bsaleurope.com/wp-content/uploads/2019/07/Leaflet-veterinarians-2019_DEF.pdf
- on *Bsal* identification, how to report suspected cases, how to prevent spread of *Bsal*: http://bsaleurope.com/recognize-sick-animals/
- on disinfection of heavy machinery: http://bsaleurope.com/wp-content/uploads/2018/04/Protocol-Heavy-Machinery-UK-2018_DEF.pdf

EU *Bsal* project website link and project information on RAVON website http://sossalamander.nl/facts/fungal-disease

Created a *Bsal* Facebook page "B salamandrivorans": https://www.facebook.com/B.salamandrivorans/

Created and moderate a *Bsal* project Twitter account: https://twitter.com/BsalEurope?ref_src=twsrc%5Etfw&ref_url=http%3A%2F%2Fbsaleurope.com%2Fpublic-awereness-material%2F

News items published on the website: NatureToday.com:

- 27 nov. 2018 Threatened Limburg species in the spotlights [https://www.naturetoday.com/intl/nl/nature-reports/message/?msg=24765]
- 21 nov. 2018 Doctorate thesis provides information on required mitigation measures upon amphibian disease outbrek [https://www.naturetoday.com/intl/nl/nature-reports/message/?msg=24753]
- 28 march 2018Call for the public to be aware of dead amphibians and request to collect them [https://www.naturetoday.com/intl/nl/nature-reports/message/?msg=24233]
- 29 March 2017 Where is the salamander fungus? [https://www.naturetoday.com/intl/nl/nature-reports/message/?msg=23361]

Articles and reports (in Dutch)

- Wrote a RAVON-final report: 'Surveillance naar *Batrachochytrium salamandrivorans* in de handel' for a governemental institute (Nederlandse Voedsel- en Warenautoriteit *Ministerie van Economische Zaken*): https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/publicaties/surveillance-naar-batrachochytrium-salamandrivorans-in-de-handel
- Gilbert, M. and A. Spitzen - van der Sluijs (2019). De toekomst van de Vuursalamander in Limburg. Natuurhistorisch Maandblad 3(108): 59-63.
- Spitzen–van der Sluijs, A. M., et al. (2019). Verslag activiteiten in 2018 en workshop 2018. Batrachochytrium salamandrivorans in Nederland. Stichting RAVON, Nijmegen.
- Gilbert, M. J., et al. (2019). Surveillance naar *Batrachochytrium salamandrivorans* in de handel. Eindrapportage 2018. Stichting RAVON, Nijmegen.
- Gilbert, M., et al. (2019). Early Warning System Chytride in Noord-Brabant. RAVON. Nijmegen.
- Gilbert, M. and A. Spitzen - van der Sluijs (2019). De toekomst van de Vuursalamander in Limburg. Natuurhistorisch Maandblad 3(108): 59-63.
- Spitzen, A., et al. (2018). Het onzichtbare zichtbaar gemaakt. Kijk op Exoten September 2018: 10-11.
- Gilbert, M.J., et al. (2018). Surveillance naar *Batrachochytrium salamandrivorans* in de handel. Tussenrapportage 2017, Stichting RAVON, Nijmegen
- Spitzen, A., et al. (2017). De effecten van *Batrachochytrium salamandrivorans* op salamanders in Nederland. Tussenrapportage 2017, RAVON.

Articles (in English)

- Spitzen - van der Sluijs, A., et al. (2018). Post-epizootic salamander persistence in a disease-free refugium suggests poor dispersal ability of *Batrachochytrium salamandrivorans*. Scientific Reports 8(1): 3800.
- Gilbert, M. and A. Spitzen - van der Sluijs (2018). Health and biosecurity from an amphibian perspective. OFI Journal 88: 10-13.
- Spitzen - van der Sluijs, A. (2018). It takes three to tango. The impact of chytridiomycosis on native amphibians in the Netherlands. Laboratory of Veterinary

Bacteriology and Mycology, Faculty of Veterinary Medicine Ghent University. PhD-thesis.

- Spitzen, A., et al. (2018). Collaboration for Fire Salamander conservation. AArk Newsletter 45: 9-11.
- Gilbert, M. and A. Spitzen - van der Sluijs (2017). Amphibian fungal disease in the live animal trade. OFI Journal 84 (May 2017): 16-18.

Presentations 2019 (in Dutch)
- Stark, T. Spitzen –van der Sluijs, Gilbert, M. Early Warning System chytride Noord-Brabant. Lezing bij KNNV Oisterwijk, 16 maart 2019, Oisterwijk.
- Stark, T. Spitzen –van der Sluijs, Gilbert, M. Early Warning System chytride Overijssel. Lezing bij KNNV/IVN Steenwijk , Steenwijk, 18 maart 2019.
- Stark, T. Spitzen –van der Sluijs, Gilbert, M. Early Warning System chytride Noord-Brabant. Lezing bij Staatsbosbeheer (professionals en vrijwilligers) , 19 maart 2019, Bezoekers centrum Grote Peel.
- Stark, T. Spitzen –van der Sluijs, Gilbert, M. Early Warning System chytride Noord-Brabant. Lezing bij Poelenwerkgroep Eindhoven, 25 maart 2019, Eindhoven.
- Stark, T. Spitzen –van der Sluijs, Gilbert, M. Early Warning System chytride Noord-Brabant. Lezing en excursie bij Poelenwerkgroep Brabantse Wal, 1 april 2019, Roosendaal.
- Stark, T. Spitzen –van der Sluijs, Gilbert, M. Early Warning System chytride Noord-Brabant. Lezing bij KNNV Oisterwijk, 16 maart 2019, Oisterwijk.
- Stark, T. Spitzen –van der Sluijs, Gilbert, M. Early Warning System chytride Noord-Brabant. Lezing en excursie bij poelenwerkgroep Veldhoven, 19 april 2019, Veldhoven.
- Stark, T. Spitzen –van der Sluijs, Gilbert, M. Early Warning System chytride Noord-Brabant. Lezing bij poelenwerkgroep Zundert, 20 april 2019, Zundert.
- Stark, T. Spitzen –van der Sluijs, Gilbert, M. Early Warning System chytride Noord-Brabant. Lezing en excursie bij Poelenwerkgroep Laarbeek, 4 mei 2019, Laarbeek.

Presentations 2019 (in English)
- Spitzen-van der Sluijs, A., Stark. T. How (and why) to detect emerging infectious diseases? Lezing op het ' ZSL Mitigating single pathogen and co-infections that threaten amphibian biodiversity' symposium, 25 april 2019, London.
- Spitzen-van der Sluijs, A., Stark. T. How (and why) to detect emerging infectious diseases? Lezing op 20th European Congress of Herpetology (SEH), 3 september 2019, Milaan.
- Spitzen-van der Sluijs, A., Gilbert, M. Batrachochytrium salamandrivorans in the Netherlands: past, present and future perspectives. 20th European Congress of Herpetology (SEH), 4 september 2019, Milaan.
- Spitzen-van der Sluijs, A., Stark. T. How (and why) to detect emerging infectious diseases? Lezing op het ' ZSL Mitigating single pathogen and co-infections that threaten amphibian biodiversity' symposium, 25 april 2019, London.
- Spitzen-van der Sluijs, A., Stark. T, Gilbert, M. How (and why) to detect emerging infectious diseases? Karch herpetologische conferentie, 8 december 2019, Bern.

Presentations 2018 (in Dutch)

- Spitzen-van der Sluijs, A.M., 2018. Vuursalamanderonderzoek, Bsal en beheer. RAVON-dag 2018. Nijmegen. 10 November.
- Stark, T., 2018. Lezing over amfibieënziektes. Noord-Brabant. 8 February.
- Stark, T., 2018. Lezing over amfibieënziektes. Vechtdal, Hardenberg. 20 February.
- Stark, T., 2018. Lezing over amfibieënziektes. Zuid Twente, Hardenberg. 23 February
- Stark, T., 2018. Lezing over amfibieënziektes voor natuurbeschermingsvereniging. IJhorst. 5 March.
- Stark, T., 2018. Lezing over amfibieënziektes voor KNNV Twente. Almelo. 15 March.

Presentations 2017 (in Dutch)

- Stark, T., 2017. De paddenvlieg in Nederland. Taxonomie en ecologie. RAVON-dag 2017. Nijmegen. 11 November.

Presentations 2017 (in English)

- Stark, T., 2017. A bumpy business: effects of dermocystid infections on fitness of two newt populations in the Netherlands. Amphibian Conservation Research Symposium (ACRS). Canterbury. 23-25 June.

UK

- <u>Scientific communication addressed to any audience, including governmental authorities and NGO</u>

Joint Conference: 69th Wildlife Disease Association (WDA) and 14th European Wildlife Disease Association (EWDA): 'Managing Wildlife Diseases for Sustainable Ecosystems': "First global symposium and workshops on *Batrachochytrium salamandrivorans* (*Bsal*)",
- Symposium organised by Pasmans F., Gray M., Martel A., Bletz M., Cunningham AA., Miller D. (31st of August 2020)
- Workshops organised by Pasmans F., Martel A., Pessier A., Grant E., Canessa S., Briggs C., Wilber M. (31st of August 2020)

ZSL Symposium. Mitigating single pathogen and co-infection that threaten amphibian biodiversity. April 2019.

Project website information included on partner's website:

www.gardenwildlifehealth.org

www.zsl.org

Promoted EU *Bsal* project via social media (Twitter and Facebook) https://www.facebook.com/wildlifehealth/

Sharing posts from Garden Wildlife Health on Facebook https://www.facebook.com/wildlifehealth/

Highlighting availability of evidence-based public awareness material on Garden Wildlife Health:

https://www.facebook.com/B.salamandrivorans/photos/pcb.1949609445259314/1949608531926072/?type=3&theater

https://www.facebook.com/B.salamandrivorans/photos/pcb.1949609445259314/1949608528592739/?type=3&theater

https://www.facebook.com/wildlifehealth/

Shared Spitzen-van de Sluijs et al. 2018 publication on social media: https://www.facebook.com/B.salamandrivorans/photos/a.1938209236399335.1073741828.1938038339749758/2000653956821529/?type=3&theater

Provided a summary of Spitzen-van de Sluijs et al. 2018 to DEFRA

Advised DEFRA on how it can increase public awareness of *Bsal* and spread information to the amphibian industry and the public

Media attention

Expressed as Altmetric scores of key papers that received the widest attention:

1. Martel, A., Vila-Escale, M., Fernández-Giberteau, D., Martinez-Silvestre, A., Canessa, S., Van Praet, S., Pannon, P., Chiers, K., Ferran, A., Kelly, M., Picart, M, Piulats, D., Li, Z., Pagone, V., Pérez-Sorribes, L., Molina, C., Tarragó-Guarro, A., Velarde-Nieto, R., Carbonell, F., Obon, E., Martínez-Martínez, D., Guinart, D., Casanovas, R., Carranza, S., Pasmans, F. (2020). Integral chain management of wildlife diseases. Conservation Letters. DOI:10.1111/conl.12707

Altmetric Score – 21

Sources	Number of citations/reads
Tweets	31
Facebook pages	1

2. Canessa, S., Spitzen-van der Sluijs, A., Stark, T., Allen, B.E., Bishop, P.J., Bletz, M., Briggs, C.J., Daversa, D.R., Gray, M.J., Griffiths, R.A., Harris, R.N., Harrison, X.A., Hoverman, J.T., Jervis, P., Muths, E., Olson, D.H., Price, S.J., Richards-Zawacki, C.L., Robert, J., Rosa, G.M., Scheele, B.C., Schmidt, B.R., Garner, T.W.J. (2020) Conservation decisions under pressure: Lessons from an exercise in rapid response to wildlife disease. Conservation Science and Practice. https://doi.org/10.1111/csp2.141

Altmetric Score – 27

Sources	Number of citations/reads
Tweets	40

3. Cunningham, A.A., Smith, F., McKinley, T.J., Perkins, M.W., Fitzpatrick, L.D., Wright, O.W., Lawson, B. (2019). Apparent absence of *Batrachochytrium salamandrivorans* in wild urodeles in the United Kingdom. Scientific Reports. DOI:10.1038/s41598-019-39338-4

Altmetric Score – 64

Sources	Number of citations/reads
News outlets	5
Tweets	27
Facebook pages	3
Reads on Mendeley	7

4. Scheele, B.C., Pasmans, F., Skerratt, L.FL., Berger, L., Martel, A., Beukema, W., Acevedo, A.A., Burrowes, P.A., Carvalho T., Catenazzi, A., De la Riva, I., Fisher, M.C., Flechas, S.V., Foster, C.N., Frías-Álvarez, P., Garner, T.W.J., Gratwicke, B., Guayasamin, J.M., Hirschfeld, M., Kolby, J.E., Kosch, T.A., La Marca, E., Lindenmayer, D.B., Lips,

K.R., Longo, A.V., Maneyro, R., McDonald, C.A., Mendelson III, J., Palacios-Rodriguez, P., Parra-Olea, G., Richards-Zawacki, C.L., Rödel, M.-O., Rovito, S.M., Soto-Azat, C., Toledo, L.F., Voyles, J., Weldon, C., Whitfield, S.M., Wilkinson, M., Zamudio, K.R., Canessa S. (2019) Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science. DOI: 10.1126/science.aav0379

Altmetric Score – 1862

Sources	Number of citations/reads
News outlets	118
Blogs	20
Tweets	1188
Facebook pages	14
Wikipedia pages	2
redditor	1
Research highlight platform	1
Citations	60
Reads on Mendeley	283

5. Sabino-Pinto, J., Veith, M., Vences, M., & Steinfartz, S. (2018). Asymptomatic infection of the fungal pathogen *Batrachochytrium salamandrivorans* in captivity. Scientific Reports. doi:10.1038/s41598-018-30240-z

Altmetric Score – 20

Sources	Number of citations/reads
Tweets	27
Facebook pages	1
Citations	4
Reads on Mendeley	25

6. Fitzpatrick, L. D., Pasmans, F., Martel, A., & Cunningham, A. A. (2018). Epidemiological tracing of *Batrachochytrium salamandrivorans* identifies widespread infection and associated mortalities in private amphibian collections. Scientific Reports. DOI:10.1038/s41598-018-31800-z

Altmetric Score – 75

Sources	Number of citations/reads
News outlets	2
Policy sources	1
Tweets	74
Facebook pages	3
Wikipedia page	1
Citations	4
Reads on Mendeley	25

7. Beukema, W., Martel, A., Nguyen, T.T., Goka, K., Schmeller, D.S., Yuan, Z., Laking, A.E., Nguyen, T.Q., Ling, C., Shelton., Loyau, A., Pasmans, F. (2018). Environmental context and differences between native and invasive observed niches of *Batrachochytrium salamandrivorans* affect invasion risk assessments in the Western Palaearctic. Diversity and Distributions. https://doi.org/10.1111/ddi.12795

Altmetric Score – 22

Sources	Number of citations/reads
Tweets	31
Facebook pages	2
Citations	4
Reads on Mendeley	36

8. Smith., H. K., Pasmans, F., Dhaenens, M., Deforce, D., Bonte, D., Verheyen, K., Lens, L., Martel, A.(2018). Skin mucosome activity as an indicator of *Batrachochytrium salamandrivorans* susceptibility in salamanders. PLoS ONE. https://doi.org/10.1371/journal.pone.0199295

Altmetric Score – 33

Sources	Number of citations/reads
News outlets	1
Blogs	1
Tweets	28
Facebook pages	1
Citations	4
Reads on Mendeley	34

9. Spitzen-van der Sluijs, A., Stegen, G., Bogaerts, S., Canessa, S., Steinfartz, S., Jannsen, N., Bosman, W., Pasmans, F., Martel. A. (2018). Post-epizootic salamander persistence in a disease-free refugium suggests poor dispersal ability of *Batrachochytrium salamandrivorans*. Scientific Reports. https://doi.org/10.1038/s41598-018-22225-9

Altmetric Score – 25

Sources	Number of citations/reads
Tweets	36
Facebook pages	3
Citations	5
Reads on Mendeley	43

10. Yuan, Z., Martel, A,, Wu, J., Van Praet, S., Canessa, S., Pasmans, F. (2018) Widespread occurrence of an emerging fungal pathogen in heavily traded Chinese urodelan species. Conservation Letters. https://doi.org/10.1111/conl.12436

Altmetric Score – 41

Sources	Number of citations/reads
News outlets	1
Blogs	1
Tweets	42
Facebook page	1
Citations	8
Reads on Mendeley	27

11. Canessa, S., Bozzuto, C., Campbell Grant, E. H., Cruickshank, S. S., Fisher, M. C., Koella, J. C., Lötters, S., Martel, A., Pasmans, F., Scheele, B. C., Spitzen-van der Sluijs, A., Steinfartz, S. and Schmidt, B. R. (2018). Decision making for mitigating wildlife diseases: from theory to practice for an emerging fungal pathogen of amphibians. Journal of Applied Ecology. DOI:10.1111/1365-2664.13089

Altmetric Score – 87

Sources	Number of citations/reads
Tweets	127
Facebook page	3
Google+ users	1
Citations	16
Reads on Mendeley	51

12. Van Rooij, P., Pasmans, F., Coen, Y., Martel, A. (2017) Efficacy of chemical disinfectants for the containment of the salamander chytrid fungus *Batrachochytrium salamandrivorans*. *PloS ONE*. https://doi.org/10.1371/journal. pone.0186269

Altmetric Score – 43

Sources	Number of citations/reads
Tweets	56
Facebook pages	12
Citations	6
Reads on Mendeley	49

13.   Nguyen, T.T., Nguyen, T.V., Ziegler, T., Pasmans, F., Martel, A. (2017). Trade in wild anurans vectors the urodelan pathogen *Batrachochytrium salamandrivorans* into Europe. Amphibia-Reptilia**.** DOI:10.1163/15685381-00003125

Altmetric Score –135

Sources	Number of citations/reads
News outlets	1
Blog	1
Tweets	178
Facebook pages	8
Wikipedia pages	1
Citations	12
Reads on Mendeley	37

14.   Stegen, G., Pasmans, F., Schmidt, B. R., Rouffaer, L. O., Van Praet, S., Schaub, M., Canessa, S., Laudelout, A., Kinet, T., Adriaensen, C., Haesebrouck, F., Bert, W., Bossuyt, F., Martel, A. (2017). Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. Nature. http://dx.doi.org/10.1038/nature22059

Altmetric Score – 347

Sources	Number of citations/reads
News outlets	26
Blogs	3
Policy sources	1
Tweets	205
Facebook pages	12
Google+ users	1
Citations	69
Reads on Mendeley	183

15.   Farrer, R.A., Martel, A., Verbrugghe, E., Abouelleil, A., Ducatelle, R., Longcore, J.E., James, T.Y., Pasmans, F., Fisher, M.C., Cuoma, C.A. (2017) Evolutionary innovations underpin niche-specific infection strategies across emerging pathogenic chytrid fungi. Nature Communications. https://doi.org/10.1038/ncomms14742

Altmetric Score – 63

Sources	Number of citations/reads
News outlets	2
Tweets	77
Facebook page	1
Citations	26
Reads on Mendeley	101

16.   Laking A.E., Ngo, H.N., Pasmans, F., Martel, A., Nguyen, T.T. (2017). *Batrachochytrium salamandrivorans* is the predominant chytrid fungus in Vietnamese salamanders. Scientific Reports. https://doi.org/10.1038/srep44443

Altmetric Score – 73

Sources	Number of citations/reads
Tweets	97
Facebook page	9
Citations	27
Reads on Mendeley	73

Scientific publications

addressed to readers with scientific background, such as governmental authority and NGO's

1. Martel, A., Vila-Escale, M., Fernández-Giberteau, D., Martinez-Silvestre, A., Canessa, S., Van Praet, S., Pannon, P., Chiers, K., Ferran, A., Kelly, M., Picart, M, Piulats, D., Li, Z., Pagone, V., Pérez-Sorribes, L., Molina, C., Tarragó-Guarro, A., Velarde-Nieto, R., Carbonell, F., Obon, E., Martínez-Martínez, D., Guinart, D., Casanovas, R., Carranza, S., Pasmans, F. (2020). Integral chain management of wildlife diseases. Conservation Letters. DOI:10.1111/conl.12707

2. Canessa, S., Spitzen-van der Sluijs, A., Stark, T., Allen, B.E., Bishop, P.J., Bletz, M., Briggs, C.J., Daversa, D.R., Gray, M.J., Griffiths, R.A., Harris, R.N., Harrison, X.A., Hoverman, J.T., Jervis, P., Muths, E., Olson, D.H., Price, S.J., Richards-Zawacki, C.L., Robert, J., Rosa, G.M., Scheele, B.C., Schmidt, B.R., Garner, T.W.J. (2020) Conservation decisions under pressure: Lessons from an exercise in rapid response to wildlife disease. Conservation Science and Practice. https://doi.org/10.1111/csp2.141

3. Verbrugghe, E., Pasmans, F., Martel, A. (2019) Reference gene screening of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* for quantitative real-time PCR studies. Scientific Reports. DOI:10.1038/s41598-019-54582-4

4. Wagner, N., Schulz, V., Steinfartz, S., Reinhardt, T., Vences, M., Lötters, S., Dalbeck, L., Düssel-Siebert, H., Guschal, M., Ohlhoff, D., Wegge, J., Veith, M. (2019) Current findings on the status of the salamander plague in Germany. Natur und Landschaft. DOI:10.17433/11.2019.50153747.463-471

5. Thomas, V., Wang, Y., Van Rooij, P., Verbrugghe, E., Baláz, V., Bosch, J., Cunningham, A.A., Fischer, M.C., Garner, T.W.J., Gilbert, M.J., Grasselli, E., Kinet, T., Laudelout, A., Lötters, S., Loyau, A., Miaud, C., Salvidio, S., Schmeller, D.S., Schmidt, B.R., Spitzen-van der Sluijs, A., Steinfartz, S., Veith, M., Vences, M., Wagner, N., Canessa, S., Martel, A., Pasmans, F. (2019) Mitigating *Batrachochytrium salamandrivorans* in Europe. Amphibia-Reptilia. https://doi.org/10.1163/15685381-20191157

6. Canessa, S., Spitzen-van der Sluijs, A., C., Martel, A., Pasmans, F. (2019) Mitigation of amphibian disease requires a stronger connection between research and management. Biological Conservation. https://doi.org/10.1016/j.biocon.2019.05.030

7. Grasselli, E., Bianchi, G., Dondero, L., Machiano, V., Carafa, M., Perrone, M., Salvidio, S. (2019). First screening for *Batrachochytrium salamandrivorans* (*Bsal*) in wild and captive salamanders from Italy. Salamandra. 55(2), 124-126. ISSN 0036-3375

8. Sabino-Pinto, J., Martel, A., Pasmans, F., Steinfartz, S., Vences, M. 2019. Pooling skin swabs does not inhibit qPCR detection of amphibian chytrid infection. Plos One. https://doi.org/10.1371/journal.pone.0214405

9. Cunningham, A.A., Smith, F., McKinley, T.J., Perkins, M.W., Fitzpatrick, L.D., Wright, O.W., Lawson, B. (2019). Apparent absence of *Batrachochytrium salamandrivorans* in

wild urodeles in the United Kingdom. Scientific Reports. DOI:10.1038/s41598-019-39338-4

10. Marquis, O., Miaud, C., Gibault, C., Chai, N. (2019). A first screening of chytrid fungus Batrachochytrium in amphibians in French zoos. International Zoo Yearbook. https://doi.org/10.1111/izy.12210

11. Scheele, B.C., Pasmans, F., Skeratt, L.FL., Berger, L., Martel, A., Beukema, W., Acevedo, A.A., Burrowes, P.A., Carvalho T., Catenazzi, A., De la Riva, I., Fisher, M.C., Flechas, S.V., Foster, C.N., Frías-Álvarez, P., Garner, T.W.J., Gratwicke, B., Guayasamin, J.M., Hirschfeld, M., Kolby, J.E., Kosch, T.A., La Marca, E., Lindenmayer, D.B., Lips, K.R., Longo, A.V., Maneyro, R., McDonald, C.A., Mendelson III, J., Palacios-Rodriguez, P., Parra-Olea, G., Richards-Zawacki, C.L., Rödel, M.-O., Rovito, S.M., Soto-Azat, C., Toledo, L.F., Voyles, J., Weldon, C., Whitfield, S.M., Wilkinson, M., Zamudio, K.R., Canessa S. (2019) Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science. DOI:10.1126/science.aav0379

12. Canessa, S., Bozzuto, C., Pasmans, F., Martel, A. (2019). Quantifying the burden of managing wildlife diseases in multiple host species. Conservation Biology. https://doi.org/10.1111/cobi.13313

13. Bozzuto, C., & Canessa, S. (2019). Impact of seasonal cycles on host-pathogen dynamics and disease mitigation for *Batrachochytrium salamandrivorans*. Global Ecology and Conservation, https://doi.org/10.1016/j.gecco.2019.e00551

14. Verbrugghe, E., Adriaensen, C., Martel, A., Vanhaecke, L., & Pasmans, F. (2019). Growth regulation in amphibian pathogenic chytrid fungi by the quorum sensing metabolite tryptophol. Frontiers in Microbiology. https://doi.org/10.3389/fmicb.2018.03277

15. Lötters, S., Wagner, N., Kerres, A., Vences, M., Steinfartz, S., Sabino-Pinto, J., Seufer, L., Preissler, K., Schulz, V., Veith, M. (2018). First report of host co-infection of parasitic amphibian chytrid fungi. SALAMANDRA, 54(4), 287-290. ISSN 0036-3375

16. Sabino-Pinto, J., Veith, M., Vences, M., & Steinfartz, S. (2018). Asymptomatic infection of the fungal pathogen *Batrachochytrium salamandrivorans* in captivity. Scientific Reports. DOI:10.1038/s41598-018-30240-z

17. Fitzpatrick, L. D., Pasmans, F., Martel, A., & Cunningham, A. A. (2018). Epidemiological tracing of *Batrachochytrium salamandrivorans* identifies widespread infection and associated mortalities in private amphibian collections. Scientific Reports. DOI:10.1038/s41598-018-31800-z

18. Bletz, M. C., Kelly, M., Sabino-Pinto, J., Bales, E., Van Praet, S., Bert, W., Boyen, F., Vences, M., Steinfartz, S., Pasmans, F., Martel, A. (2018). Disruption of skin microbiota contributes to salamander disease. Proc. R. Soc. B. https://doi.org/10.1098/rspb.2018.0758

19. Pasmans, F., Canessa, S., Martel, A. (2018). The eye of the storm: silent infections driving amphibian declines. Animal Conservation. https://doi.org/10.1111/acv.12411

20. Martel, A., Pasmans, F., Fisher, M.C., Grogan, L.F., Skerratt, L.F., Berger, L. (2018) Chytridiomycosis. In: Seyedmousavi S., de Hoog G., Guillot J., Verweij P. (eds) Emerging

and Epizootic Fungal Infections in Animals. Springer, Cham. https://doi.org/10.1007/978-3-319-72093-7_14. ISBN: 978-3-319-72093-7

21. Beukema, W., Martel, A., Nguyen, T.T., Goka, K., Schmeller, D.S., Yuan, Z., Laking, A.E., Nguyen, T.Q., Ling, C., Shelton., Loyau, A., Pasmans, F. (2018). Environmental context and differences between native and invasive observed niches of *Batrachochytrium salamandrivorans* affect invasion risk assessments in the Western Palaearctic. Diversity and Distributions. https://doi.org/10.1111/ddi.12795

22. Sabino-Pinto, J., Krause, E. T., Bletz, M. C., Martel, A., Pasmans, F., Steinfartz, S., Vences, M. (2018). Detectability vs. time and costs in pooled DNA extraction of cutaneous swabs: a study on the amphibian chytrid fungi. Amphibia-Reptilia. https://doi.org/10.1163/15685381-20181011

23. Smith., H. K., Pasmans, F., Dhaenens, M., Deforce, D., Bonte, D., Verheyen, K., Lens, L., Martel, A.(2018). Skin mucosome activity as an indicator of *Batrachochytrium salamandrivorans* susceptibility in salamanders. PLoS ONE. https://doi.org/10.1371/journal.pone.0199295

24. EFSA Panel on Animal Health and Welfare (AHAW), More, S., Angel Miranda, M., Bicout, D., Bøtner, A., Butterworth, A., Calistri, P., Depner, K., Edwards, S., Garin-Bastuij, B., Good, M., Michel., V., Raj, M., Saxmose Nielsen, S., Sihvonen, N., Spoolder, H., Arend Stegeman, J., Thulke, H.H., Verlarde, A., Willeberg, P., Winckler, C., Baláž , V., Martel, A., Murray, K., Fabris, C., Munoz-Gerjardo, I., Gogin, A., Verdonck, F., Gortázar Schmidt, C. (2018) Risk of survival, establishment and spread of Batrachochytrium salamandrivorans (Bsal) in the EU. Efsa Journal, https://doi.org/10.2903/j.efsa.2018.5259

25. Dalbeck, L., Düssel-Siebert, H., Kerres, A., Kirst, K., Koch, A., Lötters, S., Ohlhoff, D., Sabino-Pinto, J., Preißler, K., Schulte, U., Schulz, V., Steinfartz, S., Veith, M., Vences, M., Wagner, N., Wegge, J. (2018). Die Salamanderpest und ihr Erreger *Batrachochytrium salamandrivorans* (Bsal): aktueller Stand in Deutschland. Zeitschrift für Feldherpetologie 25: 1–22

26. Spitzen – van der Sluijs, A., Stegen, G., Bogaerts, S., Canessa, S., Steinfartz, S., Janssen, N., Bosman, W., Pasmans, F., Martel, A. (2018). Post-epizootic salamander persistence in a disease-free refugium suggests poor dispersal ability of *Batrachochytrium salamandrivorans*. Scientific Reports. https://doi.org/10.1038/s41598-018-22225-9

27. Šunje, E., Pasmans, F., Maksimović, Z., Martel, A., & Rifatbefović, M. (2018). Recorded mortality in the vulnerable Alpine salamander, *Salamandra atra prenjensis* (Amphibia: Caudata), is not associated with the presence of known amphibian pathogens. SALAMANDRA. 54(1): 75-79. ISSN 0036-3375

28. Yuan, Z., Martel, A., Wu, J., Praet, S., Canessa, S., Pasmans, F. (2018). Widespread occurrence of an emerging fungal pathogen in heavily traded Chinese urodelan species. Conservation Letters. https://doi.org/10.1111/conl.12436

29. Thomas, V., Blooi, M., Van Rooij, P., Van Praet, S., Verbrugge, E., Grasselli, E., Lukac, M., Smith, S., Pasmans, F., Martel, A. (2018). Recommendations on diagnostic tools for *Batrachochytrium salamandrivorans*. Transboundary and Emerging Diseases. https://doi.org/10.1111/tbed.12787

30. Pasmans, F., Bogaerts, S., Braeckman, J., Cunningham, A.A., Hellebuyck, T., Griffiths, R.A., Sparreboom, M., Schmidt, B.R., Martel, A. (2017). Future of keeping pet reptiles and amphibians: towards integrating animal welfare, human health and environmental sustainability. Veterinary Record. http://dx.doi.org/10.1136/vr.104296

31. Canessa, S., Bozzuto, C., Campbell Grant, E. H., Cruickshank, S. S., Fisher, M. C., Koella, J. C., Lötters, S., Martel, A., Pasmans, F., Scheele, B. C., Spitzen-van der Sluijs, A., Steinfartz, S. and Schmidt, B. R. (2018). Decision making for mitigating wildlife diseases: from theory to practice for an emerging fungal pathogen of amphibians. J Appl Ecol. DOI:10.1111/1365-2664.13089

32. Van Rooij, P., Pasmans, F., Coen, Y., Martel, A. (2017). Efficacy of chemical disinfectants for the containment of the salamander chytrid fungus *Batrachochytrium salamandrivorans*. PLoS ONE. https://doi.org/10.1371/journal. pone.0186269

33. Nguyen, T.T., Nguyen, T.V., Ziegler, T., Pasmans, F., Martel, A. (2017). Trade in wild anurans vectors the urodelan pathogen *Batrachochytrium salamandrivorans* into Europe. Amphibia-Reptilia. DOI:10.1163/15685381-00003125

34. Stegen, G., Pasmans, F., Schmidt, B.R., Rouffaer, L.O., van Praet, S., Schaub, M., Canessa, S., Laudelout, S., Kinet, T., Adriaensen, C., Haesebrouck, F., Bert, W., Bossuyt, F., Martel A. (2017). Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. Nature. http://dx.doi.org/10.1038/nature22059

35. Grant, E.H.C., Muths, E., Katz, R.A., Canessa, S., Adams, M.J., Ballard, J.R., Berger, L., Briggs, C.J., Coleman, J.T.H., Gray, M.J., Harris, M.C., Harris, R.N., Hossack, B., Huyvaert, K.P., Kolby, J., Lips, K.R., Lovich, R.E., McCallum, H.I., Mendelson J.R. III., Nanjappa, P., Olson, D.H., Powers, J.G., Richgels, K.L.D., Russell, R.E., Schmidt, B.R., Spitzen-van der Sluijs, A., Watry, M.K., Woodhams, D.L. & White, C.L. (2017). Using decision analysis to support proactive management of emerging infectious wildlife diseases. Frontiers in Ecology and the Environment. https://doi.org/10.1002/fee.1481

36. Farrer, R. A., Martel, A., Verbrugghe, E., Abouelleil, A., Ducatelle, R., Longcore, J. E., James, T.Y., Fisher, M.C., Cuomo, C. A. (2017). Genomic innovations linked to infection strategies across emerging pathogenic chytrid fungi. Nature Communications. https://doi.org/10.1038/ncomms14742

37. Laking, A.E., Ngo, H.N., Pasmans, F., Martel, A., Nguyen, T.T. (2017). *Batrachochytrium salamandrivorans* is the predominant chytrid fungus in Vietnamese salamanders. Scientific Reports. https://doi.org/10.1038/srep44443

38. Schmidt, B.R., Bozzuto C., Lötters, S., Steinfartz, S. (2017). Dynamics of host populations affected by the emerging fungal pathogen *Batrachochytrium salamandrivorans*. Royal Society Open

Science. https://doi.org/10.1098/rsos.160801

39. EFSA (European Food Safety Authority), Balàž, V., Schmidt, C.G., Murray, K., Carnesecchi, E., Garcia, A., Gervelmeyer, A., Martino, L.,Munoz, G.I.,Verdonck, F., Zancanaro, G., Fabris, C. (2017). Scientific and technical assistance concerning the survival, establishment and spread of *Batrachochytrium salamandrivorans* (*Bsal*) in the EU. EFSA Journal. DOI:10.2903/j.efsa.2017.4739

Informative Leaflets

Veterinarian informative leaflet: How to recognize and treat an infecton with *Bsal*

How to recognize and treat an infection with *Bsal*

The emerging infectious chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) causes mass mortality events in both captive collections of salamanders and newts as well as in wild living populations of at least fire salamanders (*Salamandra salamandra*). Swift and accurate detection of the pathogen is of utmost importance to prevent further expansion of this pathogen. This leaflet provides veterinarians an overview of macroscopic and microscopic lesions, the required diagnostic tests to confirm diagnosis, and the proper treatment.

Typical lesions, although not pathognomonic, consist of multifocal epidermal erosions and ulcera, often characterized by a black margin. The extent and size of the lesions range from asymptomatic (at the onset of the infection) 1-2 mm circular and localized lesions to large skin ulcera affecting the whole body. Dysecdysis, anorexia and ataxia may be present. Ultimately the animal dies

Microscopy

Microscopy includes wet mount preparations, histology, and immunohistochemistry, and requires pieces of whole or shed skin.

Histology/histopathology reveals keratinocytes with eosinophilic necrosis and marginated nuclei at the periphery of the erosions/ulcerations. Within these keratinocytes (mostly colonial) thalli can be present.

Immunohistochemistry is used to stain the chytrid fungus (no distinction between *Bd* and *Bsal*).

Wet mounts may reveal the presence of motile zoospores.

PCR/qPCR

Real-time PCR is a sensitive method to show the presence of Bsal ante- and post-mortem and can be applied to skin swabs or skin samples.

The Bsal- and Bd species-specific duplex real-time PCR allows simultaneous quantification of both chytrid fungi in amphibian samples. When used as a post-mortem diagnostic tool, the detection limit should be 1.0 GE of Bsal to prevent false positives.

Molecular diagnostic tools should be used in conjunction with histology or histopathology and clinical signs, where applicable.

Treatment

Exposing infected amphibians to temperatures of 25℃ for a 10-day period will result in clearance of infection and the healing of associated lesions. This is of course taking into consideration the clinical stage of the disease and the amphibians' thermal tolerance (many urodeles tolerate these relatively high temperatures poorly).

Alternatively: a treatment protocol of a combination of Voriconazole 12.5 µg/ml, Polymyxin E 2000 IU/ml at a temperature of 20°C clears the infection in infected salamanders in 10 days.

More information, literature, diagnostic and reference labs are available via: www.BsalEurope.com and Ghent University, Wildlife Health Ghent, Merelbeke (Belgium).

Photo credits: A. Martel & F. Pasmans (Ghent University)

Batrachochytrium salamandrivorans (*Bsal*) recognition leaflet

Batrachochytrium salamandrivorans (Bsal)

This leaflet can be used to recognize Bsal in the amphibian host. Important: the symptoms are variable and can be difficult to detect at an early infection stage. It is often that lesions become evident at a relatively late stage of infection with Bsal.

Symptoms

The fungus has not yet been seen to be able to infect larvae. It may infect frogs and toads, but these hosts are not susceptible to disease, hence they don't get sick, but will act as vectors and transmit the fungus as to salamanders and newts.

Metamorphosed salamanders and newts often show multifocal superficial erosions (holes in the skin) and extensive epidermal ulcerations (ulcers on the skin) all over the body. The animal may also suffer from anorexia (stop eating) and ataxia (muscle spasms) and show excessive shedding of the skin. Ultimately the animal dies.

This fire salamander suffers extensive shedding of the skin

In this heavily infected fire salamander, the erosions and shedding is clearly visible

This alpine newt is severely infected and shows skin erosions and apathy

This fire salamander died due to Bsal and the ulceration and skin erosion is evident

The erosion of the skin is obvious in this fire salamander

A suspicious mass mortality event of fire salamanders in a forest

UNIVERSITEIT GENT · CENTRE D'ÉCOLOGIE FONCTIONNELLE & ÉVOLUTIVE · Universität Trier · CSIC Consejo Superior de Investigaciones Científicas · natagora · ZSL INSTITUTE OF ZOOLOGY · RAVON · European Environment Agency · cnrs · UNIVERSITÀ DEGLI STUDI DI GENOVA

Photo credits: Frank Pasmans, An Martel, Martha van Diepenbeek, Gwij Stegen

Amphibian diseases

Diseases and death are part of the circle of life. However, currently there are some emerging infectious diseases that pose an existential threat to European amphibians. Here we describe chytridiomycosis caused by *Batrachochytrium salamandrivoran* (Bsal), and answer the most frequently asked questions.

What do I do?

You are in the field and encounter sick or dead amphibians. Now what?
- take as many photos as you can,
- note down the location (or write on a map)
- note the time and date
- the species and number of animals
- your own contact information

If you are allowed to, bring as many dead animals as you can. Place them in separate plastic bags and store them frozen or in ethanol. Make sure you label all individuals separately.

Is monitoring safe?

Monitoring and studying amphibians is and remains important. You can still go out into the field and collect your data, but please be alert and implement a disinfection protocol to be sure you're not transferring pathogens from one site to another.

Who do I contact?

Contact your local research institute for advice and help. You can find their addresses on this website:
www.BsalEurope.com

For captive animals you can also contact your veterinarian. They can advice you on the proper treatment. Please report cases of Bsal in captive collections to the research institutes!

Captive collection?

If you have a captive collection, make sure that when you are introducing new animals to your collection they have a health certificate. Implement a quarantine period of at least 6 weeks before you place your new animal with others. Report diseases to your vet and local research institute.

Do not deposit your waste water in the environment, but pour it directly into a drain connected to the sewerage system.

Disinfection protocol

It is strongly advised to disinfect your field gear (boots, buckets, dipnets etc) to prevent the spread of pathogens to yet naïve populations. Site managers are also advised to disinfect large machinery between sites.

The website www.BsalEurope.com
provides information that will help you with this!

Photo credits: Jelger Herder, Rolf van Leeningen

Disinfection protocol heavy machinery leaflet

Disinfection protocol heavy machinery

UNIVERSITEIT GENT · Centre d'Écologie Fonctionnelle & Évolutive · Universität Trier · CSIC Consejo Superior de Investigaciones Científicas · natagora · ZSL INSTITUTE OF ZOOLOGY · RAVON · European Environment Agency · cnrs · UNIVERSITÀ DEGLI STUDI DI GENOVA

Background

This document provides simple, but effective measures in order to minimize the spill over of infectious amphibian pathogens to disease free areas. The advice listed below is meant for heavy machinery that is used for work in- and around water bodies like ponds, canals and streams that are home to amphibians. This includes (for example) tractors, excavators, loaders, mowers, harvesters, dredgers, etc. For ecological fieldwork we refer to our disinfection protocol fieldwork.

What kind of work?

This advice applies to activities with heavy machinery in areas where amphibians can occur.

Example
A tracked excavator that has been used to excavate a pond often has large amounts of substrate sticking to its tires. This machinery is often immediately needed at another location. Contaminated substrate can unintentionally be introduced to another area. Therefore, it is very important that prior to driving to another location to first clean (hose down) the equipment.

This is not only necessary when the equipment is moved from a contaminated to a clean site but should be a routine procedure.

Human role

Emerging infectious diseases such as chytridiomycosis and ranavirosis pose a significant threat to amphibians in Europe. The spread of the infectious agents causing these diseases can be facilitated by humans.

Humans can spread fomites over very long distances and in large amounts in comparison to potential natural vectors such as amphibians or wading birds.

Avoid direct contact

The disinfection solutions described in this document may be harmful for humans but also for amphibians, fish and other organisms. Use carefully.

Dispose of disinfectant solution

Dispose of the disinfectant solution via the prescribed means. Preferably take it with you. Never dispose of it in nature.

Ideally equipment is cleaned with a disinfectant. If such a cleaning procedure is not possible, the minimum effort should include removing as much substrate as possible with clean water. By doing so the amount of pathogens that can be transported is significantly reduced.

Photo credits: Jelger Herder, Tariq Stark

Bsal

Disinfection protocol heavy machinery

Cleansing and disinfection

1. Remove plant residues and muddy soil with a shovel, broom, brush or high pressure water spray. Rinse the materials or part of the machine with clean water and make sure the surface is as clean as possible
2. Always disinfect materials as follows: from a long distance of any water bodies (ponds, streams, etc.) and try not to contaminate the environment with the residue.
3. Virkon S (1% solution) is the preferred disinfectant. Other effective disinfectants are bleach (at least 1.6% sodium hypochlorite), Nolvasan (0.75% solution) and 70% ethanol or spirit (85% alcohol content). Always use "fresh" solutions as their disinfectant properties may be lost over time.
4. Place the materials in the solution or spray the solution on the materials. Let materials soak for at least five minutes.
5. Rinse the materials after disinfection with clean (tap) water.
6. At some locations it may not be preferable or allowed to use certain disinfectants. Then clean and disinfect in the workshop or storage depot, or clean without the use of these solutions as thorough as possible.
7. Don't forget to disinfect smaller materials and equipment, tools and boots. Please consult our disinfection protocol for fieldwork.

Advice

- It should be aimed for to keep or process potentially contaminated soil, mud, plants, etc. within the source area.
- If the equipment has been in contact with water or moist soil it needs to be disinfected.
- All materials used on a location[1] need to be disinfected before using them at other sites
- Avoid unnecessary contact of material with surface water and/or with damp river- and stream banks.
- Target the disinfection measures on the parts of the materials/machine that have been in direct contact with water of (moist) soil near river-, stream and pond banks. For example: excavator buckets on bulldozers, mowing buckets, tires and caterpillar tracks, etc.
- It is important that the amount of transported material is limited as much as possible. Is disinfection not possible? Then remove as much muddy soil and vegetation of the machine as possible.

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

More information

For the most recent version of this hygiene protocol and additional information please visit:
www.BsalEurope.com

Disinfection protocol fieldwork leaflet

Disinfection protocol fieldwork

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 salamandrivorans* and *B. dendrobatidis*, but also ranavirosis, 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 location[1] 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 location[1] 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.

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

Disinfect your field material

Handle dead amphibians with disposable gloves.

Help to prevent mass mortality.

Photo credits: Claude Miaud, Martha van Diepenbeek, Jelger Herder, Rolf van Leeningen, Tariq Stark

Disinfection protocol fieldwork

Cleansing and disinfection

1. Remove plant residues and muddy soil from boots, field materials, etc.
2. Rinse with water. Water from a pond is sufficient. Make sure the materials are as clean as possible.
3. Always disinfect materials as follows: from a long distance of any surface water (ponds, streams, etc.) and try not to contaminate the environment with the residue. Use a bucket or large container to disinfect your materials. Dispose the disinfectant at home (as prescribed). It is preferable to use two or more sets of field materials in order to limit the the use of chemical disinfectants.
4. Virkon S (1% solution) is the preferred disinfectant. Other effective disinfectants are bleach (at least 1.6% sodium hypochlorite), Nolvasan (0.75% solution) and 70% ethanol or spirit (85% alcohol content). Always use "fresh" solutions as their disinfectant properties may be lost over time.
5. Place the materials in the solution or spray the solution on the materials. Let materials soak for at least five minutes.
6. Rinse the materials after disinfection with clean (tap) water.
7. If cleaning the materials on site is not possible, then remove mud and plant residues and rinse with water. Take the material home in plastic bags (separately) and clean/disinfect them at home.
8. Wash your hands with a disinfectant or disinfect them with a hand sanitizer with disinfectant properties.

Dispose of disinfectant solution

Dispose of the disinfectant solution via the prescribed means. Preferably take it with you. Never dispose of it in nature.

Avoid direct contact

The disinfection solutions described in this document may be harmful for humans but also for amphibians, fish and other organisms. Use carefully.

Checklist

- Heavy duty brush
- Bucket
- Sponge
- Disinfectant
- Plastic bags
- Disinfectant hand sanitizer
- Disposable gloves
- Spray bottle

More information

For the most recent version of this hygiene protocol and additional information please visit:
www.BsalEurope.com

UNIVERSITEIT GENT · Centre d'Écologie Fonctionnelle & Évolutive · Universität Trier · natagora · CSIC Consejo Superior de Investigaciones Científicas · ZSL INSTITUTE OF ZOOLOGY · RAVON · European Environment Agency · cnrs · UNIVERSITÀ DEGLI STUDI DI GENOVA

Photo credits: Jelger Herder, Rolf van Leeningen, Tariq Stark

Hygiene protocol translated to French on LPO Comté website

https://cdnfiles1.biolovision.net/franche-
comte.lpo.fr/userfiles/proteger/Maladiesamphibiens/ProtocoleHygineluttecontrepathognesam
phibiensVersionFCVF.pdf

Protocole d'hygiène
Lutte contre la dissémination des pathogènes des amphibiens dans la nature

UNIVERSITEIT GENT — Université Trier — CSIC — RAVON — natagora la nature avec nous — European Environment Agency — cnrs — ZSL INSTITUTE OF ZOOLOGY — UNIVERSITÀ DEGLI STUDI DI GENOVA

Contexte

Ce document fournit des mesures simples et efficaces pour limiter la dissémination des champignons et des virus pathogènes des amphibiens dans la nature.

Les maladies infectieuses émergentes telles que la Chytridiomycose et la Ranavirose menacent les populations d'amphibiens d'Europe.
Par nos activités, nous pouvons être d'importants vecteurs de dissémination des agents infectieux à l'origine de ces maladies dans la nature. Comparativement aux « vecteurs naturels » (amphibiens, oiseaux d'eau, etc.) nous pouvons disperser les agents pathogènes sur de bien plus longues distances (commerce international d'animaux vivants) et en grandes quantités (déplacements de matériaux d'extraction à partir des masses d'eau).

Nous encourageons tous les opérateurs de terrain à appliquer rigoureusement le protocole d'hygiène. Nous pourrons ainsi réduire la dissémination des agents pathogènes et « gagner » du temps en alimentant la recherche pour trouver de nouvelles solutions conservatoires et permettre aux amphibiens de s'adapter.

Conseils

- Ne capturez les amphibiens et autres animaux/végétaux aquatiques qu'en cas de nécessité absolue.
- Toujours relâcher les amphibiens sur leur site* de capture.
- En cas de manipulation d'amphibiens, utilisez des gants à usage unique non poudrés. Les gels désinfectants non parfumés, virucides, fongicides et bactéricides sont aussi efficaces.
- L'ensemble du matériel utilisé dans un site* doit être désinfecté avant réutilisation sur un site différent.
- Chaussures, vêtements, outils de chantier (manuels et engins) en contact direct avec l'eau et la boue doivent être désinfectés rigoureusement.
- Garez votre véhicule sur des chemins carrossables ; évitez la boue.
- Les amphibiens potentiellement malades (morts ou vivants) représentent un haut risque écologique. Si besoin, ne les manipulez qu'avec des gants à usage unique et appliquez la procédure de vigilance et d'alerte. Leurs capture, transport, détention sont interdits par la loi.

*Site = point d'eau stagnant ou cours d'eau qui n'est pas directement connecté à d'autres zones d'eau au sein d'une surface donnée. Dans un site on considère que les animaux sont déjà en contact étroit les uns avec les autres.

Procédure de vigilance et d'alerte

1_
Prenez des photos et des notes (nombre d'animaux, espèce, endroit précis, date et heure, etc.)

2_
Saisissez-les sur le **formulaire dédié** du site **alerte-amphibien.fr** et **sur notre base de données Obsnatu la Base franche-comte.lpo.fr** via le **formulaire mortalité** et avec les mots clés « **alerte amphibien** » dans le champ **remarque.**

3_
Cherchez bien dans les environs de votre découverte car si le pathogène est présent, il est très probable qu'il y ait plus d'un animal infecté. **Les talus et bords de chemin** sont généralement de bons endroits où chercher.

Source : https://bsalinfoeurope.wixsite.com/eubsalmitigation2017/

Protocole d'hygiène
Lutte contre la dissémination des pathogènes des amphibiens dans la nature

Nettoyage et désinfection

1. Avec une brosse, enlevez l'ensemble des résidus de végétaux et de sol de vos chaussures et autre matériel de terrain.

2. Rincez à l'eau. L'eau d'une mare est suffisante. Assurez-vous que le matériel est aussi propre que possible.

3. Désinfectez toujours votre matériel comme suit : préparez votre solution désinfectante en petite dose (250 mL) dans un pulvérisateur dédié. Pulvérisez votre matériel à distance de toutes zones humides (mares, cours d'eau, etc.) et évitez au maximum de contaminer l'environnement. Idéalement, utilisez des bacs, a minima, désinfection sur chemin carrossable.

4. Privilégiez l'utilisation du Virkon S (dilution 1 %). D'autres désinfectants plus impactants sont efficaces : eau de javel à 4 % de NaCl (dilution 1:5) ou éthanol à 70 % non dilué.

Toujours utiliser des solutions désinfectantes « fraîches » : les préparer sur place, en petit volume (250 mL) avant votre intervention (propriétés désinfectantes perdues au cours du temps).

Pour les vêtements : machine à >60°C, >30 minutes.

5. Laissez le matériel sécher au moins 5 minutes (respect du temps d'action des désinfectants).

6. Si la mise en œuvre du protocole d'hygiène n'est pas possible sur place enlevez l'ensemble des résidus de végétaux et de sol et rincez à l'eau votre matériel. Transportez votre matériel à domicile dans des sacs poubelles. Appliquez le protocole à votre domicile et jetez les sacs/gants usagés.

8. Lavez vos mains à l'aide d'un gel désinfectant.

Evitez tout contact direct

Les solutions désinfectantes décrites dans ce document peuvent être dangereuses pour les humains mais aussi les amphibiens, les poissons et autres organismes. Utilisez-les avec précaution.

Check-list matériel d'hygiène

- ✓ Brosse
- ✓ Gants à usage unique non poudrés
- ✓ Pulvérisateur
- ✓ Désinfectant
- ✓ Gel désinfectant non parfumé à action virucide, fongicide, bactéricide
- ✓ Sacs poubelle
- ✓ Bouteille d'eau
- ✓ Bacs plastiques (désinfection/rangement)

Approvisionnement et élimination des produits désinfectants

Pour obtenir du Virkon S (1 %) en poudre et vous débarrasser du produit brut périmé contactez la LPO Franche-Comté.

Conserver le désinfectant dans un endroit sec, frais, à l'abri du soleil, dans un contenant hermétique (couvercle toujours clos).

Les restes de solution désinfectante doivent être versés dans les réseaux d'eaux usées ; ne jamais les verser dans le milieu.

Le produit périmé et son contenant doivent être éliminés comme produits spéciaux dans une déchetterie.

Detailed *Bsal* Hygiene Protocol for Fieldwork and Amphibian Husbandry

Materials for disinfection:

·Brushes

·Buckets

·Disinfection liquid

·Hand soap

·Spray bottles/pump sprayers

·Disposable powder-free gloves

·Plastic bags

·Trash bags

Top Tips for *Bsal* Hygiene protocols in the field

1. All organic material (soil, plants, small invertebrates, debris, biological material and secretions etc.) should be removed from equipment before any chemical disinfectants are used. Organic material will inactivate chemical disinfectants and/or render the concentration of these solutions ineffective to kill pathogens. The presence of any material organic or inorganic may impede the contact of the disinfectant with the surface which is meant to work on.

2. The following chemical disinfectants can be used in the field to disinfect fomites from *Bsal*:

***Bsal* can be killed using most of the common disinfectants (Table 1). This table was taken from a paper that has been submitted for publication (Van Rooij et al., 2017).** Hydrogen peroxide shows poor activity against *Bsal*. Heat treatment is to be expected to result in fast killing of all life stages of *Bsal* but needs further study. The fungus tolerates high temperatures poorly: *Bsal* cultures are killed after incubation for 5 days at 25°C (Blooi et al., 2015). If Bsal responds to heat as its sister species *B. dendrobatidis*, exposing materials to 60°C for 5 minutes or 100°C for 1 minute should be efficient (Johnson et al., 2003). Several of the disinfectants mentioned may cause harm to humans, animals, the environment and to materials (including clothes). Please always carefully consult the disinfectant's manual. Virkon S is widely used (relatively safe, highly efficient) but its use in the field may require derogations from existing legislation. Disposal of disinfectants in the natural environment should be avoided.

Disinfectant	Concentration	Minimal exposure time for 100% killing of *Bsal*
Ethanol (EtOH)	70%	**30s**
Disolol®	undiluted	**30s**
Hibiscrub®	0.25, 0.5, 0.75%	**30s**
Chloramine-T®	0.5%	**5 min**
	1%	**2 min**
Bleach	1:5 dilution	**5 min**
	4%	**30s**
Kickstart®	0.05%	**5 min**
	0.1%	**2 min**
Potassium permanganate (KMnO$_4$)	1%	**10 min**
	2%	**5 min**
Virkon S®	0.5%	**5 min**
	1%	**2 min**
Dettol medical®	1:20 dilution	**5 min**
Biocidal®	undiluted	**30s**
Safe4®	undiluted	**30s**
F10 ®	1:100 dilution	**30s**
	1:250 dilution	**30s**
	1:500 dilution	**30s**
	1:1000 dilution	**30s**
Sodium chloride (NaCl)	10%	**10 min**

Table 1: Minimal exposure time for 100% killing of *Bsal* spores and sporangia at room temperature

3. Please ensure that you utilize the disinfectant in the concentrations and manner stipulated by the references above and/or the manufacturer in order to achieve the intended results.

Hygiene protocol for:

1. Swabbing amphibians in one or multiple site(s)/population(s)

Non-invasive sampling of amphibians using skin swabs is the preferred method to survey for *Bsal* infections. This procedure can be done after minimal training, does not result in animal injury when properly done and produces a usable sample.

a. Ensure that all equipment has already been disinfected prior arrival on the site.

b. Use powder-free vinyl (not latex) disposable gloves when handling amphibians. Gloves should be changed for each individual amphibian handled then discarded.

c. Remove cotton swab from protective case, be careful that you only touch the upper part of the swab that was meant for holding so as to avoid contamination.

d. Place the tip of the cotton swab on amphibian skin and rub firmly on the point of contact with the skin (especially areas where there are obvious lesions), ideally 10 times the abdomen, 10 times the ventral tail and 10 times the underside of a foot. Samples should be done in duplicate.

e. Reinsert swab into protective case.

f. Label the swab's protective case with identifying information such as date, site, sample number and species.

g. Place used gloves in a trash bag for disinfection and disposal later.

h. Put on a fresh pair of gloves.

i. Repeat steps c-g.

j. Store all samples from one site/population together. Swabs should be stored dry and preferably frozen. Frozen samples can be stored for long periods (several months). If swabs have to be stored longer than one week and cooling is not available, they may be stored in a small volume (200 µl) of 70% ethanol.

k. When moving to a new site or population please follow the hygiene protocol described for "Hygiene protocol used between sites or populations and Hygiene protocol used on equipment".

2. Tissue Sampling in amphibians

Tissue sampling is not recommended for surveying amphibian populations for the presence of *Bsal*, since this is an invasive sampling method that may produce serious health issues in the animal sampled (wound infections, mechanical hindrance). Non-invasive sampling using skin swabs as described above should be preferred. In case tissue samples are being collected (e.g. for reasons of genetic research), these can be used for *Bsal* detection when properly collected. Collecting tissue samples from live amphibians requires advanced skills and should be approved by an ethical committee.

a. Ensure that all equipment has already been disinfected or utilize disposables.

b. Use powder-free vinyl (not latex) disposable gloves when handling amphibians. Gloves should be changed for each individual amphibian handled then discarded.

c. Clip small portion of amphibian skin, particularly in location where there are lesions.

d. Preserve skin sample in 70% EtOH or frozen in a cryotube.

e. Put on a fresh pair of gloves.

f. Store all samples from one site/population together.

g. Repeat steps a-f. Disinfect clipper between animals.

h. When moving to a new site or population please follow the hygiene protocol described for "Hygiene protocol used between sites or populations and Hygiene protocol used on equipment".

3. Hygiene protocol for equipment and clothes used between sites or populations

When sampling multiple sites in one field visit, in order to minimize the potential for transmission or spread of disease it is important to establish a plan as to the order in which these sites will be visited. It is good biosecurity practice to first visit sites/populations where there have been no cases or no suspected incidence of disease. Later, where there is no information on the status of sites/populations, one may visit these next. Finally, the sites or populations with known cases of disease may be visited. All material, including footwear and clothes should be subject to proper disinfection procedures between visits to different sites.

a. Ideally, when visiting multiple locations, travel with as many bags of clothes, shoes and other equipment necessary to organize a dedicated set of clothes, shoes, equipment for each site/population etc. or to store dedicated clothing and equipment for each site/population on each actual site. The use of disposable materials effectively prevents disease transmission. Alternatively, all materials must be thoroughly cleaned and disinfected between sites as outlined below.

b. All mud, soil, leaves and other organic and non-organic material should be brushed off of all equipment and field gear.

c. The equipment should then be rinsed to remove all residues of organic material or any inorganic material which impedes contact of the disinfectant with the fomites which need to be disinfected.

d. Small equipment should then be submerged in disinfection liquid (prepared according to the manufacturer's instructions) or larger equipment should be thoroughly covered with disinfection liquid sprayed on.

e. Rinse the disinfectant from the equipment or allow mixture to dry in case of volatile disinfectants (e.g. ethanol).

f. It is important to get all disinfectant residues off the equipment as they can be harmful to amphibian skin. At the new site/population, water from any body of water present can be used to rinse the equipment. This should be done at least 50m from the body of water and preferably on an impermeable surface.

g. Place all gloves and other waste material in trash bags so that they can be disinfected and disposed of properly at the office or other suitable location.

h. Vehicles used during sampling or carrying out surveillance for amphibians may have residual soil or water that could contain *Bsal* zoospores. Therefore, the following steps may be required to ensure that no *Bsal* can be spread to other sites via the vehicles:

   - clean wheels and tyres

   - disinfect wheels and tyres

i. Hands, arms and any other body parts which have come into contact with water, soil or debris should be:

   - cleansed of all organic material

   - disinfected with suitable solution. Not all disinfectants are appropriate for use on human skin.

Please carefully consult the manufacturer's instructions. Frequent use of disinfectants on skin may produce side effects.

Hygiene protocol for amphibian husbandry

English

The cornerstone of avoiding spill-over of pathogens from amphibians from captivity to the wild is certified absence of *Bsal* (and other pathogens like *Bd* and ranaviruses) from the captive animals. *Bsal* infections can be treated rather easily (by housing the infected animals at 25°C for 10 days, with subsequent assessment of absence of *Bsal*). Absence of *Bsal* can be achieved by having:

1) all animals present tested using a skin swab for the presence of *Bsal*

2) all newly arrived animals maintained under strict quarantine for at least 40 days. During this period, the animals should be sampled for *Bsal, Bd* and ranaviruses. If positive, the animals should be treated until total clearance of any *Bsal* infection. During this quarantine period, all materials and terrarium contents should be disinfected thoroughly before disposal or use elsewhere. Before discarding any wastewaters or terrarium contents which may have been contaminated by salamanders or newts, these should be

disinfected. Heat treatment is the preferred way of treatment given its relative ease of application and absence of environmental contamination. All waste should be treated for at least 30 minutes at, at least, 60°C before disposal.

3) ill or dead animals examined by a competent veterinarian.

Captive salamanders and newts kept as pets should neither be released into the wild nor have direct or indirect contact with native salamanders or newts (direct contact: for example, in outside terraria, indirect contact: for example, by using dipnets, containers, buckets etc. both for terrarium animals and for wild animals). Even in the absence of obvious signs of clinical illness, some amphibians are still able to carry and shed the pathogen. If pet owners are no longer able to care for their pets then they should contact their local herpetology society, zoo, a local veterinarian, or local animal welfare organization to care for them.

Trade in captive salamanders and newts should be restricted to certified *Bsal* free animals.

French
Protocole d'hygiène pour l'élevage des amphibiens

La pierre angulaire pour éviter que des agents pathogènes ne se répandent des amphibiens de captivité aux amphibiens sauvages est l'absence certifiée de *Bsal* (et d'autres agents pathogènes comme *Bd* et ranavirus) chez les animaux captifs. Les infections à *Bsal* peuvent être traitées assez facilement (en exposant les animaux infectés à 25 °C pendant 10 jours, en vérifiant ensuite l'absence de *Bsal*). L'absence de *Bsal* peut être obtenue en ayant:

1) tous les animaux présents sont analysés à l'aide d'un frottis cutané de peau pour vérifier la présence de *Bsal*

2) tous les animaux nouvellement arrivés sont maintenus sous quarantaine stricte pendant au moins 40 jours. Au cours de cette période, les animaux devraient être testés pour *Bsal*, *Bd* et les ranavirus. Si un cas positif est observé, les animaux doivent être traités jusqu'à la disparition totale de toute infection à *Bsal*. Au cours de cette période de quarantaine, tous les matériaux et le contenu du terrarium doivent être désinfectés soigneusement avant leur élimination ou leur utilisation ultérieure. Les eaux usées et matériaux du terrarium qui ont pu être contaminés par des salamandres ou des tritons doivent être désinfectés. Le traitement thermique est recommandé du fait sa relative facilité d'application et de l'absence de contamination environnementale. Tous les déchets doivent être traités pendant au moins 30 minutes à au moins 60 ° C avant leur élimination.

3) Les animaux malades ou morts sont examinés par un vétérinaire compétent.

Les salamandres et les tritons de captivité ne doivent pas être libérés dans la nature, ni mis en contact direct ou indirect avec des salamandres ou des tritons sauvages (contact direct, par exemple dans un enclos en pleine air, contact indirect en utilisant par exemple des épuisettes, bacs et seaux à la fois pour les animaux de captivité et sauvages). Certains amphibiens sont

capables de porter et transmettre le pathogène sans présenter de signes évidents de maladie. Si les propriétaires d'animaux en captivité ne sont plus en mesure de prendre soin de leurs animaux, ils doivent contacter la société herpétologique de France (lashf.fr) afin de trouver une solution d'accueil pour ces animaux.

Le commerce des salamandres et des tritons de captivité doit être restreint aux animaux certifiés sans *Bsal*.

Italian
Protocollo sanitario per il mantenimento degli anfibi in ambiente controllato

Per evitare la diffusione di agenti patogeni dagli anfibi tenuti cattività all'ambiente naturale è fondamentale certificare che gli animali in allevamento siano esenti dal fungo Batrachochytrium salamandrivorans (*Bsal*) e da altri patogeni come *Batrachochytrium dendrobatidis* (*Bd*) e ranavirus. L'infezione da *Bsal* può essere trattata abbastanza facilmente (mantenendo gli animali per 10 giorni a 25°C, e successiva verifica di assenza di *Bsal*). L'assenza di *Bsal* si accerta in questi modi:

1) Prelevando e analizzando tamponi cutanei di tutti gli animali

2) Mantenendo in quarantena tutti i nuovi arrivi per almeno 40 giorni. Durante questo periodo, gli animali dovrebbero essere analizzati per *Bsal*, *Bd* e ranavirus. Dopo la quarantena, il terrario e tutto il materiale utilizzato deve essere sterilizzato prima di poter essere riutilizzato. Trattare col calore anche l'acqua e ogni altro materiale venuto a contatto con salamandre o tritoni. Si consiglia di mantenere il materiale ad almeno 60°C per 30 minuti prima di riutilizzarlo o eliminarlo

3) Far esaminare animali morti o malati da un veterinario competente

Inoltre, non liberate mai anfibi mantenuti in acquario o terrario in ambiente naturale; evitate contatti diretti tra anfibi esotici e anfibi autoctoni (mantenendoli temporaneamente negli stessi ambienti); evitate anche contatti indiretti, sterilizzando sempre il materiale usato (retini, secchi, contenitori etc.). Anche in assenza di evidenti segni clinici, alcuni anfibi possono trasmettere patogeni. Se un proprietario non può più mantenere i propri animali, dovrebbe contattare chi possa prendersene cura: un veterinario, un centro di recupero fauna, associazioni animaliste locali, giardini zoologici o acquari. Infine, sarebbe sempre meglio acquistare solo salamandre certificate esenti da *Bsal*.

Spanish
Protocolo de higiene para el manejo de anfibios

La clave para evitar la dispersión de patógenos desde ejemplares mantenidos en cautividad a la naturaleza pasa por tener la certeza de la ausencia total de *Bsal* (y otros patógenos como *Bd* y ranavirus) en dichos animales. Las infecciones por *Bsal* pueden tratarse con bastante facilidad

(manteniendo a los animales infectados a 25°C durante 10 días, con una evaluación posterior de la ausencia de *Bsal*). La ausencia de *Bsal* en ejemplares en cautividad se puede lograr:

1. analizando la presencia de *Bsal* en todos los ejemplares existentes mediante hisopados de la piel.

2. sometiendo a los nuevos ejemplares a una cuarentena estricta durante al menos 40 días. Durante ese período, los ejemplares recién llegados deben ser analizados para *Bsal*, *Bd* y ranavirus. Si el resultado es positivo, los ejemplares deben ser tratados hasta la eliminación total de la infección por *Bsal*. Durante este período de cuarentena, todos los materiales y el contenido de los terrarios deben desinfectarse completamente antes de ser desechados o reutilizados. El agua usado, y cualquier contenido de los terrarios que pudiera estar contaminado por haber estado en contacto con las salamandras o tritones, también deben ser desinfectados antes de ser desechados o reutilizados. Un tratamiento térmico es el método más recomendable ya que es fácil de aplicar y no produce contaminación ambiental. Para ello, todos los residuos y materiales deben ser tratados como mínimo durante 30 minutos a, al menos, 60°C.

3. mediante un veterinario especializado que examine a los animales enfermos o muertos.

Las salamandras y los tritones mantenidos en cautividad como mascotas no deben ser liberados en la naturaleza, ni tener contacto directo o indirecto con salamandras o tritones autóctonos (contacto directo: por ejemplo, en un terrario en el exterior, contacto indirecto: por ejemplo, usando mangas, recipientes, cubos etc. tanto para los ejemplares de terrario como en la naturaleza). Algunos anfibios pueden ser portadores y dispersar el patógeno, aunque no presenten síntomas evidentes de enfermedad clínica. Si los dueños de las mascotas no desean seguir teniéndolas, deben ponerse en contacto con una asociación herpetológica local, un zoológico, un veterinario local, o una organización local para el bienestar animal para que cuiden de ellas.

El comercio de mascotas de salamandras y tritones debe restringirse a ejemplares para los que se ha certificado la ausencia de *Bsal*.

Educational videos

http://bsaleurope.com/videos/"

Bsal information

Disease-free amphibian collection

Hygiene protocol Fieldwork

Etiology and Epidemiology of *Bsal*

Clinical signs, Pathology and Pathogenesis of *Bsal*

Diagnosis of *Bsal*

Treatment of amphibians infected with *Bsal*

Prevention of *Bsal* introduction and dispersal for pet keepers

Prevention of *Bsal* introduction and dispersal by naturalists

Papers and poster on the *Bsal* project

<u>Papers</u>

- Stark, T., Martel, A., Pasmans, F., Thomas, V., Gilbert, M., Spitzen-van der Sluijs, A. 2018, A European early warning system for a deadly salamander pathogen. FrogLog. 26(1): 42-43. https://www.iucn-amphibians.org/wp-content/uploads/2019/02/FL120high.pdf

- Stark, T., Martel, A., Pasmans, F., Thomas, V., Gilbert, M., Spitzen-van der Sluijs, A. 2018. A European early warning system for a deadly salamander pathogen. AArk Newsletter. 42: 12-14.

  English: http://www.amphibianark.org/Newsletters/AArk-newsletter-42.pdf; http://bsaleurope.com/wp-content/uploads/2018/06/AArk-Newsletter-42-pages-12-14.pdf

  Spanish: http://bsaleurope.com/wp-content/uploads/2018/06/AArk-Newsletter-42-pages-12-14.pdf

Annex 7: *Bsal* Action Plan

Mitigating *Batrachochytrium salamandrivorans* in Europe

Batrachochytrium salamandrivorans Action Plan for European urodeles

European Commission

Colophon

Contents

Bsal

Reading guide

This document describes the *in situ* and *ex situ* actions that need to be taken to mitigate the effects of *Batrachochytrium salamandrivorans* (Bsal) in nature in order to safeguard current European urodelan diversity. Following the Introduction (Chapter 1), this document contains four main sections:

- A Bsal risk assessment for all European urodelan species and subspecies (Chapter 2)

- An overview of the current European legislative regulations with regard to Bsal (Chapter 3).

- A general Bsal mitigation action plan for all European urodeles, which describes the actions needed prior to, during and after an incursion of Bsal into a new country, region or area (Chapter 4)

- A species-specific action plan for the mitigation of Bsal for each European urodelan species, providing details about Bsal susceptibility, the risk Bsal poses to the persistence of the particular species and Bsal risk mitigation, as well as a proposal for the delineation of conservation units for each European urodelan species (Chapter 5)

Tables 2 and 3 provide a clear overview of the known or expected susceptibility to Bsal for each species, the risk that Bsal poses to these species and the urodelan diversity by country. In the species-specific protocols (Chapter 5), recommended actions are listed in brief. More information regarding these actions can be found in Chapter 4.

Executive summary (English)

The fungal pathogen *Batrachochytrium salamandrivorans* (Bsal) causes chytridiomycosis, a lethal ulcerative skin disease, in urodeles (salamanders and newts). Bsal is closely related to *B. dendrobatidis* (Bd), which has already caused declines and extinctions of at least 500 amphibian species all over the world, including at least 90 global species extinctions. Bsal originates from East Asia and it likely reached, and spread internationally within Europe via the pet trade. Incursion of the pathogen in the European ecosystem coincides with urodele declines in Belgium, Germany, the Netherlands and Spain.

The risk assessment in this Bsal action plan shows that Bsal threatens the survival of populations of at least 30 out of 40 European urodelan species and even the survival of at least 10 entire species over a 10-year time frame. The combination of Bsal's propensity to cause severe urodele population declines, its erratic spread due to unpredictable human-mediated Bsal introductions and the presence of several high-risk urodelan taxa, renders Bsal an unprecedented threat to Europe's urodele diversity.

The European Union (EU) has an obligation to preserve and protect its biodiversity against such threats, based on international agreements, including the United Nations Convention on Biological Diversity (CBD), Bern Convention and Habitats Directive. In addition, the World Organisation for Animal Health (OIE) listed infection with Bsal in its Aquatic Animal Health Code. Spontaneous elimination of Bsal from Europe is highly unlikely and the pathogen is slowly expanding its range. With approximately 25 known disease outbreaks, Bsal incursion is still in a relatively early stage of invasion. At this stage, to avert further establishment of Bsal and future loss of European urodele diversity, disease eradication should be envisaged, which requires a clear and long-term commitment of the EU and its member states.

The European Bsal Action Plan defines urodelan conservation priorities in the face of the Bsal threat and aims to guide the European Commission and the EU member states in their response to the Bsal incursion with phase-specific actions for the pre-invasion, invasion and endemic phases of Bsal invasion. Immediate implementation of this Action Plan at European and member state level could result in the elimination of the Bsal threat from Europe. The most urgently needed general actions are:

At EU level:

- The establishment and maintenance of an Early Warning System (EWS)
- Implementing measures to obtain a "Clean Trade" in live amphibians: absence of Bsal throughout the whole chain
- The funding of targeted studies to improve efficient and effective Bsal mitigation as well as eradication
- The establishment and maintenance of a European Bsal Working Group (BWG), to provide advice to the EU and national governments

At member state level:

- The establishment and implementation of a national Action Plan (AP)
- The establishment and maintenance of an Early Warning System (EWS)
- The ability to rapidly respond to Bsal incursion with subsequent monitoring and evaluation, which will minimize ecological damage and financial costs on the long-term
- The immediate and effective removal of any non-native amphibian species

Zusammenfassung (Deutsch)

Der Hautpilz *Batrachochytrium salamandrivorans* (Bsal) verursacht Chytridiomykose, eine tödliche Krankheit bei Salamandern und Molchen. Bsal ist nah verwandt mit dem Hautpilz *B. dendrobatidis* (Bd), der weltweit bei mindestens 500 Amphibien-Arten Populationszusammenbrüche verursacht hat und durch den mindestens 90 Arten bereits ausgestorben sind. Bsal stammt aus Ost-Asien und ist wahrscheinlich durch den globalen Tierhandel nach Deutschland verschleppt worden. Der Eintrag des Pathogens in europäische Ökosysteme verursachte lokale Aussterbeereignisse bei Molchen und Salamandern in Belgien, Deutschland, den Niederlanden und Spanien.

Die Risikobewertung in diesem Aktionsplan zeigt, dass Bsal mindestens 30 von 40 europäischen Molch- und Salamanderarten bedroht und mindestens 10 davon sogar innerhalb der nächsten 10 Jahre aussterben könnten. Dieses Bsal-Bedrohungspotenzial, in Kombination mit dem unvorhersehbaren Ausbreitungsmuster des Pathogens durch menschliches Handeln sowie generell die Existenz stark bedrohter Amphibien, macht Bsal zu einer außergewöhnlich massiven Bedrohung für Europas Salamander und Molche.

Die Europäische Union (EU) steht in der Verantwortung, ihre Biodiversität zu erhalten und gegen derartige Bedrohungen zu schützen. Dies basiert auf internationalen Vereinbarungen, wie der Biodiversitätskonvention CBD der Vereinten Nationen, der „Bern Convention" und der FFH-Direktive. Außerdem wird Bsal von der „World Organisation for Animal Health" gelistet (OIE Aquatic Animal Health Code).

Eine spontane Elimination von Bsal in Europa ist höchst unwahrscheinlich. Im Gegenteil, Bsal breitet sich weiter aus. Mit ungefähr 25 bekannten Ausbrüchen, ist Bsal noch in einem frühen Stadium der Ausbreitung. Um die weitere Ausbreitung zu unterbinden und um in der Zukunft den Verlust von Salamander- und Molchdiversität zu verhindern, sollten Eliminierungsmaßnahmen getroffen werden. Dies erfordert ein klares und langfristiges Engagement der EU und ihrer Mitgliedstaaten.

Der Europäische Bsal-Aktionsplan definiert die Prioritäten für den Salamander- und Molchschutz angesichts der Bedrohung durch Bsal und zielt darauf ab, die Europäische Kommission und die EU-Mitgliedstaaten bei ihren Maßnahmen in Bezug auf die Bsal-Bedrohung mit phasenspezifischen Maßnahmen zu begleiten und zwar für jeweils die Phase vor der Invasion, während der Invasion und während der Endemie. Die sofortige Umsetzung dieses Aktionsplans auf europäischer Ebene und auf Ebene der Mitgliedstaaten könnte zur Eliminierung der Bsal-Bedrohung in Europa führen. Die am dringendsten benötigten allgemeinen Maßnahmen sind:

Auf EU-Ebene:

- Die Einrichtung und der Unterhalt eines Frühwarnsystems (Early Warning System, EWS)
- Die Implementierung von Maßnahmen zum a "sauberen Handel" mit lebenden Amphibien: Bsal-frei durch die gesamte Handelskette hindurch
- Die Finanzierung gezielter Studien zur Verbesserung der effizienten und wirksamen Bsal-Bekämpfung und Eradikation
- Die Einrichtung und den Unterhalt einer europäischen Arbeitsgruppe (European Bsal Working Group, BWG), zur Beratung der EU und der nationalen Regierungen

Auf Ebene der Mitgliedsstaaten:

- Die Einrichtung und den Unterhalt national Aktionspläne (AP)
- Die Einrichtung und den Unterhalt eines Frühwarnsystems (Early Warning System, EWS)
- Die Möglichkeit, schnell auf das plötzliche Auftreten von Bsal zu reagieren und anschließend ein Monitoring zu etablieren und zu bewerten, wodurch ökologische Schäden und finanzielle Kosten langfristig minimiert werden. Eine Bsal-Arbeitsgruppe (BWG) berät die EU und die nationalen Regierungen
- Die sofortige und wirksame Eliminierung nicht-heimischer Amphibienarten

Resumen operativo (Espanol)

El hongo patógeno *Batrachochytrium salamandrivorans* (Bsal) es el causante de la quitridiomicosis en urodelos (salamandras y tritones), una enfermedad letal ulcerativa de la piel. Bsal está estrechamente emparentado con *B. dendrobatidis* (Bd), responsable del declive de más de 500 especies de anfibios en todo el mundo y la extinción de, al menos, 90 especies. Bsal es originario del este de Asia, pero se ha expandido por Europa a través del comercio internacional de mascotas. La llegada de este patógeno a ecosistemas europeos coincide con declives poblacionales de urodelos en Bélgica, Alemania, Países Bajos y España.

La evaluación de riesgos de este plan de acción de Bsal indica que este patógeno compromete la supervivencia de las poblaciones de, al menos, 30 de las 40 especies de urodelos europeos, e incluso la supervivencia de, al menos, 10 especies en un plazo de 10 años. La combinación de su capacidad para provocar declives severos de poblaciones de urodelos, su errática dispersión debido a las impredecibles introducciones provocadas por el hombre, así como la existencia de varios taxones de urodelos muy amenazados, convierten a Bsal en un peligro sin precedentes para la diversidad de urodelos europeos.

La Unión Europea (UE), en base a acuerdos internacionales como el Convenio de Naciones Unidas sobre la Diversidad Biológica (CDB), el Convenio de Berna y la Directiva Hábitats, tiene la obligación de preservar y proteger su biodiversidad contra estas amenazas. Además, la Organización Mundial de Sanidad Animal (OIE) ha incluído a la infección por Bsal en su Código Sanitario para los Animales Acuáticos. La desaparición espontánea de Bsal de Europa es altamente improbable, y su rango de distribución se está expandiendo lentamente. Con unos 25 brotes conocidos, la introduciión de Bsal permanece en una etapa relativamente temprana de la invasión. En esta etapa, y para evitar el establecimiento de Bsal y pérdidas futuras de diversidad de urodelos europeos, la erradicación de la enfermedad debería preverse, lo que requiere un compromiso claro y a largo plazo de la UE y de sus estados miembros.

El Plan de Acción europeo contra Bsal establece las prioridades de conservación de los urodelos ante la amenaza de Bsal, y pretende guiar a la Comisión Europea y a los estados miembros de la UE en su respuesta ante la aparición de Bsal con acciones específicas para las distintas fases de pre-invasión, invasión, y fase endémica de la invasión de Bsal. La inmediata implementación de este Plan de Acción a nivel europeo y de los estados miembros podría evitar la amenaza de Bsal en Europa. Las acciones generales necesarias mas urgentes son:

A nivel de la Unión Europea:

- El establecimiento y mantenimiento de un Sistema de Alerta Temprana (SAT)
- La implementación de medidas para conseguir un 'comercio limpio' de anfibios vivos: ausencia de Bsal en toda la cadena
- La financiación de estudios destinados a aumentar la eficiencia y efectividad de la mitigación y erradicación de Bsal
- El establecimiento y mantenimiento de un Grupo Europeo de Trabajo (GET) sobre Bsal que asesore a la Unión Europea y a los gobiernos nacionales.

A nivel de estado miembro de la Unión Europea:

- El establecimiento y mantenimiento de un Plan de Acción nacional (PA)
- El establecimiento y mantenimiento de un Sistema de Alerta Temprana (SAT)
- La capacidad de responder rápidamente a la introducción de Bsal con labores de seguimiento y evaluación que reducirían a largo plazo los daños ecológicos y los costes económicos de la introducción
- La retirada efectiva e inmediata del medio natural de cualquier especie de anfibio no nativa

Résumé exécutif (Français)

Le champignon pathogène *Batrachochytrium salamandrivorans* (Bsal) provoque la chytridiomycose, une maladie cutanée ulcéreuse mortelle, chez les urodèles (salamandres et tritons). Bsal est étroitement lié à *B. dendrobatidis* (Bd), qui a déjà provoqué le déclin d'au moins 500 espèces d'amphibiens dans le monde entier, dont au moins 90 extinctions globales d'espèces. Bsal est originaire d'Asie de l'Est et il s'est probablement répandu en Europe via le commerce des animaux de compagnie. L'introduction de l'agent pathogène dans les écosystèmes européens coïncide avec le déclin d'urodèles en Belgique, en Allemagne, aux Pays-Bas et en Espagne.

L'évaluation des risques de ce plan d'action envers Bsal montre que le champignon menace la viabilité des populations d'au moins 30 des 40 espèces européennes d'urodèles, et même la survie d'au moins 10 espèces sur une période de 10 ans. La combinaison de la propension de Bsal à provoquer de graves déclins des populations d'urodèles, de sa propagation erratique due à des introductions imprévisibles dues à l'homme et de la présence de plusieurs taxons d'urodèles à haut risque, fait de Bsal une menace sans précédent pour la diversité des urodèles d'Europe.

L'Union européenne (UE) a l'obligation de préserver et de protéger la biodiversité contre ces menaces, sur la base d'accords internationaux, notamment la Convention des Nations unies sur la diversité biologique (CDB), la Convention de Berne et la directive "Habitats". En outre, l'Organisation mondiale de la santé animale (OIE) a inscrit l'infection par Bsal dans son Code sanitaire pour les animaux aquatiques. L'élimination spontanée Bsal en Europe est très peu probable et l'agent pathogène étend lentement son aire de répartition. Avec environ 25 foyers de maladie connus, l'incursion du Bsal est encore à un stade relativement précoce d'invasion. À ce stade, pour éviter l'établissement de Bsal et la perte de la diversité de l'Europe en urodèles, il convient d'envisager l'éradication de la maladie, ce qui nécessite un engagement clair et à long terme de l'UE et de ses États membres.

Le plan d'action européen envers Bsal définit les priorités de conservation des urodèles face à la menace de Bsal, et vise à guider la Commission européenne et les États membres de l'UE dans leur réponse à l'incursion de Bsal par des actions spécifiques aux phases de pré-invasion, d'invasion et d'endémie de Bsal. La mise en œuvre immédiate de ce plan d'action au niveau européen et des États membres pourrait permettre d'éliminer la menace Bsal en Europe. Les actions générales les plus urgentes sont les suivantes:

Au niveau de l'UE :

- Mise en place et maintenance d'un Système d'Alerte Précoce (SAP)
- Mise en œuvre de mesures visant à obtenir un "commerce propre" pour les amphibiens vivants: absence de Bsal tout au long de la chaîne
- Le financement d'études ciblées pour améliorer l'efficacité et l'efficience de l'atténuation et de l'éradication du Bsal
- La création et le maintien d'un Groupe de Travail européen sur Bsal (GTB), chargé de conseiller l'UE et les gouvernements nationaux

Au niveau des États membres :

- L'établissement et la mise en œuvre d'un Plan d'Action national (PA)
- La mise en place et la maintenance d'un Système d'Alerte Précoce (SAP)
- La capacité de répondre rapidement à l'introduction de Bsal avec un suivi et une évaluation ultérieurs, ce qui permettra de minimiser les dommages écologiques et les coûts financiers à long terme
- L'élimination immédiate et effective de toute espèce d'amphibien non indigène dans la nature

Riepilogo operativo (Italiano)

Il fungo patogeno *Batrachochytrium salamandrivorans* (Bsal) può causare la chitridiomicosi, una malattia letale che provoca lesioni della pelle negli urodeli (salamandre e tritoni). Bsal è strettamente imparentato con *B. dendrobatidis* (Bd), che ha già causato il declino di oltre 500 specie di anfibi in varie parti del mondo, incluse almeno 90 estinzioni. Il fungo Bsal ha origini in Asia orientale e probabilmente ha raggiunto l'Europa, dove si sta diffondendo, con animali importati per la terraristica e l'acquariologia. La presenza di questo patogeno negli ecosistemi naturali europei ho coinciso con il declino di popolazioni di salamandre in Belgio, Germania, Olanda e Spagna.

La valutazione del rischio effettuata nel presente Piano d'Azione, indica che Bsal può mettere in pericolo la sopravvivenza a lungo termine di almeno 30 delle 40 specie di urodeli europei e causare l'estinzione di circa 10 specie, in soli 10 anni dal possibile contagio. La capacità di Bsal di causare forti declini delle popolazioni di salamandre, la sua facilità di diffusione mediata dall'uomo in modo imprevedibile e l'esistenza di numerose specie di urodeli altamente vulnerabili, rende la presenza di Bsal in ambiente naturale una minaccia senza precedenti per la diversità delle salamandre in Europa.

Il Piano d'Azione Europeo per Bsal stabilisce le priorità di conservazione per gli urodeli nei confronti di Bsal, e ha lo scopo di informare la Commissione Europea e gli Stati Membri della UE sulle risposte alla diffusione di Bsal con azioni specifiche per le fase precedente la diffusione, quella di diffusione e quella di stabilizzazione di Bsal. La realizzazione immediata di questo Piano d'Azione a livello europeo e in ogni Stato Membro, potrebbe permettere l'eliminazione di questa minaccia in Europa. Pertanto, le azioni più urgenti da intraprendere sono:

A livello europeo

- L'istituzione e il mantenimento di un Sistema di Sorveglianza Precoce (SSP)
- L'implementazione di misure atte a ottenere un "Commercio Sicuro" per gli anfibi vivi, con assenza di Bsal lungo tutto il loro percorso commerciale
- Il finanziamento di studi mirati a migliorare la mitigazione e l'eradicazione effettiva e totale di Bsal
- L'istituzione e il mantenimento di un gruppo di lavoro internazionale su Bsal, al fine di fornire linee guida e indirizzi all'UE e ai governi nazionali

A livello degli stati membri

- L'istituzione e l'implementazione di un Piano d'Azione Nazionale (PA)
- L'istituzione e il mantenimento di un Sistema di Sorveglianza Precoce (SSP)
- La capacità di fornire una risposta rapida alla diffusione di Bsal tramite monitoraggio e valutazione, che possa minimizzare i danni ecologici e i costi finanziari sul lungo periodo
- L'immediata ed effettiva rimozione delle specie esotiche di anfibi dagli ecosistemi naturali

Managementsamenvatting (Nederlands)

De pathogene chytrideschimmel *Batrachochytrium salamandrivorans* (Bsal) veroorzaakt chytridiomycose, een dodelijke huidziekte van land- en watersalamanders. De schimmel Bsal is nauw verwant aan *B. dendrobatidis* (Bd), die wereldwijd de afname van ten minste 500 amfibiesoorten heeft veroorzaakt, waaronder ook het uitsterven van minstens 90 amfibiesoorten. Bsal is afkomstig uit Oost-Azië en is waarschijnlijk via de dierhandel in Europa terecht gekomen en internationaal verspreid. In de gebieden in Europa waar Bsal is vastgesteld bij wild levende salamanders (België, Duitsland, Nederland en Spanje) gaan besmette salamanderpopulaties drastisch achteruit.

De risicobeoordeling in dit Bsal actieplan toont aan dat Bsal een bedreiging vormt voor het voortbestaan van populaties van ten minste 30 van de 40 Europese salamandersoorten op lange termijn, en voor het voortbestaan van ten minste 10 salamandersoorten op korte termijn (10 jaar). De ongekende bedreiging van Bsal voor het behoud van de diversiteit van salamanders in Europa komt door de combinatie van Bsal's mogelijkheid om in korte tijd salamanderpopulaties drastisch te reduceren en een onvoorspelbaar verspreidingspatroon. Door introducties in naïeve gebieden en populaties, mede gefaciliteerd door de mens, kan de schimmel snel en onverwachts toeslaan.

De Europese Unie (EU) heeft een verplichting om haar biodiversiteit te behouden en beschermen tegen dergelijke bedreigingen. Deze verplichtingen zijn gebaseerd op internationale overeenkomsten, zoals de 'Convention on Biological Diversity' (CBD) van de Verenigde Naties, de Bern Conventie en de Habitatrichtlijn. Daarnaast heeft de Wereldorganisatie voor diergezondheid (OIE) Bsal-infecties opgenomen in de 'Aquatic Animal Health Code'. Spontane eliminatie van Bsal uit Europa is zeer onwaarschijnlijk en het verspreidingsgebied van de pathogeen breidt zich langzaam uit. Met ongeveer 25 gekende uitbraken bevindt de invasie van Bsal zich in een relatief vroeg stadium. Ter voorkoming van verdere vestiging van Bsal en het toekomstig verlies van salamanderdiversiteit, is uitroeiing van de ziekte een vereiste. Dit vereist een duidelijke inzet op lange termijn van de EU en haar lidstaten.

Het Europese Bsal Actieplan definieert beschermingsprioriteiten voor salamanders in het kader van de bedreiging door Bsal. Het Actieplan geeft de Europese Commissie en de EU-lidstaten richtlijnen voor het tegengaan van Bsal introductie, alsmede fase-specifieke adviezen wanneer Bsal wel is geïntroduceerd. Onmiddellijke implementatie van dit Actieplan op Europees niveau en het niveau van de individuele lidstaten zou eliminatie van de Bsal-bedreiging mogelijk kunnen maken. De meest urgente benodigde acties zijn:

Op EU-niveau:

- Het bewerkstelligen en onderhouden van een 'Early Warning System' (EWS)
- Implementatie van maatregelen om een 'schone handel' in amfibieën te bewerkstelligen met als doel de afwezigheid van Bsal door de gehele keten
- Het bekostigen van gerichte studies ter verbetering van efficiënte en effectieve Bsal mitigatie, alsmede eliminatie
- Het bewerkstelligen en onderhouden van een Europese Bsal Werkgroep (BWG), om de EU en nationale regeringen van advies te voorzien

Op lidstaatniveau:

- Het bewerkstelligen en implementeren van een nationaal Actieplan (AP)
- Het bewerkstelligen en onderhouden van een 'Early Warning System' (EWS)
- Het vermogen om snel te kunnen reageren op de introductie van Bsal met bijbehorende monitoring en evaluatie, wat ecologische schade en financiële kosten op de lange termijn zal minimaliseren
- De onmiddellijke en effectieve verwijdering van niet-inheemse amfibiesoorten

Technical summary

Background

The fungal pathogen *Batrachochytrium salamandrivorans* (Bsal) primarily infects urodeles (salamanders and newts) in which it can cause chytridiomycosis, a lethal ulcerative skin disease. Bsal is closely related to *B. dendrobatidis* (Bd), which has already caused declines and extinctions of at least 500 amphibian species all over the world, including at least 90 global species extinctions. Bsal originates from East Asia and it likely reached, and spread internationally within Europe via the pet trade. It has been detected in urodeles traded and kept by hobbyists. Incursion of the pathogen in the European ecosystem coincides with urodele declines in Belgium, Germany, the Netherlands and Spain. Here it causes mortality and population declines in a range of urodelan species, most notably the fire salamander (*Salamandra salamandra*). The combination of Bsal's propensity to cause severe urodele population declines, its erratic spread due to unpredictable human-mediated Bsal introductions and the presence of several high-risk urodelan taxa, render Bsal an unprecedented threat to Europe's urodele diversity.

The European Union (EU) has an obligation to preserve and protect its urodelan biodiversity against such threats, based on international agreements, including the United Nations Convention on Biological Diversity (CBD), Bern Convention and Habitats Directive. In addition, the World Organisation for Animal Health (OIE) listed infection with Bsal in its Aquatic Animal Health Code. Spontaneous elimination of Bsal from Europe is highly unlikely and the pathogen is slowly expanding its range. With approximately 25 known disease outbreaks, Bsal incursion is still in a relatively early stage of invasion. At this stage, to avert further establishment of Bsal and future loss of European urodele diversity, disease eradication should be envisaged, which requires a clear and long-term commitment of the EU and its member states.

The European Bsal Action Plan defines urodelan conservation priorities in the face of the Bsal threat and aims to guide the European Commission and the EU member states in their response to the Bsal incursion with phase-specific actions for the pre-invasion, invasion and endemic phases of Bsal invasion. Immediate implementation of this Action Plan at European and member state level could result in the elimination of the Bsal threat from Europe.

According to the prevailing taxonomic insights at the time of writing, this Action Plan covers 40 urodelan species belonging to the families Salamandridae (30 species), Plethodontidae (8 species), Hynobiidae (1 species) and Proteidae (1 species), which occur naturally in (geographical) Europe, including all EU member states.

Risk assessment

To define conservation priorities a risk assessment was performed based on available knowledge and expert judgement for all European urodelan species and subspecies in order to assess the likely impact of Bsal on the persistence of these taxa.

The risk that Bsal poses to distinct intraspecific lineages may be different from the risk it presents to the species as a whole; therefore, subspecies have been used as a proxy for intraspecific diversity in the risk assessment.

Overall, Bsal risk is defined as '*the predicted impact of Bsal introduction on the persistence of native European urodelan biodiversity*'. Here, the risk that Bsal poses to the total urodelan diversity for a given country or region is also considered.

The risk of Bsal at urodelan population level is defined as '*risk of population extinction upon introduction of Bsal for a given species, subspecies or lineage*'. The risk of Bsal at urodelan species, subspecies and lineage level is defined as '*risk of species or subspecies extinction upon introduction of Bsal*'.

Based on published and non-published evidence of Bsal susceptibility, the risk that Bsal poses to a particular urodelan taxon at the population level could be assessed with a certain degree of confidence, dependent on the availability of information. To assess the degree of risk at species and subspecies level, the species/subspecies distribution range size was combined with the population level risk. The resulting risk on species and subspecies level increases with decreasing range sizes for Bsal susceptible taxa. Outcomes were assessed by expert judgment, explaining slightly deviant risk categories for some taxa.

We assessed the risk of Bsal at species and subspecies level over two time frames (10 years and 100 years post-incursion of Bsal) and we categorized the degree of risk as low, medium or high. The selected time frames reflect the immediately required short-term actions and the long-term risk for urodelan biodiversity when restraining from actions.

- **Low** The (sub)species shows no response (no infection, no disease) or a tolerant response (infection, no disease) to exposure with Bsal. For laboratory trials, this corresponds to <20% mortality after experimental exposure.
- **Moderate** The (sub)species is moderately susceptible, upon infection disease occurs, but infection may not always be lethal, and may be dose dependent. For laboratory trials, this corresponds to 20-80% mortality after experimental exposure.
- **High** The species is highly susceptible and upon infection, fatal disease occurs. For laboratory trials, this corresponds to >80% mortality after experimental exposure.

Of the 40 European urodelan species, 30 (75.0%) are considered to be at high risk, five (12.5%) are considered to be at medium risk and five (12.5%) are considered to be at low risk at the population level (Table 2). At the species level over a 10-year time frame, ten (25.0%) are considered to be at high risk of extinction, six (15.0%) are considered to be at medium risk and 24 (60.0%) are considered to be at low extinction risk. Over a time frame of 100 years, 16 (40.0%) species are considered to be at high risk of extinction, 16 (40.0%) are considered to be at medium risk and eight (20.0%) are considered to be at low extinction risk. For many of the assessed subspecies, the Bsal risk category is identical to, or higher than, the species-level risk category. The latter is apparent, as the range sizes of subspecies are smaller than for species.

To preserve urodelan biodiversity at the European or national scale, the taxon-level (species or subspecies) risk over a 10-year time frame is preferred to prioritize conservation actions, with the taxa categorized as high risk deserving immediate, proactive Bsal mitigation actions. Using the 10-year time frame allows to focus on the taxa which need conservation actions in the short-term, as this time period reflects the short-term expected effects of Bsal at urodelan conservation units. To preserve urodelan biodiversity at the local scale, the risk at the population level is the preferred metric for prioritising conservation actions.

Current legislative regulations

At European **legislative level** it is recommended to:
- Implement enforcement of EU decision 2018/320 ubiquitously
- Expand EU decision 2018/320 to include vectoring anurans
- Expand EU decision 2018/320 to include all urodeles kept in captivity in the EU
- Implement a specific CN-code for amphibians
- Implement stringent biosecurity measures for all traded amphibians, which are currently not covered by EU decision 2018/320

General Action Plan

This general Action Plan describes the general actions, which are needed to preserve the European urodelan biodiversity with regard to Bsal, and is the suggested basis for each national Action Plan.

Phase-specific actions have been devised for the pre-invasion, invasion and endemic phases of Bsal invasion. The most urgently needed general **pre-invasion phase** actions are:

- For each European country to establish its own national Action Plan (AP)
- The establishment and maintenance of national and regional Early Warning Systems (EWS) for early and rapid identification of Bsal infection in the wild. These should be based on a combination of active (targeted) and passive infection surveillance.
- Set up long-term population monitoring for at least the high risk conservation units, particularly at locations with high likeliness of exposure to Bsal
- Ability to immediately respond to Bsal incursion (e.g. removal and collection of animals, imposition of sanitary and biosecurity measures in the wild, closing areas to the general public). An immediate response will reduce ecological damage and financial costs on the long-term
- Increased regulation of traded amphibian species, and the implementation of additional biosecurity regulations
- The immediate and effective removal of any non-native amphibian species. Apart from sites of Bsal incursion, good practice dictates that this should be done elsewhere too, as it is likely to decrease the risk of non-native pathogen incursion
- Support for effective monitoring and evaluation of mitigation actions at sites of Bsal incursion

- Convey scientific outputs on Bsal mitigation measures to the relevant authorities, conservation managers and to the public
- Preparation for, and initiation of, *in situ* and *ex situ* management for high risk conservation units
- Promotion of, and support for, targeted scientific studies to fill the knowledge gaps that prevent efficient or effective Bsal mitigation
- The establishment and maintenance of a European Bsal Working Group, to provide advice to the EU and national governments with regard to Bsal to ensure biodiversity conservation targets are met

When Bsal has entered the population or country, either by natural spread or human-facilitated, a mitigation response must be implemented as rapidly as possible. Communication, active surveillance and monitoring must be established early and maintained throughout the **invasion (epidemic) phase**.

The aims in the invasion phase should be to:

- Eliminate Bsal
- Prevent establishment of Bsal
- Prevent the spread of Bsal
- Ensure population persistence

If implemented measures are insufficient to eliminate Bsal, infection might become endemic within the affected population **(established (endemic) phase)**. In this situation there is the continuous risk of the spread of Bsal to other naïve populations.

Member states should strive for the eradication of Bsal to:

- Prevent pathogen spread to naïve populations
- Prevent new disease outbreaks
- Conserve biodiversity

Endemic pathogen presence requires the following actions:

- If feasible, long-term effort to consistently remove amphibians from the site until confirmed eradication of Bsal
- Continuously monitor urodelan populations, Bsal prevalence and spread via monitoring, active and passive surveillance
- Invest in scientific research that seeks the elimination of Bsal given the current situation
- Do not restock Bsal positive populations
- Ensure good quality habitat for amphibians
- Maintain high standards of biosecurity
- Isolate the area as effective as possible (fence or other barriers) and restrict access
- Prevent the introduction of new pathogens

In the case of the risk of conservation unit **extinction** due to Bsal, member states should:

- Safeguard an *ex situ* population
- Identify potential release areas for *ex situ* animals that were caught prior to Bsal incursion or that were translocated from an uninfected population
- Monitor areas for the absence of Bsal - consider using a sentinel species for at least a year
- Follow the IUCN criteria for reintroductions and the mitigation of infectious disease threats (e.g. have the appropriate professionals conduct a Disease Risk Analysis)
- Initiate potential reintroduction only in case of *confirmed* absence of Bsal
- Be vigilant for novel threats (such as novel pathogen introductions, including those which may be present in animals destined for reintroduction)

Species-specific protocols

Species-specific protocols have been devised for each European urodelan species, including for proposed intraspecific conservation units where these have been identified. For each species, species-specific information relevant to Bsal-related conservation are provided, including epidemiological relevant data, Bsal susceptibility and risk status, species distribution, proposed conservation units, species-specific actions and *ex situ* management information.

In all cases, upon definitive diagnosis of a Bsal outbreak, disease eradication must be envisaged.

At least for high risk conservation units, the following general actions are required:

- Implement biosecurity measures to prevent the human-facilitated Bsal incursion
- Ensure proper habitat management
- Set up long-term population monitoring
- Set up active and passive Bsal surveillance
- Prepare and initiate *ex situ* measures

Glossary

AIS	Alien Invasive Species
Bd	*Batrachochytrium dendrobatidis*
Biodiversity	The variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems.
Bsal	*Batrachochytrium salamandrivorans*
Bsal risk (general)	The predicted impact of Bsal introduction on the persistence of native European urodelan biodiversity.
Bsal risk (population level)	Risk of population extinction upon introduction of Bsal for a given species, subspecies or lineage.
Bsal risk (taxon level)	Risk of species or subspecies extinction upon introduction of Bsal.
CBD	Convention on Biological Diversity
Chytridiomycosis	Amphibian disease caused by the fungus *Batrachochytrium dendrobatidis* and *B. salamandrivorans*. Animals that test positive for the presence of Bd/Bsal may show no signs of the disease.
Conservation unit	An evolutionarily significant unit that is considered distinct for purposes of conservation, including species, subspecies and intraspecific lineages.
Effective population size	The average number of individuals in a population that contribute genes to the next generation.
EID	Emerging Infectious Disease; Infectious disease that has increased in incidence recently and could increase in the near future.
Endemic	Infection is maintained at low or non-detrimental levels.
Epidemic	Describes pathogens that are increasing in frequency, that is, have not reached a stable equilibrium.
EWS	Early Warning System
Exotic species	Introduced non-native species that occurs in an area where it did not evolve, but causes no harm to the local ecosystem.
Ex situ	Off-site. *Ex situ* conservation refers to the management of a captive population outside the natural habitat.
Functional extinction	The decline of the population to a level at which it is no longer viable in the long-term, or at which it no longer plays a role in ecosystem function.
In situ	On-site. *In situ* conservation is the conservation of species diversity within normal and natural habitats and ecosystems.
Invasive species	Non-native species that causes major ecological, health or economic problems.

IUCN	International Union for Conservation of Nature and Natural Resources
Lethal	The host becomes infected, infection results in fatal disease, no recovery from disease.
Pandemic	The worldwide spread of a new infectious disease
Pathogenicity	The ability of an organism to cause disease.
Pathogen pollution	Human-mediated introduction of a pathogen to a new host or region.
Persistence	The indefinite existence of the current diversity in European urodelan (sub)species.
Phylogeny	The evolutionary development or history of a species or of a taxonomic group of organisms.
Population	All the organisms of the same species, which live in a particular geographical area, and have the capability of interbreeding.
Population extinction	The complete or functional extinction of the population.
Resistant	Host does not become infected, there is no disease.
Susceptible	The host becomes infected, and infection leads to clinical diseases with the possibility of recovery from disease.
Susceptibility	The response of the host species to exposure to Bsal.
Tolerant	The host becomes infected, but there is no disease or mortality.
Urodeles	Salamanders and newts.
Virulence	The degree to which an organism can cause damage to a host.

1   Introduction

Following an enigmatic 99.9% decline of a fire salamander (*Salamandra salamandra*) population in the Netherlands, it was discovered in 2013 that the newly described fungal pathogen *Batrachochytrium salamandrivorans* (Bsal), a chytrid fungus, was the cause of this decline (Martel et al. 2013). Subsequent research showed that Bsal specifically affects salamanders and newts (collectively called urodeles), while frogs and toads (collectively called anurans), remain unaffected (Martel et al. 2014), although infection of the latter can occur in the absence of disease. By causing a lethal ulcerative skin disease known as chytridiomycosis, Bsal literally eats away the skin of urodeles, and infection can be lethal for many urodelan species.

Bsal is closely related to another fungal pathogen, *Batrachochytrium dendrobatidis* (Bd), which has already caused population declines and extinctions of at least 500 amphibian species all over the world, including the global extinction of at least 90 species (Scheele et al. 2019). The fear is that Bsal will have a similar impact on urodeles in Europe. In laboratory trials, Bsal was found to be lethal for all North American newt species and nearly all European, North African, and Middle Eastern urodelan species tested. In particular, urodelan species of the Salamandridae family, comprising the majority of all European species, were found to be susceptible to lethal infection (Martel et al. 2014). Bsal was shown to be lethal to 8 of 10 European urodelan species experimentally tested (Martel et al. 2014), although there seems to be a dose-dependent relationship regarding outcome of disease for at least some species (Bates et al. 2019, Stegen et al. 2017).

Some urodelan species are tolerant to Bsal infection and can spread Bsal unnoticed. East Asian salamanders, the presumed original hosts for Bsal, including species of the genera *Cynops* and *Paramesotriton* which were widely available in the pet trade, may be asymptomatic carriers of Bsal (Martel et al. 2014, Laking et al. 2017). These species are likely to have co-evolved with Bsal for millions of years and hence may be infected with Bsal, but with no noticeable health effects. Based on large-scale screenings of wild urodeles in China and Vietnam, Bsal was detected from species of the genera *Cynops*, *Pachytriton*, *Paramesotriton*, *Tylototriton*, and *Andrias,* with an estimated prevalence of between 2 and 4% (Laking et al. 2017, Yuan et al. 2018). In addition, it has been shown that anuran species (i.e. frogs and toads) can act as asymptomatic carriers for Bsal (Stegen et al. 2017, Nguyen et al. 2017). Besides the infection in nature, Bsal has been detected in captive-held urodeles in Germany, the Netherlands, Spain and the United Kingdom (Sabino-Pinto et al. 2015, Fitzpatrick et al. 2018). Captive urodeles and anurans are considered a potential reservoir for Bsal and present a serious risk of Bsal spillover from captivity to the wild via direct and indirect routes, thus threatening native species (Cunningham et al. 2019, Martel et al. 2020).

 Bsal has been detected in multiple locations across Europe. Currently, disease outbreaks have been detected in the Netherlands, Germany, Spain and in Belgium, including a location close to the French border (Spitzen-van der Sluijs et al. 2016, Beukema et al. 2018, Dalbeck et al. 2018, Martel et al. 2020). To date, the infection is thought to be absent from the wild in the United Kingdom, although it is known to be present in captive populations in that country (Fitzpatrick et al. 2018, Cunningham et al. 2019). Once in the wild, Bsal is likely to have a large impact on urodelan populations. It is of importance to emphasize that the possibility exists that disease outbreaks in other EU countries may be present, but, especially in sparsely populated areas, are yet undetected.

The urgency for each EU country to establish and implement a national Action Plan for the mitigation of Bsal is underlined by the combination of the erratic spread of the pathogen due to unpredictable human-mediated Bsal introductions and the presence of rare and range-restricted urodelan taxa, which may face extinction if Bsal reaches their populations. Therefore, the prevention of the introduction and spread of Bsal is of the utmost importance. Should Bsal be detected in the wild there should be no hesitation with regard to implementation of effective and appropriate control actions.

The European Bsal Action Plan presented here provides guidelines for countries at the general and species-specific levels in order to help the development and implementation of pro-active and reactive responses to Bsal incursion.

1.1 Considered species and geographic area

According to the prevailing taxonomic insights at the time of writing, 40 urodelan species belonging to the families Salamandridae (30 species), Plethodontidae (8 species), Hynobiidae (1 species) and Proteidae (1 species) occur naturally in (geographical) Europe (Table 1). *Lissotriton vulgaris* sensu lato has been shown to be a species complex of five different species (Pabijan et al. 2017, Wielstra et al. 2018), of which three occur within Europe as defined below. All except two (*Triturus karelinii* and *Salamandrella keyserlingii*) of the species considered within the document occur within EU territory.

The risk that Bsal poses to distinct intraspecific lineages may be different from the risk it presents to the species as a whole; therefore, in order to protect urodelan biodiversity, intraspecific lineages are also covered. While subspecies have been used as a proxy for intraspecific diversity in the risk assessment (see §2.1), such diversity often extends beyond the subspecies level. Where required, therefore, intraspecific lineages have been proposed as the conservation units in certain cases described in Chapter 5.

We used the geographic area for Europe as the European continent bordered by the Arctic Ocean to the north, the Atlantic Ocean to the west, and the Mediterranean Sea to the south. The eastern boundaries are formed by the Ural Mountains, the Ural River, and the Caspian Sea. In the southeast, the boundaries are formed by the Black Sea and the waterways connecting the Black Sea to the Mediterranean Sea, excluding the Caucasus region. All EU member states are included as are a number of states that are not members of the EU (Figure 1).

For (sub)species which also occur outside Europe, only the distribution ranges within the area described above are considered here.

Figure 1. Map of the considered geographic area.

Table 1. List of European urodelan species, including their IUCN Red List Category (www.iucnredlist.org; accessed May 21, 2019) and Habitats Directive Annex listing.

	Family	Species		IUCN Red List Category [1]	Habitats Directive Annexes
1	Hynobiidae	*Salamandrella keyserlingii* [2]	Siberian salamander	LC	n/a
2	Plethodontidae	*Speleomantes ambrosii*	Ambrosi's cave salamander	NT	II/IV
3	Plethodontidae	*Speleomantes flavus*	Monte Albo cave salamander	VU	II/IV
4	Plethodontidae	*Speleomantes genei*	Gené's cave salamander	VU	II/IV
5	Plethodontidae	*Speleomantes imperialis*	Imperial cave salamander	NT	II/IV
6	Plethodontidae	*Speleomantes italicus*	Italian cave salamander	NT	IV
7	Plethodontidae	*Speleomantes sarrabusensis*	Sette Fratelli cave salamander	VU	II/IV
8	Plethodontidae	*Speleomantes strinatii*	Strinati's cave salamander	NT	II/IV
9	Plethodontidae	*Speleomantes supramontis*	Supramonte cave salamander	EN	II/IV
10	Proteidae	*Proteus anguinus*	Olm	VU	II/IV
11	Salamandridae	*Calotriton arnoldi*	Montseny brook newt	CR	IV
12	Salamandridae	*Calotriton asper*	Pyrenean brook newt	NT	IV
13	Salamandridae	*Chioglossa lusitanica*	Golden-striped salamander	VU	II/IV
14	Salamandridae	*Euproctus montanus*	Corsican brook newt	LC	IV
15	Salamandridae	*Euproctus platycephalus*	Sardinian brook newt	EN	IV
16	Salamandridae	*Ichthyosaura alpestris*	Alpine newt	LC	n/a
17	Salamandridae	*Lissotriton boscai*	Bosca's newt	LC	n/a
18	Salamandridae	*Lissotriton graecus*	Greek smooth newt	NE	n/a
19	Salamandridae	*Lissotriton helveticus*	Palmate newt	LC	n/a
20	Salamandridae	*Lissotriton italicus*	Italian newt	LC	IV
21	Salamandridae	*Lissotriton montandoni*	Montandon's newt	LC	II/IV
22	Salamandridae	*Lissotriton schmidtleri*	Schmidtler's smooth newt	NE	n/a
23	Salamandridae	*Lissotriton vulgaris* [3]	Smooth newt	LC	n/a [4]
24	Salamandridae	*Lyciasalamandra helverseni*	Karpathos salamander	VU	II/IV
25	Salamandridae	*Lyciasalamandra luschani*	Luschan's salamander	VU	II/IV
26	Salamandridae	*Pleurodeles waltl*	Sharp-ribbed newt	NT	n/a
27	Salamandridae	*Salamandra atra*	Alpine salamander	LC	IV [5]
28	Salamandridae	*Salamandra corsica*	Corsican fire salamander	LC	n/a
29	Salamandridae	*Salamandra lanzai*	Lanza's salamander	VU	IV
30	Salamandridae	*Salamandra salamandra*	Fire salamander	LC	n/a
31	Salamandridae	*Salamandrina perspicillata*	Northern spectacled salamander	LC	II/IV
32	Salamandridae	*Salamandrina terdigitata*	Southern spectacled salamander	LC	II/IV
33	Salamandridae	*Triturus carnifex*	Italian crested newt	LC	II/IV
34	Salamandridae	*Triturus cristatus*	Great crested newt	LC	II/IV
35	Salamandridae	*Triturus dobrogicus*	Danube crested newt	NT	II
36	Salamandridae	*Triturus ivanbureschi*	Buresch's crested newt	NE	II/IV
37	Salamandridae	*Triturus karelinii* [2]	Karelin's crested newt	LC	II/IV
38	Salamandridae	*Triturus macedonicus*	Macedonian crested newt	NE	II/IV
39	Salamandridae	*Triturus marmoratus*	Marbled newt	LC	IV
40	Salamandridae	*Triturus pygmaeus*	Southern marbled newt	NT	IV

[1] *LC, Least Concern; NT, Near Threatened; VU, Vulnerable; EN, Endangered; CR, Critically Endangered; NE, Not Evaluated.* [2] *Species does not naturally occur within any EU member state.* [3] *Lissotriton vulgaris sensu stricto (Pabijan et al. 2017).* [4] *Subspecies L. v. ampelensis is listed on Annexes II/IV.* [5] *Subspecies S. a. aurorae is listed on Annexes II/IV.*

2 Species and subspecies-specific Bsal risk assessment

Based on factors such as their susceptibility to Bsal, range, habitat preference, exposure and biology, European urodelan species show variable risks of becoming infected with Bsal and of the impact of infection at the individual, population and species levels (Martel et al. 2014, Stegen et al. 2017, Beukema et al. 2018). To define conservation priorities, therefore, a risk assessment was performed for each European urodelan species and subspecies in order to assess the likely impact of Bsal on the persistence of these taxa.

Definitions

Overall, Bsal risk is defined as '*the predicted impact of Bsal introduction on the persistence of native European urodelan biodiversity*'. Here, the risk that Bsal poses to the total urodelan diversity for a given country or region is also considered. It includes intraspecies diversity, as defined by the Convention on Biological Diversity (CBD): 'Biological diversity means the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems.' While subspecies are used as a proxy for intraspecific diversity in the risk assessment (see §2.1), such diversity often extends beyond the subspecies level. Where required, therefore, intraspecific lineages have been proposed as the conservation units in certain cases described in Chapter 5. Persistence is defined as the indefinite existence of the current diversity in European urodelan (sub)species.

The risk of Bsal at urodelan population level is defined as '*risk of population extinction upon introduction of Bsal for a given species, subspecies or lineage*'. The risk of Bsal at urodelan species, subspecies and lineage level is defined as '*risk of species or subspecies extinction upon introduction of Bsal*'.

Population extinction is defined as the complete or functional extinction of the population. Functional extinction is the decline of the population to a level at which it is no longer viable in the long-term, or at which it no longer plays a role in ecosystem function.

2.1 Risk assessment methodology

A risk assessment based on available knowledge was performed for all European urodelan species and subspecies based on their estimated susceptibility to Bsal (§ 2.1.1) and their range size (§ 2.1.2).

Based on evidence of Bsal susceptibility, when available, the risk that Bsal poses to a particular urodelan species or subspecies at the population level could be assessed with a certain degree of confidence. To assess the degree of risk at species and subspecies (taxon) level, the species/subspecies distribution range size was combined with the population level risk. The resulting risk on species and subspecies level increases with decreasing range sizes for Bsal susceptible taxa, as shown in Box 1.

Box 1. Example of how the population level risk of extinction and range size relate to the taxon level risk of extinction.

Based on the Bsal susceptibility of a given taxon (species or subspecies), the risk of extinction is determined at population level, and is categorised as high, medium or low. This population level risk was combined with the range size (1-5, 6-25 or >25 50x50 km UTM squares) to obtain the taxon level risk, also categorised as high, medium or low. The taxon level risk was assessed over 10 years and 100 years post-incursion of Bsal, to reflect the short-term (immediate) risk and the long-term risk. The table below provides the applied scheme for risk categorization. The resulting risk on taxon level increases with decreasing range sizes, and increases over time (10 to 100 years), for Bsal susceptible taxa. For example, if a taxon (e.g. the fire salamander (*S. salamandra*)) has a high population level risk, but has a large distribution range (>25 50×50 km UTM squares), then the taxon level risk of extinction is rated low at the short-term (10 years), but increases to medium when Bsal infection persists (100 years).

Population level risk of extinction	Range size	Taxon level risk of extinction	
		10 years	**100 years**
High	1-5	High	High
High	6-25	Medium	High
High	>25	Low	Medium
Medium	1-5	Medium	Medium
Medium	6-25	Low	Medium
Medium	>25	Low	Low
Low	1-5	Low	Low
Low	6-25	Low	Low
Low	>25	Low	Low

We assessed the risk of Bsal at species and subspecies level over two time frames (10 years and 100 years post-incursion of Bsal) and we categorized the degree of risk as low, medium or high (§ 2.2). The 10-year and 100-year time frames were chosen based on expert judgment and are intended to reflect the short- and long-term expected effects of Bsal at urodelan conservation units. These two time frames allow the distinction between the taxa which need conservation actions in the short-term and those which need conservation actions in the long-term. As the degree of confidence is based on the susceptibility to Bsal, the confidence at the taxon level and at the population level are the same. Outcomes were assessed by expert judgment of the project partners, explaining slightly deviant risk categories for some taxa in comparison to the table in Box 1. For example, *Calotriton asper*, which has been placed in a higher risk category at the 100-year time frame due to uncertainty regarding Bsal susceptibility and high susceptibility of the closely related *C. arnoldi*.

While the parameters we used for the risk assessment have been validated for some species (e.g. *Salamandra salamandra*), they have not yet been thoroughly assessed for most urodelan species, which introduces a level of uncertainty in the assessment. Additionally, it needs to be stressed that our current knowledge of Bsal is limited, and as research is ongoing novel insights may change our perception of Bsal-related risks.

2.1.1 Estimated susceptibility

Susceptibility of a urodele to Bsal infection may vary based on environmental conditions, level of exposure and intraspecific variation, and therefore the degree of susceptibility can be context-specific such that a species which might appear to be e.g. tolerant under some circumstances and susceptible under other circumstances.

The estimated host susceptibility of a given taxon to Bsal was based on three lines of evidence:

- **Laboratory trials** Bsal susceptibility tested after experimental exposure in a controlled setting.
- **Field outbreaks** Bsal susceptibility based on outbreaks known from the field.
- **Captivity** Bsal susceptibility based on outbreaks known from captivity (exluding laboratory trials).

For many taxa the Bsal susceptibility can only be deduced, if not known from experimental or field data. Inference of susceptibility from phylogeny is justified for the clade that contains the genera *Salamandra, Chioglossa* and *Lyciasalamandra* as laboratory experiments (Martel et al. 2014, Martel and Pasmans, unpublished data) and data from disease outbreaks in captivity (Fitzpatrick et al. 2018, Sabino-Pinto et al. 2018) consistently show similar susceptibility for species within these genera, but less so for others such as the genera *Lissotriton* and *Triturus*. For these latter genera host response to Bsal infection is less uniform (Martel et al. 2014, Bates et al. 2019, Martel et al. 2020). As a precautionary principle, where the susceptibility of a given taxon is not known, its susceptibility was predicted to be similar to the highest degree of susceptibility of its close relatives. The degree of risk Bsal presents at the population level was then determined with a confidence level based on the amount of evidence available for host susceptibility (see below).

The Bsal susceptibility of urodelan taxa was classified into three categories based on the known or expected response to Bsal:

- **Low** The (sub)species shows no response (no infection, no disease) or a tolerant response (infection, no disease) to exposure with Bsal. For laboratory trials, this corresponds to <20% mortality after experimental exposure.
- **Moderate** The (sub)species is moderately susceptible, upon infection disease occurs, but infection may not always be lethal, and may be dose dependent. For laboratory trials, this corresponds to 20-80% mortality after experimental exposure.
- **High** The species is highly susceptible and upon infection, fatal disease occurs. For laboratory trials, this corresponds to >80% mortality after experimental exposure.

Susceptibility to Bsal has been assessed in the laboratory for the following European urodelan species: *Calotriton arnoldi*, *Calotriton asper*, *Chioglossa lusitanica*, *Euproctus platycephalus*, *Ichthyosaura alpestris*, *Lissotriton boscai*, *Lissotriton helveticus*, *Lissotriton italicus*, *Lissotriton vulgaris*, *Lyciasalamandra helverseni*, *Pleurodeles waltl*, *Proteus anguinus*, *Salamandra salamandra*, *Salamandrella keyserlingii*, *Salamandrina perspicillata*, *Speleomantes genei*, *Speleomantes imperialis*, *Speleomantes strinatii*, *Triturus cristatus* and *Triturus marmoratus* (Martel et al. 2014, Bates et al. 2019, Martel et al. 2020, Martel and Pasmans, unpublished data). Additional susceptibility information has been derived from mortality events that occurred in captivity (Sabino-Pinto et al. 2015, Chytridiomycose *Batrachochytrium salamandrivorans* (Bsal), Actieplan - België, 2017, Fitzpatrick et al. 2018) or in the wild (Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018, Martel et al. 2020) for the following species: *Chioglossa lusitanica, Ichthyosaura alpestris*, *Lissotriton helveticus*, *Lissotriton vulgaris*, *Salamandra atra*, *Salamandra corsica*, *Salamandra salamandra*, *Triturus cristatus*, *Triturus dobrogicus*, *Triturus ivanbureschi*, *Triturus karelinii*, *Triturus macedonicus* and *Triturus marmoratus*.

As not all species and subspecies data on susceptibility is available from laboratory or field data, there is a variable level of confidence on the impact of a Bsal-infection on the sustainable persistence of a population or a (sub)species. This level of confidence was categorised as high or low.

- **High**   Susceptibility to Bsal (either low, moderate or high) has been determined based on at least two lines of evidence.
- **Low**   Susceptibility to Bsal (either low, moderate or high) has been determined based on a single line of evidence, or susceptibility is inferred from phylogeny.

2.1.2   Range size

The range size of European urodelans has been determined by Sillero et al. (2014) and Wielstra et al. (2014, 2018), based on the number of occupied 50 × 50 km UTM squares. For the risk assessment, only the range within Europe as defined in § 1.2 is considered for all urodelan (sub)species.

The range sizes of the species and subspecies have been categorised as follows:

- **Large**   >25 (50 × 50 km UTM squares)
- **Medium**   6-25 (50 × 50 km UTM squares)
- **Small**   1-5 (50 × 50 km UTM squares)

2.1.3   Excluded parameters

The exclusion of certain parameters in the risk assessment is explained below.

Conservation status

The most recent IUCN Red List categories are included to show the conservation status of each species (www.iucnredlist.org, accessed May 21, 2019). However, Red List status is not used for the risk assessment, to focus solely on the risk that Bsal poses to a particular species or subspecies. The IUCN Red List is also based on extinction risk assessments (Collen et al. 2016) and may already include the threat that Bsal poses to a particular species. In addition, IUCN Red List categories are on species level only, whereas subspecies are also included in this Bsal risk assessment, which may have a different

conservation status compared to the corresponding species. The conservation status applicable to the European urodelan species are defined as: Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR) and Not Evaluated (NE).

Probability of exposure to Bsal

The probability of exposure of the (sub)species to Bsal is not included in the risk assessment. The human-mediated spread and introduction of Bsal, even to remote sites, is unavoidable due to current poor or mostly absent biosafety regulations and enforcement. Recent findings indicate that Bsal is widely distributed amongst hobbyist urodelan collections and that human-mediated introduction and transmission (St-Hilaire et al. 2009) may be more important than previously realized (Fitzpatrick et al. 2018, Sabino-Pinto et al. 2018, Gilbert et al. 2019, Martel et al. 2020). From that perspective, geographical isolation is a less important barrier to consider with regard to introduction probability, and the likelihood of exposure is then comparable to that for most species at mainland situations.

The exact mechanisms of spread are unknown for Bsal, but considering its scattered distribution across wide areas of Europe and recent findings that Bsal has crossed geographic barriers, such as large rivers and large distances (> 1.000 km) (Dalbeck et al. 2018, Fitzpatrick et al. 2018, Martel et al. 2020), human-mediated introduction and spread of Bsal via the hobbyist trade and pathogen spillover from captive collections or via the passive transport of zoospores in water and/or fomites (e.g. boots, equipment, vehicles), are currently considered to be important. As humans easily travel long distances and to/from remote areas such as islands, Bsal can be introduced anywhere.

Assuming human transmission as an important factor, which leads to a near equal likelihood of exposure to populations, the impact of Bsal on the persistence of a urodelan (meta)population, both for isolated and connected populations, is based on the known or estimated susceptibility of the host-species, and the Bsal risk for each of the (sub)species is determined based on the parameters Bsal susceptibility of (sub)species and distribution range.

Climatic conditions

Climatic conditions are not specifically included in this risk assessment as most, if not all, urodelan species prefer a relatively cool and humid microclimate, which is likely quite homogeneous for all European species and which is also suitable for Bsal. For example, although *Lyciasalamandra* species live in hot and dry regions in Greece and Turkey, they occupy niches within this environment that are humid and relatively cool (underground in karstic areas) (Steinfartz & Mutz 1998). Furthermore, *Speleomantes* species do not tolerate temperatures higher than ~19°C, yet they live in areas that are hot and dry at the surface during summer (Lanza 2006).

2.2   Risk assessment outcomes

Of the 40 European urodelan species, 30 (75.0%) are considered to be at high risk, five (12.5%) are considered to be at medium risk and five (12.5%) are considered to be at low risk at the population level (Table 2). At the species level over a 10-year time frame, ten (25.0%) are considered to be at high risk of extinction, six (15.0%) are considered to be at medium risk and 24 (60.0%) are considered to be at low extinction risk. Over a time frame of 100 years, 16 (40.0%) species are considered to be at high

risk of extinction, 16 (40.0%) are considered to be at medium risk and eight (20.0%) are considered to be at low extinction risk. For many of the assessed subspecies, the Bsal risk category is identical to, or higher than, the species-level risk category. The latter is apparent, as the range sizes of subspecies are smaller than for species.

To preserve urodelan biodiversity at the European or national scale, the taxon-level (species or subspecies) risk over a 10-year time frame is preferred to prioritize conservation actions, with the taxa categorized as high risk deserving immediate, proactive Bsal mitigation actions. Using the 10-year time frame allows to focus on the taxa which need conservation actions in the short-term, as this time period reflects the short-term expected effects of Bsal at urodelan conservation units. To preserve urodelan biodiversity at the local scale, the risk at the population level is the preferred metric for prioritising conservation actions.

It is important to realise that lower risk category urodelan taxa may pose a risk to other Bsal-susceptible taxa by acting as vectors for Bsal. As they may carry Bsal without any visible signs, they can spread the pathogen unnoticed and act as a reservoir of infection, maintaining infection exposure of susceptible species even when those populations have declined to low levels.

The necessity of the implementation of stringent biosecurity measures is illustrated by geographically isolated species. Particularly for islands (e.g., Corsica, Sardinia), human-mediated introduction of Bsal is much more likely to occur than natural spread, especially considering that many endemic island species are rare and receive relatively more attention by researchers, herpetologists, amphibian keepers, photographers and the like, any of whom could be vectoring the pathogen, enabling it to cross geographical barriers.

Bsal risk transcends IUCN Red List categories and protection through legislation, although the majority of the European urodelan species (75% (30/40)) are also listed in Annex IV of the Habitats Directive.

Table 2. Species risk assessment based on the potential impact of Bsal for European urodelan species.

Column **'C'**: Confidence

Family	Species/subspecies	Estimated susceptibility to Bsal				Population level risk of extinction	C	Taxon level risk of extinction			Range size*	IUCN Red List Category**	Listed in Annex IV Habitats Directive
		Laboratory trial	Field outbreak	Captivity	Inferred from phylogeny			10 years	100 years	C			
Hynobiidae	*Salamandrella keyserlingii*	Low[1]	NA	NA	NA	Low	Low	Low	Low	Low	>25	LC	No
Plethodontidae	*Speleomantes ambrosii*	NA	NA	NA	High	High	Low	High	High	Low	1-5	NT	Yes
Plethodontidae	*Speleomantes ambrosii ambrosii*	NA	NA	NA	High	High	Low	High	High	Low	1-5	NA	Yes
Plethodontidae	*Speleomantes ambrosii bianchii*	NA	NA	NA	High	High	Low	High	High	Low	1-5	NA	Yes
Plethodontidae	*Speleomantes flavus*	NA	NA	NA	High	High	Low	High	High	Low	1-5	VU	Yes
Plethodontidae	*Speleomantes genei*	High[2]	NA	NA	NA	High	Low	High	High	Low	1-5	VU	Yes
Plethodontidae	*Speleomantes imperialis*	Low[2]	NA	NA	NA	Low	Low	Low	Medium	Low	1-5	NT	Yes
Plethodontidae	*Speleomantes italicus*	NA	NA	NA	High	High	Low	Medium	High	Low	6-25	NT	Yes
Plethodontidae	*Speleomantes sarrabusensis*	NA	NA	NA	High	High	Low	High	High	Low	1-5	VU	Yes
Plethodontidae	*Speleomantes strinatii*	High[1]	NA	NA	NA	High	Low	Medium	High	Low	6-25	NT	Yes
Plethodontidae	*Speleomantes supramontis*	NA	NA	NA	High	High	Low	High	High	Low	1-5	EN	Yes
Proteidae	*Proteus anguinus*	Low[2]	NA	NA	NA	Low	Low	Low	Low	Low	6-25	VU	Yes
Proteidae	*Proteus anguinus anguinus*	Low[2]	NA	NA	NA	Low	Low	Low	Low	Low	6-25	NA	Yes
Proteidae	*Proteus anguinus parkelj*	NA	NA	NA	Low	Low	Low	Low	Low	Low	1-5	NA	Yes
Salamandridae	*Calotriton arnoldi*	High[3]	NA	NA	NA	High	Low	High	High	Low	1-5	CR	Yes
Salamandridae	*Calotriton asper*	Low[4]	NA	NA	NA	Low	Low	Low	Medium	Low	>25	NT	Yes
Salamandridae	*Chioglossa lusitanica*	High[2]	NA	Yes[5]	NA	High	High	Low	Medium	High	>25	VU	Yes
Salamandridae	*Chioglossa lusitanica longipes*	High[2]	NA	NA	NA	High	Low	Low	Medium	Low	>25	NA	Yes
Salamandridae	*Chioglossa lusitanica lusitanica*	NA	NA	NA	High	High	Low	Medium	High	Low	6-25	NA	Yes
Salamandridae	*Euproctus montanus*	NA	NA	NA	High	High	Low	Medium	High	Low	6-25	LC	Yes

Family	Species												
Salamandridae	*Euproctus platycephalus*	High[1]	NA	Yes[6]	NA	High	Low	Medium	High	Low	6-25	EN	Yes
Salamandridae	*Ichthyosaura alpestris*	Moderate[1,7]	No[8,9]	NA	NA	Medium	High	Low	Low	High	>25	LC	No
Salamandridae	*Ichthyosaura alpestris alpestris*	Moderate[1,7]	No[8,9]	NA	NA	Medium	High	Low	Low	High	>25	NA	No
Salamandridae	*Ichthyosaura alpestris apuana*	NA	NA	NA	Moderate	Medium	Low	Low	Medium	Low	6-25	NA	No
Salamandridae	*Ichthyosaura alpestris cyreni*	NA	NA	NA	Moderate	Medium	Low	Low	Medium	Low	6-25	NA	No
Salamandridae	*Ichthyosaura alpestris montenegrina*	NA	NA	NA	Moderate	Medium	Low	Medium	Medium	Low	1-5	NA	No
Salamandridae	*Ichthyosaura alpestris reiseri*	NA	NA	NA	Moderate	Medium	Low	Low	Low	Low	>25	NA	No
Salamandridae	*Ichthyosaura alpestris veluchiensis*	NA	NA	NA	Moderate	Medium	Low	Low	Medium	Low	6-25	NA	No
Salamandridae	*Lissotriton boscai*	Moderate	NA	Yes[6]	NA	High	High	Low	Medium	High	>25	LC	No
Salamandridae	*Lissotriton graecus*	NA	NA	NA	Moderate	Medium	Low	Low	Low	Low	>25	NE	No
Salamandridae	*Lissotriton helveticus*	Low[1]	No[8]	NA	NA	Low	High	Low	Low	High	>25	LC	No
Salamandridae	*Lissotriton italicus*	High[1]	NA	NA	NA	High	Low	Low	Medium	Low	>25	LC	Yes
Salamandridae	*Lissotriton montandoni*	NA	NA	NA	Moderate	Medium	Low	Low	Low	Low	>25	LC	Yes
Salamandridae	*Lissotriton schmidtleri*	NA	NA	NA	Moderate	Medium	Low	Low	Low	Low	>25	NE	No
Salamandridae	*Lissotriton vulgaris*	Moderate[10]	No[8,9]	NA	NA	Medium	High	Low	Low	High	>25	LC	No
Salamandridae	*Lissotriton vulgaris ampelensis*	NA	NA	NA	Moderate	Medium	Low	Low	Medium	Low	6-25	NA	Yes
Salamandridae	*Lissotriton vulgaris meridionalis*	NA	NA	NA	Moderate	Medium	Low	Low	Low	Low	>25	NA	No
Salamandridae	*Lissotriton vulgaris vulgaris*	Moderate[10]	No[8,9]	NA	NA	Medium	High	Low	Low	High	>25	NA	No
Salamandridae	*Lyciasalamandra helverseni*	High[2]	NA	NA	NA	High	Low	High	High	Low	1-5	VU	Yes
Salamandridae	*Lyciasalamandra luschani***	NA	NA	NA	High	High	Low	High	High	Low	1-5	VU	Yes
Salamandridae	*Lyciasalamandra luschani basoglui*	NA	NA	NA	High	High	Low	High	High	Low	1-5	NA	Yes
Salamandridae	*Pleurodeles waltl*	High[1,3]	NA	NA	NA	High	Low	Low	Medium	Low	>25	NT	No
Salamandridae	*Salamandra atra*	NA	NA	Yes[6]	NA	High	Low	Low	Medium	Low	>25	LC	Yes
Salamandridae	*Salamandra atra atra*	NA	NA	NA	High	High	Low	Low	Medium	Low	>25	NA	Yes
Salamandridae	*Salamandra atra aurorae*	NA	NA	NA	High	High	Low	High	High	Low	1-5	NA	Yes
Salamandridae	*Salamandra atra pasubiensis*	NA	NA	NA	High	High	Low	High	High	Low	1-5	NA	Yes
Salamandridae	*Salamandra atra prenjensis*	NA	NA	NA	High	High	Low	Medium	High	Low	6-25	NA	Yes
Salamandridae	*Salamandra corsica*	NA	NA	Yes[6,11]	NA	High	Low	Medium	High	Low	6-25	LC	No
Salamandridae	*Salamandra lanzai*	NA	NA	NA	High	High	Low	High	High	Low	1-5	VU	Yes

Salamandridae	*Salamandra salamandra*	High[1,12]	Yes[8,9,12]	Yes[6,11]	NA	High	High	Low	Medium	High	>25	LC	No
Salamandridae	*Salamandra salamandra almanzoris*	NA	NA	Yes[11]	NA	High	Low	High	High	Low	1-5	NA	No
Salamandridae	*Salamandra salamandra bejarae*	NA	NA	NA	High	High	Low	Low	Medium	Low	>25	NA	No
Salamandridae	*Salamandra salamandra bernardezi*	NA	NA	Yes[11]	NA	High	Low	Medium	High	Low	6-25	NA	No
Salamandridae	*Salamandra salamandra crespoi*	NA	NA	NA	High	High	Low	Medium	High	Low	6-25	NA	No
Salamandridae	*Salamandra salamandra fastuosa*	NA	NA	Yes[11]	NA	High	Low	Medium	High	Low	6-25	NA	No
Salamandridae	*Salamandra salamandra gallaica*	NA	NA	Yes[11]	NA	High	Low	Low	Medium	Low	>25	NA	No
Salamandridae	*Salamandra salamandra gigliolii*	NA	NA	Yes[11]	NA	High	Low	Low	Medium	Low	>25	NA	No
Salamandridae	*Salamandra salamandra longirostris*	NA	NA	NA	High	High	Low	Medium	High	Low	6-25	NA	No
Salamandridae	*Salamandra salamandra morenica*	NA	NA	NA	High	High	Low	Low	Medium	Low	>25	NA	No
Salamandridae	*Salamandra salamandra salamandra*	NA	NA	Yes[11]	NA	High	Low	Low	Medium	Low	>25	NA	No
Salamandridae	*Salamandra salamandra terrestris*	High[12]	Yes[9,12]	Yes[11]	NA	High	High	Low	Medium	High	>25	NA	No
Salamandridae	*Salamandrina perspicillata*	High[1]	NA	NA	NA	High	Low	Low	Medium	Low	>25	LC	Yes
Salamandridae	*Salamandrina terdigitata*	NA	NA	NA	High	High	Low	Medium	High	Low	6-25	LC	Yes
Salamandridae	*Triturus carnifex*	NA	NA	Yes[2,6]	NA	High	Low	Low	Medium	Low	>25	LC	Yes
Salamandridae	*Triturus cristatus*	High[1,10]	Yes[8]	NA	NA	High	High	Low	Medium	High	>25	LC	Yes
Salamandridae	*Triturus dobrogicus*	NA	NA	Yes[6]	NA	High	Low	Low	Medium	Low	>25	NT	No
Salamandridae	*Triturus ivanbureschi*	NA	NA	Yes[6]	NA	High	Low	Low	Medium	Low	>25	NE	Yes
Salamandridae	*Triturus karelinii*	NA	NA	Yes[2,6]	NA	High	Low	High	High	Low	1-5	LC	Yes
Salamandridae	*Triturus macedonicus*	NA	NA	Yes[6]	NA	High	Low	Low	Medium	Low	>25	NE	Yes
Salamandridae	*Triturus marmoratus*	High	Yes[3]	Yes[6]	NA	High	High	Low	Medium	High	>25	LC	Yes
Salamandridae	*Triturus pygmaeus*	NA	NA	Yes[2,6]	NA	High	Low	Low	Medium	Low	>25	NT	Yes

---

*   *Based on 50 × 50 km UTM squares. Only European distribution considered.*
**   *LC, Least concern; NT, Near threatened; VU, Vulnerable; EN, Endangered; CR, Critically endangered; NE, Not evaluated; NA, Not applicable (subspecies level).*
***   *Only Lyciasalamandra luschani basoglui considered, subspecies L. luschani finikensis and L. luschani luschani do not occur in Europe.*

1, Martel et al. 2014; 2, Martel and Pasmans, unpublished data; 3, Martel et al. 2020; 4, Wang et al. in prep.; 5, Chytridiomycose Batrachochytrium salamandrivorans (Bsal), Actieplan - België, 2017; 6, Fitzpatrick et al. 2018; 7, Stegen et al. 2017; 8, Dalbeck et al. 2018; 9, Spitzen-van der Sluijs et al. 2016; 10, Bates et al. 2019; 11, Sabino-Pinto et al. 2015; 12, Martel et al. 2013.

3 Current legislative regulations

This document is not intended to provide a full overview and interpretation of the current European legislation on urodelan conservation and the emerging infectious diseases. Here a summary of the legislation active at the moment of writing is provided, with references to the original documents to get more background information. At the end of this chapter, recommendations are provided which – if implemented – should provide further legal protection to safeguard European amphibian populations against the introduction and spread of Bsal and other emerging infectious diseases.

- The member states and the EU were pressed by the Standing Committee of the Bern Convention to take measures to prevent novel introduction and the further spread of Bsal (Recommendation No. 176, 2015; Recommendation No. 197, 2017).
- In 2017, the World Organisation for Animal Health (OIE) listed infection with Bsal in its Aquatic Animal Health Code (http://www.oie.int/en/standard-setting/aquatic-code/access-online/).
- On 28 February 2018, the EU has implemented the decision (EU) 2018/320, which states that animal health protection measures need to be taken for intra-Union trade in salamanders and the introduction into the Union of such animals in relation to the fungus *Batrachochytrium salamandrivorans*. These protection measures have been prolonged until April 2021.
- Bsal is listed for Union intervention, and the species of the families Salamandridae, Plethodontidae and Hynobiidae are listed for Bsal as susceptible and reservoirs according to the criteria of the Animal Health Law (Commission Implementing Regulation (EU) 2018/1882 on the 'Animal Health Law' and Commission Delegated Regulation (EU) 2018/1629. Official Journal of the European Union L308/21 (2018)).
- The European Union has ratified the Convention on Biological Diversity (1992). In this Convention it is agreed to conserve and sustainably use biological diversity for the benefit of present and future generations. In this Convention (Article 14.1.a) it is already agreed upon that countries should promote national arrangements for emergency responses to activities or events, whether caused naturally or otherwise, which present a grave and imminent danger to biological diversity and encourage international cooperation to supplement such national efforts and, where appropriate and agreed by the States or regional economic Integration organizations concerned, to establish joint contingency plans. Additionally countries have agreed to prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species (Article 8.h).

At the EU level, many urodelan species are protected by means of the Habitats Directive (Annex IV). This implies that all EU member states have the obligation to ensure that the species listed in the Habitats Directive maintain a favourable conservation status. Individual countries may have additional legislation for the protection of indigenous urodelan species. Although many European urodelan species are covered by this legislation, some species, which may be at a high risk of being negatively impacted by Bsal, are not. An example of this is the fire salamander (*Salamandra salamandra*), which is already in strong decline locally in Belgium, the Netherlands and in Germany due to Bsal infection (Spitzen-van der Sluijs et al. 2016), but which is not specifically covered by the Habitats Directive. The favourable conservation status of such species may be seriously challenged by the presence of Bsal and additional measures may be needed to protect these species for future

generations and to ensure populations remain viable. Furthermore, while a species as a whole may not be imminently threatened by Bsal, particular genetic lineages (for which species or subspecies status may be warranted in some cases) might be threatened by Bsal. To prevent irreversible loss caused by Bsal, these genetic lineages, as described in the species-specific protocols (Chapter 5), would benefit from recognition as conservation units.

In 2018, the European Commission issued an implementing decision to ensure biosecure trade of urodeles within the EU and produced guidelines for the importation of urodeles from non-EU territories (Commission Implementing Decision (EU) 2018/320 of 28 February 2018). This decision is a binding legal act, specifically addressed to the Member States. EU decision 2018/320 has been prolonged until April 2021, after which it will be included in the Animal Health Law. It is urgently recommended that this EU decision 2018/320 is expanded in species coverage. These trade restrictions are important if the risk of Bsal spread is to be minimised and the enforcement of preventive screening and biosafety measures is crucial. Unfortunately, however, the enforcement of this directive is not ubiquitously implemented across member states. Also, the trade in Bsal vectoring anuran species is not regulated, and the lack of a unique EU trade identifier (CN-code) for amphibians makes it impossible to trace the flow of traded non-CITES listed species. Detection of consignments containing amphibians, including urodelans, therefore remains problematic (Spitzen-van der Sluijs 2018).

It is recommended to:

- Implement enforcement of EU decision 2018/320 ubiquitously
- Expand EU decision 2018/320 to include vectoring anurans
- Expand EU decision 2018/320 to include all urodeles kept in captivity in the EU
- Implement a specific CN-code for amphibians
- Implement stringent biosecurity measures for all traded amphibians, which are currently not covered by EU decision 2018/320 (see § 4.1.3)

4 General Action Plan

This general Action Plan describes the general actions, which are needed to preserve the European urodelan biodiversity with regard to Bsal, and is the suggested basis for each national Action Plan (see § 4.1.1).

The distribution of both urodelan species and Bsal transcends country borders, therefore coordinated actions between countries are needed to safeguard urodelan biodiversity. Each individual country, and the EU as a whole, has the responsibility to maintain a favourable conservation status for all urodelan species occurring within their territories (see Chapter 3). This is also part of the Convention on Biological Diversity (CBD), an international treaty, which the EU has signed up to and mandates to preserve biodiversity, including urodelan biodiversity.

Within this Action Plan, urodelan species are assessed based on the risk Bsal poses to conservation of each species. As scientific knowledge of Bsal advances, estimated risks may change. Individual countries and the EU should react as fast and flexibly as possible to these changes when needed, possibly with the support of a European Working Group for Amphibian Diseases, should one be established (see § 4.1.8).

When pathogens invade new species or geographic areas, several phases of the invasion process can be discerned (Langwig et al. 2015). This enables invasion phase-specific measures to be devised; those required in response to the emergence of Bsal are shown in Figure 2, as adapted from Spitzen-van der Sluijs (2018). Ideally, the ability to enact these measures should be put in place in advance of any Bsal incursion and decisions to implement them should be made when there still is an opportunity to act (Martin et al. 2012). The invasion of the European continent by Bsal is still at a very early stage at the time of writing, so there is still time to adopt adequate pre-emptive actions and to develop plans to prevent the future spread of the pathogen, or to mitigate its impacts should spread occur. However, disease eradication should be envisaged in all cases, which requires a clear and long-term commitment of the EU and its member states.

Invasion phase-specific measures are key for a cost-effective response to Bsal (Figure 2). Here, three invasion phases are considered: 1) pre-invasion phase (the fungus has not yet invaded the considered country or urodelan population), 2) invasion (epidemic) phase (the fungus has entered the country or population and causes either no added mortality (no or low susceptibility hosts) or the fungus causes mass mortality (high susceptible hosts) and 3) established (endemic) phase (the fungus remains present albeit possibly at a low prevalence, however it continues to cause mortality in susceptible hosts threatening species conservation).

Fundamental to informing management decisions, including the identification of the invasion phase and the defining of management actions, is data. Obtaining as much relevant, quality data as possible is required in order to reduce uncertainties about the actions required and with regard to the best and most efficient allocation of resources. Bearing in mind the destructive global impact of Bd (the fungus closely related to Bsal that also causes catastrophic declines due to chytridiomycosis), we cannot afford to wait for post-hoc crisis management (Grant et al. 2017) with regard to Bsal if amphibian biodiversity is to be protected. This means we need to translate available scientific knowledge into practical management as pragmatically as possible. The control of infectious diseases often demands rapid decision-making in the face of scarce knowledge, limited time for learning, and challenges turning the

available scientific knowledge into actions (Grant et al. 2017). Yet, complexity and uncertainty are not excuses for inaction (Lindgren et al. 2012).
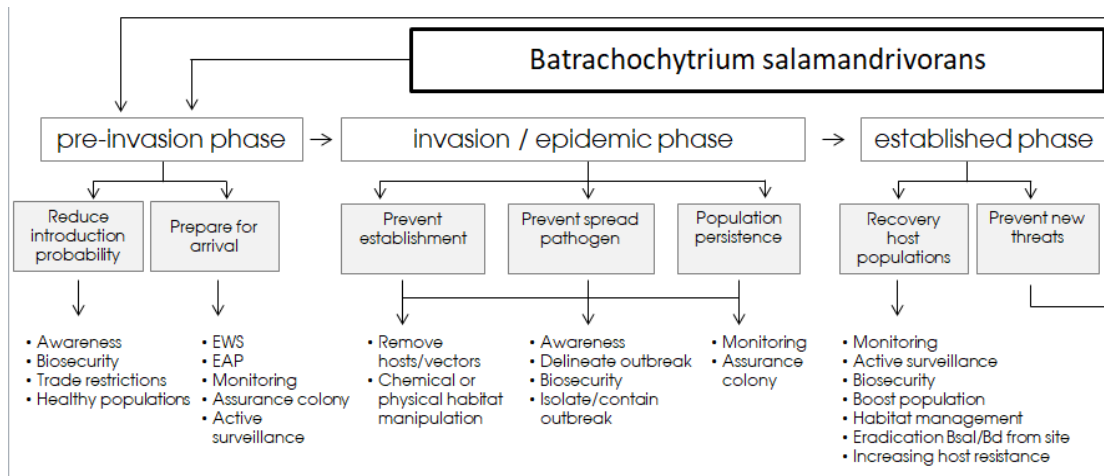

Figure 2. Illustration of the three invasive phases: pre-invasion phase (the fungus has not yet invaded the considered country or population), invasion (epidemic) phase and the established phase, in which a conservation unit might go extinct due to Bsal, or the situation could become endemic in which the pathogen is present, at low prevalence, but continues to cause mortality (from: Spitzen-van der Sluijs, 2018).

4.1 Pre-invasion phase actions

As the detection of a novel Bsal outbreak in a country or population will most likely be unforeseen, but immediate actions are required, member states need to be prepared and facilitate the below actions in advance (Canessa et al. 2020). These listed actions should preferably be initiated during the pre-invasion phase and continued during the subsequent phases (invasion and the endemic phase). Here, the actions that are recommended during all phases are also mentioned. During the pre-invasion phase, Bsal is not yet detected within a population, particular country or region. However, as Bsal can be introduced in various ways (e.g. by introduced or translocated amphibians, by contaminated materials and, once established in a region, by natural dispersal) it is important to anticipate possible routes and mechanisms of introduction of Bsal and to mitigate these as much as is feasible (Figure 2).

Areas that need to be considered are listed below and are discussed in detail in the following pages:

- National Action Plans (AP) (§ 4.1.1)
- National/regional Early Warning Systems (EWS) (§ 4.1.2)
- Biosecurity (§ 4.1.3)
- Habitat management (§ 4.1.4)
- Monitoring (§ 4.1.5)
- Passive surveillance (§ 4.1.6)
- Removal of non-native species (§ 4.1.7)
- European Bsal Working Group (BWG) (§ 4.1.8)
- Budgets and permits (§ 4.1.9)
- *Ex situ* conservation measures (§ 4.1.10)
- Scientific work (§ 4.1.11)
- Trade restrictions (§ 4.1.12)

4.1.1 National Action Plans

Prior to Bsal incursion, each European country prepares a national Action Plan (AP) detailing the required actions needed to reduce the risk of Bsal incursion (§ 4.1.3, § 4.1.7, § 4.1.12), enable early detection of Bsal (§ 4.1.2, § 4.1.5, § 4.1.6) and eradicate the pathogen as quickly as possible (§ 4.1.1.1). The recommendations provided throughout Chapter 4 can serve as guidelines for a national AP. Each national AP should allow for a thorough consideration of the allocation of resources, mitigation actions and priorities before there is an actual incursion of Bsal. This additionally allows for allocating roles to organisations and assigning their tasks and responsibilities. Additional local action plans may be preferred for areas with high urodelan diversity and/or endemism (e.g. islands). The distribution of each European urodelan species can be found in Table 3 and in the species-specific protocols.

Prior to Bsal incursion, countries should define the conservation priorities for the urodelan species, subspecies and intraspecific lineages (conservation units) occurring in the concerned country, based on the risk Bsal poses to the concerned conservation unit (Chapter 2; Table 2). This allows for a targeted and rapid response upon Bsal incursion. Measures such as active surveillance are expensive, hence prioritizing high-risk conservation units and/or areas may be required.

4.1.1.1 Actions prior to and upon Bsal incursion

The following section contains specific guidelines how to prepare for and react to Bsal incursion.

Although rapid response is essential upon Bsal incursion, rushing to unplanned or poorly considered actions must be avoided. It is essential for national and regional authorities to be well prepared prior to Bsal incursion. As Bsal may be a poorer disperser than initially believed (Schmidt et al. 2017, Spitzen-van der Sluijs et al. 2018), and some mitigation actions are drastic and may lock in efforts and resources for a long period. It may therefore be worth investing between a few days and a couple of weeks to develop a site-specific well-planned response. The foundations of any such response plan are its objectives. Therefore, allow sufficient time to clarify them to all decision-makers and stakeholders. In most cases, three broad objectives can be expected, (1) **minimise the risk of Bsal introduction**, (2) **contain/eradicate the pathogen** and (3) **preserve the affected population**. Multiple objectives are case-specific, but in most cases, actions are likely to include (4) minimize the side effects of management actions and (5) meet budget and other constraints. The overriding objective is the preservation of urodelan biodiversity.

Other context-specific objectives are likely to come into play and should be assessed locally (e.g. budget limitations, side effects of response actions on non-amphibian species, socio-economic impacts) (Spitzen-van der Sluijs 2018). These objectives are likely to compete with each other: clearly identifying decision-makers, stakeholders, legislation and priorities before the arrival of Bsal is fundamental to solving any trade-offs and to avoiding wasting precious time upon detection of the pathogen.

Therefore, identifying the **decision makers** (individuals and agencies) clearly and early and establish clear roles will help in preventing a delayed response. Because the spatial spread of the pathogen upon detection is a fundamental cause of uncertainty, be as clear as possible about the scale of the mitigation plan (local/provincial/regional/national) from the beginning. Do not overlook apparently

minor issues such as ownership of data (e.g. results of Bsal screening) and scientific roles as they can cause conflicts later. When deciding which actions to implement, try to project into the future, also considering medium- and long-term funding needs. The persistence of reservoirs (environmental and species), uncertainty surrounding population and Bsal monitoring and the degree of risk generated by Bsal mean that management programs will normally need to last for years. How long will an action need to be in place for, how will it be funded, who needs to be consulted?

Given the uncertainties surrounding Bsal, especially in a novel location or species, the use of **expert opinion** is essential. Engage multiple experts appropriately, rather than relying on the intuition of a single expert (Martin et al. 2012, Sutherland and Burgman 2015). Although expertise on amphibian species and amphibian diseases is fundamental, keep in mind that responding to Bsal is not only about amphibians, particularly when actions such as host removal, fencing or chemical disinfection are considered. For example, experts in ecotoxicology, hydrology and invasive species management can all provide important insights. Involve local, national and international experts where possible and establish a strong connection between management and research, to ensure analyses and further research can be coordinated with needs on the ground. When expert opinion is sought, it is recommended to make quantitative estimates where possible, using formal methods for expert elicitation (Martin et al. 2012, McBride et al. 2012, Hemming et al. 2018). Quantitative estimates make it easier to identify key uncertainties and disagreements, to update initial estimates when further data become available, and to report, discuss and justify decisions with third parties and with the public.

Depending on the impact of Bsal on the amphibian host, a different set of measures is required. Infection in some species is dose-dependent, but not in others (Stegen et al. 2017). Specific measures depend on species composition, landscape permeability and meta-population composition. Importantly, Bsal management cannot be restricted to high-risk or high-priority species; once the pathogen enters a country or region, pathogen management should be considered at the community/ecosystem level, including potential reservoir/carrier species, environmental reservoir and free-living pathogen states (Canessa et al. 2018; 2019).

Action plans should delineate clearly species priorities: which species are to be conserved, which are to be targeted by management, which are to be monitored (see also Chapter 5). It is strongly recommended to establish good monitoring (§ 4.1.5) practices early on, to ensure a full picture of the extent of the pathogen invasion and (if applied) the success or failure of any mitigation actions.

Listed **management actions** in the AP may target either host (remove hosts/vectors) or environment (isolate the outbreak/remove contaminated substrate) to prevent pathogen spread and establishment. As for human and livestock diseases, Bsal incursions should be hit early and hard (Diekmann et al. 2012, Martel et al. 2020) to maximize the chance of success. At the same time, site management may need to continue for several years due to the high likelihood of Bsal persistence in the environment (Stegen et al. 2017). Rigorous actions may be required and should not be shunned considering the severe long-term and large-scale threat that an unchecked incursion may present to biodiversity. Suggestions about potential longer-term management actions to mitigate chytridiomycosis are available from: Woodhams et al. (2011), Scheele et al. (2014), Garner et al. (2016), Grant et al. (2016), Canessa et al. (2018), Thomas et al. (2019). Here, we concentrate on some principles for implementing Bsal mitigation during the immediate post-detection phase.

The practical tools to allow for effective site isolation and eradication of Bsal are:

- Outbreak delineation
    - Active surveillance (eDNA and amphibian skin swabs) in concentric circles around the outbreak site, depending on landscape permeability to hosts/pathogen
    - Increased passive surveillance effort in the surrounding areas
- Host management
    - Remove hosts from the infected site (culling or treating and thereafter keeping in captivity)
    - Decide pre-outbreak to cull animals from infected sites or to treat and keep them in captivity
    - Maintain the captive collections that were set in place pre-outbreak. If proven free of Bsal, these may be released back into the site once it has been verified that Bsal has been eradicated from the site, conform the IUCN criteria.
- Site management
    - Isolate the site to prevent the spread of Bsal
    - Containment (biosecurity, fencing off, restricting access)
    - Active surveillance around and beyond the perimeter of the infected area
    - Stringent and mandatory biosecurity measures when moving anything or anyone in to or out of the designated infected area
    - Physical (e.g. draining water, removing vegetation) and chemical (e.g. disinfection) manipulation to eradicate Bsal from the designated infected area, including both aquatic and terrestrial habitats

Outbreak definition and isolation. The first action should be to immediately delineate and isolate the infected site and establish strict biosecurity (Appendix 3 and 4). The size of the perimeter within which to implement preventive or reactive measures will be uncertain and may require a precautionary approach. Species monitoring, landscape surveys, active surveillance via amphibian skin swabs (sample size should be sufficient to allow for a high level of reliability of the outcome, especially if prevalence is low) and eDNA, and passive surveillance should be combined to rapidly provide information. Because of host-pathogen seasonality and environmental longevity of the pathogen, search efforts for Bsal should not be limited to the immediate period of Bsal detection, but should be extended to at least the next year as well (Bozzuto and Canessa 2019).

Importantly, the true presence of Bsal in a given locality should be assessed before further actions take place. To prevent an animal to be wrongly designated as Bsal positive (false positive), it is recommended to use a detection limit of 1.0 GE for the duplex real-time PCR, which is widely used for the detection of Bsal (Thomas et al. 2018). Furthermore, it is strongly recommended that molecular diagnostic tools, such as PCR, should be used in conjunction with independent diagnostics that demonstrate Bsal colonization and/or disease such as histology or histopathology as generally recommended by the World Organisation for Animal Health (OIE). Conversely, wrongly designating an animal as Bsal negative (false negative) should also be prevented. Apparently healthy animals may carry Bsal unnoticed, especially in early stages of infection or in tolerant species. In these cases, Bsal may not be detected. This is also particularly important when animals are translocated or reintroduced. A quarantine period of at least six weeks, followed by testing for Bsal is recommended. However, in some cases Bsal may be carried in low doses for long periods, lowering the chances on reliable Bsal testing using any test that aims at detecting the fungus or its DNA at the animal's body surface. Addressing this would require developing novel diagnostics using complementary methods.

Host removal. There is no single optimal choice about which host species to remove/restrict/manage. Consider the potential impact of their removal on the system, and whether to make a conservative/precautionary choice. If removal of hosts is considered, then it should encompass all potential hosts including species that are not of conservation interest but which can carry and maintain Bsal (Canessa et al. 2019). If removal is chosen as a management strategy, under the current limited knowledge, the precautionary approach is to try to remove as high a proportion of hosts as possible, with the aim of complete eradication (Canessa et al. 2019). Such removal should be as rapid and intensive as possible to minimize chances of pathogen spread: carry out as many intensive removal sessions as possible, in the shortest possible time frame. Seasonal cycles obviously influence the effectiveness of management, because both hosts and pathogen have periods of greater activity and/or easier detection and management; repeated surveys may be needed at different times of the year (Bozzuto and Canessa 2019). If host removal is considered, the infected region should be strictly separated from the surrounding area, preventing natural repopulation after host removal.

Ex situ conservation. Ex situ conservation strategies are best planned in advance (see § 3.2.1), but they can become an expensive long-term undertaking with uncertain conservation benefits. *Ex situ* measures should not be rushed, as they are expensive and complex, and a plan should be prepared prior to any Bsal incursion. It is unlikely to be necessary or cost effective to conduct *ex situ* conservation breeding for common species. However, for both rare and common species, sick individuals collected from the wild may be treated and kept *ex situ* if this is available and feasible. It is recommended to discuss all options early in the action plan to avoid instinctive, non-evidence-based reactions, and always keeping in mind the ultimate conservation objectives (Canessa et al. 2016).

Site management. Bsal spores can persist in water: consider carefully how to manage/dispose of water. Do not simply drain waterbodies downstream as this might facilitate spore dispersal. Consider whether to allow the water to dry up naturally or to remove it, or to treat it chemically and/or physically and return it. Moreover, consider whether draining waterbodies entirely might trigger host dispersal to other sites and facilitate pathogen dispersion. Instead, use the characteristics of the waterbody and the surrounding landscape to assist management (for example, in some areas, removing vegetation can increase sun exposure and increase temperatures beyond the optimal Bsal survival window) and monitoring (for example using pitfall traps surrounding a pond).

A shortlist of the actions an AP should preferably list:

- Define conservation priorities; high-risk conservation units and/or areas should be prioritized
- Responsibilities, tasks and network of collaborating stakeholders
  - Identify project managers, diagnostic laboratories, etc.
- Define the entire required set of actions to be taken when Bsal is discovered at a particular site, e.g.:
  - Prohibit entry
  - Fence off the infected area
    - Address the ecological impact on other species
  - Remove all Bsal hosts and vectors (i.e. organisms which may carry Bsal)
    - Address the ethical and animal welfare considerations
  - Application of chemical substances to kill off Bsal

- ▪ Address the ethical, environmental, nature conservation and animal welfare considerations
    - o Monitor the site and its surroundings (set perimeter)
    - o Identify the entire network of potentially affected locations (and demarcate the perimeter)
    - o Actions should be based on the best available scientific knowledge (Canessa et al. 2018; Martel et al. 2020)
- Prepare all legislative requirements to prevent any delay in intervention, e.g.:
    - o Fencing off an area
    - o Prohibiting the public to enter
    - o The potential use of chemicals in the environment
    - o The complete removal of hosts and vectors (vertebrate and invertebrate)
- List the agreements made on financial responsibility
    - o Sufficient budget should be allocated
    - o It should be possible to immediately have access to this budget
    - o There should be agreement on which institution(s) is/are eligible for payment

4.1.2 Early Warning System

An Early Warning System (EWS) that maximizes the probability of early detection of Bsal infection in wild urodeles allows mitigation measures to be implemented in the most cost-effective way (Reinhardt et al. 2003).

An EWS should preferably aim to encompass both wild and captive populations. It is advised that in captive collections it becomes commonplace to have animals tested for the presence of Bsal infection and to share the information if a positive animal has been detected in order to warn others and have animals treated as necessary. Spillover from captive populations to wild ones is a realistic, yet preventable, threat.

When the pathogen enters a wild population, generally its control becomes increasingly difficult over time, creating a limited window of opportunity for cost-efficient action. An EWS therefore, should be set up to maximize the chances of early detection of Bsal incursion. Active surveillance is expensive and should prioritize high-risk species/population/areas (§ 4.1.1); a broader surveillance system can benefit from involvement of the public (Lawson et al. 2015, Cunningham et al. 2019). Because human-mediated introduction can theoretically occur anywhere within the EU, creating awareness of the Bsal threat (http://bsaleurope.com/public-awereness-material/) and stimulating reporting of potential Bsal cases is of utmost importance. It should be clear to whom people should report their findings of sick or dead urodeles from the field or from captive collections. These animals should be retrieved and analysed. Therefore, regional hotlines should be established or maintained (in case of the existing hotlines in Belgium, the Netherlands, France, Germany, UK, Italy and Spain). These hotlines can set up and maintain a passive surveillance system by spreading information about Bsal to regional stakeholders (including research institutions, administrations, NGOs, relevant scientific societies, associations of animal breeders, pet shops and people with an interest in herpetofauna) and collecting suspect cases. The hotlines will be the first selection point of suspect cases and should select the animals they will accept for analysis (for example excluding victims of traffic, predation, drowning, etc.) to ascertain only relevant specimens are being diagnosed. The EWS should indicate clearly diagnostic laboratories where samples can be tested for the presence of Bsal

(http://bsaleurope.com/laboratories/). Hotlines can collect and store suspect cases (frozen at -20°C) and send samples for Bsal detection to these laboratories. Sufficient budget should be allocated to these hotlines for operation costs and analysis (§ 4.1.9).

Involvement of the general public can increase coverage and detection rates while minimizing extra costs. Surveillance for sick or dead amphibians by the general public can be used to recognize Bsal-induced mortality in wild (and captive) amphibians. For this purpose recognition sheets have been developed to support identification of Bsal-infected urodeles (Appendix 1). Using the available channels, awareness should be raised to assure that whenever a sick or dead urodele is found, the finder knows that it should at least be reported. However, as sick and dead urodeles are often not evident in the wild even during periods of epidemic mortality, complementary indirect measures to assess the presence of Bsal, such as the monitoring of urodele abundance, are valuable.

The set-up and maintenance of an EWS should preferably encompass:

- A passive surveillance network (see 'passive surveillance' § 4.1.6)
- A network to monitor urodelan population dynamics (see 'monitoring' § 4.1.5)
- A central organisation (hotline) that collects and analyses the data and reports to the government
- A legal framework that allows people and institutions to collect dead amphibians
- A long-term budget to allow for creating awareness (see 'passive surveillance') and for contact with the public
- The infrastructure to ensure that collected samples are quickly sent to the appropriate laboratory
- A list of diagnostic laboratories trained to detect Bsal to allow for a fast diagnosis

4.1.3 Biosecurity

Human-facilitated introduction of Bsal is unpredictable and potentially devastating for both island and mainland populations, underlining the necessity of implementing measures to prevent the human-facilitated incursion of Bsal, especially to isolated populations.

Preventing novel introductions or further spread of Bsal is the most effective way to reduce further impacts. It is important to create awareness at a broad level, introducing and enforcing high standards of biosecurity at border customs posts, in the amphibian trade (including non-commercial trade) and during fieldwork (Thomas et al. 2019).

Standard preventive biosecurity measures need to be taken to avoid human-mediated spread of Bsal. This starts with informing the public, customs officers, zoos and private owners, and increasing awareness about the risks of Bsal and biosecurity measures needed to avoid human-mediated spread of Bsal. Compliance with hygiene protocols in the field, especially for people who regularly come in close contact with amphibians and/or the water bodies which contain amphibians is important. Hygiene protocols for field workers and for people working with heavy machinery are available (Appendix 3 and 4) and processes should be in place to encourage these to be implemented. In addition, whenever possible, restricting human access to areas where Bsal has been detected is recommended.

As a minimum, European countries should:

- Introduce mandatory health certificates for traded amphibians (for both the commercial trade and the non-commercial exchange of animals between owners), after being tested for the presence of Bsal. Visual inspection is insufficient as animals which appear healthy may carry Bsal (Stegen et al. 2017).
- Disseminate disinfection protocols for the disposal of waste products from terraria/aquaria to amphibian retailers, pet owners and hobbyists
- Introduce mandatory disinfection protocols for all field workers working with urodeles and/or in their (potential) habitat
- Be extremely reserved with amphibian translocations; limit translocations only to those that are strictly necessary and are following the IUCN criteria (IUCN/SSC 2013)
- Ensure that all translocations follow the conservation translocation guidelines (IUCN/SSC 2013), even when they are over short distances, and include mandatory Bsal screening of amphibians
- If Bsal is discovered in the wild, the national Action Plan should be activated, and it is advised to start all actions to contain and eliminate the infection
- Discourage, and if possible, prohibit the release of pet amphibians

4.1.4 Habitat management

In situ habitat management can strengthen amphibian populations, which may increase population resilience to events such as disease outbreaks. Hence, proper habitat management is key during all invasion phases. During the epidemic phase, populations of susceptible urodeles can be very low, making them vulnerable to other stochastic events. Optimal habitat may increase the chance of survival of a particular population, for instance by providing a disease-free refuge. Yet, despite the positive effects of habitat management, the protection of habitat in itself offers no full barriers to threats such as climate change and infectious diseases (Bosch et al. 2018). Proper habitat management may help to mitigate the effects of the pathogen, but cannot prevent a disease outbreak.

Countries should:

- Ensure that large, robust and stable populations of their native urodelan species exist and are maintained in order to minimize risks of population extirpations

4.1.5 Population monitoring

Long-term baseline monitoring following standardized protocols of urodelan populations, particularly for high-risk species (Table 2), is necessary to (1) detect changes in population trends that may alert to the presence of Bsal infection and associated mortality, (2) estimate the effects of Bsal infection once diagnosis is confirmed, (3) evaluate the effectiveness of response actions.

It has been shown that even a mass mortality event in relatively large and populous urodeles such as fire salamanders can be hard to detect (Spitzen-van der Sluijs et al. 2013), even in areas with high human population density. Animals may die in their underground shelters, may be predated or decompose quickly and are therefore not always found, or dead findings are not reported.

Baseline monitoring ideally encompasses:

- A national covering grid that is monitored for all amphibian conservation units with a sufficient frequency and intensity over multiple years. Long-term monitoring is crucial in order to enable the detection of population changes over time.
- A national organisation that collects, analyses and validates the population monitoring data, calculates trends and provides feedback to the national government. Such an organisation is at the forefront of detecting anomalies and should be part of the early warning system.

4.1.6 Passive pathogen surveillance

Passive surveillance comprises the detection of Bsal suspect cases (sick and dead urodeles) by public sightings. For a proper assessment of the current threat, countries need to be aware of the present distribution of Bsal and need to participate in Bsal surveillance, especially along the borders of the currently known Bsal range and other high-risk areas (Lawson et al. 2015; § 4.1.2).

To set up passive surveillance it is advised to:

- Distribute information as widely as possibly with a high frequency (social media, local presentations, television and radio, magazines, etc.)
- Allow for the legal framework to collect dead urodeles and swab samples for this purpose
- Allow for sufficient, long-term budget to collect and analyse dead urodeles and swab samples of Bsal-suspect urodeles by the national institutions or central organisation (hotlines) and laboratories (§ 4.1.2, § 4.1.9)
- Provide feedback to the people who found and reported the animal or provided the swab sample

4.1.7 Removal of non-native species

Populations of introduced species indicate points of potential high risk of Bsal entry, particularly when linked to releases from captive collections. Monitoring, disease surveillance and eradication of such high-risk situations are highly recommended.

Upon detection of an introduced non-native species it is recommended to:

- Remove the entire population of the introduced species as soon as possible
- Allow for a monitoring, visually or via eDNA, of the site for consecutive years to ensure absence of the alien invasive species
- Conduct Bsal screening of the removed animals (as part of a disease screening following IUCN guidelines)

4.1.8 European Bsal Working Group

It is suggested to establish a knowledgeable European Bsal Working Group (BWG). This BWG can serve four goals:

- Have an objective/unprejudiced overview of all European (suspected) Bsal cases
- Collate experience from several countries with regard to Bsal eradication or incursion

- Provide advice to national governments for management decisions that are recommended to be taken, even when concrete evidence-based information is scarce or unavailable, to guide rapid responses to new detections of Bsal
- Provide advice to the EU with regard to tools that can aid in Bsal incursion

In this BWG, a small group of relevant stakeholders can be invited to participate so to have an inclusive group, consisting of for instance government employees, scientists, conservationists and/or individuals with expertise in keeping and breeding urodelan species. This European Working Group will allow for an overarching proactive approach, as this BWG will have the full overview of what is happening in the EU and will be able to provide objective suggestions to countries with regard to the chosen course and required set of actions.

4.1.9 Budget and permits

To allow for a swift and targeted approach, prior allocation and reservations of budgets for the national Action Plans, the national and regional Early Warning Systems and the European Bsal Working Group, as well as for the potentially required *in situ* and *ex situ* conservation measures is required. This pre-incursion consideration of the needed resources is also recommended in the consideration for the relevant permits. If a dead amphibian is reported via the EWS, it should be possible to legally collect and store this animal for analysis. Also, if Bsal instantly threatens a highly susceptible and range-restricted species, then costly time can be lost if permits for the collection and *ex situ* conservation of individuals need to be applied for. The prior consideration of the required budget and permits will allow for a decisive and efficient response.

Prior allocation of budgets and permits is required for:

- The set-up, start and maintenance of the EWS and AP (§ 4.1.1, § 4.1.2, § 4.1.6)
- The immediate response to an outbreak (e.g. removal and collection of animals, imposing sanitary measures in habitats, closing areas for the general public)
- Increased regulation of traded species, and the implementation of additional biosecurity regulations (§ 4.1.3, § 4.1.12)
- The immediate and effective removal of any non-native species (§ 4.1.7)
- The set-up and maintenance of the Bsal Working Group (§ 4.1.8)
- *Ex situ* management (§ 4.1.10)
- Promote and stimulate targeted scientific studies to fill the knowledge gaps that prevent efficient or effective mitigation (§ 4.1.11)
- Convey scientific outputs with regard to Bsal mitigation measures to the relevant authorities, conservation managers and the public

4.1.10 Ex situ conservation measures

Once Bsal incursion has taken place, further spread within a country or region is likely to occur via both natural and human-mediated means. Bsal is therefore expected to spread erratically across Europe in the near future. Many small-ranged and highly susceptible European salamander species, such as *Calotriton arnoldi* and *Salamandra lanzai* are at a high risk of extinction if Bsal reaches their populations (Table 2; Martel et al. 2014, Martel et al. 2020).

Ideally, for medium- and high-risk species, subspecies or genetic lineages (Chapters 2 and 5; Table 2) that have been identified as being of conservation importance (conservation units), *ex situ* protocols should be prepared in advance of Bsal incursion. *Ex situ* protocols include genetic management, captive breeding and the development of the appropriate husbandry guidelines (see Appendix 5 and 6; Chapter 5). For high-risk conservation units with very small ranges *ex situ* efforts should be initiated before Bsal is introduced or detected. Being well prepared can enable smooth and clear decision-making once Bsal incursion has taken place, and avoid extinction of a species or other conservation unit. As *ex situ* measures can be expensive, sufficient budget for multiple years of captive management should be reserved (Spitzen-van der Sluijs 2018; § 2.1.9). It is critically important that captive assurance (*ex situ*) colonies are maintained under biosecure conditions (Appendix 5), in order to ensure the captured animals, or their offspring, are suitable for release back into the wild should the threat of Bsal be abated.

To anticipate if bringing animals into captivity should be prioritised, the genetic diversity of the species concerned needs to be determined, both to determine major intraspecific lineages and genetic (allelic) diversity within those lineages (Valbuena-Ureña et al. 2017), and conservation priorities need to be agreed amongst expert stakeholders. This information is crucial to define the make-up of any *ex situ* populations to ensure they capture the genetic diversity of the species/population concerned.

To prepare for effective *ex situ* conservation it is suggested that countries:

- Define the appropriate conservation units
- Develop best practice guidelines for the keeping and breeding of a species
- Obtain experience in the keeping and breeding of a species
  o Consider including both zoos and captive breeders/organisations
- Allow for the appropriate permits and long-term financial support
- Make clear agreements on legal and financial responsibilities and tasks
- Set a clear goal and start in a timely fashion

4.1.11 Scientific work

Conservation measures must be evidence based. Countries should therefore fund research on Bsal. Equally, countries should stimulate the translation of scientific findings into conservation measures, ensuring that this information is accessible to conservation managers. The derived knowledge will allow for better targeted conservation measures, better value for money and improved conservation outcomes.

Some urgent key questions are:

- Can susceptible species develop host tolerance or resistance to Bsal infection or to Bsal-induced chytridiomycosis?
- What is the environmental reservoir for Bsal and how can Bsal be eradicated from the environment while minimising environmental impacts?
- Can we develop a safe and effective treatment for use under natural (*in situ*) conditions?
- What are the Bsal transmission routes, at individual, population and landscape level?
- Is Bsal evolving as it infects amphibians in Europe and, if so, is it becoming more or less virulent to a wider or narrower range of host species?

4.1.12 Trade restrictions

A ban on the trade of all salamanders and anuran vector species have been suggested as the sole most effective mitigation action against Bsal (Grant et al. 2017). As is expanded on in Chapter 3, trade restrictions and the enforcement of preventive screening as well as biosafety measures (§ 4.1.3) are welcomed. Here, trade is defined as the commercial exchange and the non-commercial exchange of animals between owners.

It is suggested that countries and the EU:

- Introduce mandatory health certificates for traded amphibians (§ 4.1.3)
- Impose and implement trade restrictions on Bsal vectoring anurans
- Implement enforcement and extend EU decision 2018/320
- Implement a specific CN-code for amphibians (§ 3)

4.2   Invasion (epidemic) phase actions

When Bsal has entered the country, either by natural spread or human-facilitated, a mitigation response must be implemented as rapidly as possible. Communication, active surveillance and monitoring must be established early and maintained throughout the invasion period. An immediate response will reduce ecological damage and financial costs on the long-term.

The predetermined AP should provide all relevant institutions and organisations with a worked-out plan that can then be implemented immediately upon Bsal detection (see § 4.1.1).

The aims in this phase should be to:

- Prevent establishment of Bsal
- Prevent the spread of Bsal
- Ensure population persistence

Because uncertainty will surround every case of Bsal detection in novel locations, population monitoring (§ 4.1.5) and pathogen surveillance (§ 4.1.6) play a vital role. Whenever monitoring and surveillance are considered, it must be clear (1) what is the question that should be answered and how is it relevant to species management (2) how data will be collected and analysed (3) what sample sizes can be expected and whether they are meaningful. The lower the probability of detection/capture, the more surveys are needed and the less robust the inference.

Given the urgent need to respond immediately to the detection of Bsal in the wild, some actions should be implemented at the same time as initiating population monitoring and Bsal surveillance of the population known to be infected. The current extent of the pathogen at and around the detection site is the most important piece of information on which to base pathogen control measures. Therefore, we recommend initiating the permitting and subcontracting processes in parallel with host/pathogen monitoring of the area surrounding the outbreak site (1-5 km buffer, depending on host traits and site characteristics). Results of laboratory tests for infection detection should be available within 1-2 weeks, by which time implementation of mitigation actions can begin at the appropriate scale. Remember that any decision to delay action implies a trade-off: more information can lead to better

actions but gives time for the pathogen to spread. Again, time of year plays a role in this decision: periods of low host activity and/or unsuitable climatic conditions for Bsal may afford (marginally) more time for planning.

It is important to establish good data collection practices from the beginning. In particular, it is recommended to:

- Collect skin swabs and/or tissue samples following defined protocols (Hyatt et al. 2007).
- Record the following data for each animal sampled/captured: (1) individual animal identifier, (2) date, (3) code of swabs and/or tissue samples, (4) GPS coordinates of capture, (5) species sampled, (6) age and sex of the individual upon capture. Always record surveys where no animals are caught, as zeroes are a very important component of analyses.
- Handle and house all animals separately (following strict biosecurity measures), whether removing them or returning them to the site, to avoid possible cross-contamination/infection.

As mentioned previously, the focus of this document is on wild urodelan populations, but as the spillover of Bsal from captive to wild populations is a severe threat, eradication of Bsal in captive populations should be strived for.

4.3   Established (endemic) phase actions

Member states should strive for the eradication of Bsal to:

- Prevent pathogen spread to naïve populations
- Prevent new disease outbreaks
- Conserve biodiversity

The situation may arise that Bsal is detected too late for effective disease mitigation or the mitigation actions are not successful. In this case, the infection might become endemic within the affected population. In an endemic situation, the pathogen is still present, albeit often at a low prevalence, and may continue to cause mortality in its host (depending on host and context). In this situation there is the continuous risk of the spread of Bsal to other naïve populations.

Ecological theory suggests that - in the absence of reservoir hosts or an environmental reservoir of infection - susceptible species may persist in equilibrium with Bsal. Such populations, however, may remain below a sustainable threshold and become functionally extinct, or be placed at greater risk of extinction from other stochastic events (Stegen et al. 2017, Spitzen-van der Sluijs et al. 2018). The presence of reservoir species can maintain the infection and drive susceptible hosts to extinction (Brannelly et al. 2018). When Bsal infection remains in susceptible hosts at low population densities, its detection may be difficult and the infection status at a site may be uncertain. Re-stocking is not recommended in such situations. In the event Bsal is still present, increasing host densities could lead to a new disease outbreak and increase the chances of spread beyond the focal site (Canessa et al. 2018).

Endemic pathogen presence requires the following actions:

- If feasible, long-term effort to consistently remove amphibians from the site until confirmed eradication of Bsal
- Continuously monitor urodelan populations, Bsal prevalence and spread via monitoring, active and passive surveillance
- Invest in scientific research that seeks the elimination of Bsal given the current situation
- Do not restock Bsal positive populations
- Ensure good quality habitat for amphibians
- Maintain high standards of biosecurity
- Isolate the area as effective as possible (fence or other barriers) and restrict access
- Prevent the introduction of new pathogens

4.3.1 Conservation unit extinction

In the situation that the entire conservation unit has disappeared from the wild and there is a high degree of confidence of the absence of the fungus at the site and its surroundings (it has disappeared with high certainty from any vectoring hosts or substrate), and a healthy source/captive colony is available, a conservation reintroduction program could be considered, within or outside the original range depending on Bsal presence and prospects for successful re-establishment (IUCN/SSC 2013). Captive management guidelines are provided in Appendix 5 and 6.

In the case of the risk of conservation unit extinction due to Bsal, member states should:

- Safeguard an *ex situ* population
- Identify potential release areas for *ex situ* animals that were caught prior to Bsal incursion or that were translocated from an uninfected population
- Monitor areas for the absence of Bsal – consider using a sentinel species for at least a year
- Follow the IUCN criteria for reintroductions and the mitigation of infectious disease threats (e.g. have the appropriate professionals conduct a Disease Risk Analysis)
- Initiate potential reintroduction only in case of *confirmed* absence of Bsal
- Be vigilant for novel threats (such as novel pathogen introductions, including those which may be present in animals destined for reintroduction)

Table 3. Risk of population extinction upon introduction of Bsal for a given species, listing in the Annex IV of the Habitats Directive and occurrence per European country for all European urodelan species.

The presence of a particular species in a country is indicated by '1'. Country abbreviations: AD, Andorra; AL, Albania; AT, Austria; BA, Bosnia and Herzegovina; BE, Belgium; BG, Bulgaria; BY, Belarus; CH, Switzerland; CY, Cyprus; CZ, Czech Republic; DE, Germany; DK, Denmark; EE, Estonia; ES, Spain; FI, Finland; FO, Faroe Islands; FR, France; GB, United Kingdom; GI, Gibraltar; GR, Greece; HR, Croatia; HU, Hungary; IE, Ireland; IM, Isle of Man; IS, Iceland; IT, Italy; LI, Liechtenstein; LT, Lithuania; LU, Luxembourg; LV, Latvia; MC, Monaco; MD, Moldova; ME, Montenegro; MK, Macedonia; MT, Malta; NL, Netherlands; NO, Norway; PL, Poland; PT, Portugal; RO, Romania; RS, Serbia; RU, Russia; SE, Sweden; SI, Slovenia; SK, Slovakia; SM, San Marino; TR, Turkey; UA, Ukraine; VA, Vatican City; XK, Kosovo.

Family	Species	Bsal risk	Annex IV*	AD	AL	AT	BA	BE	BG	BY	CH	CY	CZ	DE	DK	EE	ES	FI	FO	FR	GB	GI	GR	HR	HU	IE	IM	IS	IT	LI	LT	LU	LV	MC	MD	ME	MK	MT	NL	NO	PL	PT	RO	RS	RU	SE	SI	SK	SM	TR	UA	VA	XK	Total
Hynobiidae	Salamandrella keyserlingii	Low																																												1								1
Plethodontidae	Speleomantes ambrosii	High	1																										1																										1
Plethodontidae	Speleomantes flavus	High	1																										1																										1
Plethodontidae	Speleomantes genei	High	1																										1																										1
Plethodontidae	Speleomantes imperialis	Low	1																										1																										1
Plethodontidae	Speleomantes italicus	High	1																										1																					1					2
Plethodontidae	Speleomantes sarrabusensis	High	1																										1																										1
Plethodontidae	Speleomantes strinatii	High	1																	1									1					1																					3
Plethodontidae	Speleomantes supramontis	High	1																										1																										1
Proteidae	Proteus anguinus	Low	1				1																	1					1							1**											1							4
Salamandridae	Calotriton arnoldi	High	1														1																																						1
Salamandridae	Calotriton asper	Low	1	1												1			1																																			3
Salamandridae	Chioglossa lusitanica	High	1														1																									1												2
Salamandridae	Euproctus montanus	High	1																	1																																		1
Salamandridae	Euproctus platycephalus	High	1																										1																									1
Salamandridae	Ichthyosaura alpestris	Medium			1	1	1	1	1		1		1	1	1					1			1	1	1				1	1		1				1	1		1	1	1		1	1			1	1			1		1	27
Salamandridae	Lissotriton boscai	High															1																									1												2
Salamandridae	Lissotriton graecus	Medium			1		1		1														1	1												1	1																	7
Salamandridae	Lissotriton helveticus	Low			1			1			1			1			1			1	1											1									1		1											11
Salamandridae	Lissotriton italicus	High	1																										1																									1
Salamandridae	Lissotriton montandoni	Medium											1																												1		1					1			1			5
Salamandridae	Lissotriton schmidtleri	Medium					1																1																											1				3
Salamandridae	Lissotriton vulgaris	Medium			1	1	1	1	1	1			1	1	1	1		1		1	1		1	1	1	1			1	1	1	1	1		1	1	1		1	1	1		1	1	1	1	1	1			1		1	36
Salamandridae	Lyciasalamandra helverseni	High	1																			1																																1
Salamandridae	Lyciasalamandra luschani	High	1																			1																																1
Salamandridae	Pleurodeles waltl	High															1																									1												2
Salamandridae	Salamandra atra	High	1			1	1	1			1									1					1				1	1						1								1			1	1						11
Salamandridae	Salamandra corsica	High																		1																																		1
Salamandridae	Salamandra lanzai	High	1																	1									1																									2
Salamandridae	Salamandra salamandra	High			1	1	1	1	1	1		1		1	1					1			1	1	1				1			1				1	1		1		1		1	1	1		1	1	1	1	1	1		1	29
Salamandridae	Salamandrina perspicillata	High	1																										1																									1
Salamandridae	Salamandrina terdigitata	High	1																										1																									1
Salamandridae	Triturus carnifex	High	1			1	1				1		1											1	1																							1	1						8
Salamandridae	Triturus cristatus	High	1			1		1	1	1	1		1	1	1	1		1		1	1										1	1	1	1		1				1	1	1		1	1	1	1	1		1			1			26
Salamandridae	Triturus dobrogicus	High					1		1															1	1										1								1	1				1				1			9
Salamandridae	Triturus ivanbureschi	High	1					1															1														1						1							1			5
Salamandridae	Triturus karelinii	High	1																																									1							1			2
Salamandridae	Triturus macedonicus	High	1		1		1																1													1	1						1									1	7
Salamandridae	Triturus marmoratus	High	1														1			1																						1												3
Salamandridae	Triturus pygmaeus	High	1														1																									1												2
	Total		**29**	3	5	6	9	5	8	2	7	0	7	5	3	2	10	2	0	12	3	0	8	8	5	1	0	0	18	4	2	5	2	1	3	7	6	0	5	2	5	7	6	8	4	2	6	6	4	3	7	0	4	**228**

* Species listed in Annex IV Habitats Directive.

** Presence based on environmental DNA (Gorički et al. 2017).

5   Species-specific protocols

This section covers Bsal-related conservation measures for all currently recognized European urodelan species. For each species, the major intraspecific lineages, often defined as subspecies, are described. Each intraspecific lineages should preferably be used as a conservation unit. Ongoing scientific work may change the species-specific protocols, therefore it is advised to check for updates prior to the set-up of management plans on http://bsaleurope.com.

According to the Bsal risk status of a given conservation unit, different general actions are needed. These actions can be on the scale of population, intraspecific lineage, subspecies or species, depending on the conservation priorities. For national conservation efforts, the Bsal risk at the (sub)species level for a 10-year time frame may be most relevant, while for local conservation efforts the Bsal risk at the population level may be most relevant.

Here, the general actions for each Bsal risk category are summarised, while in the species-specific protocols, additional species or lineage-specific actions are listed, if applicable. In all cases, upon definitive diagnosis of a Bsal outbreak, disease eradication must be envisaged.

High risk

- Implement biosecurity measures to prevent the human-facilitated Bsal incursion (§ 4.1.3).
- Ensure proper habitat management (§ 4.1.4).
- Set up long-term population monitoring (§ 4.1.5).
- Set up active and passive Bsal surveillance (§ 4.1.1, § 4.1.6).
- Prepare and initiate *ex situ* measures (§ 4.1.10).

Medium risk

- Implement biosecurity measures to prevent the human-facilitated Bsal incursion (§ 4.1.3).
- Ensure proper habitat management (§ 4.1.4).
- Set up passive Bsal surveillance (§ 4.1.6).
- Set up long-term population monitoring, at least at locations with high likeliness of exposure to Bsal.
- Prepare *ex situ* measures.

Low risk

- Implement biosecurity measures to prevent the human-facilitated Bsal incursion (§ 4.1.3).
- Ensure proper habitat management (§ 4.1.4).
- Set up passive Bsal surveillance, at least at locations with high likeliness of exposure to Bsal (§ 4.1.6).

For (sub)species which also occur outside Europe, only the distribution ranges within the area as described in § 1.2 are considered here.

Hynobiidae   *Salamandrella keyserlingii*   Siberian salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
NA	LC	None	Low	Low	Low

Epidemiology

Dispersal

A semi-aquatic species, may disperse widely over land. Most animals disperse 2-5 m from the reproduction waters, but animals have been found up to 700 m from the water. Dispersal distances of > 1.5 km have been mentioned for young animals.

Density

Abundant species. On Sachalin, at least 1 individual/m^2 has been reported during the aquatic reproduction period.

Co-occurrence

Lissotriton vulgaris and *Triturus cristatus*.

Bsal risk status

Tolerant (Bsal infection in the absence of disease) in laboratory experiments. Species has a large distribution range and co-occurs with potential reservoir species.

Conservation Unit

The level of intraspecific genetic isolation and variation is remarkably low. Based on mitochondrial DNA analyses, three major lineages can be discerned, of which two in the southeastern part of the species'

range. These lineages can be considered as conservation units. Japanese and South Kuril populations are genetically distinct and may be considered subspecies.

Currently recognized subspecies
NA

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Moderate	High

European distribution of *Salamandrella keyserlingii*.

References

Bannikov, A. G. et al. 1977. Opredelitel Zemnovodnykh i Presmykayushchikhsya Fauny SSSR. Moscow: Prosveshchenie Publ. 414 p. (in Russia,from: Borkin, 1999).

Bassarukin, A. M. and L. J. Borkin. 1984. Distribution, ecology and morphological variability of the Siberian salamander *Hynopbius keyserlingii* of the Sakhalin island. Proc. Of the Zool. Inst. USSR Acad. Of Sci. Leningrad,124: 12-54 In Russian, from: Borkin 1999.

Berman, D. I. and G. P. Sapozhnikov. 1994. Spatial structure of the population of the Siberian Newt (*Salamandrella keyserlingii*) in the Verkhnya Kolyma River basin. Ekologia (Ekaterinburg 2:78-88 (in Russian, from: Borkin 1999).

Berman, D. I., et al. 2005. Intraspecific genetic differentiation of the Siberian newt (*Salamandrella keyserlingii* , Amphibia, Caudata) and the cryptic species *S. schrenckii* from Southeastern Russia. Entomological Review 85(2): 240-253.

Borkin, L. J. 1999. *Salamandrella keyserlingii* (Dybowski, 1870) – Sibirischer Winkelzahnmolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Malyarchuk, B., et al. 2013. Phylogeny and genetic history of the Siberian salamander (*Salamandrella keyserlingii*, Dybowski, 1870) inferred from complete mitochondrial genomes. Molecular Phylogenetics and Evolution 67: 348-357.

Poyarkov, N. A. and S. L. Kuzmin. 2008. Phylogeography of the Siberian newt *Salamandrella keyserlingii* by mitochondrial DNA sequence analysis. Russian Journal of Genetics 44(8): 948-958.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Steinort, D. and M. Römhild. 2016. „Eiskalt" Überwinterung und Zucht von *Salamandrella keyserlingii*. DGHT-AG Urodela, Gersfeld.

Plethodontidae   *Speleomantes ambrosii*   Ambrosi's cave salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	NT	High	High	High	High

Epidemiology

Dispersal

Fully terrestrial species, active year round. No data on movements available.

Density

Local density in suitable habitat can be high. Likely similar to *S. strinatii*. Estimated abundance for *S. strinatii* at an Italian site was 0.86 salamanders/m^2.

Co-occurrence

Salamandra salamandra, Salamandrina perspicillata, Lissotriton vulgaris, Ichthyosaura alpestris, Triturus carnifex and *Speleomantes italicus.*

Bsal risk status

Susceptibility to Bsal has not been examined in the laboratory. Likely highly susceptible based on close relationship to the Bsal susceptible species *Speleomantes strinatii*. Risk of human-mediated introduction, and co-occurrence with species such as *Ichthyosaura alpestris* that could function as reservoir for Bsal.

Conservation Unit

Two subspecies exist. Level of subspecies can be considered as the unit of conservation. West of Magra River there is *S. a. ambrosii* and east of the Magra River *S. a. bianchii*. Genetic introgression occurs between *S. italicus* and *S. ambrosii*.

176

Currently recognized subspecies
Speleomantes ambrosii ambrosii
Speleomantes ambrosii bianchii

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4), although it is recommended to gain experience in keeping and breeding this species.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low

European distribution of *Speleomantes ambrosii*.

References

Chiari, Y., et al. 2012. Phylogeography of Sardinian cave salamanders (Genus Hydromantes) is mainly determined by geomorphology. PLoS ONE 7(3): e32332.

Ficetola, G., et al. 2019. Transgressive niche across a salamander hybrid zone revealed by microhabitat analyses. Journal of Biogeography 46(7): 1342-1354.

Lanza, B. 1999. *Speleomantes ambrosii,* Sp. *strinatii* – Ambrosis Höhlensalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Plethodontidae   *Speleomantes flavus*   Monte Albo cave salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	VU	High	High	High	High

Epidemiology

Dispersal

Presumed similar to other *Speleomantes* species, no exact data known.

Density

Local density in suitable habitat can be high. Estimates range from 0.03 individuals/m^2 – 0.06 ± 0.03 individuals/m^2.

Co-occurrence

No other urodelan species within the species' range.

Bsal risk status

Bsal susceptibility has not been tested for this species, likely lethal based on close relationship to the Bsal susceptible species *Speleomantes strinatii*. Restricted range, high impact when Bsal is introduced in its distribution range.

Conservation Unit

The level of intraspecific genetic isolation and variation is high, with two major lineages based on mitochondrial DNA analyses, which can be considered as conservation units. Endemic to northeastern Sardinia.

Currently recognized subspecies

NA

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4), although it is recommended to gain experience in keeping and breeding this species.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low
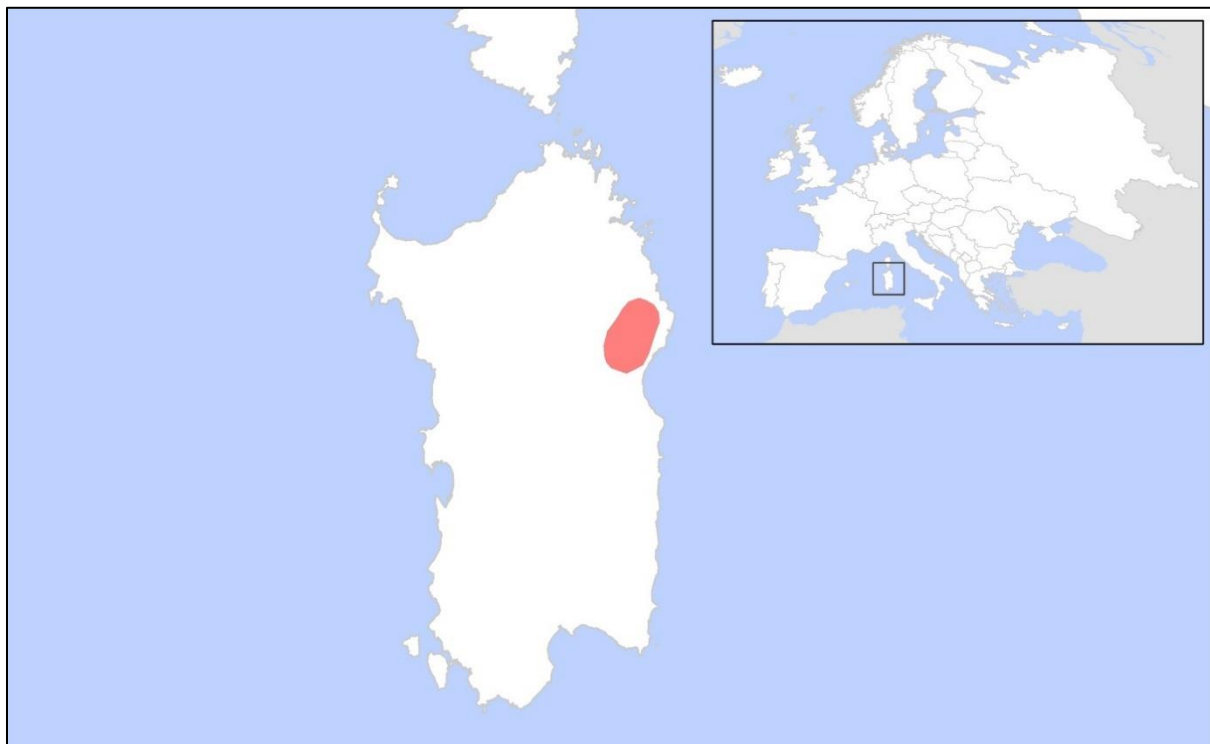
European distribution of *Speleomantes flavus*.

References

Chiari, Y., et al. 2012. Phylogeography of Sardinian cave salamanders (Genus Hydromantes) is mainly determined by geomorphology. PLoS ONE 7(3): e32332.

Lanza, B. 1999. *Speleomantes flavus* – Monte-Albo-Höhlensalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Lunghi, E., et al. 2018. Environmental suitability models predict population density, performance and body condition for microendemic salamanders. Scientific Reports 8(1).

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Voesenek, L. A. C. J., et al. 1987. Some autecological data on the Urodeles of Sardinia. Amphibia-Reptilia 8(4): 307-314

Plethodontidae   *Speleomantes genei*   Gené's cave salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	VU	High	High	High	High

Epidemiology

Dispersal

Presumed similar dispersal pattern as other *Speleomantes* species, no exact data known.

Density

Presumed similar as *S. flavus*, with estimated density of 0.03 individuals/m^2.

Co-occurrence

Euproctus platycephalus.

Bsal risk status

High Bsal susceptibility (laboratory experiments). Restricted range, high impact when Bsal is introduced in its distribution range.

Conservation Unit

The level of intraspecific genetic isolation and variation is high, with four major lineages based on mitochondrial DNA analyses, which can be considered as relevant units of conservation. Endemic to the region Sulcis-Iglesiente in southwestern Sardinia.

Currently recognized subspecies
NA

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4), although it is recommended to gain experience in keeping and breeding this species.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low
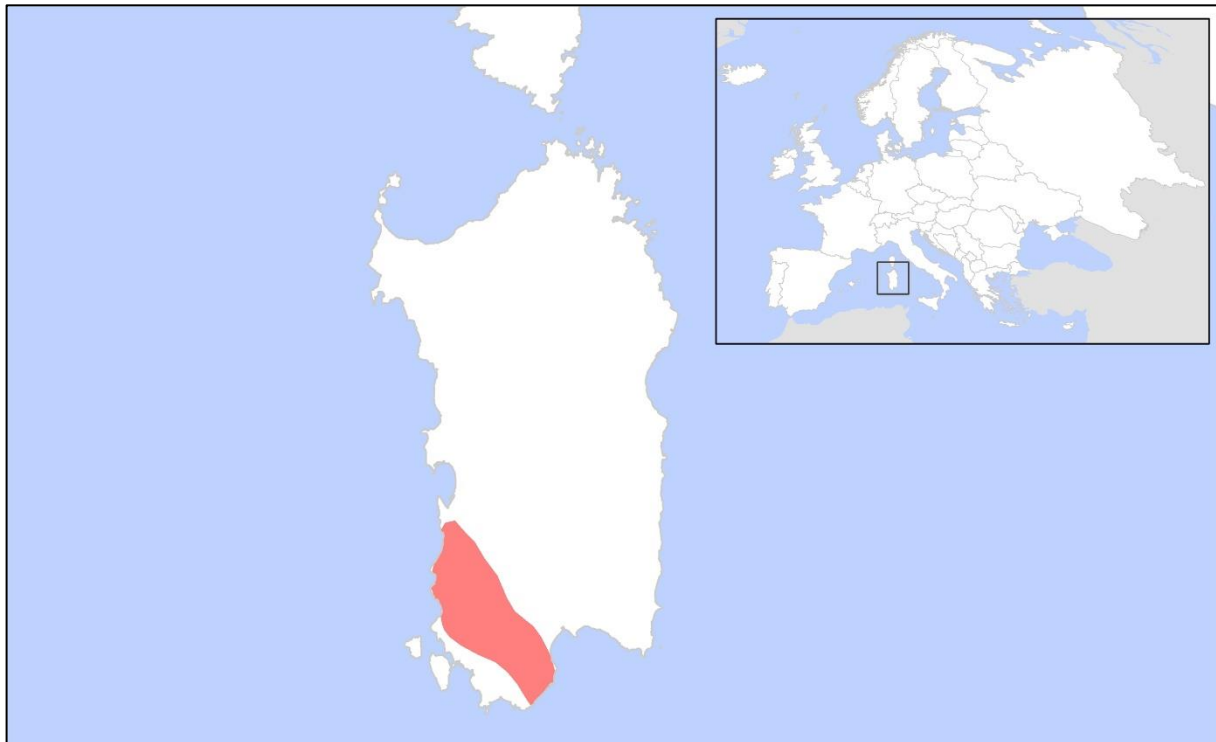
European distribution of *Speleomantes genei*.

References

Carranza, S., et al. 2008. Biogeography and evolution of European cave salamanders, Hydromantes (Urodela: Plethodontidae), inferred from mtDNA sequences. Journal of Biogeography 35(4): 724-738.

Chiari, Y., et al. 2012. Phylogeography of Sardinian cave salamanders (Genus Hydromantes) is mainly determined by geomorphology. PLoS ONE 7(3): e32332.

Lanza, B. 1999. *Speleomantes genei* – Genés Höhlensalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Van der Meijden, A. et al. 2009. Phylogenetic relationships of Sardinian cave salamanders, genus Hydromantes, based on mitochondrial and nuclear DNA sequence data. Molecular Phylogenetics and Evolution 51: 399-404.

Voesenek, L. A. C. J., et al. 1987. Some autecological data on the Urodeles of Sardinia. Amphibia-Reptilia 8(4): 307-314

Plethodontidae   *Speleomantes imperialis*   Imperial cave salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	NT	High	Low	Low	Medium

Epidemiology

Dispersal

Fully terrestrial and nocturnal species, presumed similar dispersal pattern as other *Speleomantes* species, no exact data known.

Density

Presumed similar as *S. flavus*, with estimated density of 0.03 individuals/m^2.

Co-occurrence

Euproctus platycephalus.

Bsal risk status

No Bsal susceptibility (laboratory experiments). However, restricted range, risk of human introduction and high susceptibility of other *Speleomantes* species warrant caution.

Conservation Unit

The level of intraspecific genetic isolation and variation is high, with six lineages based on mitochondrial DNA analyses, which can be considered as conservation units. Endemic to central, central eastern and southeastern Sardinia.

Currently recognized subspecies
NA

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

The only *Speleomantes* species for which captive breeding has been published.

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low
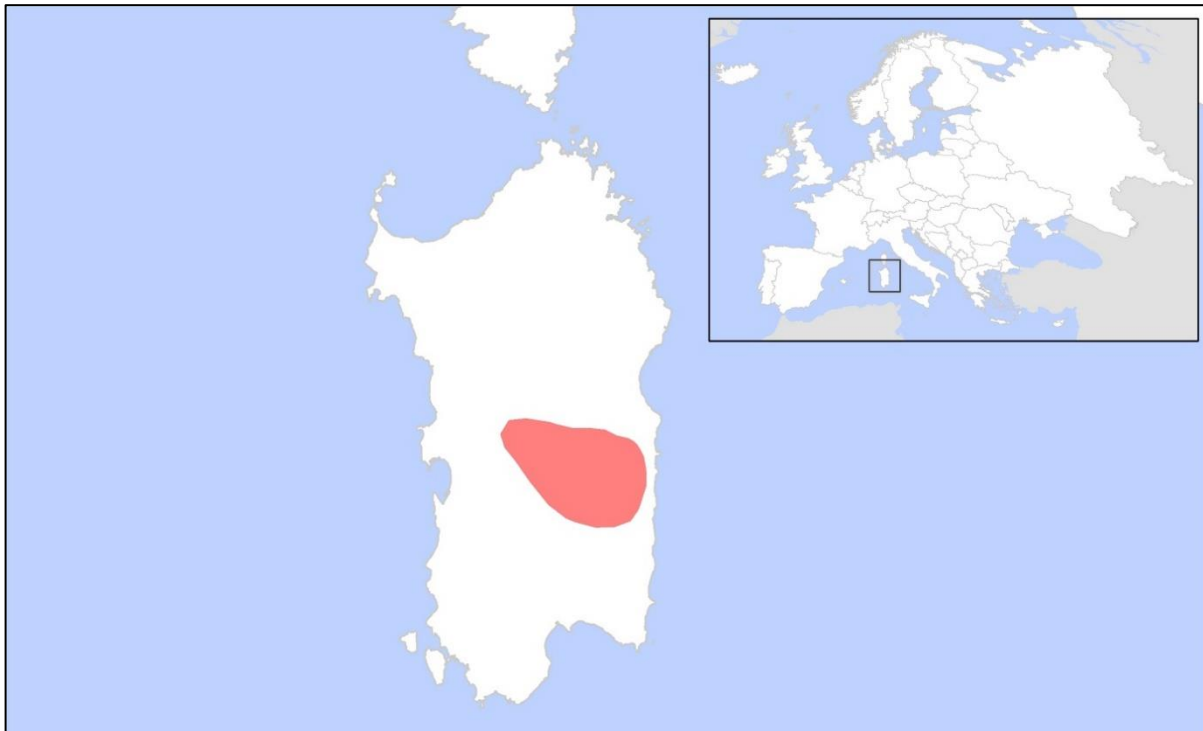
European distribution of *Speleomantes imperialis*.

References

Chiari, Y., et al. 2012. Phylogeography of Sardinian cave salamanders (Genus Hydromantes) is mainly determined by geomorphology. PLoS ONE 7(3): e32332.

Lanza, B. 1999. *Speleomantes imperialis* – Duftender Höhlensalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Mutz, T. 1998. Haltung und Zucht der Sardischen Höhlensalamanders *Hydromantes imperialis* (Stefani, 1969) und einige Beobachtungen zur Ökologie der Europäischen Höhlensalamander. Salamandra 34(2): 167–180.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Voesenek, L. A. C. J., et al. 1987. Some autecological data on the Urodeles of Sardinia. Amphibia-Reptilia 8(4): 307-314.

Plethodontidae   *Speleomantes italicus*   Italian cave salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex IV	NT	High	High	Medium	High

Epidemiology

Dispersal

Fully terrestrial species, active year round. No data on movements available.

Density

Local density in suitable habitat can be high. Likely similar to *S. strinatii*. Estimated abundance for *S. strinatii* at an Italian site was 0.86 salamanders/m^2.

Co-occurrence

Salamandra salamandra, Salamandrina perspicillata, Lissotriton vulgaris, *L. italicus, Ichthyosaura alpestris, Triturus carnifex* and *Speleomantes ambrosii.*

Bsal risk status

Susceptibility to Bsal has not been examined in the laboratory. Likely highly susceptible based on close relationship to the Bsal susceptible species *Speleomantes strinatii*. At relatively large distance to known Bsal presence, without major geographic barriers. Risk of human-mediated introduction, and co-occurrence with reservoir species such as *Ichthyosaura alpestris*.

Conservation Unit

Although *S. italicus* has the largest geographic distribution of all European *Speleomantes* species, it has a low level of genetic divergence based on mitochondrial DNA analyses. As such, the species can be considered as the relevant unit of conservation until further assessment of the genetic diversity within the species has been conducted. The observed uniformity suggests relatively rapid spread, perhaps after a restriction in range that reduced previous genetic diversity. Genetic introgression occurs between *S. italicus* and *S. ambrosii*. Endemic to northern and central Apennines.

184

Currently recognized subspecies

NA

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4), although it is recommended to gain experience in keeping and breeding this species.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low

European distribution of *Speleomantes italicus*.

References

Carranza, S., et al. 2008. Biogeography and evolution of European cave salamanders, Hydromantes (Urodela: Plethodontidae), inferred from mtDNA sequences. Journal of Biogeography 35(4): 724-738.

Chiari, Y., et al. 2012. Phylogeography of Sardinian cave salamanders (Genus Hydromantes) is mainly determined by geomorphology. PLoS ONE 7(3): e32332.

Ficetola, G., et al. 2019. Transgressive niche across a salamander hybrid zone revealed by microhabitat analyses. Journal of Biogeography 46(7): 1342-1354.

Lanza, B. 1999. *Speleomantes italicus* – Italienischer Höhlensalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Plethodontidae — *Speleomantes sarrabusensis* Sette Fratelli cave salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	VU	High	High	High	High

Epidemiology

Dispersal

Little is known about its behaviour and ecology. Presumed similar dispersal pattern as other *Speleomantes* species, no exact data known.

Density

Presumed similar as *S. flavus*, with estimated density of 0.03 individuals/m^2.

Co-occurrence

Euproctus platycephalus.

Bsal risk status

Bsal susceptibility has not been tested for this species, likely lethal based on close relationship to the Bsal susceptible species *Speleomantes strinatii and S. genei*, although *S. imperialis* was not susceptible to Bsal. Restricted range, likely high impact when Bsal when is introduced in its distribution range.

Conservation Unit

The level of intraspecific genetic isolation and variation is rather low, with no distinct phylogenetic sub-structuring based on mitochondrial DNA analyses. The species can be considered as the relevant unit

of conservation until further assessment of the genetic diversity within the species has been conducted. Endemic to southeastern Sardinia.

Currently recognized subspecies

NA

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4), although it is recommended to gain experience in keeping and breeding this species.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low

European distribution of *Speleomantes sarrabusensis*.

References

Chiari, Y., et al. 2012. Phylogeography of Sardinian cave salamanders (Genus Hydromantes) is mainly determined by geomorphology. PLoS ONE 7(3): e32332.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Voesenek, L. A. C. J., et al. 1987. Some autecological data on the Urodeles of Sardinia. Amphibia-Reptilia 8(4): 307-314.

Plethodontidae   *Speleomantes strinatii*   Strinati's cave salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	NT	High	High	Medium	High

Epidemiology

Dispersal

Fully terrestrial species, active year round. Movement of adult salamanders were generally low; some repeatedly recaptured individuals had a mean home range of 6 m^2. However, species is capable of migrating 10m/night.

Density

Local density in suitable habitat can be high. For a rock-face population in northwestern Italy, the population density varied between 0.6-1.0 individuals/m^2 of rock face (average 0.8).

Co-occurrence

Salamandra salamandra, *Salamandrina perspicillata*, *Lissotriton vulgaris*, *Ichthyosaura alpestris* and *Triturus carnifex*.

Bsal risk status

Bsal has been shown lethal for this species (laboratory experiments). Risk of human-mediated introduction in its distribution range, and co-occurrence with reservoir species such as *Ichthyosaura alpestris*.

Conservation Unit

The level of intraspecific genetic isolation and variation is high, with two highly divergent clades in the eastern and central-western part of the range, which can be considered as conservation units. Occurrence is limited to southeastern France and northwestern Italy.

Currently recognized subspecies

NA

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4), although it is recommended to gain experience in keeping and breeding this species.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low

European distribution of *Speleomantes strinatii*.

References

Costa, A., et al. 2016. European plethodontid salamanders on the forest floor: Local abundance is related to fine-scale environmental factors. Herpetological Conservation and Biology 11(2): 344–349.

Chiari, Y., et al. 2012. Phylogeography of Sardinian cave salamanders (Genus Hydromantes) is mainly determined by geomorphology. PLoS ONE 7(3): e32332.

Cimmaruta, R., et al. 2015. Persistence, isolation and diversification of a naturally fragmented species in local refugia: The case of *Hydromantes strinatii*. PLoS ONE 10(6): e0131298.

Lanza, B. 1999. *Speleomantes ambrosii,* Sp. *strinatii* – Ambrosis Höhlensalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Salvidio, S., et al. 1994. Ecology of a *Speleomantes ambrosii* population inhabiting an artificial tunnel. Amphibia-Reptilia 15: 34-35.

Salvidio, S. 1998. Estimating abundance and biomass of a *Speleomantes strinatii* (Caudata, Plethodontidae) population by temporary removal sampling. Amphibia-Reptilia **19**(2): 113-124.

Salvidio, S. 2013. Homing behaviour in *Speleomantes strinatii* (Amphibia Plethodontidae): A preliminary displacement experiment. North-Western Journal of Zoology 9(2): 429-432.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Plethodontidae   *Speleomantes supramontis*   Supramonte cave salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	EN	High	High	High	High

Epidemiology

Dispersal

Fully terrestrial and nocturnal species, presumed similar dispersal pattern as other *Speleomantes* species, no exact data known.

Density

300/ha in the forests and 98 ± 7 individuals in a cave.

Co-occurrence

Euproctus platycephalus.

Bsal risk status

Bsal susceptibility has not been tested for this species, likely lethal based on close relationship to the Bsal susceptible species *Speleomantes strinatii*. Restricted range, high impact when Bsal when is introduced in its distribution range.

Conservation Unit

The level of intraspecific genetic isolation and variation is high, with two major lineages based on mitochondrial DNA analyses, which can be considered as conservation units. Conservation units

geographically determined by isolated mountain ranges Sopramonte and Monte Tuttavista. Endemic to central-eastern Sardinia.

Currently recognized subspecies
NA

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4), although it is recommended to gain experience in keeping and breeding this species.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low

European distribution of *Speleomantes supramontis*.

References

Chiari, Y., et al. 2012. Phylogeography of Sardinian cave salamanders (Genus Hydromantes) is mainly determined by geomorphology. PLoS ONE 7(3): e32332.

Lanza, B. 1999. *Speleomantes supramonte* – Supramonte-Höhlensalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Voesenek, L. A. C. J., et al. 1987. Some autecological data on the Urodeles of Sardinia. Amphibia-Reptilia 8(4): 307-314.

Proteidae   Proteus anguinus   Olm

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	VU	Low	Low	Low	Low

Epidemiology

Dispersal

Dispersal confined to the inhabited river/cave system. Genetic admixture of populations in two interconnected cave systems indicates the ability to disperse over several kilometres of subterranean rivers.

Density

From a study in caves in Croatia population density was in cave 1: 7-11.45 individuals/10 m^2, in cave 2: 0.45-1.08 individuals/10 m^2, and in cave 3: 1.12-1.38 individuals/10m^2.

Co-occurrence

Not likely to co-occur with other urodelan species in its subterranean habitat.

Bsal risk status

Low susceptibility to Bsal (laboratory experiments). The occurrence of Bsal vectoring species within its range and risk of human introduction of Bsal warrant caution.

Conservation Unit

Phylogenetic analyses reveal that the white olm represents six clades and the black olm (*P. a. parkelj*) is deeply nested within the white olm lineages. Relevant conservation units should include all clades and subspecies. Further studies are required: in Croatia the genetic uniqueness was so distinct in four populations (Pincinova, Rupečica, Markarova, and Vedrine) that they should be treated as evolutionary significant units.

The level of intraspecific genetic isolation and variation is high, with six distinct lineages based on mitochondrial DNA analyses. Populations at close proximity may become genetically isolated and should be treated as conservation units.

> **Currently recognized subspecies**
>
> *Proteus anguinus anguinus*
>
> *Proteus anguinus parkelj**
>
> * Has distinct coloration and morphology, but may not be considered as a subspecies based on genetic data.

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ **management**

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Difficult	Difficult	Low

European distribution of *Proteus anguinus*.

References

Briegleb, W. 1962. Zur Biologie und Ökologie des Grottenolms (*Proteus anguinus* Laur. 1768)." Zeitschrift für Morphologie und Ökologie der Tiere 51(3): 271-334.

Grosse, W. R. (Ed.). 2018. Threatened Newts and Salamanders of the World – Captive Care Management. Mertensiella Vol. 2, 292 p.

Parzefall, J. et al. 1999. *Proteus anguinus* – Grottenolm. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Trontelj, P., et al. 2009. A molecular test for cryptic diversity in ground water: how large are the ranges of macro-stygobionts? Freshwater Biology 54(4): 727-744.

Vörös, J., et al. 2019. Population genetic analyses using 10 new polymorphic microsatellite loci confirms genetic subdivision within the olm, *Proteus anguinus*. Journal of Heredity 110(2): 211-218.

Salamandridae   *Calotriton arnoldi*   Montseny brook newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex IV	CE	High	High	High	High

Epidemiology

Dispersal

No animals have ever been found on land, dispersal is likely limited. Data on behaviour is absent.

Density

The few existing populations have very low densities.

Co-occurrence

Salamandra salamandra, Lissotriton helveticus and *Triturus marmoratus.*

Bsal risk status

High risk is based on high susceptibility to Bsal (laboratory experiments), the presence of potential Bsal reservoir species within its range (i.e. *Lissotriton* and *Triturus* spp.), small range and the known introduction of Bsal within 20 kilometers of the species' range.

Conservation Unit

The level of intraspecific genetic isolation and variation is considerable, with two genetically distinct populations separated by the Tordera river, which can be considered as conservation units. A LIFE project was funded in 2016 aiming to ensure the conservation of the genetic pool of the species and to expand its geographic distribution area.

Currently recognized subspecies

NA

Species-specific actions

Bsal has been introduced within 20 kilometers of the the species' range. Actions as described in § 4 have been initiated. Bsal spread to the species range must be prevented. *Ex situ* measures have been initiated prior to Bsal incursion.

Ex situ management

Captive breeding facilities were set up in the framework of the conservation plan for the species. Genetic management is set in place.

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Moderate	High

European distribution of *Calotriton arnoldi*.

References

http://lifetritomontseny.eu/en/evolucio/-/cercador/cerca#resultats-cercador

Carranza, S. and F. Amat 2005. Taxonomy, biogeography and evolution of *Euproctus* (Amphibia: Salamandridae), with the resurrection of the genus *Calotriton* and the description of a new endemic species from the Iberian Peninsula. Zoological Journal of the Linnean Society 145(4): 555-582.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Salamandridae   *Calotriton asper*   Pyrenean brook newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex IV	NT	Low	Low	Low	Medium

Epidemiology

Dispersal

Largely, but not exclusively aquatic newt, larvae may disperse by drift. Overall, dispersal is very limited (< 50 m) and distribution is linked to number of refugia.

Density

Local density can be high, particularly in shallow streambeds with sufficient aquatic vegetation and a weak current. In eastern Pyrenees between 3500 – 5000 newts in 1.5 km brook.

Co-occurrence

Salamandra salamandra, *Lissotriton helveticus*, *Ichthyosaura alpestris* and *Triturus marmoratus.*

Bsal risk status

Low risk is based on the absence of susceptibility to Bsal (laboratory experiments). However, the presence of potential Bsal reservoir species within its range (i.e. *Lissotriton* and *Triturus* spp.) and the high potential of human-mediated introduction warrant caution.

Conservation Unit

Based on mitochondrial DNA, three shallowly differentiated with low genetic diversity lineages could be discerned in the French Pyrenees. However, variation based on 382 loci was high and revealed a clear pattern of isolation by distance, consistent with long-term restriction of gene flow. Marked genetic differentiation exists at the scale of different drainages, but also between localities separated by just a few kilometres. Also, paedomorphic populations constitute evolutionary significant units.

Pending more research across the entire range, populations from different drainages can be considered as conservation units.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4)*.*

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Moderate	High

European distribution of *Calotriton asper*.

References

Clergue-Gazeau, M. 1999. *Euproctus asper* – Pyrenäen-Gebirgsmolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Milá, B., et al. 2010. Marked genetic structuring and extreme dispersal limitation in the Pyrenean brook newt *Calotriton asper* (Amphibia: Salamandridae) revealed by genome-wide AFLP but not mtDNA. Molecular Ecology 19(1): 108-120.

Montori, A., et al. 2008. Habitat features affecting the small-scale distribution and longitudinal migration patterns of *Calotriton asper* in a Pre-Pyrenean population. Amphibia-Reptilia 29(3): 371-381.

Montori, A., et al. 2012. Natural fluctuations in a stream dwelling newt as a result of extreme rainfall: a 21-year survey of a *Calotriton asper* population. Basic and Applied Herpetology 26: 43-56.

Oromi, N., et al. 2019. Genetic structure of lake and stream populations in a Pyrenean amphibian (*Calotriton asper*) reveals evolutionary significant units associated with paedomorphosis. Journal of Zoological Systematics and Evolutionary Research 57(2): 418-430.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Schultschik, G. and W. R. Grosse (eds.). 2013. Threatened Newts and Salamanders - Guidelines for Conservation Breeding. - Mertensiella 20e, 180 p.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Salamandridae   *Chioglossa lusitanica* Golden-striped salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	VU	High	High	Low	Medium

Epidemiology

Dispersal

Limited dispersal. Dependent on season and life stage between 5-30 m from brook, but may migrate > 750 m along the brook (> 350 m overnight).

Density

397-770 salamanders/ha. 4-5 adult specimens/m along brook.

Co-occurrence

Salamandra salamandra, Pleurodeles waltl, Lissotriton helveticus, L. boscai, Ichthyosaura alpestris, Triturus marmoratus and *T. pygmaeus.*

Bsal risk status

High risk is based on lethal susceptibility to Bsal (laboratory experiments) and restricted range. Human-mediated introduction can have high impact on this species.

Conservation Unit

The level of intraspecific genetic isolation and variation high, with the existence of two major lineages north (*C. l. longipes*) and south (*C. l. lusitanica*) of the Mondego River, which can be considered as conservation units for this species. A decrease in genetic variability from the Mondego northwards was associated with the Douro and Minho rivers. The species is endemic to the Iberian Peninsula.

Currently recognized subspecies

Chioglossa lusitanica lusitanica

Chioglossa lusitanica longipes

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4), although it is recommended to gain experience in keeping and breeding this species.

***Ex situ* management**

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Moderate	High

European distribution of Golden-striped salamander (*Chioglossa lusitanica*).

References

Alexandrino, J. et al. 2001. Genetic subdivision, glacial refugia and postglacial recolonization in the golden-striped salamander, *Chioglossa lusitanica* (Amphibia: Urodela). Molecular Ecology 9: 771-781.

Arntzen, J. W. 1981. Ecological observations on *Chioglossa lusitanica* (Caudata, Salamandridae). Amphibia-Reptilia 1(3-4): 187-203.

Arntzen, J. W. 1999. *Chioglossa lusitanica* – Goldstreifensalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Arntzen, J. W. 2015. Drastic population size change in two populations of the golden-striped salamander over a forty-year period-are eucalypt plantations to blame? Diversity 7(3): 270-294.

Busack, S. D. 1976. A review of the biology of the gold-striped salamander, *Chioglossa lusitanica* (amphibia: Salamandridae). Biological Conservation 10(4): 309-319.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

<u>Salamandridae</u>   *Euproctus montanus*   <u>Corsican brook newt</u>

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex IV	LC	High	High	Medium	High

Epidemiology

Dispersal

Migratory movements between land and water habitat. No data available on distances and dispersal.

Density

Relatively abundant especially between 600-1500 m. No exact data on abundancy available.

Co-occurrence

Salamandra corsica.

Bsal risk status

High risk and highly susceptibility is based on the species' close relationship to Bsal susceptible *Euproctus platycephalus* and the high potential of human-mediated introduction.

Conservation Unit

E. montanus is strongly fragmented into several reciprocally monophyletic lineages of ancient origin. The level of intraspecific genetic isolation and variation is high, with five major clades recognized,

particularly in the northern parts of Corsica, which can be considered as conservation units for this species.

Currently recognized subspecies

NA

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4), although it is recommended to gain experience in keeping and breeding this species.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Moderate	High

European distribution of *Euproctus montanus*.

References

Bisconti, R., et al. 2013. A geographic mosaic of evolutionary lineages within the insular endemic newt *Euproctus montanus*. Molecular Ecology 22(1): 143-156.

Glandt, D. 2009.Taschenlexikon der Amphibien und Reptilien Europas. Alle Arten von den Kanarischen Inseln bis zum Ural. Quelle + Meyer. 636 p.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Rimp, K., and B. Thiesmeier. 1999. *Euproctus montanus* – Korsischer Gebirgsmolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Schultschik, G. and W. R. Grosse (eds.). 2013. Threatened Newts and Salamanders - Guidelines for Conservation Breeding. - Mertensiella 20e, 180 p.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Salamandridae   *Euproctus platycephalus*   Sardinian brook newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex IV	EN	High	High	Medium	High

Epidemiology

Dispersal

Migratory movements between land and water habitat. No data available on distances and dispersal.

Density

Locally abundant, on the whole very rare. Report from Sardinian site mentions population size between 180-445 individuals/lake (lake size unknown).

Co-occurrence

Speleomantes imperialis, S. sarrabusensis and S. supramontis.

Bsal risk status

Bsal has been shown lethal for this species (laboratory experiments). The species has a restricted range, and human-mediated introduction of Bsal is probable and can have high impact on this species.

Conservation Unit

At least two conservation units. Populations of the northern region comprise an evolutionary significant unit (ESU), and while populations of the central and southern regions do not meet the stringent criteria to be classified as independent ESUs, the deep genetic divisions suggest that they too should not be considered genetically interchangeable.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

European distribution of *Euproctus platycephalus*.

References

Ball, S. E., et al. 2017. Islands within an island: Population genetic structure of the endemic Sardinian newt, *Euproctus platycephalus*. Ecology and Evolution 7(4): 1190-1211.

Lecis, R. and G. de la Conservaciò 2004. The endemic Sardinian newt *Euproctus platycephalus*: Local threats and population decline. Italian Journal of Zoology 71(SUPPL.2): 195-198.

Rimp, K., and B. Thiesmeier. 1999. *Euproctus platycephalus* – Sardischer Gebirgsmolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae,

Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Schultschik, G. and W. R. Grosse (eds.). 2013. Threatened Newts and Salamanders - Guidelines for Conservation Breeding. - Mertensiella 20e, 180 p.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Voesenek, L. A. C. J., et al. 1987. Some autecological data on the Urodeles of Sardinia. Amphibia-Reptilia 8(4): 307-314.

Salamandridae   *Ichthyosaura alpestris*   Alpine newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
NA	LC	Moderate	Medium	Low	Low

Epidemiology

Dispersal

Action radius is limited, yearly migration between hibernation site and reproduction water is usually around 400 m.

Density

Usually not numerous, 0.01-10 adult individuals/m² pond.

Co-occurrence

Salamandra salamandra, *S. atra*, *S. lanzai*, *Salamandrina perspicillata*, *S. terdigitata*, *Chioglossa lusitanica*, *Calotriton asper*, *Lissotriton vulgaris*, *L. helveticus*, *L. italicus*, *L. boscai*, *L. montandoni*, *Triturus cristatus*, *T. carnifex*, *T. macedonicus*, *T. dobrogicus*, *T. ivanbureschi*, *T. marmoratus*, *Speleomantes italicus*, *S. ambrosii* and *S. strinatii*.

Bsal risk status

The alpine newt shows a dose-dependent susceptibility to Bsal, infection is lethal when exposed to a high Bsal dose, but it has the potential to clear the infection when exposed to a low dose. The species has a large range, but co-occurs with susceptible hosts and Bsal is present within its distribution range.

Conservation Unit

The level of intraspecific genetic isolation and variation is high. Over its entire range, five clades are distinguished, which can be considered as conservation units for this species: one clade in southeastern Serbia, a second clade representing Italian populations, the third representing central

European and Iberian populations, the fourth and fifth clades represent southern and central-northern Balkan populations. Within each subspecies several Evolutionary Significant Units (ESUs) can be recognized. For instance, *I. alpestris veluchiensis* in Greece consists of two clades separated by the Gulf of Corinth.

Currently recognized subspecies

Ichthyosaura alpestris alpestris	*Ichthyosaura alpestris montenegrina*
Ichthyosaura alpestris apuana	*Ichthyosaura alpestris reiseri*
Ichthyosaura alpestris cyreni	*Ichthyosaura alpestris veluchiensis*

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

***Ex situ* management**

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High
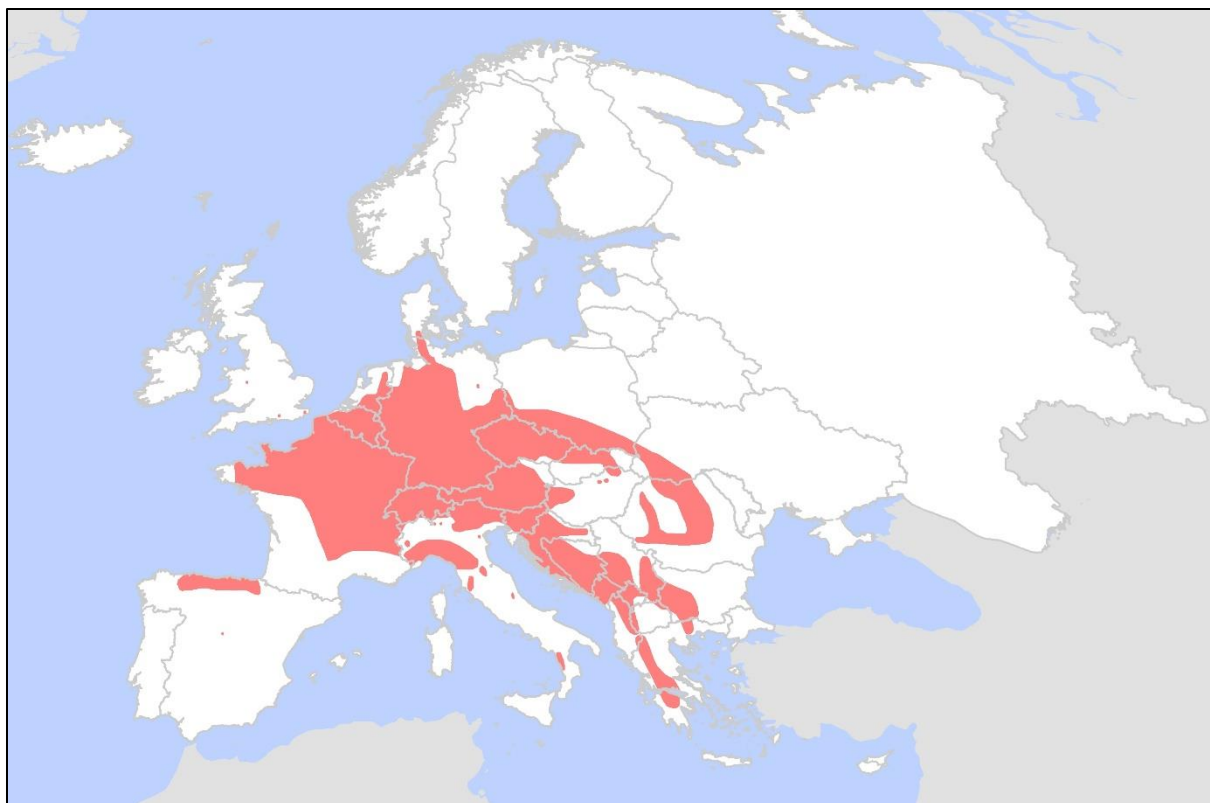
European distribution of *Ichthyosaura alpestris*.

References

AmphibiaWeb. 2019. <http://amphibiaweb.org> University of California, Berkeley, CA, USA. Accessed 1 Jul 2019.

Grosse, W. R. (Ed.). 2018. Threatened Newts and Salamanders of the World – Captive Care Management. Mertensiella Vol. 2, 292 p.

Blab, J. 1986. Biologie, Ökologie, und Schutz von Amphibien. Schriftenreihe für Landschaftspflege und Naturschutz 18, Kilda Verlag, Bonn

Roček, Z., et al. 2004. *Triturus alpestris* – Bergmolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sotiropoulos, K., et al. 2007. Phylogeny and biogeography of the alpine newt *Mesotriton alpestris* (Salamandridae, Caudata), inferred from mtDNA sequences. Molecular Phylogenetics and Evolution 45(1): 211-226.

Sotiropoulos, K., et al. 2008. Genetic structure of the alpine newt, *Mesotriton alpestris* (Salamandridae, Caudata), in the southern limit of its distribution: Implications for conservation. Biochemical Systematics and Ecology 36(4): 297-311.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Salamandridae   *Lissotriton boscai*   Bosca's newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
NA	LC	High	High	Low	Medium

Epidemiology

Dispersal

Presumed similar to other *Lissotriton* species. No data available.

Density

Local density can be high, no exact figures published.

Co-occurrence

Salamandra salamandra, *Chioglossa lusitanica*, *Pleurodeles waltl*, *Lissotriton helveticus*, *Ichthyosaura alpestris*, *Triturus marmoratus* and *T. pygmaeus*.

Bsal risk status

Bsal susceptibility is considered high for this species based on mortality events in captivity and lab experiments. Co-occurrence with Bsal reservoir hosts and the risk of human-mediated Bsal introduction warrant caution.

Conservation Unit

Two major lineages exist: a well differentiated lineage in southwestern Iberia and a major lineage comprising four sub-lineages, which show gene flow. At least these two major lineages should be considered as conservation units. New data are needed to clarify the taxonomic status of these two divergent lineages. Endemic to the western Iberian Peninsula.

Currently recognized subspecies
NA*

* The southwestern clade of *L. boscai* has previously been proposed as a separate species, *Lissotriton maltzani*, but pending more research *L. boscai* is considered monotypic.

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ **management**

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

European distribution of *Lissotriton boscai*.

References

Caetano, M. H. 2004. *Triturus boscai* – Spanischer Wassermolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Grosse, W. R. (Ed.). 2018. Threatened Newts and Salamanders of the World – Captive Care Management. Mertensiella Vol. 2, 292 p.

Martínez-Solano, I., et al. 2006. Mitochondrial DNA phylogeography of *Lissotriton boscai* (Caudata, Salamandridae): Evidence for old, multiple refugia in an Iberian endemic. Molecular Ecology 15(11): 3375-3388.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Speybroeck, J., W. Beukema, and P.-A. Crochet. 2010. A tentative species list of the European herpetofauna (Amphibia and Reptilia)—an update. Zootaxa 2492: 1–27.

Teizeira, J. et al. 2015. Genealogy of the nuclear β-fibrinogen intron 7 in *Lissotriton boscai* (Caudata, Salamandridae): concordance with mtDNA and implications for phylogeography and speciation. Contributions to Zoology. 84 (3): 193-215.

Salamandridae *Lissotriton graecus* Greek smooth newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
NA	NE	Moderate	Medium	Low	Low

Epidemiology

Dispersal

Likely similar to the previously considered conspecific *L. vulgaris*.

Density

Likely similar to *L. vulgaris*.

Co-occurrence

Salamandra salamandra, S. atra, Ichthyosaura alpestris, Lissotriton vulgaris, Triturus ivanbureschi and T. macedonicus.

Bsal risk status

Susceptibility to Bsal has not been examined in the laboratory for this species, but is likely similar to the susceptibility of the closely related species *L. vulgaris*.

Conservation Unit

Two major lineages can be discerned, one on the Peloponnese Peninsula and one in the remaining part of its range, which can be considered as conservation units. In light of the recent taxonomic revision of the smooth newt species complex, the IUCN status for the five species currently subsumed in *L. vulgaris* sensu lato should be revised.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

European distribution of *Lissotriton graecus*.

References

Kuzmin, S.L. 1999. The amphibians of the former Soviet Union. Pensoft, Sofia, Moscow.

Pabijan M, Zieliński P, Dudek K, Stuglik M, Babik W. 2017. Isolation and gene flow in a speciation continuum in newts. Mol.Phylogenet.Evol. 116:1-12.

Schmidtler, J.F. and M. Franzen. 2004. *Triturus vulgaris* (Linnaeus, 1758) – Teichmolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2, *Salamandra* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Wielstra, B., et al. 2018. The distributions of the six species constituting the smooth newt species complex (*Lissotriton vulgaris* sensu lato and *L. montandoni*) - An addition to the New Atlas of Amphibians and Reptiles of Europe. Amphibia-Reptilia 39: 252-259.

Salamandridae   *Lissotriton helveticus*   Palmate newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
NA	LC	Low	Low	Low	Low

Epidemiology

Dispersal

Young newts show high dispersal capacity of up to a few kilometers. Adults hibernate 150-400 m from reproductive water.

Density

Local density can be high, ranging from 1-388 individuals/50 m^2 pond surface.

Co-occurrence

Salamandra salamandra, *S. atra*, *Chioglossa lusitanica*, *Calotriton asper*, *C. arnoldi*, *Lissotriton vulgaris*, *L. boscai*, *Ichthyosaura alpestris*, *Triturus cristatus* and *T. marmoratus*.

Bsal risk status

Species is not susceptible to Bsal in laboratory experiments and has a large distribution range. No infection and disease in laboratory experiments, but Bsal reported in this species in the wild.

Conservation Unit

The level of intraspecific genetic isolation and variation appears to be low compared to other *Lissotriton* species, with four different mitochondrial haplotypes on the Iberian Peninsula. Nuclear genes were not geographically structured, suggesting gene flow and incomplete lineage sorting. Populations north of the Pyrenees were closely related to those from northeastern Iberia. Over the wide sympatric zone with *L. vulgaris* there is a moderate level of hybridization which does not compromise the genetic integrity of the species. The known haplotypes can be considered as units of conservation, but the genetic diversity of this species needs to be further assessed to determine conservation priorities, and hotspots of paedomorphosis should be considered.

217

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High
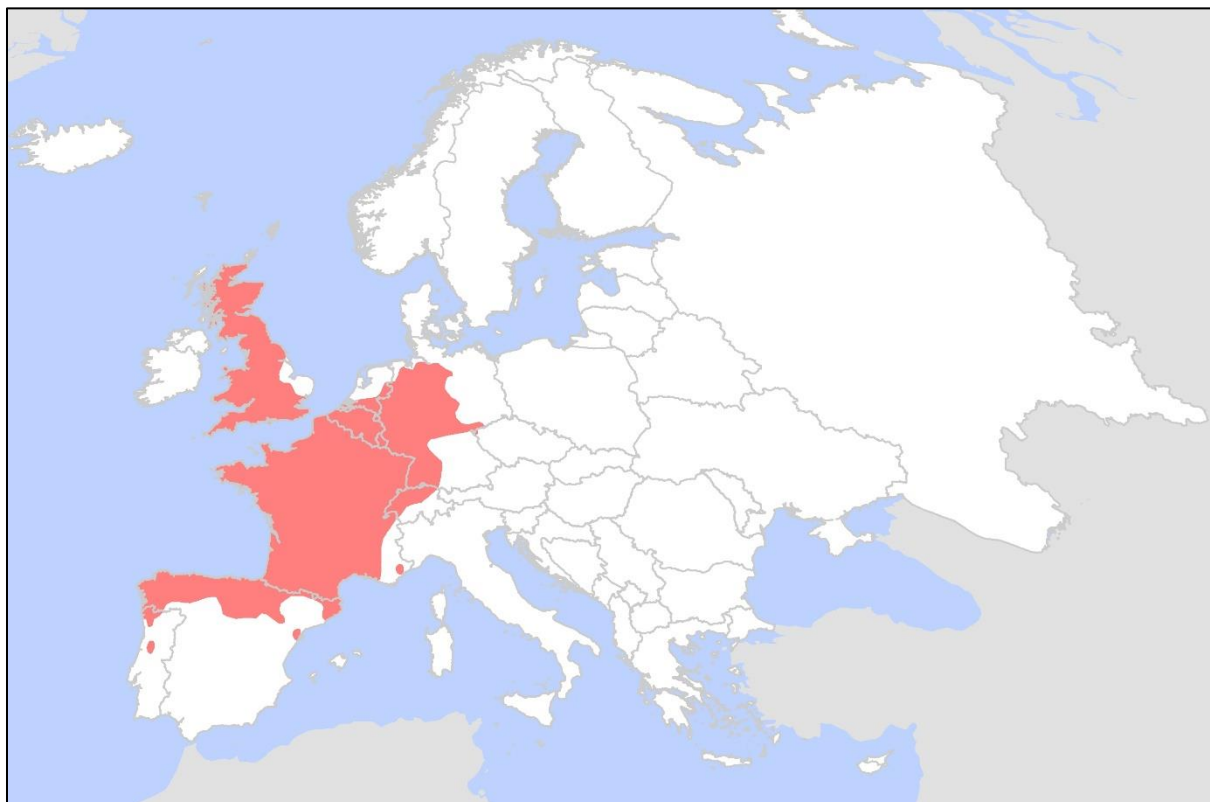
European distribution of *Lissotriton helveticus*.

References

Cayuela, H., et al. 2018. Context-dependent dispersal, public information, and heterospecific attraction in newts. Oecologia 188(4): 1069-1080.

Denoël, M. and G. F. Ficetola 2015. Using kernels and ecological niche modeling to delineate conservation areas in an endangered patch-breeding phenotype. Ecological Applications 25(7): 1922-1931.

Grosse, W. R. (Ed.). 2018. Threatened Newts and Salamanders of the World – Captive Care Management. Mertensiella Vol. 2, 292 p.

Isselin-Nondedeu, F., et al. 2017. Spatial genetic structure of *Lissotriton helveticus* L. following the restoration of a forest ponds network. Conservation Genetics 18(4): 853-866.

Johanet, A., et al. 2011. Widespread introgression does not leak into allotopy in a broad sympatric zone. Heredity 106: 962-972.

Oromi, N., et al. 2016. High gene flow between alternative morphs and the evolutionary persistence of facultative paedomorphosis. Scientific Reports 6.

Recuero, E. and M. García-París. 2011. Evolutionary history of Lissotriton helveticus: multilocus assessment of ancestral vs. recent colonization of the Iberian Peninsula. Mol Phylogenet Evol. 60(1):170-82.

Schlüpmann, M. and J. van Gelder. 2004. *Triturus helveticus* – Fadenmolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2, *Salamandra* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Salamandridae   *Lissotriton italicus*   Italian newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex IV	LC	High	High	Low	Medium

Epidemiology

Dispersal

Presumed similar to other *Lissotriton* species, although it seems more sedentary and may remain aquatic year-round in some regions.

Density

Local density can be high, no exact figures published.

Co-occurrence

Salamandra salamandra, Salamandrina perspicillata, S. terdigitata, Lissotriton vulgaris, Ichthyosaura alpestris and *Triturus carnifex*.

Bsal risk status

Bsal has been shown lethal for this species (laboratory experiments). Potential reservoir hosts co-occur, human-mediated introduction is probable and may heavily impact this species.

Conservation Unit

The level of intraspecific genetic isolation and variation is high, with two major, parapatric mitochondrial lineages, and a further eight subdivisions in the Calabrian peninsula. The two major mitochondrial lineages can be considered as units of conservation. Endemic species to central and southern Italy.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

European distribution of *Lissotriton italicus*.

References

Canestrelli, D., et al. (2012). On glacial refugia, genetic diversity, and microevolutionary processes: Deep phylogeographical structure in the endemic newt *Lissotriton italicus*. Biological Journal of the Linnean Society 105(1): 42-55.

Grosse, W. R. (Ed.). 2018. Threatened Newts and Salamanders of the World – Captive Care Management. Mertensiella Vol. 2, 292 p.

Lanza, B. et al. (eds) 2007. Fauna d'Italia. Vol. XLII, *Amphibia*. Edizioni Calderini, Bolgona.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2004. *Triturus italicus* – Italienischer Wassermolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Salamandridae   *Lissotriton montandoni*   Montandon's newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	LC	Moderate	Medium	Low	Low

Epidemiology

Dispersal

Action radius is limited, yearly migration between hibernation site and reproduction water is usually between 300-350 m.

Density

Local density can be high, 18-20 specimens/m^2 at 500-750 m altitude and in Romania a density of 1-79 specimens/km^2 was recorded.

Co-occurrence

Salamandra salamandra, *Lissotriton vulgaris*, *Ichthyosaura alpestris* and *Triturus cristatus*.

Bsal risk status

Bsal susceptibility has not been tested for this species, but assumed moderately susceptible based on susceptibility of other *Lissotriton* species. Co-occurs with reservoir hosts, and the risk of human-mediated pathogen introduction is realistic.

Conservation Unit

Two major lineages are identified, which can be considered as units of conservation: the northern group in the Western Carpathians and the western part of the Eastern Carpathians, and the southern group across the rest of the species range. Endemic species to east Carpathian and easternmost Sudetes Mountains.

Currently recognized subspecies

NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy*	Easy	High

* More sensitive than *L. vulgaris*.

European distribution of *Lissotriton montandoni*.

References

Grosse, W. R. (Ed.). 2018. Threatened Newts and Salamanders of the World – Captive Care Management. Mertensiella Vol. 2, 292 p.

Gulicka, J. 1954. K. rozšíreniu a ekológii mloka karpatského (*Triturus montandoni* Boul.) na Slovensku. Biológia, Bratislava 9 (5): 545-560 (from: Grossenbacher and Thiesmeier (eds.) Handbuch der Reptilien und Amphibien Europas. Schwanzlurche IIA. Zavadil, V., Piálek, J and Dandová, R. *Triturus montandoni* – Karpatenmolch pp 657-706.

Kuzmin, S.L. 1999. The amphibians of the former Soviet Union. Pensoft, Sofia, Moscow.

Pabijan M, Zieliński P, Dudek K, Stuglik M, Babik W. 2017. Isolation and gene flow in a speciation continuum in newts. Mol.Phylogenet.Evol. 116:1-12.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Şova, C. 1971. Researches on the population density and structure in *Triturus montandoni* (Boul.) during the period of reproductive aggregation in the eastern and northern Carpathians mountains. Stud. Com. Muz. Şti. Nat. Bacău 4: 197-205.

Wielstra, B., et al. 2018. The distributions of the six species constituting the smooth newt species complex (*Lissotriton vulgaris* sensu lato and *L. montandoni*) - An addition to the New Atlas of Amphibians and Reptiles of Europe. Amphibia-Reptilia 39: 252-259.

Zavadil, V., et al. 2004. *Triturus montandoni* – Karpatenmolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Zieliński, P., et al. 2014. Single sucleotide polymorphisms reveal genetic structuring of the Carpathian newt and provide evidence of interspecific gene flow in the nuclear genome. PLoS ONE 9(5).

Zieliński, P., et al. 2016. Divergence history of the Carpathian and smooth newts modelled in space and time. Molecular Ecology 25(16): 3912-3928.

Salamandridae   *Lissotriton schmidtleri*   Schmidtler's smooth newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
NA	NE	Moderate	Medium	Low	Low

Epidemiology

Dispersal

Likely similar to the previously considered conspecific *L. vulgaris*.

Density

Likely similar to *L. vulgaris*.

Co-occurrence

Salamandra salamandra and *Triturus ivanbureschi*.

Bsal risk status

Susceptibility to Bsal has not been examined in the laboratory for this species, but is likely similar to the susceptibility of the closely related species *L. vulgaris*.

Conservation Unit

Pending further research, the species can be considered as unit of conservation, at least in the European part of its distribution range. In light of the recent taxonomic revision of the smooth newt species complex, the IUCN status for the five species currently subsumed in *L. vulgaris* sensu lato should be revised.

Currently recognized subspecies

NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

European distribution of *Lissotriton schmidtleri*.

References

Kuzmin, S.L. 1999. The amphibians of the former Soviet Union. Pensoft, Sofia, Moscow.

Pabijan M, Zieliński P, Dudek K, Stuglik M, Babik W. 2017. Isolation and gene flow in a speciation continuum in newts. Mol.Phylogenet.Evol. 116:1-12.

Schimdtler, J.F. and M. Franzen. 2004. *Triturus vulgaris* (Linnaeus, 1758) – Teichmolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2, *Salamandra* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Wielstra, B., et al. 2018. The distributions of the six species constituting the smooth newt species complex (*Lissotriton vulgaris* sensu lato and *L. montandoni*) - An addition to the New Atlas of Amphibians and Reptiles of Europe. Amphibia-Reptilia 39: 252-259.

Salamandridae   *Lissotriton vulgaris*   Smooth newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
NA*	LC	Moderate	Medium	Low	Low

Epidemiology

Dispersal

Semi-aquatic species, can cover 600 m/48 d. Can quickly colonize new habitats. Terrestrial hibernation sites usually within a 500 m radius from the breeding water. Adults and larvae may hibernate in the water.

Density

Most widespread and ubiquitous newt of Europe. Local density can be high, up to 40/km^2.

Co-occurrence

Salamandra salamandra, S. atra, Salamandrina perspicillata, S. terdigitata, Lissotriton graecus, L. helveticus, L. montandoni, L. italicus, Ichthyosaura alpestris, Triturus cristatus, T. marmoratus and *Salamandrella keyserlingii*.

Bsal risk status

Course of infection dependent on host condition, environmental conditions and infection intensity. Infection does not always lead to disease, infected animals may develop lethal chytridiomycosis whereas others may clear an infection. Widespread distribution, co-occurrence with reservoir and susceptible hosts.

Conservation Unit

Consider at least each major intraspecific lineage/subspecies as conservation unit. A genetically distinct northern and a southern clade have been identified for *Lissotriton vulgaris vulgaris*. In light of

the recent taxonomic revision of the smooth newt species complex, the IUCN status for the five species currently subsumed in *L. vulgaris* sensu lato should be revised.

Currently recognized subspecies

Lissotriton vulgaris ampelensis*

Lissotriton vulgaris meridionalis

Lissotriton vulgaris vulgaris

* *L. v. ampelensis* is listed on Annexes II and IV of the Habitats Directive.

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High
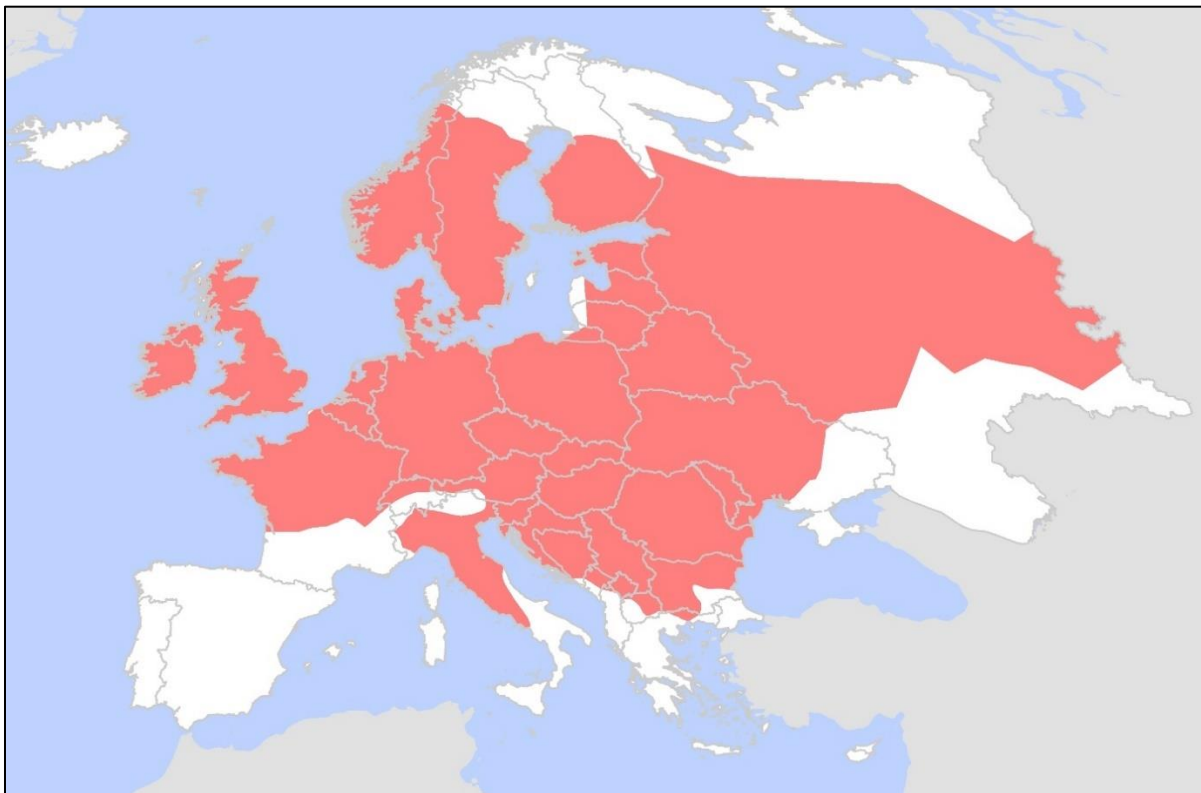
European distribution of *Lissotriton vulgaris* sensu stricto.

References

Kuzmin, S.L. 1999. The amphibians of the former Soviet Union. Pensoft, Sofia, Moscow.

Pabijan M, Zieliński P, Dudek K, Stuglik M, Babik W. 2017. Isolation and gene flow in a speciation continuum in newts. Mol.Phylogenet.Evol. 116:1-12.

Schimdtler, J.F. and M. Franzen. 2004. *Triturus vulgaris* (Linnaeus, 1758) – Teichmolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2, *Salamandra* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Wielstra, B., et al. 2018. The distributions of the six species constituting the smooth newt species complex (*Lissotriton vulgaris* sensu lato and *L. montandoni*) - An addition to the New Atlas of Amphibians and Reptiles of Europe. Amphibia-Reptilia 39: 252-259.

Salamandridae   *Lyciasalamandra helverseni* Karpathos salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	VU	High	High	High	High

Epidemiology

Dispersal

Little information is available on the ecology and biology of this species, presumably quite similar to *L. luschani*. Terrestrial and viviparous species.

Density

Exact figures unknown. Species is fairly common and abundant within its range.

Co-occurrence

No other urodelan species within the European range.

Bsal risk status

High risk is based on lethal susceptibility to Bsal (laboratory experiments) and restricted range. Human-mediated introduction can have high impact on this species. The high susceptibility is based on laboratory experiments and on its close relationship to the Bsal susceptible *Salamandra* genus.

Conservation Unit

Consistent within this genus is the occurrence of small to very small range lineages, with little overlap even at short distances, suggesting very limited gene flow between populations. Marked differentiation was shown to occur both on the islands of Karpathos and Kasos, with two major lineages on separate islands. Pending further delineation, the island of occurrence can be considered as conservation unit for this species. The species is endemic to the Greek islands of Karpathos, Kassos and Saria.

Currently recognized subspecies
NA

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4), although it is recommended to gain experience in keeping and breeding this species.

Ex situ management

This species can be kept and bred in captivity but is often short-lived and may be highly sensitive shortly after bringing to captivity. Once established, the species has been kept for over 20 years.

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low
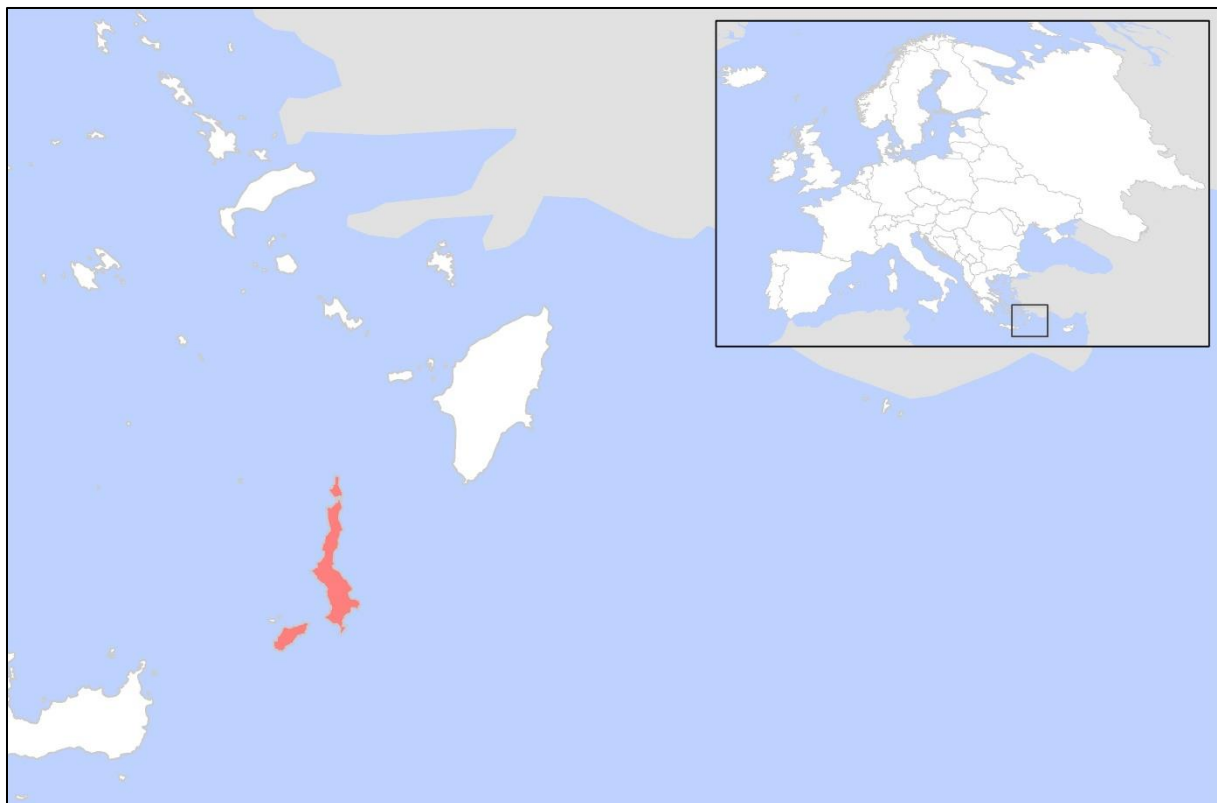
European distribution of *Lyciasalamandra helverseni*.

References

Eleftherakos, K., et al. 2007. Conservation units in the insular endemic salamander *Lyciasalamandra helverseni* (Urodela, Salamandridae). Ann. Zool. Fennici 44: 387-399.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Steinfartz, S. and Mutz T. 1999. *Mertensiella luschani* – Lykischer Salamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Veith, M. et al. 2016. Seven at one blow: the origin of major lineages of the viviparous Lycian salamanders (*Lyciasalamandra* Veith and Steinfartz, 2004) was triggered by a single paleo-historic event. Amphibia-Reptilia 37 (4): 373-387.

Salamandridae   *Lyciasalamandra luschani*   Luschan's salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	VU	High	High	High	High

Epidemiology

Dispersal

Little information is available on the ecology and biology of this terrestrial and viviparous species. Exhibits gregarious behaviour.

Density

Exact figures unknown. Species is fairly common and abundant within its range

Co-occurrence

No other urodelan species within the European range.

Bsal risk status

High risk is based on lethal susceptibility of the sister species *L. helverseni* to Bsal and its restricted range. Human-mediated introduction can have high impact on this species. The high susceptibility is also based on its close relationship to the Bsal susceptible *Salamandra* genus.

Conservation Unit

The three subspecies occur in an area little more than 100 km, and even a smaller range in Europe alone (the island of Kastellorizon, Greece). Pending further delineation, the island of occurrence can be considered as conservation unit for this species.

Currently recognized subspecies

*Lyciasalamandra luschani basoglui**

Lyciasalamandra luschani finikensis

Lyciasalamandra luschani luschani

* Only *L. l. basoglui* occurs in Europe.

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4), although it is recommended to gain experience in keeping and breeding this species.

Ex situ management

Once the animals have become accustomed to their captive environment, they are quite easy to keep, but the species responds sensitively to changes in their environment. Propagating *Lyciasalamandra* species in captivity has proven to be rather difficult.

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low

European distribution of *Lyciasalamandra luschani*.

References

Gautier, P., et al. 2006. Gregarious behaviour in a salamander: Attraction to conspecific chemical cues in burrow choice. Behavioral Ecology and Sociobiology 59(6): 836-841.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Steinfartz, S. and Mutz T. 1999. *Mertensiella luschani* – Lykischer Salamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Veith, M. et al. 2016. Seven at one blow: the origin of major lineages of the viviparous Lycian salamanders (*Lyciasalamandra* Veith and Steinfartz, 2004) was triggered by a single paleo-historic event. Amphibia-Reptilia 37 (4): 373-387.

<u>Salamandridae</u>   *Pleurodeles waltl*   Sharp-ribbed newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
NA	NT	High	High	Low	Medium

Epidemiology

Dispersal

Limited dispersal. Study over 8 years showed 0.51 % movements > 250 m.

Density

Between 407-464 individuals/ha in Spain.

Co-occurrence

Salamandra salamandra, *Chioglossa lusitanica*, *Lissotriton helveticus*, *L. boscai*, *Triturus marmoratus* and *T. pygmaeus*.

Bsal risk status

High susceptibility to Bsal (laboratory experiments). Large range, although the presence of potential Bsal reservoir species within its range (i.e. *Triturus* species) and high probability of human-mediated introduction warrant caution.

Conservation Unit

Within the two major mtDNA lineages, several sublineages with a marked geographic pattern were identified, which can be considered as the units of conservation. In the case of the western lineage, two sublineages exist: one formed by the population of the Algarve (Southern Portugal) and the other grouping the remaining populations (Atlantic). In the case of the eastern lineage, three sub-clades were recovered (Mediterranean, Southern and Morocco).

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

European distribution of *Pleurodeles waltl*.

References

Gutiérrez-Rodríguez, J. et al. 2017. Integrative inference of population history in the Ibero-Maghrebian endemic *Pleurodeles waltl* (Salamandridae). Molecular Phylogenetics and Evolution 112: 122-137.

Gutiérrez-Rodríguez, J. et al. 2017. Effective to census population size ratios in two Near Threatened Mediterranean amphibians: *Pleurodeles waltl* and *Pelobates cultripes*. Conservation Genetics 18(5): 1201-1211.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Salvador A., and M. Garcia-Paris. 1999. *Pleurodeles waltl* – Spanischer Rippenmolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Vliet, van de, M. S. et al. 2014. Genetic divergence for the amphibian *Pleurodeles waltl* in Southwest Portugal: dispersal barriers shaping geographic patterns. Journal of Herpetology 48(1).

Salamandridae   *Salamandra atra*   Alpine salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex IV	LC	High	High	Low	Medium

Epidemiology

Dispersal

A fully terrestrial species which can disperse widely over land. High degree of site fidelity. Females show a higher level of philopatry than males.

Density

Local density can be high. Population density estimates vary from 97-770 animals/ha for *S. a. aurorae* to maximally 2000-3000 animals/ha for *S. a. atra*.

Co-occurrence

Ichthyosaura alpestris, Lissotriton helveticus, L. vulgaris, Salamandra salamandra and *Triturus cristatus*.

Bsal risk status

High risk is based on high likeliness of susceptibility to Bsal, restricted range, and presence of potential Bsal reservoir species within its range (i.e. *Ichthyosaura alpestris*). Human-mediated introduction can have high impact on this species. Several genetically distinct relict populations with small to very small ranges. Introduction of Bsal in the ranges of the subspecies *S. a. aurorae* and *S. a. pasubiensis* is likely to pose an acute threat to the survival of these lineages. An infection with Bsal is likely lethal based on close relationship to the Bsal susceptible species *S. salamandra* and presumed suitability of its niche for Bsal.

Conservation Unit

For this species, at least seven distinct genetic lineages can be discerned, which can be considered as conservation units. Three subspecies occupy small and fragmented (*S. a. prenjensis*) to very small ranges (*S. a. aurorae* (12 sites); *S. a. pasubiensis* (1 site)). The validity of the subspecies *S. a. prenjensis* has recently been proven. *S. a. pasubiensis* and *S. a. aurorae* have been assessed from vulnerable to critically endangered according IUCN criteria in global, national and regional red lists. The total distribution range of *S. a. aurorae* is smaller than 50km^2, *S. a. pasubiensis* is endemic to an open high valley.

Currently recognized subspecies

Salamandra atra atra   *Salamandra atra pasubiensis*

Salamandra atra aurorae*   *Salamandra atra prenjensis*

* Listed as priority (sub)species in Habitats Directive Annex II.

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4), although for the small-ranged *S. a. aurorae* and *S. a. pasubiensis*, the set-up of a preventive *ex situ* collection and active Bsal surveillance is recommended.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Difficult	Moderate	Low

European distribution of *Salamandra atra*.

References

Bonato, L. and G. Fracasso. 2003. Movements, distribution pattern and density in a population of *Salamandra atra aurorae* (Caudata: Salamandridae). Amphibia-Reptilia 24(3): 251-260.

Guex, G. and K. Grossenbacher. 2004. *Salamandra atra* – Alpensalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Romanazzi, E. and L. Bonato. 2014. Updating the range of the narrowly distributed endemites *Salamandra atra aurorae* and *S. atra pasubiensis*. Amphibia-Reptilia 35(1): 123-128.

Seidel, U. and P. Gerhardt. 2016. The genus Salamandra. History, Biology, Systematics, Captive breeding. Chimaira Buchhandelsgesellschaft mbH, Frankfurt am Main.

Sunje, E, R. van Damme, D. Jelic, M. Müller, R. Skrijelj, V. Helfer. 2018. Morphometric characteristics of Alpine salamanders: A support for subspecies validation and conservation? Amphibia-Reptilia 40(1).

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. M.A. Oliveira, P. Sousa, F. Sousa, and L. Gonçalves-Seco. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Salamandridae _Salamandra corsica_ Corsican fire salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
NA	LC	High	High	Medium	Medium

Epidemiology

Dispersal

A largely terrestrial species which can disperse widely over land. Its biology and dispersal potential appears to be generally similar to that of _S. salamandra_. Aquatic larvae may disperse by drift when deposited in streams.

Density

Presumably similar to _S. salamandra_.

Co-occurrence

Euproctus montanus.

Bsal risk status

High risk is based on lethal susceptibility to Bsal, restricted range and presumed suitability of its niche for Bsal. Although geographical barriers make natural introduction of Bsal unlikely, human-mediated introduction can have high impact on this species. Within its range, no obvious geographic barriers separate populations. Lethal infections have been observed in captive animals, with 100% morbidity and mortality. The species' close relationship to _S. salamandra_ corroborates high susceptibility.

Conservation Unit

The species is endemic to the island of Corsica. At least seven distinctive haplotypes can be distinguished, which can be considered as conservation units.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ **management**

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Moderate	High

European distribution of *Salamandra corsica*.

References

Escoriza, D. and A. Hernandez. 2019. Using hierarchical spatial models to assess the occurrence of an island endemism: the case of *Salamandra corsica.* Ecological Processes 8(1): 15.

Mutz, T. 2004. *Salamandra corsica* – Korsischer Feuersalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Salvi, D., et al. 2016. High phylogeographical complexity within Mediterranean islands: insights from the Corsican fire salamander. Journal of Biogeography 43(1): 192-203.

Seidel, U. and P. Gerhardt. 2016. The genus Salamandra. History, Biology, Systematics, Captive breeding. Chimaira Buchhandelsgesellschaft mbH, Frankfurt am Main.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. M.A. Oliveira, P. Sousa, F. Sousa, and L. Gonçalves-Seco. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Salamandridae   *Salamandra lanzai*   Lanza's salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex IV	VU	High	High	High	Medium

Epidemiology

Dispersal

A fully terrestrial species which can disperse widely over land. High degree of site fidelity.

Density

Local density can be high. Estimates vary from 300-733 animals/ha.

Co-occurrence

Ichthyosaura alpestris and *Salamandra salamandra.*

Bsal risk status

High risk is based on high likeliness of susceptibility to Bsal, restricted range, and presence of potential Bsal reservoir species within its range (i.e. *Ichthyosaura alpestris*). Human-mediated introduction can have high impact on this species. No indication of barriers between existing populations. Introduction of Bsal in the range of this species is likely to pose an acute threat to its survival. The species' Bsal susceptibility is likely lethal based on close relationship to the Bsal susceptible species *Salamandra salamandra*.

Conservation Unit

The level of intraspecific genetic isolation and variation is very low, both within and amongst populations. For *S. lanzai*, two conservation units (a French and an Italian) may be distinguished, which are not in contact with each other and show some extent of phenotypical differentiation. Owing to its restricted occurrence and small genetic variability, *S. lanzai* is threatened in its continued existence.

Currently recognized subspecies
NA

Species-specific actions

No specific *in situ* or *ex situ* conservation actions required (see § 4), although it is recommended to gain experience in keeping and breeding this species.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Moderate	Low

There is no available information on the husbandry and propagation of *S. lanzai*, but this may be comparable to *S. atra.*

European distribution of *Salamandra lanzai*.

References

Grossenbacher, K. 2004. *Salamandra lanzai* – Lanzas Salamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Miaud, C., et al. 2001. Variations in age, size at maturity and gestation duration among two neighbouring populations of the alpine salamander (*Salamandra lanzai*). Journal of Zoology 254(2): 251-260.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Seidel, U. and P. Gerhardt. 2016. The genus Salamandra. History, Biology, Systematics, Captive breeding. Chimaira Buchhandelsgesellschaft mbH, Frankfurt am Main.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. M.A. Oliveira, P. Sousa, F. Sousa, and L. Gonçalves-Seco. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Salamandridae   *Salamandra salamandra*   Fire salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
NA	LC	High	High	Low	Medium

Epidemiology

Dispersal

Terrestrial adults may disperse up to 980 m, high site fidelity, small home ranges (130-255 m2). Aquatic larvae may disperse by drift when deposited in streams.

Density

29 – 1458 individuals/ha.

Co-occurrence

Salamandra atra, *S. lanzai*, *Salamandrina perspicillata*, *S. terdigitata*, *Chioglossa lusitanica*, *Pleurodeles waltl*, *Calotriton asper*, *C. arnoldi*, *Lissotriton vulgaris*, *L. helveticus*, *L. italicus*, *L. boscai*, *L. montandoni*, *Ichthyosaura alpestris*, *Triturus cristatus*, *T. carnifex*, *T. macedonicus*, *T. dobrogicus*, *T. ivanbureschi*, *T. marmoratus*, *T. pygmaeus*, *Speleomantes italicus*, *S. ambrosii* and *S. strinatii*.

Bsal risk status

High risk is based on confirmed lethal susceptibility to Bsal, well-documented persistent significant population declines and presence of potential Bsal reservoir species within its range (i.e. *Ichthyosaura alpestris*). Bsal has been shown lethal for this species, both after experimental inoculation in lab experiments and after natural exposure in captivity and in the wild. The course of infection can be short and Bsal may be lethal in two weeks after initial exposure.

Conservation Unit

Pending more detailed identification of conservation units, the subspecies level appears appropriate. All subspecies apart from *Salamandra salamandra terrestris* and *S. s. salamandra* should be considered

as endemics with specific conservation priorities. Genetic analyses of fire salamanders from the Balkans are needed and may yield additional conservation units.

Currently recognized subspecies

S. s. almanzoris	*S. s. fastuosa*	*S. s. morenica*
S. s. bejarae	*S. s. gallaica*	*S. s. salamandra*
S. s. bernardezi	*S. s. gigliolii*	*S. s. terrestris*
S. s. crespoi	*S. s. longirostris*	

Due its doubtful status, *S. s. werneri* is not retained here as valid subspecies.

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

While most subspecies produce larvae, which experience an aquatic phase, some Iberian subspecies (*S. s. bernardezi* and *gallaica*) can also produce fully developed young. Given proper husbandry, this species can be relatively easy propagated in captivity, although not all subspecies breed easily.
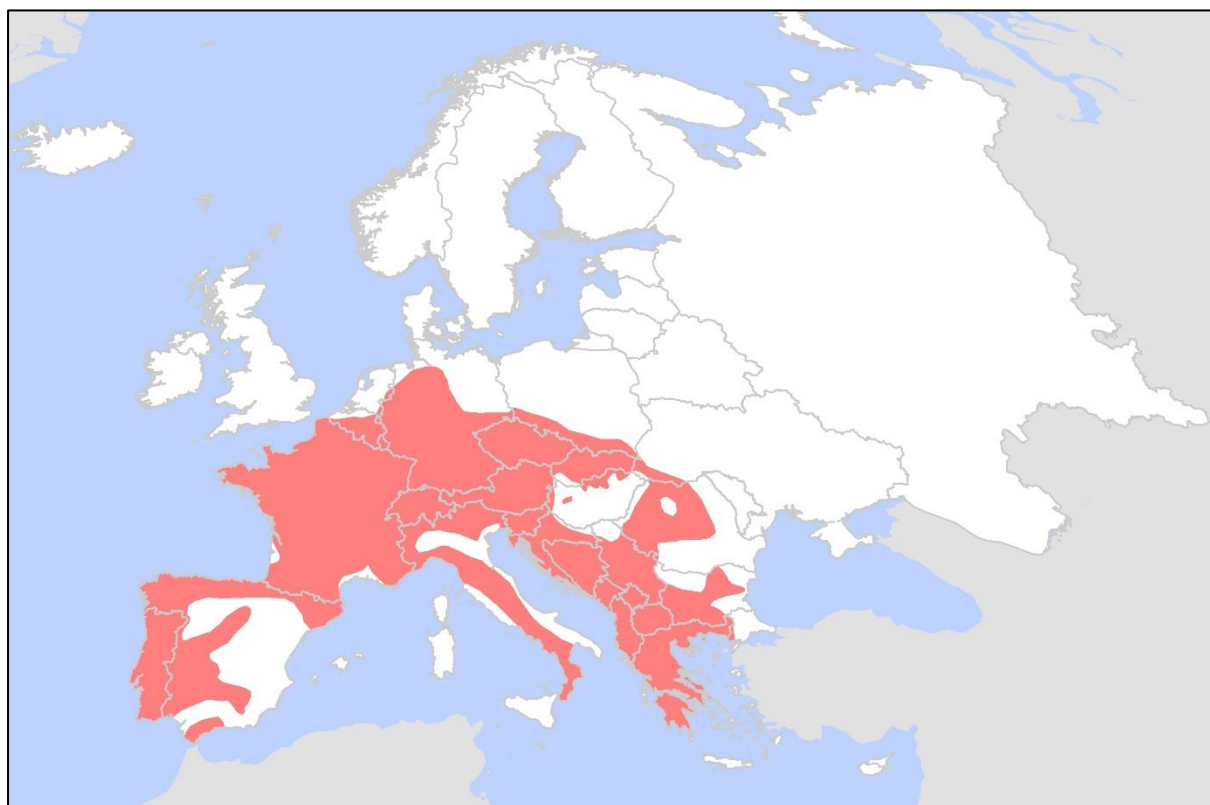
European distribution of *Salamandra salamandra*.

References

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Seidel, U. and P. Gerhardt. 2016. The genus Salamandra. History, Biology, Systematics, Captive breeding. Chimaira Buchhandelsgesellschaft mbH, Frankfurt am Main.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. M.A. Oliveira, P. Sousa, F. Sousa, and L. Gonçalves-Seco. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Thiesmeier, B. and. K. Grossenbacher. 2004. *Salamandra salamandra* – Feuersalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2, *Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Salamandridae *Salamandrina perspicillata* Northern spectacled salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	LC	High	High	Low	Medium

Epidemiology

Dispersal

Males are fully terrestrial, females deposit eggs in slow-running streams. Strong site fidelity, also to breeding sites.

Density

Local density can be high, up to 1600 individuals/ha.

Co-occurrence

Salamandra salamandra, *Lissotriton vulgaris*, *L. italicus*, *Ichthyosaura alpestris*, *Triturus carnifex*, *Speleomantes italicus*, *S. ambrosii* and *S. strinatii*.

Bsal risk status

Bsal has been shown lethal for this species in captivity (laboratory experiments). High risk is therefore based on the species' susceptibility to Bsal, its restricted range, and the presence of potential Bsal reservoir species within its range (i.e. *Ichthyosaura alpestris*). Human-mediated introduction can have high impact on this species.

Conservation Unit

For *S. perspicillata* the species level can be used as conservation unit, although southern Latium is a major genetic diversity reservoir and thus deserves particular conservation efforts. The species is endemic to Central and Northern Italy, and is widespread along the Apennine Mountains.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	High

Spectacled salamanders have been kept and bred in captivity, but they are delicate subjects. Raising larvae is not problematic, but rearing terrestrial juveniles is difficult.

European distribution of *Salamandrina perspicillata*.

References

Bogaerts, S. and F. pasmans (2002). Veldwaarnemingen en herhaalde kweek bij de brilsalamander *Salamandrina terdigatata*. Pod@rcis 3(2): 59-68.

Costa, A. et al. 2015. Assessing the abundance of *Salamandrina perspicillata* (Savi, 1821) using spatially and temporally replicated count data. In: Atti X Congresso Nazionale Societas Herpetologica Italica, p. 63-69. Doria, G. et al. (eds.) Inanieri Edizioni, Pescara.

Liuzzi, C., et al. 2011. New distribution and genetic data extend the ranges of the spectacled salamanders, genus *Salamandrina*, in the Apulia region (South Italy). Acta Herpetologica 6(2): 315-321.

Mattoccia, M. et al. 2011. Phylogeography of an Italian endemic salamander (genus Salamandrina): Glacial refugia, postglacial expansions, and secondary contact. Biological Journal of the Linnean Society 104: 903-922.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Romano, A., et al. 2009. Distribution and morphological characterization of the endemic Italian salamanders *Salamandrina perspicillata* (Savi, 1821) and *S. terdigitata* (Bonnaterre, 1789) (Caudata: Salamandridae). Italian Journal of Zoology 76(4): 422-432.

Romano, A., et al. 2018. Skewed sex ratio in a forest salamander: Artefact of the different capture probabilities between sexes or actual ecological trait? Amphibia-Reptilia 39(1): 79-86.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Zuffi, A. 1999. *Salamandrina terdigitata* – Brillensalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Salamandridae   *Salamandrina terdigitata*   Southern spectacled salamander

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	LC	High	High	Low	Medium

Epidemiology

Dispersal

Little information is available on the ecology and biology of this species, presumably quite similar to *S. perspicillata*. Males are fully terrestrial, females deposit eggs in slow-running streams, springs and small ponds.

Density

Unknown. Possibly similar to *S. perspicillata*.

Co-occurrence

Salamandra salamandra, Lissotriton vulgaris, *L. italicus, Ichthyosaura alpestris* and *Triturus carnifex*.

Bsal risk status

Bsal susceptibility has not been examined, likelihood is based on its close relationship to the Bsal susceptible species *Salamandrina perspicillata.* High risk is therefore based on the assumed species' susceptibility to Bsal, its restricted range, and the presence of potential Bsal reservoir species within its range (i.e. *Ichthyosaura alpestris*). Human-mediated introduction can have high impact on this species.

Conservation Unit

For *S. terdigitata* the species level can be used as conservation unit, although Calabria is a major genetic diversity reservoir and thus deserves particular conservation efforts. The species is endemic to southern peninsular Italy.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ **management**

| Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	High

Spectacled salamanders have been kept and bred in captivity, but they are delicate subjects. Raising larvae is not problematic, but rearing terrestrial juveniles is difficult.

European distribution of *Salamandrina terdigitata*.

References

Liuzzi, C., et al. 2011. New distribution and genetic data extend the ranges of the spectacled salamanders, genus *Salamandrina*, in the Apulia region (South Italy). Acta Herpetologica 6(2): 315-321.

Mattoccia, M., et al. 2011. Phylogeography of an Italian endemic salamander (genus Salamandrina): Glacial refugia, postglacial expansions, and secondary contact. Biological Journal of the Linnean Society 104: 903-922.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Romano, A., et al. 2009. Distribution and morphological characterization of the endemic Italian salamanders *Salamandrina perspicillata* (Savi, 1821) and *S. terdigitata* (Bonnaterre, 1789) (Caudata: Salamandridae). Italian Journal of Zoology 76(4): 422-432.

Romano, A. et al. 2018. Skewed sex ratio in a forest salamander: Artefact of the different capture probabilities between sexes or actual ecological trait? Amphibia-Reptilia 39(1): 79-86.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Zuffi, A. 1999. *Salamandrina terdigitata* – Brillensalamander. In: Handbuch der Reptilien und Amphibien Europas. Band 4/I. Schwanzlurche (Urodela) I. (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles, Salamandrina, Euproctus, Chioglossa, Mertensiella*)(eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Salamandridae   *Triturus carnifex*   Italian newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	LC	High	High	Low	Medium
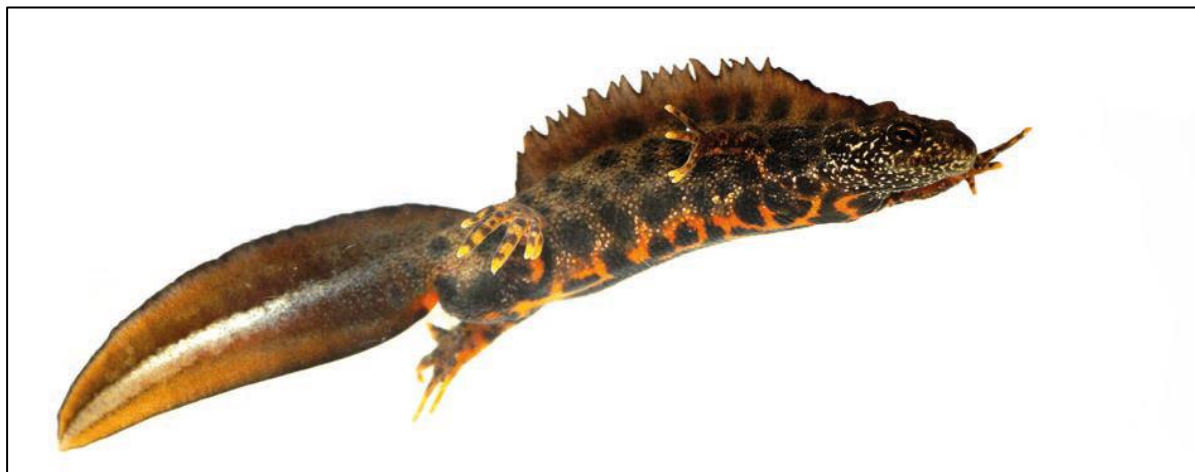
Epidemiology

Dispersal

At least 300 m, but probably equal to other *Triturus* species.

Density

Counts vary between 1-212 individuals/<30 m^2 pond.

Co-occurrence

Salamandra salamandra, Salamandrina perspicillata, S. terdigitata, Lissotriton vulgaris, L. italicus, Ichthyosaura alpestris, Triturus cristatus, T. dobrogicus, Speleomantes italicus, S. ambrosii and *S. strinatii*.

Bsal risk status

Susceptibility to Bsal has not been examined in the laboratory. Likely highly susceptible based on close relationship to the Bsal susceptible species *Triturus cristatus*. The species has a wide range, co-occurs with vectoring species and the risk of human-induced introduction of Bsal is realistic.

Conservation Unit

Three major lineages can be distinguished throughout the distribution range of *T. carnifex*, which can be considered as conservation units. One of these clades occurs south of the northern Apennine Mountains, the second along the Venetian and Po Plains and the distribution range of the third clade lies in the northern Balkans. The Balkan clade is genetically particularly distinct from all other populations.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

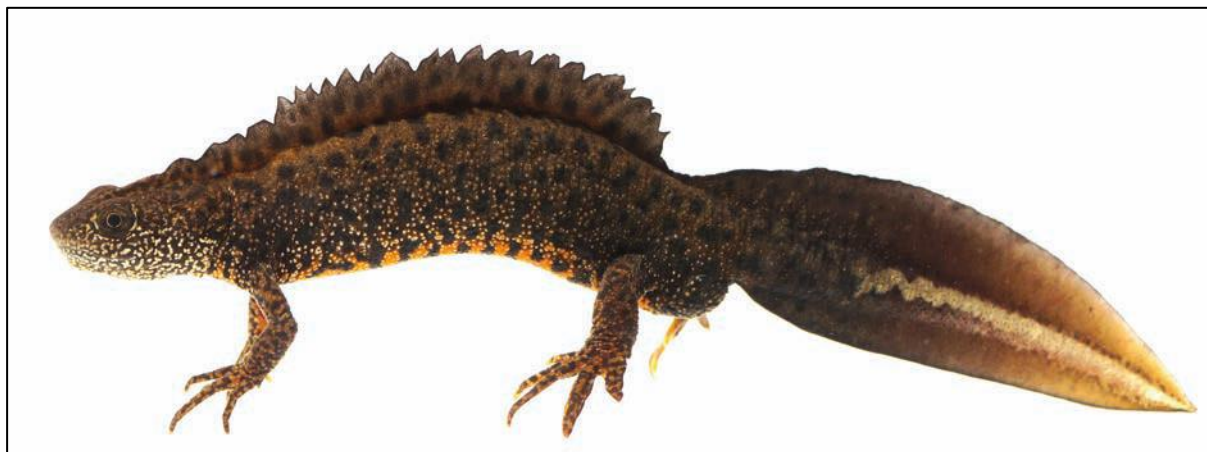| Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High
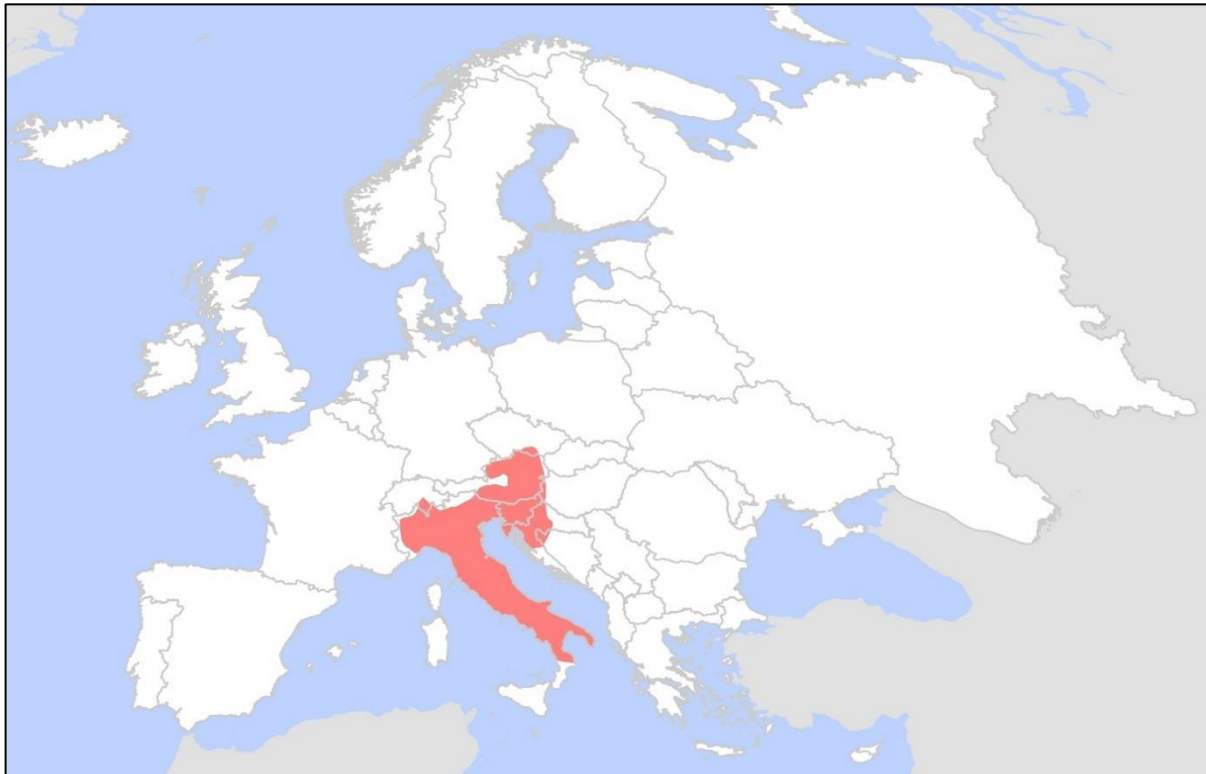
European distribution of *Triturus carnifex*.

References

Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Fahrbach, M. and W. Gerlach. 2018. The genus *Triturus*. History, Biology, Systematics, Captive Breeding. Chimaira, Frankfurt am Main.

Giacoma, C., et al. 1988. The Distribution and Habitats of the Newt (*Triturus*, Amphibia) in Calabria (Southern Italy). Monitore Zoologico Italiano - Italian Journal of Zoology 22(4): 449-464.

Grosse, W. R. (Ed.). 2018. Threatened Newts and Salamanders of the World – Captive Care Management. Mertensiella Vol. 2, 292 p.

Schabetsberger, R., et al. 2004. Delineation of terrestrial reserves for amphibians: post-breeding migrations of Italian crested newts (*Triturus c. carnifex*) at high altitude. Biological Conservation 117(1): 95-104.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

<u>Salamandridae</u>   <u>*Triturus cristatus*</u>   <u>Great crested newt</u>

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	LC	High	High	Low	Medium

Epidemiology

Dispersal

Max. dispersal ranges of 1290 m and 860 m have been reported for adults and juveniles, respectively. A range expansion of 30 km in 30 years has been recorded, corresponding to an average dispersal rate of 1 km/year.

Density

Tends to be less numerous compared to other small-bodied newts. Populations usually small, with 20-40 adults/population. Based on 50 different studies, a maximum of 1459±75 and a median of 101 individuals per population.

Co-occurrence

Salamandra salamandra, *S. atra*, *Lissotriton vulgaris*, *L. helveticus*, *L. montandoni*, *Triturus carnifex*, *T. macedonicus*, *T. dobrogicus*, *T. ivanbureschi*, *T. marmoratus* and *Salamandrella keyserlingii*.

Bsal risk status

High risk based on high susceptibility to Bsal (laboratory experiments) and suspected Bsal-related declines in nature. The species has a large range, but co-occurrence with Bsal vectoring species and high susceptibility warrant caution.

Conservation Unit

Three major lineages can be distinguished, which can be considered as conservation units. Genetically quite homogeneous across most of its range, with two distinct lineages in Eastern Europe, which result

from an extra-Mediterranean refugium in the Carpathian Basin. Hybridisation is commonplace in all regions where individual *Triturus* species encounter each other.

### Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High
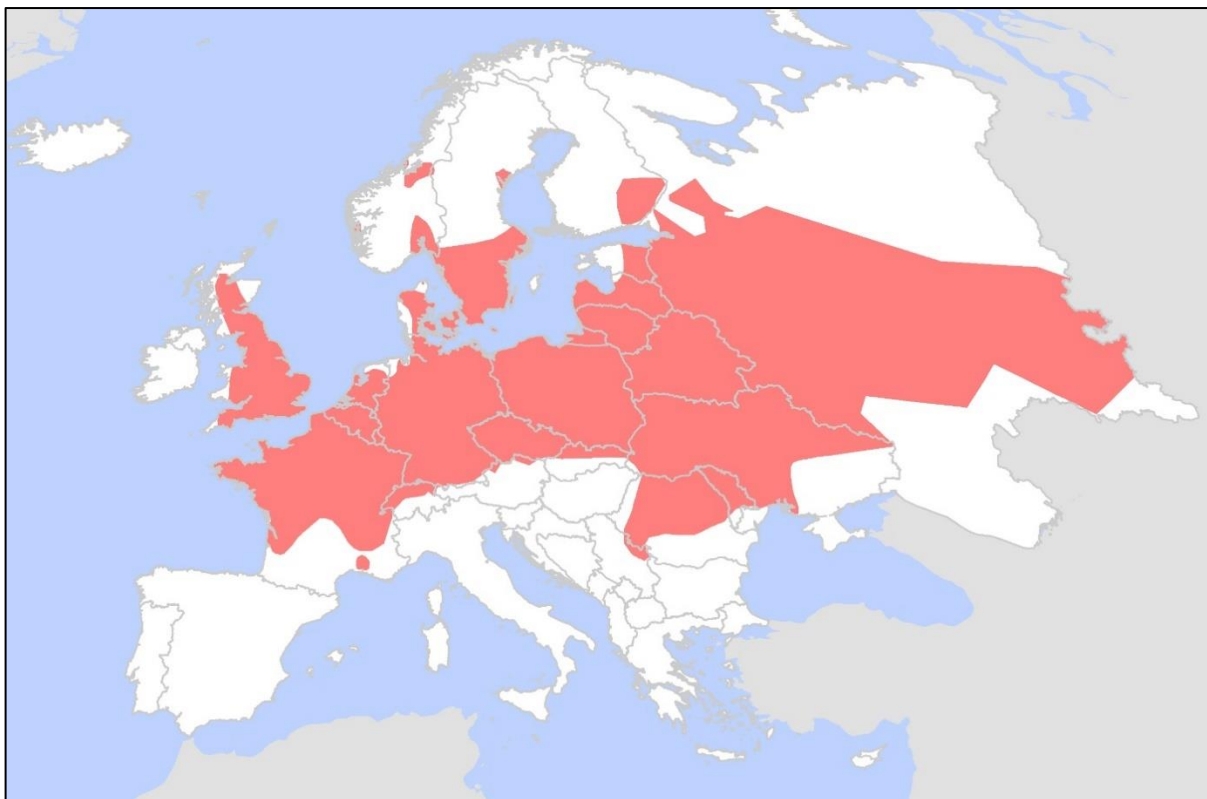
European distribution of *Triturus cristatus*.

References

Arntzen, J. W. and G. P. Wallis 1991. Restricted gene flow in a moving hybrid zone of the newts *Triturus cristatus* and *T. marmoratus* in western France. Evolution 45(4): 805-826.

Arntzen, J. W. 2004. *Triturus cristatus* Superspecies – Kammolch Artenkreis. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Fahrbach, M. and W. Gerlach. 2018. The genus *Triturus*. History, Biology, Systematics, Captive Breeding. Chimaira, Frankfurt am Main.

Kupfer, A. and S. Kneitz 2000. Population ecology of Great Crested Newts (*Triturus cristatus*) in an agricultural landscape: dynamics, pond fidelity and dispersal. Herpetological Journal 10: 165-171.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Wielstra, B., et al. 2013. Tracing glacial refugia of *Triturus* newts based on mitochondrial DNA phylogeography and species distribution modeling. Frontiers in Zoology 10(1).

Wielstra, B., et al. 2015. The crested newt *Triturus cristatus* recolonized temperate Eurasia from an extra-Mediterranean glacial refugium. Biological Journal of the Linnean Society 114: 574–587.

Salamandridae *Triturus dobrogicus* Danube crested newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II	NT	High	High	Low	Medium

Epidemiology

Dispersal

Prolonged aquatic period, strictly aquatic in some instances, comparable to *T. cristatus*.

Density

Likely similar to *T. cristatus*.

Co-occurrence

Salamandra salamandra, *Lissotriton vulgaris*, *Ichthyosaura alpestris*, *Triturus cristatus*, *T. macedonicus* and *T. ivanbureschi*.

Bsal risk status

Susceptibility to Bsal has not been examined in the laboratory. Likely highly susceptible based on close relationship to the Bsal susceptible species *Triturus cristatus*. The species co-occurs with vectoring species such as *Ichthyosaura alpestris* and the risk of human-induced introduction of Bsal is realistic.

Conservation Unit

Two major mtDNA lineages exist, which show a high level of admixture and occur over the entire species' range. As such, these cannot be used as conservation units, and the species level should considered as the unit of conservation.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ **management**

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

European distribution of *Triturus dobrogicus*.

References

Arntzen, J. W. 2004. *Triturus cristatus* Superspecies – Kammolch Artenkreis. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Fahrbach, M. and W. Gerlach. 2018. The genus *Triturus*. History, Biology, Systematics, Captive Breeding. Chimaira, Frankfurt am Main.

Schultschik, G. and W. R. Grosse (eds.). 2013. Threatened Newts and Salamanders - Guidelines for Conservation Breeding. - Mertensiella 20e, 180 p.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Vörös, J., et al. 2016. Phylogeographic analysis reveals northerly refugia for the riverine amphibian *Triturus dobrogicus* (Caudata: Salamandridae). Biological Journal of the Linnean Society 119(4): 974-991.

Wielstra, B., et al. 2016. Is the Danube crested newt *Triturus dobrogicus* polytypic? A review and new nuclear DNA data. Amphibia-Reptilia 37(2): 167-177.

Salamandridae   *Triturus ivanbureschi*   Buresch's crested newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	NE	High	High	Low	Medium

Epidemiology

Dispersal

May hibernate in breeding pond, but not in its entire range, likely similar to *T. cristatus*.

Density

Presumed similar to *T. cristatus*. In Turkey densities in ponds range between 0.2-1.3 individuals/m^2.

Co-occurrence

Salamandra salamandra, *Lissotriton graecus*, *L. schmidtleri*, *L. vulgaris*, *Ichthyosaura alpestris*, *Triturus cristatus*, *T. dobrogicus* and *T. macedonicus*.

Bsal risk status

Susceptibility to Bsal has not been examined in the laboratory. Likely highly susceptible based on close relationship to the Bsal susceptible species *Triturus cristatus*. The species co-occurs with vectoring species such as *Ichthyosaura alpestris* and the risk of human-induced introduction of Bsal is realistic.

Conservation Unit

Three major lineages exist, of which one occurs within Europe, while the other two occur in western Turkey. These lineages can be considered as conservation units.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

European distribution of *Triturus ivanbureschi*.

References

Arntzen, J. W. 2004. *Triturus cristatus* Superspecies – Kammolch Artenkreis. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Fahrbach, M. and W. Gerlach. 2018. The genus *Triturus*. History, Biology, Systematics, Captive Breeding. Chimaira, Frankfurt am Main.

Göçmen, B., et al. 2015. Population size estimates of *Lissotriton vulgaris* (L., 1758) and *Triturus ivanbureschi* Arntzen and Wielstra 2013 (Caudata: Salamandridae) from Edirne, European part of Turkey. North-Western Journal of Zoology 11(2): 342-346.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Wielstra, B., et al. 2013. A revised taxonomy of crested newts in the *Triturus karelinii* group (Amphibia: Caudata: Salamandridae), with the description of a new species. Zootaxa 3682(3): 441-453.

Wielstra, B., et al. 2013. Tracing glacial refugia of *Triturus* newts based on mitochondrial DNA phylogeography and species distribution modeling. Frontiers in Zoology 10(1).

Salamandridae   *Triturus karelinii*   Karelin's crested newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	LC	High	High	High	High
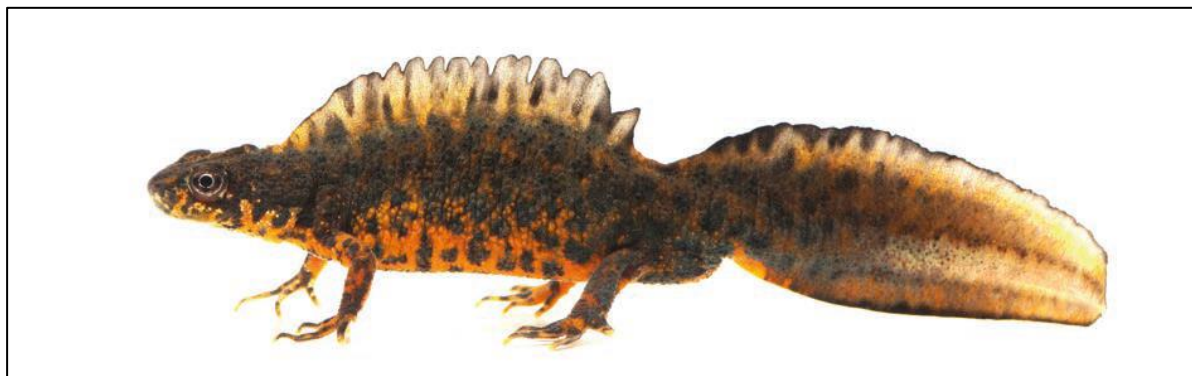
Epidemiology

Dispersal

Probably similar to *T. cristatus*, more tolerant to dry habitats than other *Triturus* species.

Density

Likely similar to *T. cristatus*.

Co-occurrence

Lissotriton vulgaris.

Bsal risk status

Susceptibility to Bsal has not been examined in the laboratory. Likely highly susceptible based on close relationship to the Bsal susceptible species *Triturus cristatus*. Risk of human-mediated introduction. High risk based on small distribution range within Europe, although the species' range is larger outside the area considered here.

Conservation Unit

Little genetic variation across the species' range. As such, the species can be considered as the unit of conservation.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ **management**

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

European distribution of *Triturus karelinii*.

References

Arntzen, J. W. 2004. *Triturus cristatus* Superspecies – Kammolch Artenkreis. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Fahrbach, M. and W. Gerlach. 2018. The genus *Triturus*. History, Biology, Systematics, Captive Breeding. Chimaira, Frankfurt am Main.

Grosse, W. R. (Ed.). 2018. Threatened Newts and Salamanders of the World – Captive Care Management. Mertensiella Vol. 2, 292 p.

Mermer, A., et al. 2008. Abundance of syntopic newts, *Triturus karelinii* (Strauch, 1870) and *Triturus vittatus* (Gray, 1835) in Uludağ National Park (Bursa, Turkey). Turkish Journal of Zoology 32(1): 59-64.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Wielstra, B., et al. 2013. A revised taxonomy of crested newts in the *Triturus karelinii* group (Amphibia: Caudata: Salamandridae), with the description of a new species. Zootaxa 3682(3): 441-453.

Wielstra, B., et al. 2013. Tracing glacial refugia of *Triturus* newts based on mitochondrial DNA phylogeography and species distribution modeling. Frontiers in Zoology 10(1).

Salamandridae   *Triturus macedonicus*   Macedonian crested newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex II/IV	NE	High	High	Low	Medium

Epidemiology

Dispersal

An ecologically flexible species, comparable to *T. carnifex*.

Density

Likely similar to *T. carnifex*.

Co-occurrence

Salamandra salamandra, *Lissotriton graecus*, *L. vulgaris*, *Ichthyosaura alpestris*, *Triturus cristatus*, *T. dobrogicus* and *T. ivanbureschi*.

Bsal risk status

Susceptibility to Bsal has not been examined in the laboratory. Likely highly susceptible based on close relationship to the Bsal susceptible species *Triturus cristatus*. The species co-occurs with vectoring species and the risk of human-induced introduction of Bsal is realistic.

Conservation Unit

At least three major lineages exist, which are separated by the Pindos mountains. These lineages can be considered as conservation units. Genetic diversity is highest along the species' southern distribution range. Exact distribution of this species needs to be determined.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

European distribution of *Triturus macedonicus*.

References

Arntzen, J. W. 2004. *Triturus cristatus* Superspecies – Kammolch Artenkreis. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIB. Schwanzlurche (Urodela) IIB. Salamandridae III: *Triturus* 2*, Salamandra.* (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Fahrbach, M. and W. Gerlach. 2018. The genus *Triturus*. History, Biology, Systematics, Captive Breeding. Chimaira, Frankfurt am Main.

Grosse, W. R. (Ed.). 2018. Threatened Newts and Salamanders of the World – Captive Care Management. Mertensiella Vol. 2, 292 p.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Wielstra, B., et al. 2013. Tracing glacial refugia of *Triturus* newts based on mitochondrial DNA phylogeography and species distribution modeling. Frontiers in Zoology 10(1).

Salamandridae   *Triturus marmoratus*   Marbled newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex IV	LC	High	High	Low	Medium

Epidemiology

Dispersal

Summer refuges are in close range of the breeding site (few meters), animals migrate up to 146 m/31 days.

Density

A study in France mentions 3-4 individuals/m^2 pond.

Co-occurrence

Salamandra salamandra, *Chioglossa lusitanica*, *Pleurodeles waltl*, *Calotriton asper*, *Lissotriton vulgaris*, *L. helveticus*, *L. boscai*, *Ichthyosaura alpestris*, *Triturus cristatus* and *T. pygmaeus*.

Bsal risk status

High risk based on high susceptibility to Bsal (laboratory experiments) and mortality in the field. Co-occurs with reservoir species such as *Ichthyosaura alpestris*. Risk of human-mediated introduction.

Conservation Unit

Little genetic variation across the species' range. As such, the species can be considered as the unit of conservation.

Currently recognized subspecies
NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ **management**

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High
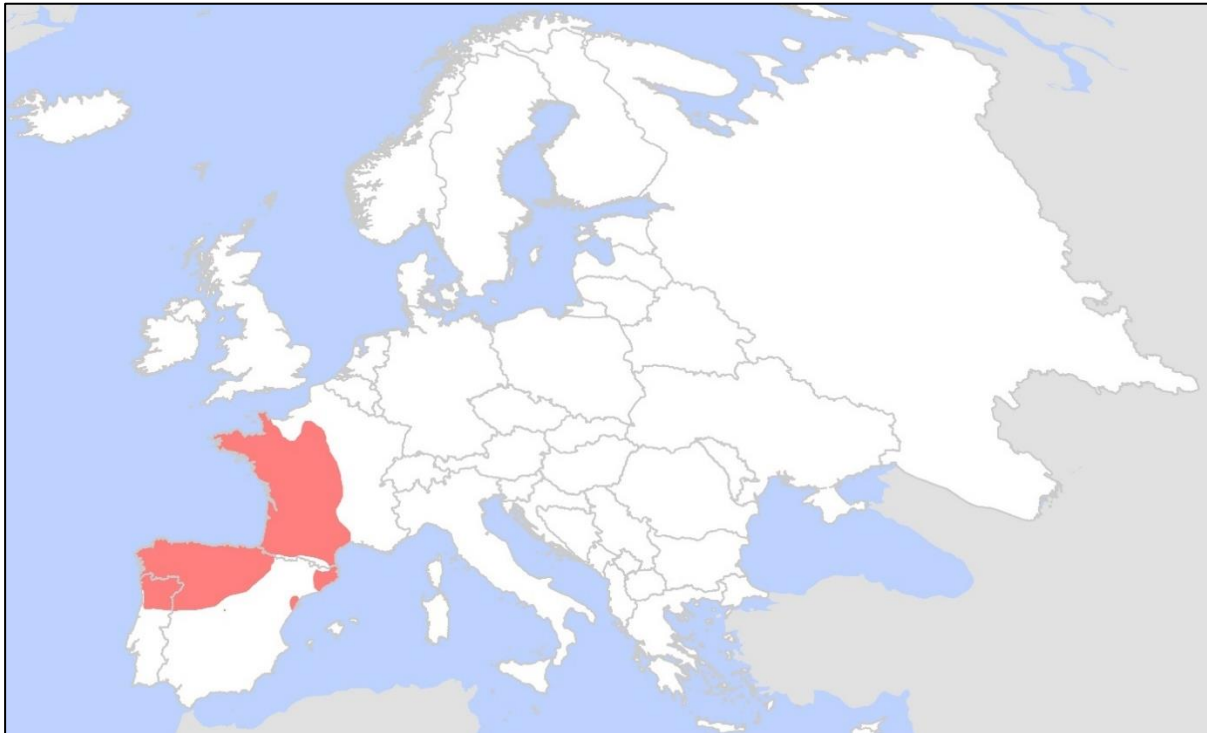
European distribution of *Triturus marmoratus*.

References

Arntzen J., et al. 2018. Morphological and molecular characters to describe a marbled newt hybrid zone in the Iberian peninsula. Contributions to Zoology 87(3):167-185.

Fahrbach, M. and W. Gerlach. 2018. The genus *Triturus*. History, Biology, Systematics, Captive Breeding. Chimaira, Frankfurt am Main.

Herrero, P., A. Montori and B. Arano. 2003. *Triturus marmoratus* (Latreille, 1800)- Nördlicher Marmormolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIA. Schwanzlurche (Urodela) IIA. Salamandridae II: *Triturus* 1 (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Jehle, R. 2000. The terrestrial summer habitat of radio-tracked great crested newts (*Triturus cristatus*) and marbled newts (*T. marmoratus*). Herpetological Journal 10(4): 137-142.

Jehle, R., et al. 2001. The annual number of breeding adults and the effective population size of syntopic newts (*Triturus cristatus*, *T. marmoratus*). Molecular Ecology 10(4): 839-850.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Rivera, X., et al. 2011. Preliminary survey of the genetic variability of the Marbled Newt *Triturus marmoratus* (Latreille 1800) (Amphibia; Caudata; Salamandridae) in Catalonia. Butll. Soc. Cat. Herp. 19: 15-20.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Wielstra, B., et al. 2013. Tracing glacial refugia of *Triturus* newts based on mitochondrial DNA phylogeography and species distribution modeling. Frontiers in Zoology 10(1).

Salamandridae   *Triturus pygmaeus*   Southern marbled newt

Habitats Directive	Red List	Bsal susceptibility	Bsal risk		
			Population level	Taxon level (10 yr)	Taxon level (100 yr)
Annex IV	NT	High	High	Low	Medium

Epidemiology

Dispersal

Limited dispersal capacity, mountainous terrain functions as a dispersal barrier.

Density

Density may be high in reproductive water, a Spanish study estimated 1000 individuals in a temporary pond (pond size varied over time between 60-880 m^2).

Co-occurrence

Salamandra salamandra, *Pleurodeles waltl*, *Lissotriton boscai* and *Triturus marmoratus*.

Bsal risk status

Susceptibility to Bsal has not been examined in the laboratory. Likely highly susceptible based on close relationship to the Bsal susceptible species *Triturus marmoratus*. At relatively large distance to known Bsal presence, without major geographic barriers. Risk of human-mediated introduction.

Conservation Unit

Little genetic variation across the species' range. As such, the species can be considered as the unit of conservation. *T. pygmaeus* and *T. marmoratus* are largely parapatric, but may hybridise. *T. pygmaeus* seems to be expanding north at the expense of *T. marmoratus*.

Currently recognized subspecies

NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

Ex situ **management**

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

European distribution of *Triturus pygmaeus*.

References

Arntzen J., et al. 2018. Morphological and molecular characters to describe a marbled newt hybrid zone in the Iberian peninsula. Contributions to Zoology 87(3):167-185.

Díaz-Paniagua, C. 1998. Reproductive dynamics of a population of small marbled newts (*Triturus marmoratus pygmaeus*) in South-Western Spain. Herpetological Journal 8: 93-98.

Herrero, P., A. Montori and B. Arano. 2003. *Triturus pygmaeus* (Wolterstorff, 1905)- Südlicher Marmormolch. In: Handbuch der Reptilien und Amphibien Europas. Band 4/IIA. Schwanzlurche (Urodela) IIA. Salamandridae II: *Triturus* 1 (eds. B. Thiesmeier and K. Grossenbacher). AULA-Verlag.

Fahrbach, M. and W. Gerlach. 2018. The genus *Triturus*. History, Biology, Systematics, Captive Breeding. Chimaira, Frankfurt am Main.

Schultschik, G. and W. R. Grosse (eds.). 2013. Threatened Newts and Salamanders - Guidelines for Conservation Breeding. - Mertensiella 20e, 180 p.

Sillero, N. et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies. Amphibia-Reptilia 35 (1): 1-31.

Sillero, N. et al. 2014. Distributed database system of the New Atlas of Amphibians and Reptiles in Europe: the NA2RE project. Amphibia-Reptilia 35 (1): 33-39.

Sparreboom, M. 2014. Salamanders of the Old World. KNNV publishing, Zeist, the Netherlands.

Themudo, G. E. and J. W. Arntzen 2007. Newts under siege: range expansion of Triturus pygmaeus isolates populations of its sister species. Diversity and Distributions 13(5): 580-586.

Themudo, G. E., et al. 2012. Is dispersal guided by the environment? A comparison of interspecific gene flow estimates among differentiated regions of a newt hybrid zone. Molecular Ecology 21(21): 5324-5335.

Wielstra, B., et al. 2013. Tracing glacial refugia of *Triturus* newts based on mitochondrial DNA phylogeography and species distribution modeling. Frontiers in Zoology 10(1).

References

Bates KA et al. 2019. Captivity and infection by the fungal pathogen *Batrachochytrium salamandrivorans* perturb the amphibian skin microbiome. Frontiers in Microbiology. 10:1834.

Beukema W et al. 2018. Environmental context and differences between native and invasive observed niches of *Batrachochytrium salamandrivorans* affect invasion risk assessments in the Western Palaearctic. Diversity and Distributions. 1-14.

Blooi M et al. 2015a. Treatment of urodelans based on temperature dependent infection dynamics of *Batrachochytrium salamandrivorans*. Scientific Reports. 5:8037.

Blooi M et al. 2015b. Successful treatment of *Batrachochytrium salamandrivorans* infections in salamanders requires synergy between voriconazole, polymyxin E and temperature. Scientific Reports. 5:11788.

Bosch J et al. 2015. Successful elimination of a lethal wildlife infectious disease in nature. Biology Letters. 11:10.1098/rsbl.2015.0874.

Bosch J, Fernández-Beaskoetxea S, Garner TW, Carrascal LM. 2018. Long-term monitoring of an amphibian community after a climate change-and infectious disease-driven species extirpation. Global Change Biology. 24:2622-2632.

Bozzuto C & Canessa S. 2019. Impact of seasonal cycles on host-pathogen dynamics and disease mitigation for *Batrachochytrium salamandrivorans*. Global Ecology and Conservation. 17:e00551.

Brannelly L et al. 2018. Non-declining amphibians can be important reservoir hosts for amphibian chytrid fungus. Animal Conservation. 21:91-101.

Byrne PG & Silla AJ. 2010. Hormonal induction of gamete release, and in-vitro fertilisation, in the critically endangered Southern Corroboree Frog, *Pseudophryne corroboree*. Reproductive Biology and Endocrinology. 8:144.

Canessa S et al. 2016. Planning for ex situ conservation in the face of uncertainty. Conservation Biology. 30:599-609.

Canessa S et al. 2018. Decision-making for mitigating wildlife diseases: From theory to practice for an emerging fungal pathogen of amphibians. J.Appl.Ecol. 55:1987-1996.

Canessa S, Bozzuto C, Pasmans F, Martel A. 2019. Quantifying the burden of managing wildlife diseases in multiple host species. Conservation Biology. 33:1131-1140.

Canessa S et al. 2020. Conservation decisions under pressure: Lessons from an exercise in rapid response to wildlife disease. Conservation Science and Practice. 2:e141.

Collen B et al. 2016. Clarifying misconceptions of extinction risk assessment with the IUCN Red List. Biology Letters. 12:20150843.

Cunningham AA et al. 2019. Apparent absence of *Batrachochytrium salamandrivorans* in wild urodeles in the United Kingdom. Scientific Reports. 9:2831.

Dalbeck L et al. 2018. Die Salamanderpest und ihr Erreger *Batrachochytrium salamandrivorans* (Bsal): aktueller Stand in Deutschland. Zeitschrift für Feldherpetologie. 25:1-22.

Diekmann, O., Heesterbeek, H. & Britton, T. (2012). *Mathematical tools for understanding infectious disease dynamics.* Princeton University Press.

EFSA Panel on Animal Health and Welfare (AHAW) et al. 2018. Risk of survival, establishment and spread of *Batrachochytrium salamandrivorans* (Bsal) in the EU. Efsa Journal. 16:e05259.

Fahrbach, M. and W. Gerlach. 2018. The genus *Triturus*. History, Biology, Systematics, Captive Breeding. Chimaira, Frankfurt am Main.

Fitzpatrick LD, Pasmans F, Martel A, Cunningham AA. 2018. Epidemiological tracing of *Batrachochytrium salamandrivorans* identifies widespread infection and associated mortalities in private amphibian collections. Scientific Reports. 8:13845.

Garner TW et al. 2016. Mitigating amphibian chytridiomycoses in nature. Philosophical Transactions of the Royal Society B: Biological Sciences. 371:20160207.

Gilbert MJ, Spitzen-van der Sluijs A, Stark T. 2019. Surveillance naar *Batrachochytrium salamandrivorans* in de handel. Eindrapportage 2018. RAVON, Nijmegen.

Gorički Š et al. 2017. Environmental DNA in subterranean biology: range extension and taxonomic implications for *Proteus*. Scientific Reports. 7:45054.

Grant EHC et al. 2016. Salamander chytrid fungus (*Batrachochytrium salamandrivorans*) in the United States—Developing research, monitoring, and management strategies. Salamander chytrid fungus (*Batrachochytrium salamandrivorans*) in the United States—Developing research, monitoring, and management strategies.

Grant EHC et al. 2017. Using decision analysis to support proactive management of emerging infectious wildlife diseases. Frontiers in Ecology and the Environment. 15:214-221.

Grosse, W. R. (Ed.). 2018. Threatened Newts and Salamanders of the World – Captive Care Management. Mertensiella. 2, 292 p.

Hemming V, Burgman MA, Hanea AM, McBride MF, Wintle BC. 2018. A practical guide to structured expert elicitation using the IDEA protocol. Methods in Ecology and Evolution. 9:169-180.

Hyatt A et al. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Dis.Aquat.Org. 73:175-192.

IUCN/SSC. 2013. Guidelines for reintroductions and other conservation translocations. Version 1.0.

Kouba AJ et al. 2013. Emerging trends for biobanking amphibian genetic resources: the hope, reality and challenges for the next decade. Biological Conservation. 164:10-21.

Laking AE, Ngo HN, Pasmans F, Martel A, Nguyen TT. 2017. *Batrachochytrium salamandrivorans* is the predominant chytrid fungus in Vietnamese salamanders. Scientific Reports. 7:44443.

Langwig KE et al. 2015. Context-dependent conservation responses to emerging wildlife diseases. Frontiers in Ecology and the Environment. 13:195-202.

Lanza B. 2006. A review of systematics, taxonomy, genetics, biogeography and natural history of the genus *Speleomantes* Dubois, 1984 (Amphibia Caudata Plethodontidae). Atti del Museo Civico di Storia Naturale di Trieste. 52:5-135.

Lawson B, Petrovan SO, Cunningham AA. 2015. Citizen science and wildlife disease surveillance. EcoHealth. 12:693-702.

Lindgren E, Andersson Y, Suk JE, Sudre B, Semenza JC. 2012. Monitoring EU emerging infectious disease risk due to climate change. Science. 336:418-419.

Martel A et al. 2013. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. Proceedings of the National Academy of Sciences of the United States of America. 110:15325-15329.

Martel A et al. 2014. Wildlife disease. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. Science. 346:630-631.

Martel A et al. 2020. Integral chain management of wildlife diseases. Conservation Letters. e12707.

Martin TG et al. 2012. Eliciting expert knowledge in conservation science. Conservation Biology. 26:29-38.

McBride MF et al. 2012. Structured elicitation of expert judgments for threatened species assessment: a case study on a continental scale using email. Methods in Ecology and Evolution. 3:906-920.

Nguyen TT, Van Nguyen T, Ziegler T, Pasmans F, Martel A. 2017. Trade in wild anurans vectors the urodelan pathogen *Batrachochytrium salamandrivorans* into Europe. Amphibia-Reptilia. 38:554-556.

Pabijan M, Zieliński P, Dudek K, Stuglik M, Babik W. 2017. Isolation and gene flow in a speciation continuum in newts. Molecular Phylogenetics and Evolution. 116:1-12.

Pasmans, F., S. Bogaerts, H. Janssen and M. Sparreboom. 2014. Salamanders. Keeping and breeding, Natur und Tier - Verlag GmbH.

Reinhardt F, Herle M, Bastiansen F, Streit B. 2003. Economic impact of the spread of alien species in Germany. Environmental Research of the Federal Ministry of the Environment, Nature Conservation and Nuclear Safety, Research Report. 201:211.

Sabino-Pinto J et al. 2015. First evidence of *Batrachochytrium salamandrivorans* from a captive salamander population in Germany. Amphibia-Reptilia. 36:411-416.

Sabino-Pinto J, Veith M, Vences M, Steinfartz S. 2018. Asymptomatic infection of the fungal pathogen *Batrachochytrium salamandrivorans* in captivity. Scientific Reports. 8:11767.

Schad K. 2008. Amphibian Population Management Guidelines.Amphibian Ark Amphibian Population Management Workshop; 2007 December 10-11; San Diego, CA, USA. Amphibian Ark.

Scheele BC et al. 2014. Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians. Conservation Biology. 28:1195-1205.

Scheele BC et al. 2017. After the epidemic: ongoing declines, stabilizations and recoveries in amphibians afflicted by chytridiomycosis. Biological Conservation. 206:37-46.

Scheele BC et al. 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science. 363:1459-1463.

Schmidt B. 2016. Import ban for salamanders and newts in Switzerland. Why? Terraria/Elaphe. 57:8-9.

Schmidt BR, Bozzuto C, Lötters S, Steinfartz S. 2017. Dynamics of host populations affected by the emerging fungal pathogen *Batrachochytrium salamandrivorans*. Royal Society Open Science. 4:160801.

Schultschik, G. and W. R. Grosse (eds.). 2013. Threatened Newts and Salamanders - Guidelines for Conservation Breeding. Mertensiella. 20e, 180 p.

Seidel, U. and P. Gerhardt. 2016. The genus Salamandra. History, Biology, Systematics, Captive breeding. Chimaira Buchhandelsgesellschaft mbH, Frankfurt am Main.

Sillero N et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe. Amphibia-Reptilia. 35:1-31.

Spitzen-van der Sluijs A et al. 2018. Post-epizootic salamander persistence in a disease-free refugium suggests poor dispersal ability of *Batrachochytrium salamandrivorans*. Scientific Reports. 8:3800.

Spitzen-van der Sluijs, A. 2018. It takes three to tango. The impact of chytridiomycosis on native amphibians in the Netherlands. PhD thesis, Ghent University, Laboratory of Veterinary Bacteriology and Mycology, Faculty of Veterinary Medicine.

Spitzen-van der Sluijs A et al. 2016. Expanding distribution of lethal amphibian fungus *Batrachochytrium salamandrivorans* in Europe. Emerging Infecious Diseases. 22:1286-1288.

St-Hilaire S, Thrush M, Tatarian T, Prasad A, Peeler E. 2009. Tool for estimating the risk of anthropogenic spread of *Batrachochytrium denrobatidis* between water bodies. EcoHealth. 6:16.

Stegen G et al. 2017. Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. Nature. 544:353.

Steinfartz, S. & Mutz, T. (1998). Mertensiella luschani (Steindachner, 1891) - Lykischer Salamander, Kleinasiatischer Salamander. *In Handbuch der Reptilien und Amphibien Europas. 4(I). Schwanzlurche (Urodela),* pp. 367-397. Edited by K. Grossenbacher & B. Thiesmeier. Wiesbaden: Aula-Verlag.

Sutherland WJ & Burgman M. 2015. Policy advice: use experts wisely. Nature News. 526:317.

Thomas V et al. 2018. Recommendations on diagnostic tools for *Batrachochytrium salamandrivorans*. Transboundary and Emerging Diseases. 65:e478-e488.

Thomas V et al. 2019. Mitigating *Batrachochytrium salamandrivorans* in Europe. Amphibia-Reptilia. 40: 265–290.

UNEP-WCMC. 2016. Review of the risk posed by importing Asiatic species of Caudate amphibians (salamanders and newts) into the EU.

US Fish and Wildlife Service. 2016. Listing salamanders as injurious due to risk of salamander chytrid fungus.

Valbuena-Ureña E et al. 2017. Getting off to a good start? Genetic evaluation of the *ex situ* conservation project of the Critically Endangered Montseny brook newt (*Calotriton arnoldi*). PeerJ. 5:e3447.

Wielstra B, Sillero N, Vörös J, Arntzen JW. 2014. The distribution of the crested and marbled newt species (Amphibia: Salamandridae: *Triturus*)–an addition to the New Atlas of Amphibians and Reptiles of Europe. Amphibia-Reptilia. 35:376-381.

Wielstra B et al. 2018. The distributions of the six species constituting the smooth newt species complex (*Lissotriton vulgaris* sensu lato and *L*. *montandoni*)–an addition to the New Atlas of Amphibians and Reptiles of Europe. Amphibia-Reptilia. 39:252-259.

Woodhams DC et al. 2011. Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. Frontiers in Zoology. 8:8.

Yuan Z et al. 2018. Widespread occurrence of an emerging fungal pathogen in heavily traded Chinese urodelan species. Conservation Letters. e12436.

Appendices

Appendix 1 - Bsal recognition leaflet

Appendix 2 - Bsal recognition leaflet veterinarians

Appendix 3 - Disinfection protocol fieldwork

Appendix 4 - Disinfection protocol heavy machinery

Appendix 5 - Amphibian *ex situ* population management guidelines

The goal of the *ex situ* measures is to safeguard a species or intraspecific lineage/subspecies from (local) extinction due to Bsal and preserve it for future reintroduction. *Ex situ* measures are particularly relevant for the high risk taxa in Table 2. Depending on the regional scale, the risk can be either at population or on taxon (species or subspecies) level. For example, when trying to conserve urodelan biodiversity on a local scale, *ex situ* conservation priorities can be based on population level risk. When trying to conserve urodelan biodiversity on a European or national scale, *ex situ* conservation priorities can be based on the taxon (species or subspecies) level risk.

Ex situ populations are preferably established in country of occurrence. However, if *ex situ* populations cannot be established in country of occurrence, these can be established in other suitable countries and/or facilities.

It is recommended to contact parties which have ample expertise and the facilities to successfully set up an *ex situ* population, such as zoos or related institutes. On http://bsaleurope.com contact information can be found of parties which can support in setting up *ex situ* populations.

Facilities and conditions

Urodelan *ex situ* populations may be housed in an indoor or outdoor enclosure, or a combination of both. Indoor enclosures (aquarium, terrarium, aquaterrarium) may be more labour and cost intensive than outdoor enclosures, but the population can be managed more efficiently. Especially when *ex situ* population sizes are small, an indoor enclosure may be recommended. Outdoor enclosures located within the natural area of occurrence of a particular species may offer the animals living conditions closely resembling those encountered in nature, but managing the population may be more difficult and biosecurity issues may arise. Depending on life stage or purpose (e.g. reintroduction) a combination of indoor and outdoor enclosures may be the best option. For example, one could choose to breed and raise the larvae, the life stage experiencing the highest mortality rate, indoor, while keeping the (sub)adult animals in an outdoor enclosure. For reintroduction purposes, keeping the animals in an outdoor enclosure prior to release may increase survival rate.

Each species has particular demands to keep them successfully in captivity. Also different life stages and purposes (e.g. breeding/non-breeding) may require different conditions. However, many general conditions are applicable to most species, especially for species with comparable biology. As such, European urodeles may be divided into three groups: (predominantly) terrestrial urodelan species, (predominantly) aquatic urodelan species and semi-aquatic urodelan species.

Predominantly terrestrial urodelan genera:
> *Chioglossa*[1], *Lyciasalamandra*, *Salamandra*[1], *Speleomantes*.

Predominantly aquatic urodelan genera:
> *Calotriton, Pleurodeles*, *Proteus*.

Predominantly semi-aquatic urodelan genera:
> *Euproctus, Ichthyosaura*[2], *Lissotriton*, *Salamandrella*, *Salamandrina*, *Triturus*[2].

[1] Includes species with an aquatic larval stage.
[2] Includes species which can be aquatic year round in captivity.

For specific information regarding the captive breeding and rearing of salamanders and newts we refer to a number of informative books on this topic such as Schultschik & Grosse (2013), Pasmans et al (2014), Seidel & Gerhardt (2016), Grosse (2018) and Fahrbach & Gerlach (2018).

Risks and diseases

Good practice is to quarantine animals in a basic enclosure for at least six weeks when setting up an *ex situ* population or adding new animals to the *ex situ* population. During that period animals need to be monitored for any sign of disease and should be tested at least for Bd, Bsal and ranavirus infection. Overall, it is highly recommended that every *ex situ* captive breeding population is considered one epidemiological unit (per conservation unit), which is kept strictly separate from other captive amphibians. Proper veterinary support is necessary for all *ex situ* programmes. Emphasis should be on disease prevention, through a combination of establishing disease free colonies, optimal husbandry and nutrition.

Genetic population management

The genetic management should aim for maintaining a maximal genetic diversity of the *ex situ* population. Based on the Amphibian Population Management Guidelines (Schad et al. 2008), there are different management strategies for *ex situ* populations based on the age to maturity and reproductive lifespan. For relatively short-lived species (reproductive lifespan 5-15 years), group management is preferred, whereas for long-lived species this shifts towards individual management (reproductive lifespan >15 years). See Appendix 5 for the Amphibian Population Management Guidelines. Guidance on which genetic lineages (conservation units) should be used for ex situ populations can be found in the species-specific protocols.

Administration

For each *ex situ* population a centralized administration needs to be created. For this the Zoological Information Management System (ZIMS) is used by many zoos. Also a studbook needs to be created to keep track of the reproduction, offspring and individual administration. For this the Single Population Analysis & Records Keeping System (SPARKS) can be used. A central administration for each species and all European *ex situ* populations is preferred.

Appendix 6 - Amphibian *ex situ* genetic population management guidelines

These amphibian *ex situ* population management guidelines for genetic goals have been adapted from the Amphibian Ark Amphibian Population Management Workshop (Schad 2008).

Age to Maturity	Reproductive Lifespan
1 - 5 years	5 - 15 years

Example Species: *Dendrobatidae, Typhlonectes, Tylototriton/Echinotriton, Theloderma, Cynops, Leptodactylus, Ceratobatrachus, Mantella, Atelopus*

Population Management Issue: These species have life histories that often start to approximate those of typical larger vertebrates, and therefore population management strategies can often be more like that used for most birds and mammals. However, although genetic management becomes easier, there may be more of a risk of demographic failure for species maintained at smaller numbers.

INDIVIDUAL MANAGEMENT

How many founders to collect?

- You want 10.10 (male.female) founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 20.20 specimens.) Try to gather as even a sex ratio as possible.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 6 years and an effective population size of 0.30.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	70*
40	110
55	150
70	190
85	225
100	265

*Note that this target size is the minimum recommended to meet genetic goals, but may be too small to meet demographic goals. In general, a population size of 100 is often considered the minimum needed to meet demographic goals.

How quickly should you grow the population to the target size?

- Grow the founding population to the target size as quickly as possible (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

- Breed according to mean kinship strategy (Lacy 1995, Pollak et al. 2005).
- Breed founders as long as possible; try to maintain equal numbers of offspring from all founders.
- Include at least some trial breeding of captive-born animals to ensure that population can be maintained when founders are gone.
- It is not necessary to keep generations discrete if animals are individually tracked.

Age to Maturity	Reproductive Lifespan
1 - 5 years	5 - 15 years

GROUP MANAGEMENT

How many founders to collect?

- You want 25.25 (male.female) founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 50.50 specimens.) Try to gather as even a sex ratio as possible.
- Keep founders in groups as small as possible (e.g., in pairs) to give equal breeding opportunity to all founders. If founders are kept in larger groups, you may need more founders to ensure 25.25 breeders.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 6 years and an effective population size of 0.15.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	140
40	225
55	300
70	370
85	450
100	530

How quickly should you grow the population to the target size?

- Grow the founding population to the target size as quickly as possible (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

Group Size

- Keep group sizes as small as is effective for the biology of the species-if possible try to maintain eight separate groups.
- Equalize family size across groups by keeping clutch sizes as equal as possible.
- If successfully breeding individuals within groups can be identified, consider removing them from the group to allow other individuals to breed.

Group Breeding Strategies: There are several strategies to retain gene diversity in populations of group-living animals:

A. Once reproduction occurs, systematically transfer individuals among groups in a "round robin" manner. We recommend one or more of these methods:

- Transfer about 5 individuals per generation – This number may need to be increased if mortality is high or fecundity is low.
- Transfer all juveniles – Move all juveniles out of their natal group to establish new next-generation groups before they reach reproductive maturity.
- Transfer all of one sex – Move all males (or females) from one group to the next group to avoid inbreeding with offspring and to mix genetic lines.

    OR

B. Keep each unique founder group together indefinitely and allow them to interbreed without mixing with other groups. This strategy is best for populations that have disease, husbandry, or logistical issues that would prohibit movement between groups.

    OR

C. Split the starting founder population in half and follow both strategies A and B (above) to increase chances of breeding success.

Age to Maturity	Reproductive Lifespan
1 - 5 years	> 15 years

Example Species: *Salamandra*, some *Ambystoma*

Population Management Issue: These species have life histories very much like those of the larger vertebrates. Population management would benefit from moving toward individual management, rather than group management, whenever feasible.

INDIVIDUAL MANAGEMENT

How many founders to collect?

- You want 10.10 (male.female) founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 20.20 specimens.) Try to gather as even a sex ratio as possible.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 7 years and an effective population size of 0.30.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	60*
40	95*
55	125
70	160
85	195
100	230

*Note that this target size is the minimum recommended to meet genetic goals, but may be too small to meet demographic goals. In general, a population size of 100 is often considered the minimum needed to meet demographic goals.

How quickly should you grow the population to the target size?

- Grow the founding population to the target size in one generation (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

- Breed according to mean kinship strategy (Lacy 1995, Pollak et al. 2005), which is based on the mean kinship of an individual relative to the mean kinship of the current population, and in which animals with a low kinship are preferred over those with high kinship for breeding.
- Breed founders as long as possible; try to maintain equal numbers of offspring from all founders.
- Include at least some trial breeding of captive-born animals to ensure that population can be maintained when founders are gone.
- It is not necessary to keep generations discrete if animals are individually tracked.

Age to Maturity	Reproductive Lifespan
1 - 5 years	> 15 years

GROUP MANAGEMENT

How many founders to collect?

- You want 25.25 (male.female) founders to survive and breed. Collect more based on your estimated rate of survival and reproductive success. (For example, if you expect 50% of the collected animals to survive and reproduce, you should collect 50.50 specimens.) Try to gather as even a sex ratio as possible.
- Keep founders in groups as small as possible (e.g., in pairs) to give equal breeding opportunity to all founders. If founders are kept in larger groups, you may need more founders to ensure 25.25 breeders.

What is the target population size?

- Target population size is defined as the minimum population size needed to meet genetic goals. This genetic target size may differ from the target size needed to meet demographic, research, or reintroduction goals.
- Target size depends on program length (e.g., short-term versus long-term) and species generation time.
- Target size was estimated using a generation time of 7 years and an effective population size of 0.15.
- These target sizes were estimated to maintain 90% gene diversity for the length of the program.

Length of Program (Years)	Minimum Genetic Target Population Size
≤ 25	115
40	185
55	250
70	320
85	390
100	455

How quickly should you grow the population to the target size?

- Grow the founding population to the target size in one generation (or at least five offspring per founder).
- After reaching the target size, each year determine the number of offspring needed to maintain the population size.

Who should breed?

Group Size
- Keep group sizes as small as is effective for the biology of the species-if possible try to maintain eight separate groups.
- Equalize family size across groups by keeping clutch sizes as equal as possible.
- If successfully breeding individuals within groups can be identified, consider removing them from the group to allow other individuals to breed.

Group Breeding Strategies: There are several strategies to retain gene diversity in populations of group-living animals:

A. Once reproduction occurs, systematically transfer individuals among groups in a "round robin" manner. We recommend one or more of these methods:
- Transfer about 5 individuals per generation – This number may need to be increased if mortality is high or fecundity is low.
- Transfer all juveniles – Move all juveniles out of their natal group to establish new next-generation groups before they reach reproductive maturity.
- Transfer all of one sex – Move all males (or females) from one group to the next group to avoid inbreeding with offspring and to mix genetic lines.
    OR
B. Keep each unique founder group together indefinitely and allow them to interbreed without mixing with other groups. This strategy is best for populations that have disease, husbandry, or logistical issues that would prohibit movement between groups.
    OR
C. Split the starting founder population in half and follow both strategies A and B (above) to increase chances of breeding success.

Annex 8. Overview of potential sustainable mitigation measures

Mitigating *Batrachochytrium salamandrivorans* in Europe

Authors: Valarie Thomas, Yu Wang, Pascale Van Rooij, Elin Verbrugghe, Vojtech Baláž, Jaime Bosch, Andrew A. Cunningham, Matthew C. Fisher, Trenton W. J. Garner, Maarten Gilbert, Elena Grasselli, Thierry Kinet, Arnaud Laudelout, Stefan Lötters, Adeline Loyau, Claude Miaud, Sebastiano Salvidio, Dirk S. Schmeller, Benedikt R. Schmidt, Annemarieke Spitzen-van der Sluijs, Sebastian Steinfartz, Michael Veith, Miguel Vences, Norman Wagner, Stefano Canessa, An Martel, Frank Pasmans

Abstract

The infectious chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) has been responsible for severe population declines of salamander populations in Europe. Serious population declines and loss of urodelan diversity may occur if appropriate action is not taken to mitigate against the further spread and impact of *Bsal*. We provide an overview of several potential mitigation methods, and describe their possible advantages and limitations. We conclude that long-term, context-dependent, multi-faceted approaches are needed to successfully mitigate adverse effects of *Bsal*, and that these approaches should be initiated pre-arrival of the pathogen. The establishment of ex situ assurance colonies, or management units, for species threatened with extinction, should be considered as soon as possible. While ex situ conservation and preventive measures aimed at improving biosecurity by limiting amphibian trade may be implemented quickly, major challenges that lie ahead are in designing in situ disease containment and mitigation post-arrival and in increasing public awareness.

1. Introduction

Infection of an amphibian host with the chytrid fungi *Batrachochytrium dendrobatidis* (*Bd*) or *B. salamandrivorans* (*Bsal*), may cause clinical chytridiomycosis, an emerging infectious disease (EID) (Berger et al., 1998; Martel et al., 2013). *Bd* was first identified in the 1990s as the prevailing cause of worldwide enigmatic declines and local extirpations of amphibian populations (Berger et al., 1998; Longcore et al., 1999). In 2013, *Bsal* was described following a population collapse of European fire salamanders (*Salamandra salamandra*) in the Netherlands from 2010 onwards, of which less than 0.1% of the original population remained in 2016 (Spitzen-van der Sluijs et al., 2013, 2016). In-depth study of a similar outbreak in Belgium in 2014 demonstrated how the interplay between host, pathogen and environment is predicted to result in the extirpation of the affected fire salamander population (Stegen et al., 2017).

Both *Bd* and *Bsal* are highly contagious and are transmitted effectively by direct contact with pathogen shedding hosts or indirectly by contact with contaminated water or substrate (Bosch and Martinez-Solano, 2006; Garmyn et al., 2012; Kolby et al., 2014; Martel et al., 2014; Courtois et al., 2017; Stegen et al., 2017). Pathogen transmission for both amphibian chytrid fungi is via aquatic, motile zoospores which infect the epidermal cells of amphibian skin. Further, *Bsal* produces an infectious, non-motile, encysted spore that manifests increased environmental resilience (Stegen et al., 2017). Although not completely understood, the release

of proteases by *Bd* zoospores and the growth of intracellular *Bd* and *Bsal* zoosporangia cause disruption of normal skin functioning which is vital to amphibian survival (Berger, Speare and Kent, 1999; Voyles et al., 2009; Brutyn et al., 2012; Martel et al., 2013; Van Rooij et al., 2015; Farrer et al., 2017).

Although *Bd* and *Bsal* belong to the same genus, they diverged an estimated 50 million years ago (Martel et al., 2014). *Bsal* is considered endemic in East Asia where it is widespread, at least in Vietnam, Japan and China, in species of the family Salamandridae (Laking et al., 2017; Yuan et al., 2018). Both species have an arsenal of virulence factors, which include a greatly expanded metalloprotease gene-family in *Bsal* (Farrer et al., 2017). Optimal growth temperatures for *Bd* range between 17°C and 25°C compared to 10-15°C for the *Bsal* type strain. Temperatures above 25°C and 30°C are lethal for *Bsal* and *Bd* respectively (Piotrowski et al., 2004; Martel et al., 2013; Blooi et al., 2015a). However, natural infections with *Bsal* were shown to occur in Asiatic newts of the genus *Tylototriton* at water temperatures up to 26°C, suggesting variation in thermal tolerance between *Bsal* isolates and, possibly, lineages (Laking et al., 2017; Beukema et al., 2018).

Bd can infect the skin of, and cause lethal disease in, a large range of anurans, urodeles and caecilians, although population declines have been observed mainly in anurans (Skerrat et al., 2007). In comparison, disease caused by *Bsal* seems to be limited to urodeles (Martel et al., 2014), even though some anurans can be infected by this fungus (Nguyen et al., 2017; Stegen et al., 2017). The currently observed niche breadth of *Bsal* in Europe appears to be only partially filled, indicating a high potential of further spread of *Bsal* (Beukema et al., 2018). The international trade of Asian salamanders and newts is suspected to be the primary route for the intercontinental spread of *Bsal* (Martel et al., 2014; Nguyen et al., 2017; Yuan et al., 2018). However, in captive collections outside Asia, infection can spread to other species, which in turn, can spread *Bsal* when traded (Fitzpatrick et al., 2018; Sabino-Pinto et al., 2018b). Eliminating this captive reservoir of *Bsal* should be a key aim in order to curtail further spillover events into natural populations of naïve amphibians.

Given the high susceptibility of salamanders to *Bsal* (Martel et al., 2014), and the infectiousness, pathogenicity and host range of the pathogen in Europe (Spitzen-van der Sluijs et al., 2016; Stegen et al., 2017; Dalbeck et al., 2018), *Bsal* poses an unprecedented threat to non-Asian salamander species (Beukema et al., 2018). Also, the prevalence of *Bsal* can be low in Asian reservoir species in captivity (Martel et al., 2014; Fitzpatrick et al., 2018) rendering detection difficult. Such pathogen reservoirs pose a formidable challenge for effectively preventing the introduction of *Bsal*, or subsequently managing a disease outbreak (Canessa et al., 2018). The development of effective mitigation strategies and measures, therefore, is crucial to maintaining amphibian biodiversity both globally and locally (Woodhams et al., 2011; Garner et al., 2016). *Bsal* abatement options have been considered before (Grant et al., 2015) and are continuously under revision by the US *Bsal* taskforce. Here, we propose a set of options we deem most feasible and efficient for the European situation given the current state of knowledge.

Two decades of research on the amphibian chytrid fungi have not yielded a single, globally effective measure for controlling *Bd* (Garner et al., 2016). Despite this, knowledge gained from these efforts is informative and has guided the development of our proposed suite of actions that are most likely to be effective in mitigating the effects of *Bsal* in Europe.

2. Pre-exposure measures

Taking actions to prevent the introduction and spread of *Bsal* into naïve regions is currently considered as the most efficient control method available ("prevention is better than cure"; Langwig et al., 2015; Richgels et al., 2016; Grant et al., 2017; Roy et al., 2017). Within Europe, the potential threat posed by *Bsal* was first recognised by the standing committee of the Bern Convention (Convention on the conservation of European Wildlife and Natural Habitats, 1979). In December 2015, the Council of Europe released recommendation No. 176 which aims to reduce the likelihood of *Bsal* expanding its range throughout Europe. This recommendation states that the signatories develop a number of precautions, including i) imposing trade restrictions on salamanders until risk assessments and prevention/mitigation protocols have been developed, ii) pre-import screening for the pathogen in the live animal trade, iii) setting up and implementing monitoring, surveillance and early-warning systems to detect *Bsal* incursion into the wild as well as the expansion of its range following its introduction, and iv) requiring biosecurity for field work, breeding sites and captive collections.

2.1. Trade restrictions and import controls

Since the international trade of salamanders and newts is suspected to be the principal route for the international spread of *Bsal*, bans/restrictions on amphibian trade, alongside controls at import pathways, are likely to be the most effective precautionary measures for preventing the introduction of *Bsal* via amphibian vectors in *Bsal*-free countries (Fitzpatrick et al., 2018; O'Hanlon et al., 2018). Wildlife trade restrictions, improved quarantine and strengthened biosecurity measures will also reduce the probability of introducing yet unknown pathogens and will thus have an impact beyond *Bsal*.

Such actions have already been implemented in several countries outside the European Union (EU) for example the United States (US Fish and Wildlife Service, 2016; EFSA, 2017a; Klocke et al., 2017) and Canada (EFSA, 2017a; Canada Gazette Part II, 2017; Wild Animal and Plant Protection Regulation of International and Interprovincial Act, 2017, updated May 12, 2018). The trade restrictions can be found summarised in supplementary Table S1.

Within the European continent, import restrictions have been implemented in Switzerland and Hungary (199/2017. (VII. 10.) Korm. Rendelet, 2017; EFSA, 2018; Stark et al., 2018) and in 2018, the European Commission issued temporary legislation (2018/320) which establishes animal health protection measures for the trade of salamanders within the EU and the importation of salamanders from non-EU territories (EFSA, 2018; Stark et al., 2018). There are omissions that weaken this regulation's relevance (Auliya et al., 2016), for example, the non-inclusion of anurans, which can act as *Bsal*carriers (Nguyen et al., 2017; Stegen et al., 2017) and not regulating animal traffic between private individuals.

Although policy-making with the aim of curtailing the spread of *Bsal* has been conducted relatively quickly in the countries mentioned above, coordinated global measures are required to regulate both the formal (e.g. commercial) and informal (e.g. hobbyists, fairs) amphibian trade (Auliya et al., 2016) in order to mitigate the spread of pathogens such as *Bsal*.

2.2. Additional control measures

Import bans of caudates alone may create a false feeling of security. They are unlikely to be 100% effective and *Bsal* is already present in captive amphibians in European regions where no *Bsal*outbreaks in the wild have been reported yet (Fitzpatrick et al., 2018; Sabino-Pinto et al., 2018b). Within the EU, the trade in captive urodeles has been shown to contribute to the international spread of *Bsal* (Fitzpatrick et al., 2018). Thorough screening of captive collections for *Bsal* (e.g. carried out in Germany; Sabino-Pinto et al., 2018b; and France; Marquis et al., 2019) and immediate treatment of these captive collections upon detection, are urgently needed to eliminate this *Bsal* reservoir, preferably supported by legislation. Based on an estimation of the number of amphibian keepers and number of pet amphibians in Europe, a total initial screening cost of the European states would be well below 1 million Euros as presented in supplementary Table S2. Clean trade, meaning the absence of known pathogens throughout the commercial chain, was promoted as a condition for sustainable exotic pet ownership (Pasmans et al., 2017). The sale of caudates in garden centers and other retail outlets should be discouraged, since this suggests suitability for release in garden ponds, which could promote the release of contaminated animals. Several stakeholders have set up campaigns to raise awareness of *Bsal* (see supplementary Fig. S1).

2.3. Biosecurity measures

It is essential to curb anthropogenic spread of *Bsal* during fieldwork, laboratory research, trade, recreational activities and amphibian husbandry by educating all including the public on appropriate biosecurity measures after use of amphibian habitats (Loyau and Schmeller, 2017). An effective measure to avoid spread of this pathogen during fieldwork is to ensure that proper disinfection protocols are utilised for hands, apparel, footwear, equipment and vehicles used in the field (EFSA, 2017b). *Bsal* can be killed using most common disinfectants (Table 1) (Van Rooij et al., 2017). Individuals involved in amphibian husbandry should ensure that captive urodeles are not housed outdoors and that captive amphibians are not released into the wild. They should also ensure that all waste is properly disinfected and disposed of (EFSA, 2017b).

Virkon S (Pfizer Limited) is used widely, relatively safe and highly efficient, but its use in the field may require derogations from existing legislation. Ethanol (and probably methanol) based commercial disinfectants can also be used effectively. Bleach is also highly effective. Soaking equipment in 10% sodium chloride for 10 minutes is potentially an effective, nontoxic and cheap alternative and its use is worth exploring further. Unfortunately, the commonly used and relatively cheap disinfectant, hydrogen peroxide, has poor activity against *Bsal* (Van Rooij et al., 2017). The efficacy of these disinfectants in table 1 against cysts is unknown, however, it is expected to be lower than for spores and sporangia.

Heat treatment can kill all life stages of *Bsal* but its routine use as a disinfectant requires further study. The fungus tolerates high temperatures poorly: *Bsal* cultures are killed after incubation

for 5 days at 25°C (Blooi et al., 2015a). If *Bsal* responds to heat in the same way as its sister species *Bd*, then exposing materials to 60°C for 5 minutes or 100°C for 1 minute should be an efficient disinfection procedure (Johnson et al., 2003). Drying may kill *Bsal*, however, since it is currently not known to what extent encysted *Bsal* spores tolerate drying, it is not recommended as the sole disinfection procedure.

In cases of *Bsal* incursion into the wild, drastic measures, such as closing areas to the public, might be required but such actions might not be compatible with local regulations, as was the case in the Netherlands and Belgium (EFSA, 2017b).

Pre-emergence measures can reduce the likelihood of introducing *Bsal* into naïve locations at a relatively low cost. Isolated populations of *Bsal*-threatened species might be considered as disease refugia and be managed by limiting human interaction. However, precautionary measures alone may not be sufficient, particularly without a full understanding of transmission routes for, and potential vectors of, *Bsal*.

2.4. Increasing host resistance

Host resistance against *Bsal* is currently poorly understood. However, adhesion to, and invasion of, the salamander skin are key events that appear to determine the outcome of infection (Martel et al., 2014). Provoking a hereditary reduction in the susceptibility of highly susceptible urodelan species may be the only sustainable measure to avert further loss of biodiversity in the long term, given the high probability that *Bsal* will not be eliminated once it has invaded an ecosystem (Feldmeier et al., 2016; Schmidt et al., 2017; Stegen et al., 2017). If we decide to assist in decreasing host susceptibility, three options may be worth exploring: vaccination, bioaugmentation using pre- or probiotics and selective breeding. Based on their close genetic compositions we tend to expect similar responses from both pathogens and the various strains to these pre-exposure mitigation methods. *Bsal*'s genome is 32.6 Mb while Bd's is 23.7 Mb (Farrer et al., 2017). Therefore, though there are commonalities, there are still differences which are reflected in the two pathogens being separate species. For example, any intervention which relies on salamanders mounting an immune response is likely to be less successful against *Bsal* (Stegen et al., 2017) than other amphibians against *Bd*.

2.4.1. Vaccination

Although there is limited evidence that the development of a *Bd* vaccine might be possible (Woodhams et al., 2011; McMahon et al., 2014), similar trials with *Bsal* have not resulted in any protection against a challenge with virulent *Bsal* (Stegen et al., 2017). There are currently no proofs of concept available for vaccination against *Bsal*. This is probably because *Bsal* severely suppresses immune response in infected hosts (Farrer et al., 2017), negating the animal's ability to mount an effective response.

Developing a vaccine is likely to be costly and any vaccine would need to be useful in a range of species. In addition, the creation of vaccines for fungal agents has proven to be much more difficult than for viruses or bacteria, as evidenced by the lack of antifungal animal vaccines. For *Bsal*, there are currently no proofs of concept available. There may also be a need to develop

appropriate policy and budget allocations to allow the vaccination of free-living wildlife (Garner et al., 2016). Finally, vaccination which requires application to individual wild salamanders would be logistically highly challenging in situ (Garner et al., 2016; Canessa et al., 2018) especially if booster doses were required.

In spite of such challenges, vaccination would be an appealing option in the event that a *Bsal* strain (or another chytrid/micro-organism) was isolated/designed that establishes self-sustaining populations in amphibian communities, is avirulent, safe for target and non-target species, yet evokes a protective response against virulent *Bsal* across host species and life stages and for different chytrid genotypes.

2.4.2. Bioaugmentation

Bioaugmentation is a method of inoculating beneficial probiotics into or on to the animal host or habitat to reduce host susceptibility by microbial defences (Woodhams et al., 2011). Probiotics have been isolated from soil, water and amphibian skin (Loudon et al., 2014). *Bd*-induced chytridiomycosis has been mitigated, although with variable success by bioaugmentation in the laboratory and in a field trial (Bletz et al., 2013), and probiotic therapy should be considered as a potential strategy for *Bsal* mitigation. Knowing any potential risks that probiotics pose to ecosystems and amphibian hosts is important prior to any application to wild populations. The risks of an uncontrolled introduction of probiotics in the wild are manifold, including disruption of nutrient cycling, which could have important cascade effects for the whole ecosystem (Schmeller et al., 2018).

In addition, a suitable probiotic for bioaugmentation should be effective across *Bsal* genotypes, should result in persistent colonisation of the urodelan skin at densities that facilitate their antifungal activity, should preferably be transmissible to conspecifics (including offspring) and should be safe and espouse qualities which would allow it to be produced in large volumes. In order to understand the bacterial community on amphibian skin and identify the effect of probiotics on *Bsal* establishment, a much better understanding will be required (Bates et al., 2018), including of the host-pathogen-environment triangle (Schmeller et al., 2018). Recent work by Bletz et al. (2018) and Bates et al. (2018) has shown that *Bsal*-induced death coincides with significant perturbation of the bacterial community, resulting in increases of opportunistic bacteria that cause septicaemic events (Bletz et al., 2018). Besides, the composition of bacterial communities on urodele skin is highly dependent on their surrounding environment, raising the possibility that laboratory trials with *Bsal* may be influenced by the mere transition of the animals to captivity (Bates et al., 2018). Currently, there are no proofs of concept that bacteria or other microbes protect susceptible salamanders against *Bsal* infection at natural microbial densities. On the contrary, Bletz et al. (2018) suggest that bioaugmentation might be impeded, at least in fire salamanders, as very low numbers of bacteria are maintained on their skin.

Although these bacterial communities on the salamander skin do contain bacterial lineages with pronounced *Bsal*-inhibiting capacity in vitro, only the repeated and consistent application of very high doses of these lineages were capable of attenuating *Bsal* infection (Bletz, et al., 2018).

2.4.3. Selective breeding

Increasing resistance against *Bsal* infection either by selective breeding (resembling natural selection by cross breeding the most resistant animals) or by genetic engineering could be an effective strategy in the mid- to long-term to permanently avert the risk of *Bsal*-induced population crashes. Based on their close genetic compositions we tend to expect similar responses from both pathogens to selective breeding. However, while there are commonalities, there are still difference in genetic composition and still a lot of important information on *Bsal* yet to be elucidated. While some frogs exposed to *Bd* and antifungals demonstrated a reduction in susceptibility (Garner et al., 2016), salamanders previously exposed to *Bsal* did not demonstrate decreased susceptibility (reduced mortality) (Stegen et al., 2017).

Further, this would require extensive resources for training staff in genetic engineering, infrastructure and genetic management. Selective breeding requires the availability of markers for resistance. Genetic engineering requires the identification of the genetic basis underpinning host resistance (with relevance for the situation in the wild). While gene editing in amphibian eggs is commonplace, genetic engineering in viviparous species of the genus *Salamandra* presents an additional challenge. For *Bd*, susceptibility has been linked to several genetic markers and modifying several of the encoding genes to decrease disease susceptibility may result in difficult to predict, severe side effects. Since a targeted approach is hindered by a lack of knowledge of the determinants of susceptibility to *Bsal*, untargeted approaches may yield usable results, yet no proof of concept (neither for *Bd* nor *Bsal*, and in fact not for any infectious disease in vertebrates) exist. For selective breeding, the slow generation time of many urodeles (typically 3-4 years) precludes the rapid evolution of resistant populations. In the current absence of suitable markers, selecting for resistant individuals in captivity will require the use of large numbers of animals in (sub-)lethal animal experiments, which may raise ethical concerns. The European Union is currently reluctant to allow the use of genetically modified organisms in agriculture and targeted modification of the urodele genome, while increasingly feasible, will have to deal with regulatory issues before any such animal can be released into the wild. In contrast with *Bd*, where response varies by species and sometimes populations (Bataille et al., 2015), there is no evidence of selection for individuals with increased disease resistance in infected, natural populations. For example, Stegen et al. (2017) demonstrated high susceptibility in the few remaining salamanders at an outbreak site and in 2018, several *Bsal*-infected salamanders were found dead at the index outbreak site in the Netherlands, where an estimated 0.1% of the animals has survived.

Selective breeding will probably be perceived by public opinion as more acceptable compared to genetic engineering (Garner et al., 2016) but 10 years of selective breeding of midwife toads has not resulted in any notable increase in their resistance against *Bd* (Bosch, unpublished). Both options could be explored but it will likely take decades before either could be shown as being successful – and probably only for a single species in that time frame. Selective breeding and genetic engineering, therefore, cannot be seen as short-term measures to address the urgency of *Bsal* mitigation, but at best as mid to long-term mitigation strategies.

3. Post-exposure measures

Bd was already widespread and had decimated many amphibian populations in several countries before its diagnosis. Epidemiological investigations of *Bd*, causative agent of

chytridiomycosis, were reported to have started 15 years after amphibian declines were initially observed, resulting in population declines, extirpations, and extinctions of approximately 200 species (Grogan et al., 2014). These measures have all been considered or research has been initiated for *Bd* mitigation. A few were implemented on various scales with varying levels of success (Woodhams et al., 2011; Garner et al., 2016). In some populations and countries affected by *Bd*, host and pathogen have reached co-existence. *Bsal* was discovered much more recently, has not been detected in many countries and has been detected in relatively small regions in those affected. Therefore, the opportunity still exists to implement measures to avoid the incursion or delay the spread of this fungus. Also, if *Bsal* enters a naïve location, the efficacy of these methods may be high as the pathogen will likely be limited to a much smaller geographical area and fewer populations. However, the presence of two different forms of the fungus with one of them being the encysted environmentally resistant spore, makes success of these individual mitigation methods less likely.

Once there has been a *Bsal* incursion to a novel site, mitigation methods should focus on: 1. reducing the impact of the pathogen on susceptible amphibian species, 2. setting up conservation strategies to prevent population extirpation, and 3. preventing further *Bsal* spread. In exceptional cases, elimination of *Bsal* from the system may be attempted. However, the presence of animal and environmental reservoirs will likely preclude eradication from most ecosystems (Stegen et al., 2017). These post-emergence approaches can be classified as measures to i) reduce the fungal load in the environment or host, and ii) safeguard populations from *Bsal*-induced extirpation. Such measures can be generally divided into in situ and ex situ approaches.

Short-term solutions are considered vital in temporarily preserving amphibian populations at risk (Garner et al., 2016). For example, as shown for *Bd*, interventions with antifungals during an epidemic can alter infection dynamics and alleviate disease (Hudson et al., 2016; Geiger et al., 2017). However, in the absence of long-term disease management in situ, any short-term measure is unlikely to result in significant conservation success. This underscores the importance of further research into potentially effective mitigation measures. Here, we will discuss captive assurance colonies, in situ treatment of animals and the environment, creating barriers to limit *Bsal* spread and bioaugmentation.

Some bacteria have been detected to decrease *Bd* in vitro and in the field (Bletz et al., 2013). In the case of *Bsal*, some bacteria found on the host's skin have been able to reduce *Bsal* in vitro (Bletz et al., 2018). Physical barriers appear to have reduced spread of both *Bd* and *Bsal* from infected populations to naïve populations located within close proximity (Rodríguez-Brenes et al., 2016; Spitzen-van der Sluijs et al., 2018). Captive assurance colonies have had mixed outcomes in the case of *Bd* (Woodhams et al., 2011) and we expect will be just as challenging for *Bsal*, especially in terms of husbandry of such varied hosts and the host-pathogen-environment triangle. In situ treatment of the environment/animals has also been carried out for *Bd* and has had some success (Woodhams et al., 2011; Garner et al., 2016). These in situ treatments of animals and the environment are expected to have some success in reducing the number of *Bsal* spores in the environment. However, they may not be as effective on the

environmentally resistant spores. In addition, since less information is currently available on *Bsal*, these mitigation measures are not likely to work better than they have for *Bd*.

3.1. Reducing the impact of Bsal
3.1.1. Reduce fungal load
3.1.1.1. Decontaminating and manipulating environments

Manipulating *Bsal*-infected environments by applying in situ intervention measures can be implemented to limit the spread of infection, reduce the impact of the pathogen and, by extension, increase amphibian survival. Environmental manipulations may be biological, physical or chemical and applying environmental interventions, such as the use of natural predators, antibiotics, fungicides, pond-drying, disinfectants and changes in ambient temperature are the most common methods used for the veterinary treatment of fungal diseases in aquaculture (Woodhams et al., 2011).

Hitherto, no environmental treatment has been applied to mitigate *Bsal* infection, but a few interventions have been shown to be effective to control *Bd*. Using aquatic invertebrate 'micropredators' for the removal of *Bd* from the aquatic environment has been identified as a potential mitigation measure for aquatic or semi-aquatic species and may also be potentially used against *Bsal* spores (Buck et al., 2011; Searle et al., 2013; Schmeller et al., 2014a). However, it is unclear to what extent the availability of other food sources influences the capacity of these micropredators to remove spores from the environment. *Bd*-removing micropredators were found to contribute to creating refuges from chytridiomycosis (Blooi et al., 2017).

Eliminating the environmental reservoir of *Bsal* can be expected to contribute to controlling *Bsal* outbreaks. Crucial information is currently lacking about whether, how and to which extent *Bsal* (but equally *Bd*) can persist in the environment in the absence of amphibian hosts. Identifying and enhancing micropredators which are able to reduce the number of *Bsal* spores in the environment may eventually lead to a reduction in the number of infected amphibians. The situation for *Bsal* is more complex compared to *Bd*, since *Bsal* produces two types of spores: zoospores and encysted spores. The latter, floating at the water-air interface, were shown to be less susceptible to predation (Stegen et al., 2017). Also, it is unclear whether a similar principle of predation is applicable to terrestrial systems. Currently, there is no proof of concept available of the impact of manipulating micropredator dynamics on amphibian chytrid dynamics in nature. Therefore, applying this approach to field situations requires caution since either selectively enhancing specific components or adding foreign organisms to ecosystems may alter foodwebs.

Physical methods, such as pond-drying and elevating the temperature of ponds, have been used to destroy *Bd* in the environment despite facing several challenges such as legal (protected species and habitats present), technical and epidemiological (for example: propensity of amphibians to escape from drying ponds, which may propagate pathogen spread). Physical methods are expected to have similar success in decreasing the *Bsal* zoospores in the environment while experiencing similar challenges as with *Bd*. *Bd* does not survive drying (Johnson et al., 2003) and the efficacy of pond-drying, in relation to *Bsal*, will depend on

how *Bsal* spores respond to desiccation. The efficacy of pond-drying and elevating the temperature of ponds will also depend on the type of *Bsal* spores present in the environment. These methods are not expected to work as efficiently on the environmentally resistant encysted form of the *Bsal* spore (Stegen et al., 2017).

Subjecting the fungus to temperatures and conditions which are unfavourable for growth and persistence of aquatic and other life stages, will result in its reduction. Johnson et al. (2003) showed in vitro that *Bd* is sensitive to desiccation and is fully cleared within 1h of drying. However, in a field study by Bosch et al. (2015), pond drying combined with the application of itraconazole did not eliminate *Bd* but merely decreased infection intensities for a short period of time. When these were combined with environmental disinfection, later, *Bd* was eradicated. Also, if pond drying is not done at an appropriate time it could result in dispersal of infected individuals, the destruction of the local ecology, including the death of tadpoles, eradication of local benign nano-, micro- and mesoplankton, which could negatively affect amphibian populations and other biodiversity. Finally, pond drying is more difficult to apply to important urodele habitats such as streams. On the other hand, for species that reproduce in ephemeral ponds, strategic artificial desiccation may result in the elimination of *Bsal* and of predators of amphibian larvae thus increasing juvenile survival and population persistence (Johnson et al., 2003; Woodhams et al., 2011). Any mitigation strategy that may potentially involve the degradation or destruction of habitat will require a careful and transparent cost-benefit analysis (where "costs" is used to encompass any side-effect, including environmental damage).

Increasing the water temperature of amphibian breeding ponds, which can be achieved by removing canopy cover, can provide an important refuge from *Bd* (Freidenburg and Skelly, 2004; Forrest and Schlaepfer, 2011; Savage, Sredl and Zamudio, 2011; Scheele et al., 2014). Decreased shading of ponds is linked to lower *Bd* infection intensities (Raffel et al., 2010; Heard, et al., 2014). While this is cost-effective and would be beneficial to amphibian species which are tolerant of or even prefer higher temperatures (Langton et al., 2001), the relevance for European urodeles can be questioned. Increasing water temperatures may be expected to be poorly tolerated by heat-sensitive species and its relevance for lotic ecosystems is very uncertain. Besides issues of feasibility, water temperatures should be higher than 25°C to kill *Bsal* (Blooi et al., 2015a), exceeding the thermal preferences of many European urodeles. While its efficacy has yet to be demonstrated, it may be worth considering the option of decreased shading of terrestrial habitats as a supportive action to reduce environmental *Bsal* loads through surface heating and desiccation. Again, competing objectives such as revegetation targets, the impact on other species and broader issues such as forestry interests will need to be taken into account.

The environmental application of chemical treatments is another option for fungal disease mitigation. Applying the disinfectant Virkon S 1% (as experimented by Bosch et al., 2015 at the breeding sites of *Alytes muletensis*) or adding sea salt to increase salinity (Stockwell, Clulow and Mahony, 2012, 2015) were able to eliminate or lower *Bd* infection in the aquatic environment and may be promising strategies for inhibiting *Bsal* growth. Fungicides have only been used in simple single-host systems and controlled, isolated habitats (Garner et al., 2016) and it remains to be demonstrated whether they could work in more complex habitats. In

addition to these potential limitations to their in situ application, preliminary studies indicate that fungicides and disinfectants are ineffective in curbing *Bsal*(Van Rooij et al., 2017).

Creating saline refuges in amphibian environments has been suggested as a feasible conservation method to control *Bd* infections in anurans, being relatively cheaper than other methods. While this method functions by disrupting chytrid growth and motility (Stockwell, Clulow and Mahony, 2015), it has been shown to have deleterious effects in aquatic organisms (Karraker, Gibbs and Vonesh, 2008; Denoël et al., 2010; Karraker and Gibbs, 2011; Tollefsen et al., 2015; Jones et al., 2016). It will also be difficult to apply to lentic systems, and like with fungicides, its effects in terrestrial systems remain unknown.

The methods used in environmental manipulation may create tolerance to, or resistance against, *Bsal* among small isolated groups of amphibians and also provide sanctuaries for focal species deemed highly vulnerable and of particular conservation concern. However, they may be less effective mitigation measures for amphibians with large ranges and their effects may be variable in complex habitats. Environmental manipulation may face many legal barriers and may conflict with other conservation priorities. For example, manipulations in protected areas or with negative effects on protected species or habitats may require environmental impact assessment and public consultation.

3.1.1.2. In situ treatment of the amphibian host

There have been no studies to date that have investigated the in situ treatment of amphibians infected with *Bsal*. Hudson et al. (2016) and Geiger et al. (2017) evaluated the impact and feasibility of in situ treatment using the antifungal drug itraconazole to mitigate *Bd*-induced amphibian chytridiomycosis. Firstly, it is easier to treat *Bd*-infection than *Bsal* using itraconazole exclusively. The results from Hudson et al. (2016) and Geiger et al. (2017), indicated that itraconazole treatment decreased the probability of *Bd* infection and the mortality rate of infected animals, however, as soon as treatment was ceased, all benefits disappeared and the infection and mortality rate increased to those of untreated individuals. This suggests treating infection does not induce any protective immune responses to *Bd* (Hudson et al., 2016) and, when based on empirical data without proper toxicity assessment, may even have detrimental effects on the survival of a species (Loyau et al., 2016). This in situ treatment method, while labour-intensive and limited to amphibian species for which recapture rates are relatively high, could be used as a short-term conservation tool to reduce the mortality caused by *Bd* or *Bsal* during periods of high disease risk or to gain time during disease outbreaks while a more permanent solution is identified (e.g. Hudson et al., 2016; Geiger et al., 2017). Effectively treating a *Bsal*-infected fire salamander population would require an almost total coverage of the population, combined with a 100% effective treatment to interrupt transmission (Canessa et al., 2018). Anything less might result in adverse effects: prolongation of the survival of infected animals would increase the potential for disease spread within and outside the focal population. Such an effective treatment can be done only ex situ, since it requires repeated and consistent application of either the use of relatively high temperatures (25°C) or a combination of the antimicrobial drugs polymyxin E and voriconazole (Blooi et al., 2015a, 2015b). In

practice, this would mean removal of all infected animals from their habitat and release after treatment.

3.1.2. Safeguard populations
3.1.2.1. Bioaugmentation and vaccination

For vaccination, see section 2.4.1. Provided a protective vaccine can be developed, this could be applied during an outbreak to limit losses. For bioaugmentation, see section 2.4.2. Besides being a preventative approach, micro-organisms, either alone or in mixtures, could potentially be used therapeutically during a *Bsal* outbreak to limit the impact of infection.

3.2. Preventing further Bsal spread
3.2.1. Reduce fungal load
3.2.1.1. Removal of hosts

The removal of infected or even of all susceptible hosts (including non-infected) from a population might be a mitigation strategy worth exploring. In susceptible species, the eradication of *Bsal* is likely to require the removal of a substantial proportion (> 90%) of the focal hosts as well as any other species in the same area that are acting as reservoirs (Canessa et al., 2018). Moreover, *Bsal* has been shown to persist in the environment in the absence of amphibian hosts. This possibly explains, at least in part, the high probability that a susceptible population will be extirpated by *Bsal* (Stegen et al., 2017). It also means that eradication from a site is unlikely, although the likelihood of this will be increased the longer the site is maintained free of amphibians. However, even if eradication cannot be achieved, removing infected animals reduces the probability of spillover of *Bsal* to neighbouring populations (Canessa et al., 2018; Spitzen-van der Sluijs et al., 2018). The reaction of the public to host removal may be expected to vary according to the fate of the animals removed. Translocation of these animals to other sites should be strongly discouraged and reintroduction at the original site is only acceptable after *Bsal* eradication has been demonstrated and maintained for a reasonable period of time. Otherwise, this may result in flare-ups of infection, with the likelihood of further spread to neighbouring sites. While culling may well be the most rational option, and is well accepted in OIE disease control programmes, this is more likely to meet adverse reactions compared to transferring the animals to captivity with subsequent treatment.

3.2.2. Safeguarding populations
3.2.2.1. Creating barriers to the spread of Bsal

Simple mathematical models suggest that *Bsal* will spread rapidly in a homogeneous landscape (Schmidt et al., 2017). Yet, this is not what was observed near the *Bsal* index site. In fact, there are indications that the natural (autonomous) spread of *Bsal* is relatively slow and can be interrupted by barriers that limit dispersal of infected salamanders such as rivers, highways, unsuitable habitat and fences (Spitzen-van der Sluijs et al., 2018). That study did not identify biotic or abiotic vectors of *Bsal*, but its results suggest that the local movement of infected hosts may be crucial in the dispersal of *Bsal* over short distances, whilst human-mediated transmission will be the most important pathway of long-distance spread. Understanding the fundamentals of range expansion would offer opportunities for developing barrier-based strategies. This may be used to protect uninfected (sub)populations through isolation, or to contain outbreaks if caught at an early stage. Such measures may be effective in the short-term

and could significantly reduce the risk of spread of *Bsal*, but their efficacy in the mid to long-term is unclear, given the non-continuous distribution pattern of *Bsal*. This pattern is characterized by often large distances between outbreak sites, which are highly unlikely to be bridged by infected salamander hosts within the observed timescales. Although human-mediated spread may at least in part explain the long distance dispersal of *Bsal*, between-site transmission is currently poorly understood and biotic (e.g. birds) and abiotic dispersers cannot be currently excluded. However, the persistence of an uninfected fire salamander population for over 8 years only 800 m from the *Bsal* index outbreak suggests that managing landscapes, exploiting existing barriers and creating meaningful barriers may be a relatively low-cost option worth exploring.

3.3. Setting up conservation strategies to prevent population extirpation
3.3.1. Safeguarding populations
3.3.1.1. Reintroduction and captive breeding

Introductions to restore original populations require prior removal or management of the *Bsal* threat in the wild (IUCN, 2013; Muths and McCallum, 2016). Affected host species could be reintroduced, either with translocations from other wild populations or using individuals that have been captured and treated or bred in captivity. Also, reinforcement of extant populations may be implemented in combination with other mitigation actions that augment resistance to infection or disease. More radical options might also include the assisted movement of threatened species to areas of lower *Bsal*risk (Gagliardo et al., 2008). Experience shows that efforts to establish captive assurance colonies should be initiated early in the mitigation process (Martin et al., 2012). Given resource limitations, prioritisation is inevitable and conservation units (from population to species level) have to be defined (see section 4.3). Establishing captive assurance colonies is currently the only effective action to preserve species with small ranges, or otherwise valuable populations, following invasion by *Bsal*. Although this is a feasible option, any such action should be planned and executed carefully and conducted from the outset, with an explicit view to future reintroduction options (Canessa et al., 2016). This includes keeping animals under high levels of biosecurity as necessary to prevent exposure to other pathogens that might eventually be released into the wild with the animals or their offspring, as was the case with the contamination of Mallorcan midwife toads by *Bd* (Walker et al., 2008). Also, captive assurance colonies need to have informed genetic and veterinary management – which often requires the involvement of multiple centres – and to be run in accordance with IUCN guidelines (Pessier et al., 2014). Protocols for such assurance colonies and resources should preferably be in place for all high-risk populations or species. Expertise to maintain and breed European urodeles is widely available, although largely limited to the private sector (e.g. DGHT, AG Urodela). Currently, only one European species is propagated consistently in the framework of a captive assurance colony, combined with reintroduction efforts: the Montseny brook newt (*Calotriton arnoldi*, LIFE-Tritó project, http://lifetritomontseny.eu/). Such captive assurance colonies would benefit from participation and collaboration of professional organisations (zoos, aquaria, represented by EAZA), research institutions and the private sector (Pasmans et al., 2017).

4. Supporting actions

The actions discussed above seek to achieve a conservation objective, namely to ensure the persistence of populations or species by preventing the introduction of *Bsal* or by mitigating its effects if it is introduced. To be effective, such actions need to be informed by knowledge of *Bsal* host-pathogen dynamics and information on *Bsal* spread, host conservation status and outcomes of any previously implemented action. Moreover, mitigation strategies will require many decisions to be taken at different levels, from the global to the local scale, with widely differing levels of available resources. Here, we detail several actions that might assist the broader mitigation process. It must be noted that these actions are only useful in supporting the mitigation actions discussed above: for example, monitoring alone will not abate the negative impacts of *Bsal*, but the data collected are vital in understanding where and how to implement conservation interventions.

4.1. Early-warning system

An early-warning system is a valuable tool for rapid *Bsal* detection and response. It consists of *Bsal* notification points that are responsible for national or regional surveillance for, and the collection of, dead amphibians (by local volunteers) and the determination of the cause of death. Early warning systems were largely unimplemented for a long period during *Bd*'s spread since the cause of amphibian mortality remained elusive. Epidemiological investigations of *Bd* were reported to have started 15 years after amphibian declines were initially observed, resulting in population declines and extirpations, and extinctions of approximately 200 species (Grogan et al., 2014). A sensitive and specific diagnostic technique that shows high interlaboratory reproducibility of results is key to an efficient early-warning system and, for *Bsal*, consists of quantifying *Bsal* genome equivalents in non-invasively collected skin swabs (Blooi et al., 2013; Thomas et al., 2018). Once more information on the disease killing amphibians became known, a sensitive and specific test with interlaboratory reproducibility for detection of *Bd* was developed by Boyle et al. (2004). Presence of *Bd* was detected on museum specimens collected over a century prior to detection of chytridiomycosis infection. As part of a project, funded by the European Commission (Tender ENV.B.3/SER/2016/0028, Mitigating a new infectious disease in salamanders to counteract the loss of biodiversity, http://bsaleurope.com/), notification points have been set up in Belgium, France, Germany, the Netherlands, Spain, Italy, and the UK. The setup of an effective early-warning system requires informing, and active involvement of all stakeholders (including the public), building sufficient diagnostic capacity and efficient data management, including proper reporting to the OIE (*Bsal* was listed in 2017 by the OIE).

Detecting environmental DNA of target organisms (eDNA) (Taberlet et al., 2012) is now widely used for biodiversity inventories, and recommended for use in the early detection of invasive species (Darling and Mahon, 2011) and aquatic pathogens (Guy et al., 2003; Huver et al., 2015). For *Bd*, eDNA detection in water was shown to be efficient in detecting occupancy of ponds by *Bd* (Walker et al., 2007; Schmidt et al., 2013). The applicability of eDNA for detecting *Bsal* is currently uncertain as it would require detecting pathogen DNA in more complex matrices such as forest soil. Standard eDNA detection in water would be useful for detection of *Bsal* during the aquatic phase of urodelan life. However, many salamanders in

Europe are terrestrial, thus the testing for eDNA would need to be carried out in matrices more complex than water.

Regardless of the diagnostic method used, an efficient early-warning system should include active and passive disease surveillance. We here use the terms pathogen and disease surveillance as ongoing recordings of *Bsal* and *Bsal*-associated disease in wild amphibian populations. "Passive pathogen and disease surveillance" is used for the recording of *Bsal* and *Bsal* disease presence as they occur (reactive) and "active pathogen and disease surveillance" for targeting individuals to detect *Bsal* and *Bsal* disease presence (proactive). Active and passive surveillance were implemented for *Bd* pathogen and disease in several countries in Europe (Garner et al., 2005). *Bd* spread to many countries before information was available on the cause of amphibian mortality in those locations therefore, many measures which have been implemented as part of the early warning system against the incursion of *Bsal* were not able to be implemented in those environments but may still be implemented in countries or regions free of *Bd*.

4.1.1. Passive disease surveillance

Passive surveillance of *Bsal* outbreaks is currently done by the reporting of opportunistically observed suspect cases to a regional hotline for further examination. Observers can be professionals or lay people. Passive surveillance can enable the detection of disease across large spatial scales, but the likelihood of detection depends on many factors such as the mere detectability of the affected species (many urodele species are secretive), the degree of observer effort (e.g. number of observers and amount of time each observer spends looking for diseased animals), the ability of observers to identify disease and the likelihood that any diseased animals detected will be reported to the relevant authority (Kéry and Schmidt, 2008; Buckland et al., 2010; Lawson, Petrovan and Cunningham, 2015). Key example of public reporting leading to disease detection in this context is detection of the index outbreak of *Bsal* in the Netherlands, the first signs of which were noted as a steep population decline in the framework of a long-term salamander monitoring campaign. Passive surveillance currently seems to be the most feasible approach for detecting the occurrence of *Bsal* disease outbreaks in Europe, at least in species with large ranges (EFSA, 2018). Through the European Union *Bsal* tender, passive surveillance for amphibian diseases recently initiated at the national level in Belgium, France, Germany, Italy, the Netherlands, Spain, Switzerland and has been ongoing at the national level in the United Kingdom since 1989 (Lawson, Petrovan and Cunningham, 2015) (http://bsaleurope.com/).

4.1.2. Active disease surveillance

Implementing a thorough system of active surveillance throughout Europe would be the most reliable way to determine the current distribution of *Bsal* infection in the wild; however, such a system would require enormous amounts of resources that may need to be diverted from other uses. It may be more efficient to concentrate active surveillance and monitoring within and around localities where a disease outbreak consistent with *Bsal* chytridiomycosis is detected (EFSA, 2018). In Austria, Belgium, Croatia, Czech Republic, France, Germany, Portugal, Slovenia, Spain, Switzerland, the Netherlands and the UK, non-systematic active surveillance

has been carried out on an *ad hoc* basis (EFSA, 2018). Active surveillance for *Bsal* is currently done by proactively sampling amphibians for presence of *Bsal* infection or for *Bsal* disease itself in a quantitatively adequate number of populations. Since *Bsal* outbreaks are characterized by collapses of urodele populations, the least costly option is to monitor sentinel populations of susceptible host species for signs of population declines. Such actions can be designed as citizen science projects (Dickinson et al., 2012) coordinated by relevant scientific entities. Integration of professional and citizen-science monitoring schemes may broaden the coverage and amount of data collected, particularly if optimised spatially and temporally (Morán-Ordoñez et al., 2018). Longitudinal monitoring of amphibian populations is key to interpret disease findings and provides the necessary baseline information to evaluate disease impact.

4.2. Monitoring of ongoing population declines and past outbreak sites

Populations already in decline and adjacent ones, require special attention via monitoring. (Grogan et al., 2014; Ficetola et al., 2018). Monitoring of the host population and the pathogen should continue well after host populations are ascertained to have declined or been extirpated, to provide information about *Bsal*'s persistence in the environment and/or in alternative hosts. In the future, this will provide useful information for the development of post-outbreak restoration protocols, such as reintroductions.

4.3. Conservation prioritisation

Scientific evidence is essential to narrow knowledge gaps and inform the decision-making process as to which species are prioritised. However, clarifying the decision context (who decides whether a species should be allocated resources, who provides those resources, who implements the action) is just as important (Game, Kareiva and Possingham, 2013). Prioritisation of *Bsal* mitigation actions at the European level would need to follow these four steps: (1) definition of priorities, based on EU, state or local legislation, or criteria describing the importance of species and subspecies in terms of e.g. genetic diversity, ecosystem function or cultural values; (2) a complete risk assessment of the impacts of *Bsal* on all species; (3) evaluation of the benefits and costs of potential actions for each species by an expert panel including scientists, managers and policy-makers; (4) identification of priority species (selection and listing of specific species that fit the criteria for prioritisation per point 1 above). In the current situation, information about species-specific risks and actions is urgently needed.

Thirty-four urodele species occur across the 27 EU member states (European Red List, 2018). Given the limited resources available, it is unlikely that full protection against *Bsal* impacts could be provided to all those species in all those countries (also considering the intraspecific variants of conservation interest). Several quantitative methods for transparent conservation prioritisation have been developed (Brooks et al., 2006; Schmeller et al., 2008; Joseph, Maloney and Possingham, 2009; Moilanen, Wilson and Possingham, 2009; Gerber et al., 2017; Grant et al., 2017). Prioritisation is the result of a trade-off between the potential for successful conservation (the actions available and their chances of success, given the risk to a species) and the preferences and constraints of the decision makers, such as the conservation value attributed to a species, its distribution range, available resources, unwanted effects on ecosystems, and

attitudes to risk (Joseph, Maloney and Possingham, 2009; Tulloch et al., 2015). Understanding these components and treating them appropriately is key to a transparent decision-making process (Game, Kareiva and Possingham, 2013).

These trade-offs are also relevant in the case of *Bsal*. First, priorities will inevitably depend on the decision context. For example, *S. salamandra* may not be considered a conservation priority at the EU level or in many countries in which it is common. Because of its restricted geographical range in the Netherlands and the fact that it has been severely affected by *Bsal*, *S. salamandra* is prioritised for conservation there (Spitzen-van der Sluijs et al., 2013). Many possible criteria for prioritisation have been suggested, from genetic representativeness (Isaac et al., 2007) to range-wide relevance of local declines (Schmeller et al., 2008, 2014b) to cultural values (Pollard et al., 2014). The object of prioritisation, is utilising feasible mitigation measures which are available for conserving species: if actions to mitigate *Bsal* are not available or feasible in practice, species priorities have little meaning (Brown et al., 2015). Also, the management of more common species that may for example serve as disease reservoirs needs to be implemented so that primary mitigation actions can be effective (Dobson, 2004; Stegen et al., 2017). This also applies to monitoring, where sentinel species might be prioritised for surveillance even though they are not conservation priorities (Halliday et al., 2007).

5. Conclusion: critical research gaps and future actions

Bsal mitigation is surrounded by a high level of uncertainty, however, this should not result in protracted decision-making periods or inaction as this will lead to certain biodiversity loss. From a pragmatic conservation perspective, the main objective of mitigating *Bsal*-induced chytridiomycosis should be to preserve susceptible amphibian species and populations and protect biodiversity, rather than the eradication of *Bsal* in the wild per se. In this sense, any single method is unlikely to accomplish the desired conservation outcome (Gagliardo et al., 2008; Garner et al., 2016). Each approach has its benefits and limitations; therefore, a combination of methods may have the best chance of success.

Given the lack of verified, reliable disease mitigation options, we advise that pre-emptive measures, aimed at reducing pathogen spread and further pathogen introductions by a combination of trade restrictions, biosecurity measures and eliminating the captive *Bsal* reservoir are enacted as a matter of urgency. The set-up of a long-term population monitoring network is key in the early recognition of changes in population sizes, which allows estimating disease impact and evaluation of population recovery. Developing and maintaining a robust early warning system based on passive surveillance will be highly beneficial for the implementation of these *Bsal* control measures. Another important supporting action is the monitoring of host population (size) and *Bsal*-infection dynamics (prevalence, mortality) in known outbreak areas with a view to making apropos conservation decisions. The final supporting action is the development of an evidence-based emergency action plan for at-risk species.

In case of a *Bsal* outbreak, actions that can be taken should focus on disease containment and preserving valuable populations or species where relevant. Disease containment may consist of a rigorous combination of:

1)   limiting opportunities for pathogen dispersal, for example by fencing off areas and restricting access to prevent entry of humans, large mammals, waterbirds and anurans.

2)   eliminating potential *Bsal* environmental reservoirs (drying and disinfection of ponds).

3)   identifying and eliminating potential *Bsal* amphibian reservoirs by consistent and repeated removal of *Bsal* hosts.

4)   delineating the outbreak by intensive monitoring of neighbouring populations for *Bsal* infection and population declines by repeated sampling using skin swabs and population monitoring.

Establishment of ex situ assurance colonies is the most immediately viable course of action and the only option available currently to preserve populations or even species at risk from *Bsal*. However, this must be implemented with the primary intention of developing a long-term protection strategy for effective and sustainable reintroduction. The latter needs applied conservation studies into sustainability, feasibility and effectiveness of mitigation actions (Table 2).

The implementation of current legislation and the above mentioned recommendations is likely to reduce introduction events of *Bsal* and may contain the disease at novel outbreak sites, but does not provide long-term, sustainable solutions for infected systems. This will require closing the following critical knowledge gaps:

1)   introduction pathways: while it is currently assumed that amphibian trade is key in the global dispersal of amphibian-infecting chytrids (Martel et al., 2014; O'Hanlon et al., 2018), proven examples of this are rare (Walker et al., 2008). Identifying crucial components of amphibian-associated pathways for introducing chytrids (not a priori excluding any biotic or abiotic vector) would increase the efficacy of measures aimed at preventing further introductions.

2)   understanding pathways of the dispersal of *Bsal* between populations. Preventing the further spread of *Bsal* in Europe from the existing outbreak sites requires knowledge of mechanisms underpinning this pathogen's spread. While dispersal through infected amphibian hosts seems important at short distances (Spitzen-van der Sluijs et al., 2018), human-mediated spread may be key on a larger spatial scale. However, the possible contribution of other biotic (e.g. migratory birds, large mammals) and abiotic (e.g. waterways, wind) vectors is not yet known.

3)   understanding *Bsal* reservoirs is crucial to any in situ control programme: an eight year follow-up of the *Bsal* index outbreak demonstrates very low prevalence, with very low infection loads in the supposed reservoir host (Alpine newt), suggesting that the existence of a different, non-amphibian reservoir of *Bsal* may be necessary to maintain *Bsal* in this ecosystem. Identifying critical components in an affected ecosystem that allow *Bsal* persistence could greatly contribute to any eradication action.

4)   understanding host susceptibility to *Bsal* infection. Any action aimed at increasing resistance against infection will benefit from a thorough understanding of the host-pathogen-environment interaction, knowledge of which is currently in its infancy. Understanding crucial events like adhesion and intra-epidermal pathogen proliferation from a host, pathogen and

environment perspective could open opportunities for vaccination, bioaugmentation, environmental augmentation and the eventual creation of more resistant host lineages.

Acknowledgements

Table 1: Minimal exposure time for 100% killing of *Bsal* spores and sporangia in water and on fomites at room temperature (Van Rooij et al. 2017)

Disinfectant	Concentration	Minimal exposure time for 100% killing of Bsal
Ethanol (EtOH)	70%	30 s
Disolol®	undiluted	30 s
Hibiscrub®	0.25, 0.5, 0.75%	30 s
Chloramine-T®	0.5%	5 minutes
	1%	2 minutes
Bleach	4%	30 s
Kickstart®	0.05%	5 minutes
	0.1%	2 minutes
Potassium permanganate ($KMnO_4$)	1%	10 minutes
	2%	5 minutes
Virkon S®	0.5%	5 minutes
	1%	2 minutes
Dettol medical®	1:20 dilution	5 minutes
Biocidal®	undiluted	30 s
Safe4®	undiluted	30 s
F10®	1:100 dilution	30 s
	1:250 dilution	30 s
	1:500 dilution	30 s
	1:1000 dilution	30 s
Sodium chloride (NaCl)	10%	10 minutes

Table 2: Advantages and limitations of mitigation and support actions against *Bsal*

Mitigation action	Advantages	Disadvantages
Trade restrictions: importation of live amphibians into the EU	Likely to greatly reduce chances of further *Bsal* introduction in EU Associated costs low in case of ban Relative ease of implementation and control	May promote illegal trade As a stand-alone measure does not prevent *Bsal* spread within EU Associated costs are significant in case of implementing quarantine and entry control measures
Trade restrictions within EU	Likely to greatly reduce chances of *Bsal* spread between EU member states Relative ease of implementation Associated costs low in case of ban	May promote illegal trade Difficult to control As a stand-alone measure does not prevent *Bsal* spread within member states Associated costs significant in case of implementing quarantine and entry control measures
Eradication of *Bsal* from captive urodeles	Elimination of *Bsal* reservoir with reduced likelihood of pathogen pollution, part of a "clean trade" programme Improves animal welfare of captive urodeles	Costs associated with education, screening, diagnosis and treatment Depends on willingness of hobby sector to cooperate
Biosecurity measures	Likely to reduce chances of spread of *Bsal* and other amphibian pathogens Protocols for disinfection already available Raises awareness Implementation of field protocols for working with amphibians already in place in many EU countries	May conflict with commercial interests Depends on willingness of all stakeholders to implement properly Considered a burden Use of chemicals may have adverse effects on humans and environment Costs associated with communication and implementation Cannot control for all potential routes of transmission (e.g. wildlife)
Vaccination	Could be used for prevention and during outbreaks Probably viewed as positive by public opinion	No vaccines available Vaccine development very expensive, long-term and uncertain Proof of concept with wild type *Bsal* strain failed Costs associated with production and application Regulatory issues Generally not transferable across generations Imperfect treatments that only create tolerance while not interrupting transmission could have adverse effects by increasing spread May need to target multiple hosts in diverse amphibian communities
Bioaugmentation	Could be used for prevention and during outbreaks May be transferable across generations Probably viewed as positive by public opinion	Currently not available Development costly and uncertain No proof of concept Regulatory issues Imperfect treatments that only create tolerance while not interrupting transmission could have adverse effects by increasing spread May need to target multiple hosts in diverse amphibian communities

Mitigation action	Advantages	Disadvantages
Selective breeding	Offers perspectives to long-term increased disease resistance	Currently no markers available for marker assisted breeding Genetic engineering fraught with regulatory and public opinion issues Development will take several generations, depending on species No proof of concept Possibly not transferable between species Requires intensive genetic population management Costs associated with producing breeding stock and genetic management
Environmental treatment with disinfectants / antimycotics	Proven effective in single host system for *Bd* May lower infection pressure and reduce likelihood of transmission	No proof of concept for *Bsal* *Bsal* may be less sensitive to disinfectants in terrestrial environment Efficacy questionable in complex systems Adverse effects on environment Not suitable for large scale application Regulatory issues Societal issue of antimycotic resistance Costs associated with products and application Feasibility dependent on application scheme Imperfect treatments that only create tolerance while not interrupting transmission could have adverse effects by increasing spread
Environmental manipulation	May reduce infection pressure and likelihood of transmission May allow host species to compensate *Bsal*-related mortality (e.g. by increased recruitment)	Potential adverse effects on environment No proof of concept for *Bsal* Environmental drivers for *Bsal* infections not known Costs associated with habitat manipulation and maintenance
In situ treatment	May eliminate *Bsal* from infected animals Probably viewed as positive by public opinion	Costs associated with treatment Labor intensive Unlikely that all infected animals are caught for treatment, which is necessary to curb infection at population level *Bsal* infection may recrudesce after treatment Imperfect treatments that only create tolerance while not interrupting transmission could have adverse effects by increasing spread
Reintroduction after Bsal eradication	Directly reinforces remnants of affected populations Probably positively viewed by public opinion	Requires ex situ captive assurance colonies (see below) Requires thorough follow up of reintroduction event, with associated costs of population and disease monitoring Risk of failure and increased pathogen proliferation if *Bsal* is not eradicated from the environment
Creating barriers to pathogen dispersal	Limits *Bsal* spread between sites May create disease free pockets Barriers (roads, canals) may be already present	Costs associated with installation and maintenance Barriers may have considerable failure rates and target only part of all potential vectors May conflict with local infrastructure May have adverse effects on non-target species Regulatory issues

Mitigation action	Advantages	Disadvantages
Culling	May reduce *Bsal* dispersal Culling of reservoir hosts may reduce community-level epidemic and assist persistence of species of conservation priority	Costs associated with culling Unlikely that a sufficient proportion of animals can be captured High likelihood of adverse reactions of public opinion *Bsal* may persist outside of managed hosts Regulatory issues May need to target multiple hosts in diverse amphibian communities
No action	No costs For broad range species with focal population declines if *Bsal* is spontaneously eradicated	Possibility of population extirpation or species extinction Potential conflict with Habitat's Directive High likelihood of adverse reactions of public opinion

Support action	Advantages	Disadvantages
Early warning system	Allows rapid detection and response	Associated costs for efficient operation: diagnostic capacity, data management, communication
Passive surveillance	Large spatial scale possible Low associated costs	Likelihood of outbreak detection highly variable Requires intensive communication efforts and sufficient diagnostic capacity
Active surveillance pre-outbreak	Opportunity to collect data on host population size and distribution	Large-scale implementation problematic Associated costs of coordination, monitoring and sampling
Active surveillance post-outbreak	Monitoring infection dynamics highly informative to mitigation (e.g. pathogen persistence versus eradication)	Associated costs of coordination, monitoring and sampling in a contaminated environment Requires rigorous application of biosecurity measures
Species prioritization	Allows efficient, evidence based allocation of resources for conservation Clarifies which species are conservation priorities (e.g. susceptible species), which are management priorities (e.g. reservoirs)	Requires the availability of detailed information on disease ecology for several urodele taxa that is currently lacking Inaccuracies may have far-reaching consequences for species conservation
Captive assurance colonies	Ensures species survival IUCN guidelines available Captive maintenance of urodeles relatively cheap	Costs associated with coordination, infrastructure and maintenance Requires genetic management Relevance questionable if no perspective for future re-introduction Regulatory issues Requires proper biosecurity Most likely requires prioritization Husbandry techniques may need to be developed for some species
Monitoring	High likelihood of detecting mortality events and population declines Detects declines regardless of the causative agent Public involvement raises awareness	Costs associated with coordination and fieldwork For financial reasons, often involves the use of volunteers, which may reduce manageability

Table S1. Import restriction legislation to prevent *Bsal* introduction and spread.

Country Restricted imports

Canada	The Canadian Wildlife Authorities prohibited the importation of all salamanders including dead Specimens, eggs, sperm, tissue culture or embryos, parts and derivatives without a permit
United States	The Lacey Act prohibits international trade of live or dead specimens and body tissue from 201 salamander species (US Fish and Wildlife Services, 2016; EFSA, 2017a; Klocke, 2017)
Hungary	Restrictions have been placed on specified species of high risk (EFSA, 2018; Stark, 2018) A modification has been made to decree 41/2010 which prohibits keeping, breeding, buying and selling of members of the family Salamandridae and Hynobidae and *Karsenia koreana* (Plethodontidae) (199/2017.(VII.10) Korm. Rendelet, 2017).
Switzerland	In December 2015, the Swiss Food Safety and Veterinary Office implemented import restrictions prohibiting the importation of all salamander species into Switzerland. After the implementation of EU decision 2018/320, Switzerland adapted its legislation accordingly.
EU and Switzerland	Temporary animal health protection measures enacted for the trade of all amphibians in the order Urodela The European Commission issued decision (EU) (2018/320) which establishes animal health protection measures for the trade of salamanders within the EU and importation of salamanders from non-EU territories. These include rejection of any salamanders with obvious signs of illness (especially skin lesions) or originating from collections where there have been positive *Bsal* diagnoses, testing salamanders to ensure that they were free from *Bsal*, restricting movement of salamanders, implementing hygiene protocols and biosecurity measures. (Commission Implementing Decision (EU) 2018/320)
Worldwide	The Convention on International Trade of Endangered Species of wild fauna and flora (CITES) was also explored as vehicle to restrict trade in Asian amphibians but not deemed appropriate

Table S2. Estimated cost of screening captive amphibian collections in Europe for *Bsal*.

1. Screening of captive collections

Scenario 1

298 members in Deutsche Gesellschaft für Herpetologie and Terrarienkunde (DGHT)	× 100 urodeles/keeper ~ 30 000 urodeles	Pool samples in groups of 5 ~ 6000 samples

With 298 urodele keepers and a theoretical 100 urodeles/keeper and pooling samples in groups of 5 (Sabino-Pinto et al., 2018a), screening would cost approximately 180 000 Euros

Scenario 2

1490 members in Deutsche Gesellschaft für Herpetologie and Terrarienkunde (DGHT)	× 100 urodeles/keeper ~ 149 000 urodeles	Pool samples in groups of 5 ~ 30 000 samples

With 5 times the urodele keepers in scenario 1 (1490) and a theoretical 100 urodeles/keeper and pooling samples in groups of 5 (Sabino-Pinto et al., 2018a), screening would cost approximately 900 000 Euros

- AG Urodela
- Salamandervereniging

1. Provided information sheets on Bsal on their website
2. Scheduled Bsal lectures during their annual events

- Ornamental Aquatic Trade Association (OATA)
- Reptile and Exotic Pet Association

1. Set up information campaigns to improve biosecurity of importers, retailers, customers in combination with several other organisations
2. Published a disease alert to prevent spread from captive to wild amphibians

Figure S1. Stakeholders (urodelan keepers and associations) promoting *Bsal* awareness.

6. References

199/2017. (VII. 10.) Korm. rendelet (2017.) a kedvtelésből tartott állatok tartásáról és forgalmazásáról szóló 41/2010. (II. 26.) Korm. rendelet és a veszélyeztetett vadon élő állat- és növényfajok nemzetközi kereskedelmét szabályozó nemzetközi és európai közösségi jogi aktusok végrehajtásának egyes szabályairól szóló 292/2008. (XII. 10.) Korm. rendelet módosításárólRetrieved from https://net.jogtar.hu/jr/gen/hjegy_doc.cgi?docid=A1700199.KOR&timeshift=ffffff4&txtrefer er=00000001.TXT

Auliya, M., Garcia-Moreno, J., Schmidt, B.R., Schmeller, D.S., Hoogmoed, M.S., Fischer, M.C., Pasmans, F., Henle, K., Bickford, D., Martel, A. (2016). The glocal amphibian trade flows through Europe: the need for enforcing and improving legislation. *Biodiversity and Conservation*. **25**(13): 2581-2595 doi: 10.1007/s10531-016-1193

Bataille A., Cashins S.D., Grogan L., Skerrat L.F., Hunter D., McFadden M., Scheele B., Branelly L.A., Macris A., Harlow P.S., Bell S., Berger L., Waldman B. (2015): Susceptibility of amphibians to chytridiomycosis is associated with MHC Class II conformation. *Proceedings of the Royal Society B Biological Sciences* 282: 20143127.

Bates, K.A., Clare, F.C., O'Hanlon, S., Bosch, J., Brookes, L., Hopkins, K., McLaughlin, E.J., Daniel, O., Garner, T.W.J., Fisher, M.C., Harrison, X.A. (2018). Amphibian chytridiomycosis outbreak dynamics are linked with host skin bacterial community structure. *Nat. Commun.* **9** 693 doi:10.1038/s41467-018-02967-w

Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C. L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B, Lips, K.R., Marantelli, G., Parkes, H. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc. Natl. Acad. Sci. U.S.A*. **95**.

Berger, L., Speare, R., Kent, A. (1999) Diagnosis of chytridiomycosis in amphibians by histologic examination. World Wide Web file. Retrieved from http://www.jcu.edu.au/school/phtm/PHTM/frogs/histo/chhisto.htm

Beukema, W., Martel, A., Nguyen,T.T., Goka, K., Schmeller, D.S., Yuan, Z., Laking, A.E., Nguyen, T.Q., Lin, C.-F., Shelton, J., Loyau, A., Pasmans, F. (2018) Environmental context and differences between native and invasive observed niches of *Batrachochytrium salamandrivorans* affect invasion risk assessments in the Western Palaearctic. *Divers. Distrib.* 24: 1788-1801.

Bletz, M. C., Loudon, A. H., Becker, M. H., Bell, S. C., Woodhams, D. C., Minbiole, K. P.C., Harris, R.N. (2013). Mitigating amphibian chytridiomycosis with bioaugmentation: Characteristics of effective probiotics and strategies for their selection and use. *Ecology Letters* **16**(6):807-20 https://doi.org/10.1111/ele.12099

Bletz, M.C., Kelly, M., Sabino-Pinto, J., Bales, E., Van Praet, S., Bert, W., Boyen, F., Vences, M., Steinfartz, S., Pasmans, F., Martel, A. (2018) Disruption of skin microbiota contributes to salamander disease. *Proc. Biol. Sci.* 285 (1885)

Blooi, M., Pasmans, F., Longcorre, J.E., Spitzen-van der Sluijs, A., Vercammen, F., Martel, A.

(2013). Duplex Real-Time PCR for Simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in Amphibian Samples. *Journal of Clinical Microbiology* **51**(12): 4173-4177

Blooi, M., Martel, A., Haesebrouck, F., Vercammen, F., Bonte, D., Pasmans, F. (2015a). Treatment of urodelans based on temperature dependent infection dynamics of *Batrachochytrium salamandrivorans*. *Sci. Rep.*, **27**(5): 80375 https://doi.org/10.1038/srep08037

Blooi, M., Pasmans, F., Rouaffer, L., Haesebrouck, Vercammen, F., Martel, A. (2015b). Successful treatment of *Batrachochytrium salamandrivorans* infections in salamanders requires synergy between voriconazole, polymyxin E and temperature. Sci. Rep. **5**:11788. doi: 10.1038/srep11788.

Blooi, M., Laking, A.E., Materl. A., Haesebrouck, F., Jocque, M., Brown, T., Green, S., Vences, M., Bletz, M.C., Pasmans, F. (2017) Host niche may determine disease-driven extinction risk. *PLoS One*. **12**(7): 30181051 https://doi.org/10.1371/journal.pone.0181051

Bosch, J., Martínez-Solano, I. (2006). Chytrid fungus infection related to unusual mortalities of *Salamandra salamandra* and *Bufo bufo* in the Peñalara Natural Park, Spain. *Oryx*, **40**(1), 84–89 https://doi.org/DOI: 10.1017/S0030605306000093

Bosch, J., Sanchez-Tomé, E., Fernández-Loras, A., Oliver, J. A., Fisher, M. C., & Garner, T.W.J. (2015). Successful elimination of a lethal wildlife infectious disease in nature. *Biol. Lett.*, **11**(11), 20150874. https://doi.org/10.1098/rsbl.2015.0874

Brooks, T.M., Mittermeier, R.A., da Fonseca, G.A., Gerlach, J., Hoffmann, M., Lamoreux, J.F., Mittermeier, C.G., Pilgrim, J.D., Rodrigues, A.S.L. (2006) Global biodiversity conservation priorities. *Science* **313**(5783): 58-61 doi: 10.1126/Science.1127609

Brown, C.J., Bode, M., Venter, O., Barnes, M.D., McGowan, J., Runge, C.A., Watson, J.E.M., Possingham, H.P. (2015). Effective conservation requires clear objectives and prioritizing actions, not places or species. *Proc.   Natl. Acad. Sci. U.S.A.*   **112**(32)e4342 doi: 10.1073/pnas.1509189112

Brutyn, M., D'Herde, K., Dhaenens, M., Van Rooij, P., Verbrugghe, E., Hyatt, A.D., Croubels, S., Deforece, D., Ducatelle, R., Haesebrouck, F., Martel, A., Pasmans, F. (2012). Batrachochytrium dendrobatidis zoospore secretions rapidly disturb intercellular junctions in frog skin. *Fungal Genet. Biol.* **49**(10): 830-7 doi: 10.1016/j.fgb.2012.07.002.

Buck, J.C., Truong, L., Blaustein, A.R. (2011). Predation by zooplankton on *Batrachochytrium dendrobatidis*: biological control of the deadly amphibian chytrid fungus ? *Biodiversity and Conservation*. **20**(14): 3549-3553 doi: 10.1007/s10531-011-0147-4

Buckland, S.T., Studeny, A.C., Magurran, A., Newson, S. (2010) Biodiversity monitoring: the relevance of detectability. In A.E. Magurran, & B.J. McGill (Eds.), *Biological Diversity: Frontiers in Measurement and Assessment* pp25-36. Oxford University press, Oxford

Canada   Gazette   Part   II   (2017,   May   31).   **151**(11)   Retrieved   from www.ecology.com/daily_images/1003988625-1003990018.pdf

Canessa, S., Converse, S.J., West, M., Clemann, N., Gillespie, G., McFadden, M. et al. (2016). Planning for ex-situ conservation in the face of uncertainty. *Conserv. Biol.* **30**, 599-609 doi: 10.1111.cobi.12613

Canessa, S., Bozzuto, C., Grant, E.H.C., Cruickshank, S., Fisher, M., Koella, J. et al. (2018). Decision making for mitigating wildlife diseases: from theory to practice for an emerging fungal pathogen of amphibians. *J. Appl. Ecol.* **55**(4): 1987-1996 doi:10.1111/1365-2664.13089

Commission Implementing Decision (EU) 2018/320 of 28 February 2018 on certain animal health protection measures for intra-Union trade in salamanders and the introduction into the Union of such animals in relation to the fungus *Batrachochytrium salamandrivorans (notified under document C(2018) 1208)* (1) Retrieved from http://eur-lex.europa.eu/eli/dec_impl/2018/320/oj

Convention on the Conservation of European Wildlife and Natural Habitats (1979) Council of Europe. Retrieved from https://www.coe.int/en/web/conventions/full-list/-/conventions/treaty/104

Courtois, E.A., Loyau, A., Bourgoin, M., Schmeller, D.S. (2017) Initiation of *Batrachochytrium dendrobatidis* infection in the absence of physical contact with infected hosts – a field study in a high altitude lake. *Oikos* 126: 843-851.

Dalbeck, L., Düssel-Siebert, H., Kerres, A., Kirst, K., Koch, A., Lötters, S., Ohlhoff, D., Sabino-Pinto, J., Preißler, K., Schulte, U., Schulz, V., Steinfartz, S., Veith, M., Vences, M., Wagner, N., Wegge, J. (2018). Die Salamanderpest und ihr Erreger *Batrachochytrium salamandrivorans* (*Bsal*): aktueller Stand in Deutschland *Zeitschift für Feldherpetologie* **25**: 1-22

Darling, J.A., Mahon, A.R. (2011) From molecules to management: adopting DNA-based methods for monitioring biologcal invasions in aquatic environments. *Environmental Research.* **111** (7): 978-988.

Denöel, M., Bichot, M., Ficetola, G.F., Delcourt, J., Ylieff, M., Kestemont, P., Poncin, P.(2010). Cumulative effects of road deicing on amphibian behaviour. *Aquat. Toxicol.* **99**: 275-280 https://doi.org/10.1016/j.aquatox.2010.05.0

Dickinson, J.L., Shirk, J., Bonter, D., Bonney, R., Crain, R.L., Martin, J., Phillips, T., Purcell, K. (2012) The current state of citizen science as a tool for ecological research and public engagement. *Front. Ecol. Environ.* **10**: 291-297.

Dobson, A. (2004). Population dynamics of pathogens with multiple host species. *Am. Nat.* 164 s64-s78 doi: 10.1086/424681

Earl, J.E., & Gray, M.J. (2014). Introduction of Ranavirus to Isolated Wood Frog Populations Could Cause Local Extinction. *EcoHealth*, **11**(4), 581–592. https://doi.org/10.1007/s10393-014-0950-y

EFSA (European Food Standard Agency) Balàž, V., Schmidt, C.G., Murray, K., Carnesecchi, E., Garcia, A., Gervelmeyer, A., Martino, L., Guajardo, I.M., Verdonck, F., Zancanaro, G., Fabris, C. (2017a) Scientific and technical assistance concerning the establishment, survival and spread of *Batrachochytrium salamandrivorans* (*Bsal*) in the EU. *EFSA J.* 2017 **15**(2):4739 doi: 10.2903/j.efsa.2017.4739

EFSA(European Food Safety Authority) More, S., Bøtner, A., Butterworth, A., Calistri, P., Depner, K., Edwards, S., Garin-Bastuji, A., Good, M., Schmidt, C.G., Micehl, V., Miranda, M.A., Nielsen, S.S., Raj, M., Sihvonen, L., Spoolder, H., Stegeman, J.A., Thulke, H., Velarde, A., Willeberg, P., Winckler, C., Baldinelli, F., Broglia, A., Candiani, D., Fabris, C., Zancanaro, G., Beltrán-Beck, B., Kohnle, L., Bicout, D. (2017b). Assessment of listing and categorisation of animal disease within the framework of the Animal Health Law (Regulation (EU) No 2016/429): *Batrachochytrium salamandrivorans (Bsal). EFSA J.* 2017;15(11): 5071 doi: 10.2903/j.efsa.2017.5071

EFSA Panel on Animal Health and Welfare (AHAW) More, S., Miranda, M.A., Bicout, D., Bøtner, A., Butterworth, A., Calistri, P., Depner, K., Edwards, S., Garin-Bastuji, B., Good, M., Michel, V., Raj, M., Nielson, S.S., Sihvonen, L., Spoolder, H., Stegemen, J. A., Thulke, H., Velarde, A., Willeberg, P., Winckler, C., Balaz, V., Martel, A., Murray, K., Munoz-Gajardo, I., Gogin, A., Verdonck, F., Schmidt, C.G. (2018) Risk of survival establishment, spread of *Batrachochytrium salamandrivorans* (*Bsal*) in the EU. *EFSA J.* 16(5):5259 doi: 10.2903/j.efsa.2018.5259

Farrer, R. A., Martel, A., Verbrugghe, E., Abouelleil, A., Ducatelle, R., Longcore, J. E., James, T.Y., Pasmans, F., Fisher, M.C., Cuomo, C.A. (2017). Genomic innovations linked to infection strategies across emerging pathogenic chytrid fungi. *Nat. Commun.* **8,** 14742 doi: 10.1038/ncomms14742 (2017).

Feldmeier, S., Schefczyk, L., Wagner, N., Heinemann, G., Veith, M., Lötters, S. (2016). Exploring the Distribution of the Spreading Lethal Salamander Chytrid Fungus in Its Invasive Range in Europe – A Macroecological Approach. *PLoS ONE*, *11*(10), e0165682. http://doi.org/10.1371/journal.pone.0165682

Ficetola, G.F., Poulenard, J., Sabatier, P., Messager, E., Gielly, L., Le Loup, A., Ettiene, D., Bakke, J., Mallet, E., Fanget, B., Storet, E., Reyys, J., Taberlet, P., Arnaud, F. (2018). DNA from lake sediments reveal patterns of ecosystem changes after a biological invasion. *Science Advances* **4**(5): eaar486-108292.

Fitzpatrick, L.D., Pasmans, F., Martel, A., Cunningham, A.A. (2018). Epidemiological tracing of *Batrachochytrium salamandrivorans* identifies widespread infection and associated mortalities in private amphibian collections. *Sci. Rep.* **8**: 13845.

Forrest, M. J., Schlaepfer, M.A. (2011). Nothing a hot bath won't cure: Infection rates of amphibian chytrid fungus correlate negatively with water temperature under natural field settings. *PLoS ONE*, **6**(12). https://doi.org/10.1371/journal.pone.0028444

Freidenburg, L.K., Skelly, D.K. (2004). Microgeographical variation in thermal preference by an amphibian. *Ecology Letters*, **7**(5), 369–373. https://doi.org/10.1111/j.1461-0248.2004.00587.x

Gagliardo, R., Crump, P., Griffith, E., Mendelson, J., Ross, H., Zippel, K. (2008). The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. *Int. Zoo. Yearb.*, **42**. doi:10.1111/j.1748-1090.2008.00043.x

Game, E.T., Kareiva, P., Possingham, H.P. (2013) Six Common Mistakes in Conservation

Priority Setting. Conservation Biology. **27**(3) 480-485 https://doi.org/10.1111/cobi.12051

Garmyn, A., van Rooij, P., Pasmans, F., Hellebuyck, T., van den Broeck, W., Haesebrouck, F., Martel, A. (2012). Waterfowl: Potential environmental reservoirs of the chytrid fungus *Batrachochytrium dendrobatidis*. *PLoS ONE*, *7*(4). https://doi.org/10.1371/journal.pone.0035038

Garner, T.W.J., Schmidt, B.R., Martel, A., Pasmans, F., Muths, E., Cunningham, A.A., Weldon, C., Fisher, M.C., Bosch, J. (2016) Mitigating amphibian chytridiomycoses in nature. *Phil. Trans. R. Soc. B. Lond. B. Biol. Sci.* 371: 20160207

Garner, T.W.J., Walker, S., Bosch, J., Hyatt, A.D., Cunningham, A.A., FisherM.C. (2005). Chytrid fungus in Europe. *Emerging Infect. Dis.* **11**: 1639-1641.

Geiger, C.C., Bregnard, C., Maluenda, E., Voordouw M.J., Schmidt, B.R. (2017) Antifungal treatment of wild amphibian populations caused a transient reduction in the prevalence of the fungal pathogen *Batrachochytrium dendrobatidis. Sci. Rep.* **7** (5956)

Gerber B.D., Converse, S.J., Muths, E., Crockett, H.J., Mosher, B.A., Bailey, L.L. (2017). Identifying species conservation strategies to reduce disease associated declines. *Conserv. Lett.* **11** (2): e12393.

Grant, E.H.C., Muths, E., Katz, R.A., Canessa, S., Adams, M.J., Harris, M.C., Harris, R.N., Hossack, B., Huyvaert, K.P., Kolby, J., Lips, K.R., Lovich, R.E., McCallum, H.I., Mendelson III, J.R., Nanjappa, P., Olson, D.H., Powers, J.G., Richgels, K.L.D., Russel, R.E., Schmidt, B.R., Spitzen-van der Sluijs, A., Watry, M.K., Woodhams, D.C., White, C.L. (2017). Using decision analysis to support proactive management of emerging infectious wildlife diseases. *Ecol. Soc. Am.* **15**(4): 214-221

Grant, E.H.C., Muths, E.L., Katz, R.A., Canessa, S., Adams, M.J., Ballard, J.R., Berger, L., Briggs, C.J., Coleman, J., Gray, M.J., Harris, M.C., Harris, R.N., Hossack, B.R., Huyvaert, K.P., Kolby, J.E., Lips, K.R., Lovich, R.E., McCallum, H.I., Mendelson, IIIJ., R., Nanjappa, P., Olsen, D.H., Powers, J.G., Richgels, K.L.D., Russel, R.E., Schmidt, B.R., Spitzen-van der Sluijs, A., Watry, M.K., Woodhams, D.C., White, C.L. (2015). Salamander chytrid fungus (Batrachochytrium salamandrivorans) in the United States – developig research monitoring and management strategies: U.S. Geological Survey Open File Report 2015-1233 16 p. DOI:10.3133/ofr20151233.

Grogan, L.F., Berger, L., Rose, K., Grillo, V., Cashins, S.D., Skerratt, L.F. (2014). Surveillance for Emerging Biodiversity Diseases of Wildlife. *PLoS Pathology* **10**: e1004015.

Guy, R.A., Payment, P., Krull, U.J., Horgen, P.A. (2003). Real-time PCR for quantification of Giardia and Cryptosporidium in environmental water samples and sewage. *Appl. Environ. Microbiol.* 69: 5178-5185.

Halliday, J.E. ., Meredith, A.L., Knobel, D.L., Shaw, D.J., Bronsvoort, B.M.d.C., Cleaveland, S. (2007). A framework for evaluating animals as sentinels for infectious disease surveillance. *J. R. Soc., Interface*, **4**(16), 973–984. https://doi.org/10.1098/rsif.2007.0237

Heard, G.W., Scroggie, M.P., Clemann, N., Ramsey, D.S.L. (2014). Wetland characteristics influence disease risk for a threatened amphibian. *Ecological Applications*, **24**(4), 650–662.

https://doi.org/10.1890/13-0389.1

Hudson, M.A., Young, R.P., Lopez, J., Martin, L., Fenton, C., McCrea, R., Griffiths, R.A., Adams, S., Gray, G., Garcia, G., Cunningham, A.A. (2016). In-situ itraconazole treatment improves survival rate during an amphibian chytridiomycosis epidemic. *Biological Conservation*, **195**, 37–45. https://doi.org/10.1016/j.biocon.2015.12.041

Huver, J.R., Koprivnikar, J., Johnson, P.T.J., Whyard, S. (2015). Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. *Ecol. Appl.* **25**: 991-1002.

Isaac, N.J., Turvey, S.T., Waterman, C., & Baillie, J.E. (2007) Mammals on the Edge:conservation priorities based on threat and phylogeny. *PLoS One* **2**(3): e296 doi: 10.1371/journal.pone.0000296

IUCN (2013). Guidelines for reintroductions and other conservation translocations. IUCN Species Survival Commission, Gland, Switzerland. retrieved from https://portals.iucn.org/library/efiles/documents/2013-009.pdf

Johnson, M.L., Berger, L., Philips, L., & Speare, R. (2003). Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid, *Batrachochytrium dendrobatidis*. *Dis. Aquat. Org.*, **57**(3):255-60. doi: 10.3354/dao057255

Jones, D.K., Mattes, B.M., Hintz, W.D., Schuler M.S., Stoler, A.B., Lind, L.A., Cooper, R.O., Relyea, R.A. (2016) *Environ Pollut.* **221**:159-167 doi:10.1016/j.envpol.2016.11.060

Joseph, L.N., Maloney, R.F., Possingham, H.P. (2009). Optimal allocation of resources among threatened species: a project prioritization protocol. *Conservation Biology* **23**(2): 328-338) doi:10.1111/j.1523-1739.2008.01124.x.

Karraker, N.E., Gibbs, J.P., Vonesh, J.R. (2008) Impacts of Road Deicing salt on the Demography of Vernal Pool-Breeding Amphibians. *Ecological Applications*. **18**(3): 724-734 https://doi.org/10.1890/07-1644.1

Karraker, N.E., Gibbs, J.P., (2011) Road deicing salt irreversibly disrupts os moregulation of salamander egg clutches. *Environmental Pollution.* **159**(3):833-5. doi:10.1016/j.envpol.2010.11.019

Kéry, M. & Schmidt, B. (2008) Imperfect detection and its consequences for monitoring for conservation. *Community Ecology.* **9**(2): 207-216 doi:10.1556/ComEc.9.2008.2.10

Klocke, B., Becker, M., Lewis, J., Fleischer R.C., Muletz, C.R., Rockwood, L., Aguirre A.A., Gratwicke, B. (2017) Batrachochytrium salamandrivorans not detected in U.S. survey of pet salamanders. *Sci. Rep.* **7**:13132 doi:10.1038/s41598-017-13500-2

Kolby, J.E., Smith, K.M., Berger, L., Karesh, W.B., Preston, A., Pessier, A.P., & Skerratt, L.F. (2014). First evidence of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) and ranavirus in Hong Kong amphibian trade. *PLoS ONE*, **9**(3). https://doi.org/10.1371/journal.pone.0090750

Laking A.E., Ngo, H.N., Pasmans, F., Martel, A., Nguyen, T.H. (2017). Batrachochytrioum salamandrivorans is the predominant chytrid fungus in Vietnamese salamanders. *Sci. Rep.* **7**,

44443; doi:10.1038/srep44443 (2017)

Langton, T.E.S., Beckett, C.L., Foster, J.P. (2001). Great Crested Newt Conservation Handbook Froglife Halesworth. Retrieved https://www.abebooks.co.uk/first-edition/Great-Crested-Newt.Conservation-Handbook-Tom-Langton.Catherine/7077169734/bd.

Langwig, K.E., Voyles, J., Wilber, M.Q., Frick, W.F., Murray, K.A., Bolker, B.M., Collins, J.P., Cheng, T.L., Fisher, M.C., Hoyt, J.R., Lindner, D.L., McCallum, H.I., Puschendorf, R., Rosenblum, E.B., Toothman, M., Willis, C.K.R., Briggs, C.J., Kilpatrick, A.M. (2015) Context-dependent conservation responses to emerging wildlife diseases. *Frontiers in Ecology and the Environment* **13**(4) 195-202, doi:10.1890/140241

Lawson, B., Petrovan, S.O., Cunningham, A.A. (2015). Citizen science and wildlife disease surveillance. *EcoHealth* **12**: 693-702.

Longcore, J.E., Pessier, A.P., Nichols, D.K. (1999). *Batrachochytrium dendrobatidis* gen. et. sp. nov, a chytrid pathogenic to amphibians. *Mycologia* **2** (91): 219-227.

Loudon, A.H., Woodhams, D.C., Parfrey, L.W., Archer, H., Knight, R., McKenzie, V., Harris, R.N. (2014). Microbial community dynamics and effect of environmental microbial reservoirs on red-backed salamanders (*Plethodon cinereus*). *The ISME Journal*, **8**(4), 830–840. https://doi.org/10.1038/ismej.2013.200

Loyau, A., Cornuau, J.H., Clare, F.C., & Schmeller, D.S. (2016) Side effects of itraconazole on post-metamorphic Alytes obstetricans after a cold stress. *Amphibian-Reptilia*. **37**(4)345-357 doi: 10.1163/15885381-00003064

Loyau, A., & Schmeller, D.S. (2017) Positive sentiment and knowledge increase tolerance toward conservation actions. *Biodiversity and Conservation*. **26**(2): 461-478 https:doi.org/10.1007/s10531-016-1253.0

Marquis, O., Miaud, C., Gibault, C., Chai, N. (2019). A first screening of chytrid fungus *Batrachochytrium* in amphibians in French zoos. *Int. Zoo Yearb.* **53**: 1-10.

Martel, A., Spitzen-van der Sluijs, A., Blooi, M., Bert, W., Ducatelle, R., Fisher, M. C., Woeltjes, A., Bosman, W., Chiers, K., Bossuyt, F., Pasmans, F. (2013). *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proc. Natl. Acad Sci*, U.S.A. **110**(38), 15325–15329. https://doi.org/10.1073/pnas.1307356110

Martel, A., Blooi, M., Adriaensen, C., Van Rooij, P., Beukema, W., Fisher, M.C., Farrer, R.A., Schmidt, B.R., Tobler, U., Goka, K., Lips, K.R., Muletz, C., Zamudio, K.R., Bosch, J., Lötters, S., Wombwell, C., Garner, T.W.J., Cunningham, A.A., Spitzen-van der Sluijs, A., Salvidio, S., Ducatelle, R., Nishikawa, K., Nguyen, T.T., Kolby, J.E., Van Bocxlaer, I., Bossuyt, F., Pasmans, F. (2014). Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science*, **346**(6209), 630–631. https://doi.org/10.1126/science.1258268

Martin, T.G., Nally, S., Burbidge, A.A., Arnall, S., Garnett, S.T., Hayward, M.W., Lumsden, L.F., Menkhorst, P., McDonald-Madden, E., Possingham, H.P., (2012). Acting fast helps avoid extinction. *Conservation Letters*, **5**, (4)274-280. https:doi.org/10.1111/j.155-263X.2012.00239.x

McMahon, T. A., Sears, B. F., Venesky M. D., Bessler, S. M., Brown, J. M., Deutsch, K., Halstead, N.T., Lentz, G., Tenouri, N., Young, S., Civitello, D.J., Ortega, N., Fites, J.S., Reinert, L.K., Rollins-Smith, L.A., Raffel, T.R., Rohr, J.R. (2014) Amphibians acquire resistance to live and dead fungus overcoming fungal immunosuppression. *Nature* **511**(7508): 224–227. doi: 10.1038/nature13491

Moilanen, A., Wilson, K.A., & Possingham, H.P. (2009) *Spacial conservation prioritization: quantitative methods and computational tools*. Moilanen, A., Wilson, K.A., & Possingham, H.P. (Eds.) Oxford University Press, Oxford

Morán-Ordóñez, A., Canessa, S., Bota, G., Brotons, L., Herrando, S., Hermoso, V. (2018). Efficiency of species survey networks can be improved by integrating different monitoring schemes in a spatial prioritization design. *Conserv. Lett.* **11**: e12591.

Muths, E., McCallum, H. (2016). Why you cannot ignore disease when you reintroduce animals in reintroduction of fish and wildlife populations. In: *Reintroduction of Fish and Wildlife Populations*. Jachowski, D.S., Millspaugh, J.J., Angermeier, P.L., Slotow, J., Eds University of California PressCalifornia.

Nguyen, T.T., Nguyen, T.V., Ziegler, T., Pasmans, F., Martel, A. (2017). Trade in wild anurans vectors the urodelan pathogen Batrachohcytrium salamandrivorans into Europe. *Amphibia-Reptilia* **38**(4) 554-556 doi: 10.1163/15685381-00003125

O'Hanlon, S.J., Rieux, A., Farrer, R.A., Rosa, G.M., Waldman, B., Bataille, A., Kosch, T.A., Murray, K.A., Brankovics, B., Fumagalli, M., Martin, M.D., Wales, N., Alvarado-Rybak, M., Bates, K.A., Berger, L., Böll, S., Brookes, L., Clare, F., Courtois, E.A., Cunningham, A.A., Doherty-Bone, T.M., Ghosh, P., Gower, D.J., Hintz, W.E., Höglund, J., Jenkinson, T.S., Lin, C., Laurila, A., Loyau, A., Martel, A., Meurling, S., Miaud, C., Minting, P., Pasmans, F., Schmeller, D.S., Schmidt, B.R., Shelton, J.M.G., Skerrat, L.F., Smith, F., Soto-Azat, C., Spagnoletti, M., Tessa, G., Toledo, L.F., Valenzuela-Sánchez, A., Verster, R., Vörös, J., Webb, R.J., Wierzbicki, C.,Wombwell, E., Zamudio, K.R., Aanensen, D.M., James, T.Y., Gilbert, M.T.P., Weldon, C., Bosch, J., Balloux, F., Garner, T.W.J., Fisher, M.C.(2018). Recent Asian origin of chytrid fungi causing global amphibian declines. *Science*. **360** (3689): 621-627 doi: 10.1126/Science.aart1965

Pasmans, F., Bogaerts, S., Braeckman, J., Cunningham, A.A., Hellebuyck, T., Griffiths, R.A., Sparreboom, M., Schmidt, B.R., Martel, A. (2017) The future of keeping pet reptiles and amphibians: towards integrating animal welfare, human health and environmentall sustainability. *Vet. Rec.* **181**(17)450 doi:10.1136/vr.104296

Pessier, A.P., Baitchman, E.J., Crump, P., Wilson, B., Griffith, E., Ross, H. (2014). Causes of Mortality in Anuran Amphibians From An Ex Situ Survival Assurance Colony in Panama. *Zoological Biology* **33**(6), 516–526. https://doi.org/10.1002/zoo.21166

Piotrowski, J.S., Annis, S.L., Longcore, J.E. (2004). Physiology of *Batrachochytrium dendrobatidis,* a chytrid pathogen of amphibians. *Mycologia*, **96**(1), 9–15. https://doi.org/10.2307/3761981

Pollard, e., Brodie, G., Thaman, R., Morrison, C. (2014) The use of herpetofauna and cultural

values to identify priority conservation forests on Malaita, Solomon Islands. *Pacific Conservation Biology* **20** (4)354-362

Raffel, T.R., Michel, P.J., Sites, E.W., Rohr, J.R. (2010). What drives chytrid infections in newt populations? Associations with substrate, temperature, and shade. *EcoHealth*, **7**(4), 526–536. https://doi.org/10.1007/s10393-010-0358-2

Richgels, K.L.D., Russel, R.E., Adams, M.J., White, C.L., Grant, E.H.C. (2016) Spatial variation in risk and consequence of Batrachochytrium salamandrivorans introduction in the USA. R. Soc. Open Sci. **3** 150616 doi: 10.1098/rsos.150616

Rodríguez-Brenes, S., Rodriguez, D., Ibáñez, R., Ryan, M.J. (2016). Spread of amphibian chytrid fungus across lowland populations of túngara frogs in Panamá. *PLoS ONE* **11**: e0155745.

Roy, H.E., Rabitsch, W., Scalera, R., Stewart, A., Belinda, G., Genovesi, P., Essl, F., Adriaens, T., Bacher, S., Booy, O., Branquart, E., Brunel, S., Copp, G.H., Dean, H., D'hondt, B., Josefsson, M., Kenis, M., Kettunen, M., Linnamagi, M., Lucy, F., Martinou, A., Moore, N., Nentwig, W., Nieto, A., Pergl, J., Peyton, J., Roques, A., Schindler, S., Schönrogge, K., Solarz, W., Stebbing, P.D., Trichkova, T., Vanderhoeven, S., Van Valkenburg, J., Zenetos, A. (2017). Developing a framework of minimum standards for the risk assessment of alien species. *J. Appl. Ecol.* **55**(2) 526-538. https://doi.org/10.1111/1365-2664.13025

Sabino-Pinto, J., Krause, E.T., Bletz, M.C., Martel, A., Pasmans, F., Steinfartz, S., Vences, M. (2018a). Detectability vs. time and costs in pooled DNA extraction of cutaneous swabs: a study on the amphibian chytrid fungi. *Amphibia-Reptilia* **39**.

Sabino-Pinto, J., Veith, M., Vences, M., Steinfartz, S. (2018b). Asymptomatic infection of the fungal pathogen *Batrachochytrium salamandrivorans* in captivity. *Sci. Rep.* **8**: 11767.

Savage, A.E., Sredl, M.J., & Zamudio, K.R. (2011). Disease dynamics vary spatially and temporally in a North American amphibian. *Biological Conservation* **144**(6) 1910-1915. https://doi.org/10.1016/j.biocon.2011.03.018

Scheele, B.C., Hunter, D.A., Grogan, L.F., Berger, L., Kolby, J., McFadden, M.S., Marantelli, G., Skerratt L.F., Driscoll, D.A. (2014). Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians. *Conservation Biology*, **28**, 1195–1205 doi.org/10.1111/cobi.12322

Schmeller, D.S., Gruber, B., Budrys, E., Framstead, E., Lengyel, S., Henle, K. (2008). National responsibilities in European species conservation: a methodological review. *Conservation Biology* **22**(3):593-601 doi:10.1111/j.1523-1739.2008.00961.x.

Schmeller, D.S., Blooi, M., Martel, A. Garner, T.W.J., Fisher, M.C., Azemar, cgF., Clare, F.C., Leclerc, C., Jäger, L., Guevara-Nieto, M., Loyau, A., Pasmans, F. (2014a) Microscopic Aquatic Predators Strongly Affect Infection Dynamics of a Globally Emerged Pathogen. *Curr. Biol*. **24**(2) 176-180. doi: http://dx.doi.org/10.1016/j.cub.2013.11.032

Schmeller, D.S., Evans, D., Lin, Y.P., Henle, K. (2014b). The national responsibility approach to setting conservation priorities – recommendations for its use. *J. Nat. Conserv.* **22**: 349-357.

Schmeller, D.S., Loyau, A., Bao, K., Brack, W., Chatzinotas, A., De Vleeschouwer, F., Friesen, J., Gandois, L., Hansson, S.V., Haver, M., Le Roux, G., Shen, J., Teisserenc, R., Vredenburg, V.T (2018). People pollution and pathogens- Global change impacts in mountain freshwater ecosystems. *Sci. Total Environ.* **622-623**,756-763) doi:10.1016/j.scitotenv.2017.12.006.

Schmidt, B.R., Bozzuto, C., Lötters, S., Steinfartz, S. (2017). Dynamics of host populations affected by the emerging fungal pathogen *Batrachochytrium salamandrivorans. R. Soc. Open Sci.* **4**, 160801 doi.org/10.1098/rsos.160801

Searle, C., Mendelson III, J.R., Green, L.E., Duffy, M.A. (2013). Daphnia predation on the amphibian chytrid fungus and its impact on disease risk in tadpoles. *Ecology and Evolution*, **3**(12), 4129-4138 doi: 10.1002/ece3.777

Skerratt, L.F., Berger, L., Speare, R., Cashins, S., McDonald, K.R., Philpot, A.D., Hines, H.B., Kenyon, N. (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* **4**: 125.

Spitzen-van der Sluijs, A., Spikmans, F., Bosman, W., de Zeeuw, M., van der Meij, T., Goverse, E., Kik, M., Pasmans, F., Martel, A. (2013). Rapid enigmatic decline drives the fire salamander (*Salamandra salamandra*) to the edge of extinction in the Netherlands. *Amphibia-Reptilia*, **34**(2), 233–239. https://doi.org/https://doi.org/10.1163/15685381-00002891

Spitzen-van der Sluijs, A., Martel, A., Asselberghs, J., Bales, E.K., Beukema, W., Bletz, M.C., Dalbeck, L., Goverse, E., Kerres, A., Kinet, T., Kirst, K., Laudelout, A., Marin da Fonte, L.F., Nöllert, A., Ohlhoff, D., Sabino-Pinto, J., Schmidt, B.R., Speybroeck, J., Spikmans, F., Steinfartz, S., Veith, M., Vences, M., Wagner, N., Pasmans, F., Lötters, S. (2016). Expanding Distribution of Lethal Amphibian Fungus *Batrachochytrium salamandrivorans* in Europe. *Emerging Infect. Dis.*, **22** (7), 1286-1288. https://dx.doi.org/10.3201\eid2207.160109.

Spitzen-van der Sluijs, A., Stegens, G., Bogaerts, S., Canessa, S., Steinfartz, S., Jannsen, N., Bosman, W., Pasmans, F., Martel. A. (2018). Post-epizootic salamander persistence in a disease-free refugium suggests poor dispersal ability of *Batrachochytrium salamandrivorans*. *Sci. Rep.* **8**:3800 doi:10.1038/s41598-018-22225-9

Stark, T., Martel, A., Pasmans, F., Thomas, V., Gilbert, M., Spitzen, A. (2018). A European early warning system for a deadly salamander pathogen. *Amphibian Ark* Newsletter **42**:12-14

Stegen, G., Pasmans, F., Schmidt, B. R., Rouffaer, L. O., Van Praet, S., Schaub, M., Canessa, S., Laudelout, A., Kinet, T., Adriaensen, C., Haesebrouck, F., Bert, W., Bossuyt, F., Martel, A. (2017). Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. *Nature*, **544**(7650), 353–356. Retrieved from http://dx.doi.org/10.1038/nature22059

Stockwell, M.P., Clulow, J., Mahony, M.J. (2012). Sodium chloride inhibits the growth and infective capacity of the amphibian chytrid fungus and increases host survival rates. *PLoS One*. https://doi.org/10.1371/journal.pone.0036942

Stockwell, M.P., Clulow, J., Mahony, M.J. (2015). Evidence of a salt refuge: chytrid infection loads are suppressed in hosts exposed to salt. *Oecologia*, **177**(3), 901–910. https://doi.org/10.1007/s00442-014-3157-6

Taberlet, P., Coissac, E., Hajibabaei, M., Rieseberg, L.H. (2012). Environmental DNA. *Mol. Ecol.* **21**: 1789-1793.

Thomas, V., Blooi, M., Van Rooij, P., Van Praet, S., Verbrugghe, E., Grasselli, E., Lukac, M., Smith, S., Pasmans, F., Martel, A. (2018) Recommendations on diagnostic tools for *Batrachochytrium salamandrivorans*. *Transboundary and Emerging Dis*. **65**(2) e478-e488 doi: 10.1111/tbed.12787

Tollefsen, E., Song, Y., Kleiven, M., Mahrosh, U., Meland, S., Rosseland, B.O., Teien, H. (2015). Transcriptional changes in atlantic salmon (Salmo salar) after embryonic exposure to road salt. *Aquat. Toxicol.* **169**:58-68. doi: 10.1016/j.aquatox.2015.10.003.

Tulloch, A.I.T., Maloney, R.F., Joseph, L.N., Bennett, J.R., Di Fonzi, M.M., Probert, W.J., O'Connor, S.M., Densem, J.P., Possingham, H.P. (2015) Effect of risk aversion on prioritizing conservation projects. *Conservation Biology* **29**(2): 513-524 https://doi.org/10.1111/cobi.12386

United States Fish and Wildlife Services. (2016) Listing Salamanders as Injurious Due to Risk of Salamander Chytrid Fungus. Retrieved from https://www.fws.gov/injurious wildlife/salamanders.html

Van Rooij, P., Martel, A., Haesebrouck, F., Pasmans, F. (2015). Amphibian chytridiomycosis: a review with focus on fungus-host interactions. *Vet. Res.* **46**, 137. https://doi.org/10.1186/s13567-015-0266-0

Van Rooij, P., Pasmans, F., Coen, Y., Martel, A. (2017)Efficacy of chemical disinfectants for the containment of the salamander chytrid fungus *Batrachochytrium salamandrivorans. PloS One* **12**(10) e0186269. https://doi.org/10.1371/journal. pone.0186269

Voyles J., Young, S., Berger, L., Campbell, C., Voyles, W. F., Dinudom, A., Cook, D., Webb, R., Alford, R.A., Skerrat, L.F., Speare, R. (2009). Pathogenesis of Chytridiomycosis, a cause of Catastrophic Amphibian Declines. *Science* **326**(5952): 582-5 doi:10.1126/science.1176765

Walker, S.F., Bosch, J., James, T.Y., Litvintseva, A.P., Oliver Valls, J.A., Piña, S., Garcia, G., Rosa, G.A., Cunningham, A.A., Hole, S., Griffiths, R., Fisher, M.C. (2008). Invasive pathogens threaten species recovery programs. *Curr. Biol.* **18**: R853-R854.

Walker, S.F., Salas, M.B., Jenkins, D., Garner, T.W.J., Cunningham, A.A., Hyatt, A.D., Bosch, J., Fisher, M.C. (2007). Environmental detection of *Batrachochytrium dendrobatidis* in a temperate climate. *Dis. Aquat. Organ.* **77**: 105-112.

Wild Animal and Plant Protection and Regulation of International and Interprovincial trade Act (2017). Retrieved https://www.canlii.org/en/ca/laws/regu/sor-96-263/latest/sor-96-263.html.

Woodhams, D.C., Bosch, J., Briggs, C.J., Cashins, S., Davis, L.R., Lauer, A., Muths, E., Puschendorf, R., Schmidt, B.R., Sheafor, B.,Voyles, J. (2011). Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. *Front. Zool.*, **8**(1), 8. https://doi.org/10.1186/1742-9994-8-8

Yuan, Z., Martel, A., Wu, J., Van Praet, S., Canessa, S., Frank Pasmans, F. (2018) Widespread occurence of an emerging fungal pathogen in heavily traded Chinese urodelan species. *Conservation Letters* e12436. https://doi.org/10.1111/conl.12436

Annex 9: Proof of concept that the application of this measure protects a salamander community in an experimental setup.

Disruption of skin microbiota contributes to salamander disease

Molly C. Bletz, Moira Kelly, Joana Sabino-Pinto, Emma Bales, Sarah Van Praet, Wim Bert, Filip Boyen, Miguel Vences, Sebastian Steinfartz, Frank Pasmans and An Martel

Abstract

Escalating occurrences of emerging infectious diseases underscore the importance of understanding microbiome–pathogen interactions. The amphibian cutaneous microbiome is widely studied for its potential to mitigate disease-mediated amphibian declines. Other microbial interactions in this system, however, have been largely neglected in the context of disease outbreaks. European fire salamanders have suffered dramatic population crashes as a result of the newly emerged *Batrachochytrium salamandrivorans* (*Bsal*). In this paper, we investigate microbial interactions on multiple fronts within this system. We show that wild, healthy fire salamanders maintain complex skin microbiotas containing *Bsal*-inhibitory members, but these community are present at a remarkably lowabundance. Through experimentation, we show that increasing bacterial densities of *Bsal*-inhibiting bacteria via daily addition slowed disease progression in fire salamanders. Additionally, we find that experimental-*Bsal* infection elicited subtle changes in the skin microbiome, with selected opportunistic bacteria increasing in relative abundance resulting in septicemic events that coincide with extensive destruction of the epidermis. These results suggest that fire salamander skin, in natural settings, maintains bacterial communities at numbers too low to confer sufficient protection against *Bsal*, and, in fact, the native skin microbiota can constitute a source of opportunistic bacterial pathogens that contribute to pathogenesis. By shedding light on the complex interaction between the microbiome and a lethal pathogen, these data put the interplay between skin microbiomes and a wildlife disease into a new perspective.

1. Introduction

Advances in the knowledge of symbiotic microbiomes are changing our understanding of vertebrate host biology and ecology [1,2]. Resident microbiotas of metazoans are intricately linked to host health, whether it be through participation in energy metabolism, immune system development, or contributing to defence against pathogens [1,3–6]. Symbiotic microbes can occupy a central role in host–pathogen interactions, eliciting protective effects against invading pathogens through space and nutrient competition, production of anti-pathogen compounds as well as immuno-modulatory stimulation [4,7]. Elucidating the role of the microbiota within host–pathogen systems is critical for understanding the ecology of the disease.

Pathogenic fungi are unprecedented players in emerging infectious diseases [8]. From plants to vertebrates, infectious diseases caused by fungi are threatening food resources and leading to biodiversity loss [8–10]. Wheat stem rust and rice blast disease threaten important crops upon which humans depend (*Puccinia graminis, Magnaporthe oryzae* [11]). Bees responsible for

pollination are being devastated by colony collapse disorder (*Nosema* species [12]). Bat populations in North America are collapsing due to White-nose syndrome (*Geomyces destructans* [13]). Furthermore, amphibian chytridiomycosis, originally caused by the sole fungus, *Batrachochytrium dendrobatidis* [14,15], has been marked as a main culprit of amphibian declines within the so-called sixth mass extinction [16]. This cutaneous pathogen is considered the largest disease threat to the world's biodiversity as it has ravaged amphibian communities globally [16,17].

The recent emergence of a second amphibian-infecting chytrid, *Batrachochytrium salamandrivorans* (*Bsal* [18]) adds to the disease threat to these animals. *Bsal* poses a significant threat to western Palearctic salamanders, and, in particular, is responsible for severe declines of European fire salamanders, *Salamandra salamandra* [19,20]. *Bsal* invades keratinized amphibian skin, leading to superficial erosions and numerous deep ulcerations across the body of susceptible host species [18]. As infection escalates and induces chytridiomycosis it can result in death in less than one month [18–20]. Salamander hosts appear to have little ability to fight back against *Bsal* through host-based defences. Expression of immune genes remained unchanged during experimental infection of an Asian species, *Tylototriton wenxianensis* [21]. Furthermore, fire salamanders, *S. salamandra*, mounted no immune response after five cycles of exposure-clearance regimes [20].

To date, limited work has been conducted on the role of host microbiota in *Bsal*-infection dynamics [22], yet a thorough understanding of salamander-microbiome-*Bsal* interactions is clearly essential. It is plausible that resident skin microbiota contributes to the host's mucosal defences against *Bsal*, which would open new horizons for disease mitigation [23]. However, the massive destruction of the epidermis during *Bsal* infection may equally predispose opportunistic pathogens to cause fatal septicemia. These juxtaposing ideas raise the pivotal question: What is the role of skin bacteria in *Bsal* infection of fire salamanders? Are they a friend, foe or bystander?

Here, we combine evidence from field studies and laboratory experiments to understand the role of bacteria in *Bsal* infection dynamics of the highly susceptible fire salamander. We surveyed healthy populations of wild fire salamanders to determine their natural skin bacterial densities, and performed laboratory experiments to investigate the response of salamander skin microbiota to *Bsal* infection. We further evaluated the function of cultivable resident bacteria against *Bsal* and the ability of these bacteria to alter infection dynamics in vivo.

2. Methods

A) field sampling

Healthy, adult European fire salamanders (*Salamandra salamandra*) were sampled at multiple locations across Germany in 2015 (electronic supplementary material, tables S1 and S2). At the time of sampling, all amphibians screened for *Bsal* from these locations, including the fire salamanders in our study, were *Bsal*-negative [24]. Adults were captured with gloved hands and skin swabs were taken following standard methods [25,26] for either DNA-based analyses (qPCR estimation of bacterial abundance and 16S amplicon sequencing of bacterial community) or cultivation of skin microbes. Swabs for DNA-based analyses were stored dry,

and swabs for cultivation were stored in 20% glycerol to maintain bacterial cell integrity. All samples were stored in ice and immediately frozen upon return to the laboratory.

(b) Culturing of skin bacteria

Skin bacteria were cultured from fire salamander populations across Germany (electronic supplementary material, table S2). Culturable skin swabs were processed as explained in [26].

(c) Bsal growth inhibition assays

A total of 708 bacterial isolates from fire salamander skin were tested in *Bsal* growth inhibition assays. Assay methodology followed amodified version of the 96-well assaymethod described in [27] (see electronic supplementary material, Methods). Inhibitory function against *Bsal* was determined by comparison of *Bsal* growth rate in the presence of bacterial CFS with that of the nutrient- depleted control (*Bsal* zoospores grown without additional nutrients) with FDR corrections. Enhancing function against *Bsal* was determined by comparison of *Bsal* growth rate in the presence of bacterial CFS with that of the positive control (*Bsal* zoospores growth in TGHL media) with FDR corrections. Selected bacteria (electronic supplementary material, table S3) were tested multiple times to explore functional consistency.

(d) Liver isolate cultivation

Bsal infection results in deep ulcerations of the skin surface. This breaching of the integrity of the skin barrier may result in bacterial invasion of internal organs and the blood. To investigate this, bacterial isolates from the livers of nine fire salamanders that died due to experimental *Bsal* infection and nine non-*Bsal* infected salamanders were cultured on Columbia agar with sheep blood. Cultures were incubated at 158C and isolated into pure culture. Morphologically distinct bacteria were identified using Sanger sequencing of the 16S rRNA gene.

(e) Bsal infection experiment for microbiome analysis

Twelve captive-bred fire salamanders (six control, six *Bsal*-exposed) were housed individually at 15 ±1°C on moist tissue, with access to a hiding place (PVC pipe) and a water container. Salamanders were exposed to *Bsal* (AMFP13/01; 5000 zoospores ml^{-1}) by dripping 1 ml of a zoospore suspension onto the salamander. Controls received 1 ml of artificial pond water. Animals were fed twice weekly with crickets. Individuals were swabbed as described in Bletz et al. [25] prior to exposure and 10 days post-exposure.

(f) Bacterial addition experiments

The bacterial addition experiment was conducted to evaluate the function of bacteria in an in vivo context and to understand if bacteria on the skin can alter *Bsal*-infection dynamics. Bacterial isolates for addition experiments were selected from in vitro *Bsal* growth assay results. Selection criteria are outlined in the electronic supplementary material, Methods.

Twenty-six captive-bred fire salamanders were housed individually and fed as explained above for a duration of 11 weeks. The following treatments were used: (i) daily addition of a *Stenotrophomonas* sp. (*Bsal*-enhancing, n = 7), (ii) daily addition of a *Pseudomonas* sp. (*Bsal*-enhancing, n = 7), (iii) daily addition of a sham treatment of 1 ml of sterile water with an agar

swab (n = 7), and (iv) no treatment (n = 5). Once the bacteria had grown on the agar plate, a swab was used to collect bacterial cells from the plate. The collected cells were then suspended in 10 ml of sterile artificial pond water. The sham treatment with the agar swab was included as a control for this process. Bacterial cellswere quantified by spectrophotometry that was verified by CFU counts. Bacterial treatments were administered daily by adding 1×10^8 bacterial cells suspended in 1 ml of sterile artificial pond water. Daily administration of treatments was completed to ensure the continual presence of these bacteria on the skin for the duration of the experiment (11 weeks/77 days). *Bsal* exposure was given as described above (except 1×10^4 zoospores ml^{-1}). Individuals were swabbed on day 0 prior to any treatment, on day 3 prior to *Bsal* exposure, and at weekly intervals following *Bsal* exposure for 11 weeks or until animals were removed from the experiment. Swabs were used to quantify bacterial load, *Bsal* load, and to culture skin bacteria for Matrix-Assisted Laser Desorption Ionization- Time-of-Flight Mass Spectrometry (MALDi-TOF) identity confirmation. Animals were removed from the experiment when clinical signs indicated lethal disease (high *Bsal* load and morbidity). At this time, skin swabs were taken, and individuals were subsequently euthanized with MS-222 overdose.

(g) DNA extraction

Bacterial DNA was isolated following protocols in previous studies: for microbiome analyses of field swabs, and the analysis of microbiome response in the *Bsal* exposure experiment, we used the MoBio PowerSoil DNA Isolation kit [25]; for skin bacterial isolates we used a Chelex protocol [26]; and for the bacterial addition experiment, Prepman was used [18]. MoBio extraction methods were used for samples where exploration of the microbial community composition was the goal. Prepman extraction was used in the bacterial addition experiment because it is more cost-effective and microbiome analysis was not being performed. Importantly, no comparisons were made among samples from different extraction methods.

(h) Identification of bacterial isolates

A fragment of the 16S rRNA gene from cultured isolates was PCR-amplified with the primers 27F (AGAGTTTGATCCTGGCTCAG) and 907R (CCGTCAATTCMTTTGAGTTT). PCR products were sequenced atLGCGenomics in Berlin, Germany. Sequencing produced approximately 500–800 bp for each bacterial isolate. Sequences were cleaned in CodonCode Aligner, and aligned with PyNAST. Taxonomy was assigned with RDP in Quantitative Insights Into Microbial Ecology (QIIME) [28] and a phylogenetic tree was built with fasttree [29].

(i) Quantification of bacterial abundance and Bsal

Total bacterial abundance and *Bsal* infection intensities were estimated with qPCR. Total bacteria were quantified using a SYBR Green qPCR assay using the universal bacterial primers described in [30]. qPCR conditions were 10 min at 95°C, followed by 39 cycles of 60 s at 94°C, 60 s at 50°C, 60 s at 60°C, and a final elongation for 15 min at 60°C. Primer concentration was 0.5 mM. Within the experiments, bacterial densities were determined by calculating the surface area swabbed from measurements of the trunk length and width. *Bsal* infection intensities were determined using the qPCR assay described in [31]. qPCRs were performed using the CFX384 Bio-Rad detection system.

(j) 16S rRNA characterization of communities

16S rRNA gene amplicon sequencing was performed on field samples and on samples collected during the *Bsal* exposure experiment. The V4 region of the 16S rRNA gene was PCR amplified with dual-indexed primers as described in [32]. Pooled amplicons were sequenced on an Illumina MiSeq with 2 x 250 paired-end technology at the Helmholtz Center for Infectious Research in Braunschweig, Germany. QIIME [33] was used to demultiplex and quality-filter the sequence data, and sequences were clustered into sub-operational taxonomic units (sOTUs) using Deblur [34]. Detailed methodologies for processing the amplicon sequencing data are provided in the electronic supplementary material, Methods.

(k) MALDi-TOF

During the bacterial addition experiment, MALDI-TOF MS direct cell profiling was used to confirm the presence of the administered bacteria [35]. More specifically, bacteria were re-isolated from the skin of treated fire salamanders and MALDI-TOF was used to compare the profile of each morphologically distinct, re-cultured isolate to that of the original inoculum. MALDITOF was performed using an Autoflex Biotyper MALDI-TOF mass spectrometer (Bruker Daltonik) using the direct transfer method and a-cyano-4-hydroxycinnamic acid (HCCA) as a matrix, according to the manufacturer's guidelines. Detailed methodologies are provided in the electronic supplementary materials.

(l) Scanning electron microscopy

Scanning electron microscopy (SEM) of the skin of *Bsal*-free fire salamanders (3) was carried out to provide a visual assessment of the density of skin microbes. SEM was performed after 2% paraformaldehyde and 2.5% glutaraldehyde fixation in 0.1 M phosphate buffer [18].

(m) Statistical analyses

All statistical analyses were performed in R (v. 3.4, [36]) unless otherwise stated. For the *Bsal* exposure experiment looking at microbiomes, bacterial density and alpha diversity were analysed with general linear mixed effect models (GLMMs, lmer4; [37]). Treatment, time, and the time by treatment interaction were included in GLMMs along with individual as a random factor to account for repeated sampling. Data were normalized with log transformations as needed. We used ADONIS2 [38] to perform a permutational multivariate analysis of variance to assess whether time and *Bsal* exposure explained significant portions of the observed variation in microbial community structure (i.e. beta diversity). Time, treatment, and the time by treatment interaction were included as explanatory variables. We used Linear discriminant analysis effect size method (LEfSe) to identify differentially abundant bacterial taxa between the microbial communities of *Bsal*-exposed and control salamanders at the post-exposure time point [39].

In the bacterial addition experiment, all analyses of bacterial density and *Bsal* infection loads were performed on samples collected from the first 28 days of the experiment when all salamanders were still in the experiment. Bacterial density was statistically analysed with GLMMs as described above. To explore main effects and significant interactions we performed pairwise post hoc comparisons among treatments and time points as necessary. Rate of change

in *Bsal* infection intensity was calculated as the slope of the estimated raw *Bsal* loads for each individual between day 14 (first time point where *Bsal* was detected) and 28 (last time point when all individuals were still in the experiment). These calculated rates were then compared across treatments with a Kruskal–Wallis test. Host survival was evaluated with a Cox log-rank test using the survival package [40]. Boxplot and survival graphs were created with ggplot2 and the survminer package [41].

3. Results

(a)   *Bacterial diversity and density on Salamandra skin*

Skin bacterial communities from 275 wild, healthy fire salamanders in Germany were characterized using 16S amplicon sequencing. The richness of sub-operational taxonomic units (sOTUs) on individuals across populations averaged 212.6 ± 9.56 s.e. The skin microbiota predominantly comprised *Proteobacteria* (48.8%), *Bacteriodetes* (24.8%), *Actinobacteria* (8.2%), *Firmicutes* (6.7%), *Cyanobacteria* (4.1%), *Acidobacteria* (2.8%) and *Verrucomicrobia* (2.7%) (electronic supplementary material, appendix, figure S1). Furthermore, we found that microbial community structure differed across locations (PERMANOVA, Pseudo-F = 4.18, p = 0.001; electronic supplementary material, figure S2).

The average bacterial abundance on fire salamander skin (n = 94) across populations was $3.5 \times 10^4 \pm 6.7 \times 10^3$ s.e. rRNA copies/swab (figure 1b). The average bacterial abundance for the four populations was $2.34 \times 10^4 \pm 4.8 \times 10^3$ s.e., $1.43 \times 10^4 \pm 3.0 \times 10^3$, $2.63 \times 10^4 \pm 4.5 \times 10^3$ and $6.39 \times 10^4 \pm 1.3 \times 10^3$ for Fleischbach (Fi), Zweifallshammer (KAZ), Kallerbach (KB) and Solchbachtal (SO), respectively. A similarly low bacterial abundance on the skin surface was shown through SEM visualization, where often no or only a very limited number of bacterial cells can be seen (figure 1a).

(b) Bsal *infection elicits subtle changes in Salamandra skin microbiome, which are associated with septicemic events*

We characterized the cutaneous microbiome response to *Bsal* invasion using experimental infection of fire salamanders (*Bsal*-exposed: n = 6, control: n = 6) and 16S amplicon sequencing. *Bsal* infection had no effect on skin bacterial abundance, bacterial richness and diversity, or community structure. Average bacterial density on the salamander skin was 5.7×10^3 rRNA copies mm22 (s.e. $\pm$ 1990.5) prior to exposure. There was no significant change as a result of *Bsal* infection or through time (GLMM: Treatment-F= 0.25, p = 0.63; Time- F = 0.63, p = 0.45; Interaction-F = 0.43, p = 0.53; figure 2a). Skin bacterial richness and diversity on fire salamanders before exposure averaged 142 ± 6.9 s.e., 81.7 ± 21.2, and 15.7 ± 1.1 for sOTU richness, effective number of sOTUs (exp(Shannon Index)), and Faith's phylogenetic diversity (PD), respectively. *Bsal* infection also had no significant effect on bacterial richness or diversity of skin communities; however, there was a significant increase through time in effective number of sOTUs (GLM: Effective number of sOTUs: Treatment-F = 0.02, p = 0.89; Time-F = 5.76, p = 0.04, Interaction-F = 1.35, p = 0.27; figure 2b; electronic supplementary material, figure S3). Bacterial community structure (i.e. beta diversity) also did not change as a result of *Bsal* infection, but significantly shifted through time (PERMANOVA: weighted Unifrac: Treatment–Pseudo-F = 0.67, p = 0.72; Time–Pseudo-F = 2.78, p = 0.01; Interaction–Pseudo-F

= 1.016, p = 0.374; unweighted Unifrac: Treatment–Pseudo-F = 0.68, p = 0.86 Time–Pseudo-F = 1.73, p = 0.03; Interaction– Pseudo-F = 1.1119, p = 0.325; figure 2c).

While *Bsal* exposure elicited no significant change in alpha and beta diversity of fire salamander skin microbiota, particular bacterial taxa were found to be differentially abundant on infected versus control individuals using LEfSe. Seven bacterial sOTUs exhibited greater relative abundance on infected individuals, and three exhibited greater relative abundance on non-infected individuals at the post-exposure time point (figure 2d; electronic supplementary material, table S4). An *Aeromonadaceae* sp., a *Chryseobacterium* sp., a *Fusobacteriacaeae* sp., a *Lactococcus* sp. and *Stenotrophomonas acidominiphila* were differentially associated with *Bsal*-infected salamanders, whereas an *Actinomycetales* sp., a *Dyadobacter* sp., a *Luecobacter* sp. and a *Pedobacter* sp. were more common on control salamanders.

Moreover, *Bsal*-induced chytridiomycosis resulted in septicemic events, likely resulting from the breaching of the skin barrier by *Bsal*. Forty-five bacterial isolates were cultured and successfully identified from livers of nine infected fire salamanders. These liver-colonizing bacteria were from three phyla: *Proteobacteria* (21 isolates) *Bacteriodetes* (20 isolates) and *Actinobacteria* (four isolates) (electronic supplementary material, table S5). Most notably there were 15 isolates identified as *Acinetobacter* johnsonii and nine isolates identified as *Chryseobacterium* sp. (electronic supplementary material, table S5). No bacteria were isolated from the livers of fire salamanders that were not infected with *Bsal*.

(c) Increased density of Bsal-*inhibitory bacteria dampens* Bsal *infection, but only marginally changes overall outcome*

We evaluated the function of cutaneous bacteria on fire salamanders using in vitro culture-based approaches as well as in vivo experimentation. The cultured isolates (n ¼ 708) were from the following phyla: *Actinobacteria* (44.6%), *Proteobacteria* (37.6%), *Bacteriodetes* (12.4%) and *Firmicutes* (5.4%) (figure 3a). We found that these resident skin bacteria exhibited a range of functional capacities against *Bsal*. Of the 708 isolates tested with in vitro growth assays, 30% inhibited *Bsal* growth, 13% enhanced *Bsal* growth and 57% had no effect (figure 3b). It is important to note, not all bacteria consistently exhibited the same function against *Bsal*. Re-testing of multiple skin bacterial isolates (n = 17) against *Bsal* resulted in variable functionality. This finding is potentially due to differences in the cell density of cultures when bacterial products were collected (electronic supplementary material, table S3).

Bacterial density at the start of the experiment (prior to experimental treatments) did not differ between experimental groups (n = 7 per treatment; KW-x^2 = 0.7851, p = 0.853). After bacterial addition began, bacterial density differed significantly among treatments (LMM, F = 12.67, p < 0.001) and through time (LMM, F = 49.57, p < 0.001) (figure 3c). There was also a significant interaction between time and treatment (LMM, F = 4.96, p = 0.003). To explore these main effects and the interaction we performed pairwise post hoc comparisons among treatments and time points. Daily addition of bacteria to fire salamander skin in both the inhibitory and enhancing treatment increased bacterial density in comparison to the no-treatment control (pseudo: t = 23.95, p < 0.001; steno: t = 25.23, p < 0.001), however, not in comparison to the agar-wash control (pseudo: t = 2.12, p < 0.152; steno: t = 0.69, p = 0.89). The mechanism for

bacterial increase in agartreated animals remains unclear. It is possible, for example, that the minimal agar media present in the inocula promoted bacterial growth; alternatively, the dilution of host skin factors could remove inhibition; to decipher this future work could consider including a water-only treatment control. Furthermore, this effect diminished over time; by day 21 there were no differences in bacterial density among treatments (KW x^2 = 1.95, p = 0.58). Re-isolation success of the added bacteria to confirmtheir presence on the skin proved highly inconsistent through time on a given individual. Nevertheless, MALDi-TOF identification of re-isolated bacteria confirmed the presence of the administered bacteria on the skin of 12 of 14 individuals on at least one time point throughout the experiment (electronic supplementary material, table S6).

The artificial addition of selected bacteria to salamander skin affected *Bsal* infection dynamics and survival. There was some evidence that the rate of increase in *Bsal* infection intensity throughout the first 28 days differed among treatments (KW x^2 = 7.24, p = 0.06; figure 3e). More specifically, when comparing the two bacterial treatments to address the hypothesis that bacteria can differentially affect infection dynamics, therewas a significant difference among treatments, with the *Bsal*-inhibitory bacteria group exhibiting a lower rate of increase in infection intensity compared to the *Bsal*-enhancing bacteria group (KW x^2 = 3.92, p = 0.047). There was also evidence for a difference in survival probability among treatments (Cox log-rank test, x^2 = 7.76, p = 0.05; figure 3d); the *Bsal*-inhibitory bacteria group exhibited slower rates of mortality compared to the control treatment (pairwise log rank: p = 0.018, all other comparisons p > 0.1). One individual within this treatment group also survived the duration of the experiment, and was no longer positive for *Bsal* after 42 days.

4. Discussion

Advances in the understanding of symbiotic microbiomes are changing our perception of animal biology, including the ecology of disease in host–pathogen systems [3,5,42]. Our study investigated fire salamander skin microbial communities in the context of the emerging pathogen, *Bsal*, finding that microbial interactions can both elicit protection from disease and contribute to disease pathogenesis. While daily addition of *Bsal*-inhibiting bacteria was able to slow disease progression, the markedly low densities of cutaneous bacteria in unmanipulated settings likely limit their protective capacity. Moreover, selected bacteria that became more abundant following *Bsal* infection coincide with bacteria involved in septicemic events, suggesting a contributing role in disease pathogenesis.

Our study suggests that the abundance of bacteria on fire salamander skin is relatively low. There is limited knowledge in the literature on densities of skin microbiota on hosts, even for human skin; however, our estimates for salamander skin are some orders of magnitude lower than the available estimated density on human skin: 5.7 x 10^3 rRNA copies mm^{-2} versus , 10^{11} m^{-2} (approximately 10^8 mm^{-2}) [43]. Interestingly, this estimate of bacterial density on human skin is derived solely from an old culture-based study (1987), which likely underestimates abundances. To date, few novel systematic studies have attempted to thoroughly address cutaneous microbial density. It is striking how scarce current data on cutaneous microbial density is, for human and amphibian systems alike, and clearly this topic warrants thorough

study. It is also likely that these densities will differ among amphibian host species, further meriting comparative investigation.

Bacterial abundance on salamander skin may be low as a result of host investment into alternative defence strategies. A host's microbial community can be seen as a trait that is an extension of the host immune system [1,44]. Selective pressure on the host may lead to evolution of a mucosal environment that is particularly suitable for protective symbionts [45]. While microbial defences can be a significant component of a host's defence strategy, at times even replacing host-produced defences, investing in them can be costly for the host [7,46]. In this context, a host may only be able to invest resources in maintaining either defensive microbiota or host-based defences. The extent and nature of the epidermal mucosal layer undoubtedly differ among amphibian species, and likely shape density and potentially spatial distribution of skin microbes. Fire salamanders have a relatively minimal mucus layer on their skin in comparison to, for example, ranid frogs, which may not favour substantial microbial colonization and proliferation on the skin due to low resource availability. Alternatively, fire salamanders may maintain a strict, active control of microbial populations on their skin, investing resources in host-based defences rather than microbial-based defences, and thus limiting the development of a cutaneous environment that is conducive to microbial establishment and persistence.

Bacterial density and their spatial distribution can have strong implications on the function of these microbial residents [47,48]. In fact, microbial abundances rather than taxonomic shifts of particular taxa in the human gut microbiome have been found to be a fundamental driver of disease [49]. Amphibian skin microbiota are known to produce antifungal compounds [50] which can inhibit colonization and growth of fungal pathogens [51]. Many microbial taxa communicate via a form of cell-to-cell communication known as quorum sensing [52]. Such communication relies on signal build up from many bacterial cells and facilitates group behaviours that can lead to inhibition of colonization by other microbes [42,53]. Likewise, antibiotic activity against pathogens can be seen as a byproduct of bacterial interference competition [54], and to participate in such competition bacteria most likely need to be in close proximity [48]. If microbial density is key, the low observed density on fire salamander skin could be inadequate to modulate quorum sensing or interference competition. Thus, the natural microbiota of these amphibians may be insufficient in eliciting large-scale effects on *Bsal* infection dynamics. Such a density-dependent effect is further supported by the artificial addition of *Bsal*-inhibitory bacteria to fire salamander skin instigating changes in infection dynamics. On the other hand, we do not fully know the spatial distribution of the bacterial residents; if spatially aggregated within specific skin regions, bacteria could engage in quorum sensing and/or interference competition. Even if the overall density is low, it may still be possible for bacteria to quickly proliferate under certain conditions, becoming locally abundant on particular skin regions and able to exert an important defensive effect.

Pathogen invasion is known to alter host microbial communities [55,56]. Cutaneous infection by the related amphibian fungus, Bd, has been documented to alter the diversity and composition of skin-associated microbiota on anuran hosts [57,58]. Experimental *Bsal* infection of fire salamanders counters this and did not alter total bacterial density, alpha diversity or

community structure (i.e. beta diversity) of the skin bacterial communities as a whole. Despite the lack of overall effects on beta diversity, selected bacterial taxa were found to exhibit differential relative abundance between *Bsal*-exposed and control salamanders. In fact, a *Chryseobacterium* sp. found to be differentially abundant on the skin of *Bsal*-exposed individuals matched cultured liver isolates associated with observed septicemic events in *Bsal*-infected salamanders.

Septicemia may be a mechanism of mortality in *Bsal*-induced chytridiomycosis. Deep ulcerations induced by *Bsal* infection [18] result in breaching of the skin barrier, which likely allows bacteria to invade internal organs. The observed septicemic events may be a result of typically commensal skin bacteria becoming opportunistic pathogens and invading internal organs. Most of the bacteria isolated from the liver are not commonly known pathogens. Many were common residents of environmental substrates as well as in host skin and gut microbiomes; however, several of them have previously been documented as opportunistic pathogens, associated with cases of infection and bacteremia in fish, plants and humans (e.g. [59–62]; electronic supplementary material, table S2). Specifically, Acinetobacter, the most common bacterium isolated from the liver, has been associated with bloodstream infections (e.g. [42]), and also with skin lesions of another amphibian, the Hellbender [63].

Host-associated microbial communities are also known to affect disease dynamics [4,64–66]. In vitro, we found that cultured resident bacteria displayed a range of functional capacities against *Bsal*, including inhibition and enhancement. Such interactions between invading pathogens and host symbionts have been documented across multiple amphibian systems aswell as other host systems [26,64,67–70]. In the conducted bacterial addition experiment we were able to, in part, replicate the in vitro function of these bacteria in vivo. We found that *Bsal*-enhancing bacteria had no effect on salamander survival. In general, *Bsal*-induced chytridiomycosis manifests itself very quickly on fire salamanders [19]. The average time until [death] in controls was 29.5 days, which is only 3–4 generations of *Bsal*; thus, in this hyper-susceptible host it is likely to be rather difficult to hasten the onset of disease, even if *Bsal*-enhancing bacteria are present in high abundance. This fact may explain the observed findings for the *Bsal*-enhancing treatment. On the other hand, the *Bsal*-inhibitory bacteria resulted in a slower build-up of *Bsal* infection intensity in comparison to the *Bsal*-enhancing treatment and a marginal increase in survival compared to the controls. These findings demonstrate that bacteria can differentially affect infection at least under certain conditions, which has also been observed in selected hosts infected with *Bd* (e.g. [47]). The inhibitory bacterium used was a Pseudomonas sp.; pseudomonads are known in multiple systems to be antifungal and have disease-suppressing properties [71–73]. Importantly, within the conducted experiment investigating in vivo function, skin bacterial densities on salamanders in bacterial addition groups were greater than those found to occur naturally on salamanders in this study; thus, density may be key for bacteria to elicit a protective function [47,49]. It is also worth highlighting that there was one individual within the *Bsal*-inhibitory bacteria group that survived the duration of the experiment (77 days), which has never been observed in untreated fire salamanders so far, neither in laboratory trials, nor in the wild.

The conducted experiment demonstrates that bacteria can shift infection dynamics within certain frameworks. The potential of bacteria to affect *Bsal* infection dynamics leaves the door to exploring probiotics open. In the sole survivor, *Bsal* infection was no longer present after 42 days, suggesting that clearance had occurred; such a prolonged course of infection, however, also involves prolonged pathogen shedding,which has been shown to be highly unfavourable to overall outcomes of disease outbreaks [74]. Any future work to develop probiotics, single species or probiotic mixtures, should focus on bacteria (or consortia) that prevent pathogen colonization or result in more rapid pathogen clearance. Such probiotics could cull disease progression and minimize transmission. The feasibility of achieving this on a large scale and thereby shifting disease dynamics at biologically meaningful levels, however, will certainly be a challenge.

Are skin bacteria on fire salamanders friends, foes or bystanders? Our data suggest that bacteria living on fire salamander skin may simply be bystanders, unable to provide sufficient protection against *Bsal*, perhaps due to low bacterial numbers combined with *Bsal's* ability to disseminate inside salamander skin, thus averting bacterial defences. Furthermore, disease-induced septicemia by potentially opportunistic pathogens within the skin microbiota presents selected bacteria as a clear foe to fire salamanders. However, dampening of *Bsal* infection by the addition of *Bsal* inhibitory bacteria suggests that skin bacteria can be a friend to the salamander under certain conditions. The multifaceted nature of host microbiota highlights the complex relationship of hosts, their microbiomes and disease, and underscores the importance for continued research in this field.
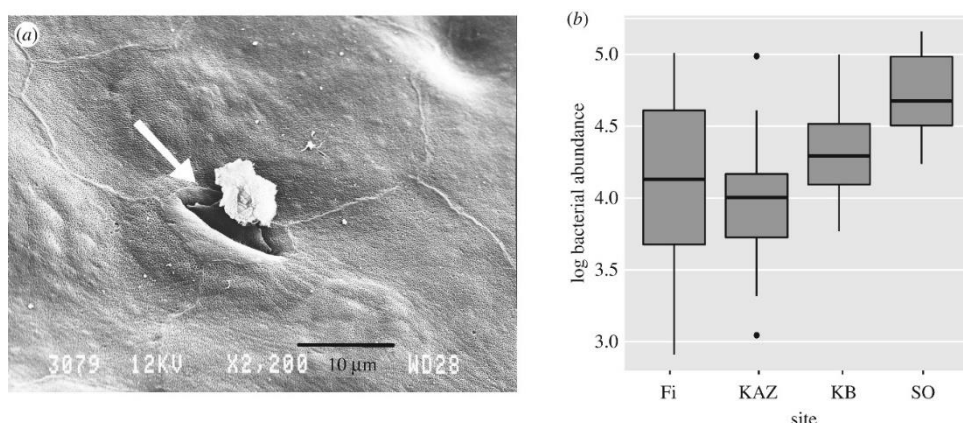
Figure 1. Natural densities of bacteria on the skin of fire salamanders. (a) Scanning electron microscopy image of salamander skin, showing a skin gland opening (arrow) and an overall lack of epidermal cell-associated bacteria. (b) Box plot showing qPCR estimates of bacterial abundance on fire salamander skin at four locations within the Eifel in western Germany. The upper and lower limits of the box represent the first and third quartiles, with the bold line representing the median. Whiskers extend to the minimum and maximum values, and points represent outliers.
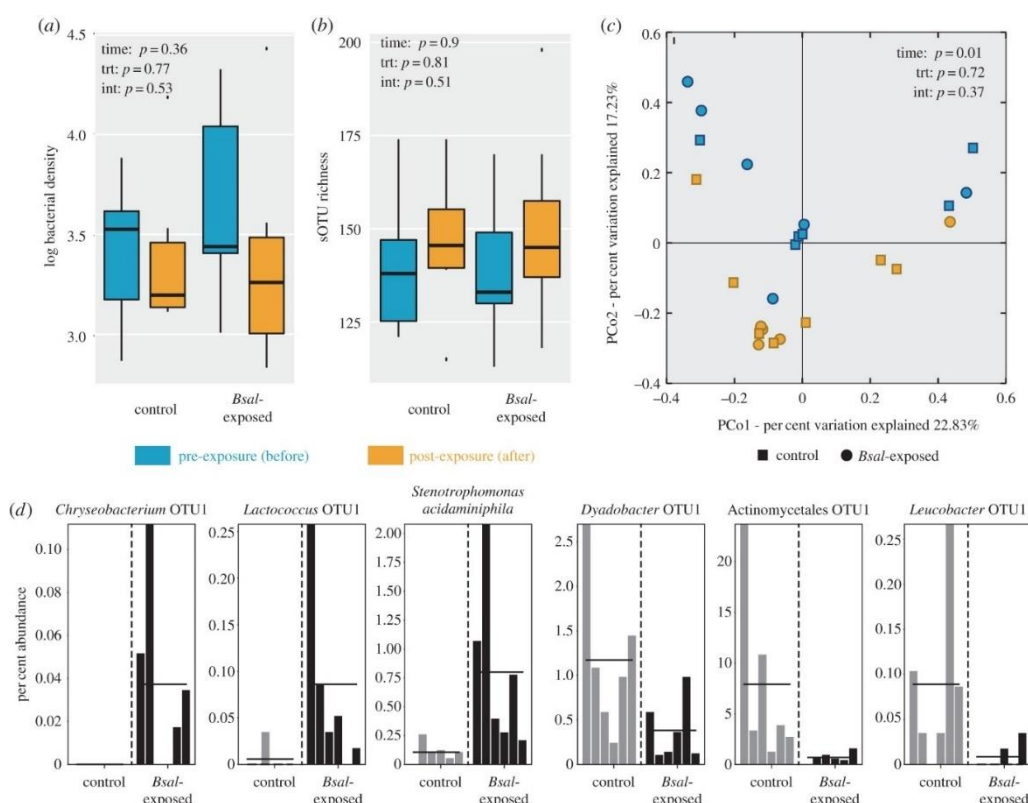

Figure 2. Response of fire salamander skin microbiota to *Bsal* infection. (a) qPCR estimates of bacterial density (rRNA copies/mm2), (b) species richness (sOTU richness) of skin bacterial communities and (c) bacterial community structure on control and Bsal-exposed salamanders. Bacterial community structure is visualized with Principal coordinate analysis of the weighted Unifrac distances. (d) Six representative sOTUs identified by LEfSe to be differentially abundant on Bsal-exposed individuals and control individuals.
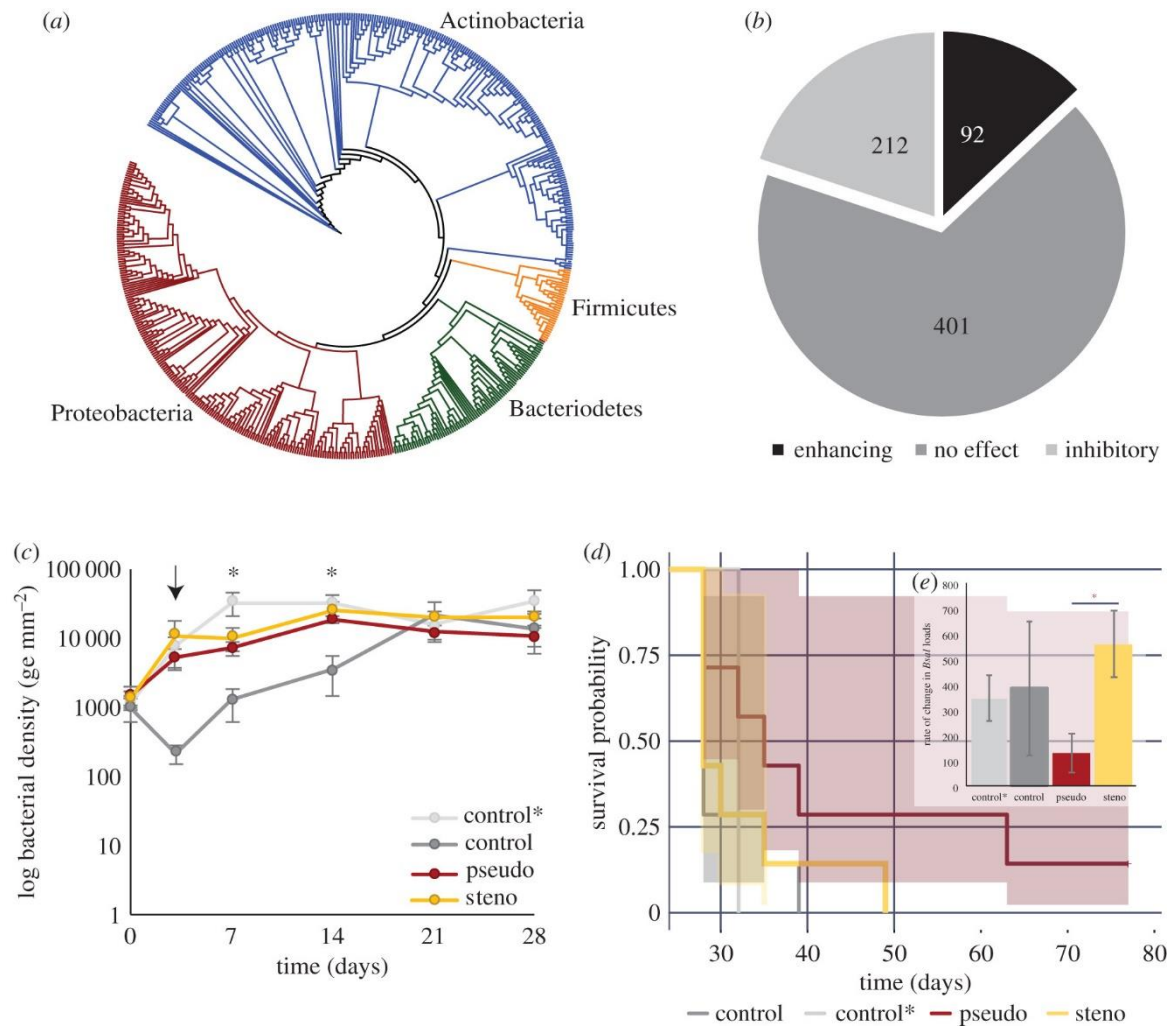
Figure 3. Phylogenetic distribution and function of cultured skin bacteria from all sampled salamanders and the effects of bacterial addition on Bsal-infection dynamics. (a) Phylogenetic distribution of skin bacterial isolates. The colours indicate bacterial phyla. (b) Distribution of functional capability of cultured skin bacterial isolates against *Bsal*. The numbers represent the total number of isolates with the respective function. (c) Bacterial density on salamander skin throughout the first 28 days of the experiment. The arrow indicates time of Bsal exposure. Treatments are labelled as follows: control* = daily addition of a sham treatment of 1 ml of sterile distilled water with an agar swab, control = no treatment, pseudo = daily addition of a *Pseudomonas* sp., and steno = daily addition of a *Stenotrophomonas* sp. Asterisks are used to denote time points where significant differences among treatments were detected. (d) Survival probability curves across experimental treatments. The pseudo treatment exhibited significantly greater survival compared to the control. (e) (Inset) Rate of change in *Bsal* infection intensity throughout the first 28 days (zoospores/day). The asterisk denotes significant differences among treatments. Error bars represent standard error of the mean (c and inset e), and shaded regions represent 95% confidence intervals (d).

References

1. McFall-Ngai M, Hadefield M, Bosch TCG. 2013 Animals in a bacterial world, a new imperative for the life sciences. Proc. Natl Acad. Sci. USA 110, 3229–3236. (doi:10.1073/pnas.1218525110)

2. Shapira M. 2016 Gut microbiotas and host evolution: scaling up symbiosis. Trends Ecol. Evol. 31, 539–549. (doi:10.1016/j.tree.2016.03.006)

3. Selber-Hnativ S et al. 2017 Human gut microbiota: toward an ecology of disease. Front. Microbiol. 8, 1265. (doi:10.3389/fmicb.2017.01265)

4. Stecher B, Hardt WD. 2008 The role of microbiota in infectious disease. Trends Microbiol. 16, 107–114. (doi:10.1016/j.tim.2007.12.008)

5. Sanford JA, Gallo RL. 2013 Functions of the skin microbiota in health and disease. Semin. Immunol. 25, 370–377. (doi:10.1016/j.smim.2013.09.005)

6. Belden LK, Harris RN. 2007 Infectious diseases in wildlife: the community ecology context. Front. Ecol. Environ. 5, 533–539. (doi:10.1890/060122)

7. King KC, Bonsall MB. 2017 The evolutionary and coevolutionary consequences of defensive microbes for host-parasite interactions. BMC Evol. Biol. 17, 190. (doi:10.1186/s12862-017-1030-z)

8. Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ. 2012 Emerging fungal threats to animal, plant and ecosystem health. Nature 484, 186–194. (doi:10.1038/ nature10947)

9. Smith KF, Sax DF, Lafferty KD. 2006 Evidence for the role of infectious disease in species extinction and endangerment. Conserv. Biol. 20, 1349–1357. (doi:10.1111/j.1523-1739.2006.00524.x)

10. Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P. 2004 Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends Ecol. Evol. 19, 535–544. (doi:10.1016/j.tree.2004.07.021)

11. Pennisi E. 2010 Armed and dangerous. Science 327, 804–805. (doi:10.1126/science.327.5967.804)

12. Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL. 2011 Patterns of widespread decline in North American bumble bees. Proc. Natl Acad. Sci. USA 108, 662–667. (doi:10. 1073/pnas.1014743108)

13. Gargas A, Trest M, Christensen M, Volk TJ, Blehert DS. 2009 *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. Mycotaxon 108, 147–154. (doi:10.5248/108.147)

Bsal

14. Longcore JE, Pessier AP, Nicholes DK. 1999 *Batrachochytrium dendrobatidis* gen. et sp. Nova chytrid pathogenic to amphibians. Mycologia 91, 219–227. (doi:10.2307/3761366)

15. Fisher MC, Garner TW, Walker SF. 2009 Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. Annu. Rev. Microbiol. 63, 291–310. (doi:10. 1146/annurev.micro.091208.073435)

16. Wake DB, Vredenburg VT. 2008 Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proc. Natl Acad. Sci. USA 105, 11 466–11 473. (doi:10.1073/pnas. 0801921105)

17. Hoffmann M et al. 2010 The impact of conservation on the status of the world's vertebrates. Science 330, 1503–1509. (doi:10.1126/science.1194442)

18. Martel A et al. 2013 *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. Proc. Natl Acad. Sci. USA 110, 15 325–15 329. (doi:10.1073/pnas. 1307356110)

19. Martel A et al. 2014 Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. Science 6209, 630–631. (doi:10.1126/science. 1258268)

20. Stegen G et al. 2017 Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. Nature 544, 353–356. (doi:10.1038/nature22059)

21. Farrer RA et al. 2017 Genomic innovations linked to infection strategies across emerging pathogenic chytrid fungi. Nat. Commun. 8, 14742. (doi:10.1038/ ncomms14742)

22. Muletz-Wolz CR, Almario JG, Barnett SE, DiRenzo GV, Martel A, Pasmans F, Zamudio KR, Toledo LF, Lips KR. 2017 Inhibition of fungal pathogens across genotypes and temperatures by amphibian skin bacteria. Front. Microbiol. 8, 1–10.

23. Bletz MC, Loudon AH, Becker MH, Bell SC, Woodhams DC, Minbiole KPC, Harris RN. 2013 Mitigating amphibian chytridiomycosis with bioaugmentation: characteristics of effective probiotics and strategies for their selection and use. Ecol. Lett. 16, 807–820. (doi:10.1111/ele.12099)

24. Spitzen-van der Sluijs A et al. 2016 Expanding distribution of lethal amphibian fungus *Batrachochytrium salamandrivorans* in Europe. Emerg. Infect. Dis. 22, 1286. (doi:10.3201/eid2207. 160109)

25. Bletz MC, Archer H, Harris RN, McKenzie VJ, Rabemananjara FCE, Rakotoarison A, Vences M. 2017 Host ecology rather than host phylogeny drives amphibian skin microbial community structure in the biodiversity hotspot of Madagascar. Front. Microbiol. 8, 1530. (doi:10.3389/fmicb.2017. 01530)

26. Bletz MC, Myers J, Woodhams DC, Rabemananjara FCE, Rakotonirina A, Weldon C, Edmonds D, Vences M, Harris RN. 2017 Estimating herd immunity to amphibian

chytridiomycosis in Madagascar based on the defensive function of amphibian skin bacteria. Front. Microbiol. 8, 1751. (doi:10.3389/ fmicb.2017.01751)

27. Bell SC, Alford RA, Garland S, Padilla G, Thomas AD. 2013 Screening bacterial metabolites for inhibitory effects against *Batrachochytrium dendrobatidis* using a spectrophotometric assay. Dis. Aquat. Organ. 103, 77–85. (doi:10.3354/dao02560)

28. Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007 Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267. (doi:10.1128/ AEM.00062-07)

29. Price MN, Dehal PS, Arkin AP. 2010 FastTree 2 - Approximately maximum-likelihood trees for large alignments. PLoS ONE 5, e9490. (doi:10.1371/ journal.pone.0009490)

30. Lee DH, Zo YG, Kim SJ. 1996 Nonradioactive method to study genetic profiles of natural bacterial communities by PCR-single-strand-conformation polymorphism. Appl. Environ. Microbiol. 62, 3112–3120.

31. Blooi M, Pasmans F, Longcore JE, Spitzen-Van Der Sluijs A, Vercammen F, Martel A. 2013 Duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in amphibian samples. J. Clin. Microbiol. 51, 4173–4177. (doi:10. 1128/JCM.02313-13)

32. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013 Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the Miseq Illumina sequencing platform. Appl. Environ. Microbiol. 79, 5112–5120. (doi:10.1128/AEM. 01043-13)

33. Caporaso JG et al. 2010 QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336. (doi:10.1038/nmeth.f.303)

34. Amir A et al. 2017 Deblur rapidly resolves singlenucleotide community sequence patterns. mSystems 2, e00191-16.

35. Singhal N, Kumar M, Kanaujia PK, Virdi JS. 2015 MALDI-TOF mass spectrometry: An emerging technology for microbial identification and diagnosis. Front. Microbiol. 6, 1–16. (doi:10.3389/fmicb.2015.00791)

36. R Core Team. 2016 R: A language and environment for statistical computing. Vienna, Austria: Foundation for Statistical Computing.

37. Bates DM, Machler M, Bolker BM, Walker SC. 2014 Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. (doi:10.18637/jss.v067. i01).

38. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB. 2017 vegan: Community Ecology Package. R package verion 2.4-2.

39. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. 2011 Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60. (doi:10.1186/gb-2011-12-6-r60)

40. Therneau TM, Grambsch PM. 2000 Modeling survival data: extending the cox model. New York: NY: Springer.

41. Wickham H. 2009 Ggplot2: elegant graphics for data analysis. New York: NY: Springer.

42. Cogen AL, Nizet V, Gallo RL. 2008 Skin microbiota: a source of disease or defence? Br. J. Dermatol. 158, 442–455. (doi:10.1111/j.1365-2133.2008. 08437.x)

43. Sender R, Fuchs S, Milo R. 2016 Revised estimates for the number of human and bacteria cells in the body. PLoS Biol. 14, 1–14. (doi:10.1371/journal. pbio.1002533)

44. Abt M, David A. 2013 The dynamic influence of commensal bacteria on the immune response to pathogens. Curr. Opin. Microbiol. 16, 4–9. (doi:10. 1016/j.mib.2012.12.002)

45. Burke C, Steinberg P, Rusch DB, Kjelleberg S, Thomas T. 2011 Bacterial community assembly based on functional genes rather than species. Proc. Natl Acad. Sci. USA 108, 14 288–14 293. (doi:10. 1073/pnas.1101591108)

46. Kueneman JG, Woodhams DC, Van Treuren W, Archer HM, Knight R, McKenzie VJ. 2015 Inhibitory bacteria reduce fungi on early life stages of endangered Colorado boreal toads (*Anaxyrus boreas*). ISME J. 10, 934–944. (doi:10.1038/ismej. 2015.168)

47. Yasumiba K, Bell S, Alford R. 2016 Cell density effects of frog skin bacteria on their capacity to inhibit growth of the chytrid fungus, *Batrachochytrium dendrobatidis*. Microb. Ecol. 71, 124–130. (doi:10.1007/s00248-015-0701-9)

48. Raynaud X, Nunan N. 2014 Spatial ecology of bacteria at the microscale in soil. PLoS ONE 9, e87217. (doi:10.1371/journal.pone.0087217)

49. Vandeputte D et al. 2017 Quantitative microbiome profiling links gut community variation to microbial load. Nature 551, 507.

50. Brucker RM, Baylor CM, Walters RL, Lauer A, Harris RN, Minbiole KPC. 2008 The identification of 2,4- diacetylphloroglucinol as an antifungal metabolite produced by cutaneous bacteria of the salamander *Plethodon cinereus*. J. Chem. Ecol. 34, 39–43. (doi:10.1007/s10886-007-9352-8)

51. Becker MH, Brucker RM, Schwantes CR, Harris RN, Minbiole KPC. 2009 The bacterially produced metabolite violacein is associated with survival of amphibians infected with a lethal fungus. Appl. Environ. Microbiol. 75, 6635–6638. (doi:10.1128/ AEM.01294-09)

52. Nunes-Alves C. 2015 Microbiome: taking advantage of quorum sensing. Nat. Rev. Micro 13, 252. (doi:10. 1038/nrmicro3477)

53. Thompson JA, Oliveira RA, Xavier KB. 2016 Chemical conversations in the gut microbiota. Gut Microbes 7, 163–170. (doi:10.1080/19490976.2016.1145374)

54. Scheuring I, Yu DW. 2012 How to assemble a beneficial microbiome in three easy steps. Ecol. Lett. 15, 1300–1307. (doi:10.1111/j.1461-0248.2012. 01853.x)

55. Hajishengallis G et al. 2011 Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. Cell Host Microbe 10, 497–506. (doi:10.1016/j.chom.2011.10.006)

56. Koskella B, Meaden S, Crowther WJ, Leimu R, Metcalf CJE. 2017 A signature of tree health? Shifts in the microbiome and the ecological drivers of orse chestnut bleeding canker disease. New Phytol. 215, 737–746. (doi:10.1111/nph.14560)

57. Jani AJ, Briggs CJ. 2014 The pathogen *Batrachochytrium dendrobatidis* disturbs the frog skin microbiome during a natural epidemic and experimental infection. Proc. Natl. Acad. Sci. USA 111, E5049–E5058. (doi:10.1073/pnas. 1412752111)

58. Becker CG, Longo AV, Haddad CFB, Zamudio KR. 2017 Land cover and forest connectivity alter the interactions among host, pathogen and skin microbiome. Proc. R. Soc. B 284, 20170582. (doi:10. 1098/rspb.2017.0582)

59. Loch TP, Faisal M. 2015 Emerging flavobacterial infections in fish: a review. J. Adv. Res. 6, 283–300. (doi:10.1016/j.jare.2014.10.009)

60. Tronel H, Plesiat P, Ageron E, Grimont PAD. 2003 Bacteremia caused by a novel species of *Sphingobacterium*. Clin. Microbiol. Infect. 9, 1242–1244. (doi:10.1111/j.1469-0691.2003.00801.x)

61. Abro AH, Reza M, Shahmirzadi R, Jasim LM, Deesi Z Al. 2016 *Sphingobacterium multivorum* bacteremia and acute meningitis in an immunocompetent adult patient: a case report. Iran. Red Crescent Med. J. 18, 10–12.

62. Seifert H, Strate A, Schulze A, Pulverer G. 1993 Vascular catheter-related bloodstream infection due to *Acinetobacter johnsonii* (Formerly *Acinetobacter calcoaceticus* var. lwoffii): Report of 13 Cases. Clin. Infect. Dis. 17, 632–636.

63. Herna´ndez-Go´mez O, Kimble SJA, Briggler JT, Williams RN. 2016 Characterization of the cutaneous bacterial communities of two giant salamander subspecies. Microb. Ecol. 73, 445–454. (doi:10. 1007/s00248-016-0859-9)

64. Fraune S, Anton-Erxleben F, Augustin R, Franzenburg S, Knop M, Schro¨der K, Willoweit-Ohl D, Bosch TC. 2014 Bacteria-bacteria interactions within the microbiota of the ancestral metazoan Hydra contribute to fungal resistance. ISME J. 9, 1543–1556. (doi:10.1038/ismej.2014.239)

65. Becker MH et al. 2015 Composition of symbiotic bacteria predicts survival in Panamanian golden frogs infected with a lethal fungus. Proc. R. Soc. B 282, 20142881. (doi:10.1098/rspb.2014.2881)

66. Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I. 2007 The role of microorganisms in coral health, disease and evolution. Nat. Rev. Microbiol. 5, 355–362. (doi:10.1038/nrmicro1635)

67. Krediet CJ, Ritchie KB, Alagely A, Teplitski M. 2013 Members of native coral microbiota inhibit glycosidases and thwart colonization of coral mucus by an opportunistic pathogen. ISME J. 7, 980–990. (doi:10.1038/ismej.2012.164)

68. Lau CSM, Chamberlain RS. 2016 Probiotics are effective at preventing *Clostridium difficile* -associated diarrhea: a systematic review and meta-analysis. Int. J. Gen. Med. 9, 27–37.

69. Becker MH et al. 2015 Phylogenetic distribution of symbiotic bacteria from Panamanian amphibians that inhibit growth of the lethal fungal pathogen *Batrachochytrium dendrobatidis*. Mol. Ecol. 24, 1628–1641. (doi:10.1111/mec.13135)

70. Woodhams DC et al. 2015 Antifungual isolates database of amphibian skin-associated bacteria and function against emerging fungal pathogens. Ecology 96, 595. (doi:10.1890/14-1837.1)

71. Haas D, De´fago G. 2005 Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat. Rev. Microbiol. 3, 307–319. (doi:10.1038/ nrmicro1129)

72. Kim JD, Kim B, Lee CG. 2007 Alga-lytic activity of *Pseudomonas fluorescens* against the red tide causing marine alga *Heterosigma akashiwo* (*Raphidophyceae*). Biol. Control 41, 296–303. (doi:10.1016/j.biocontrol.2007.02.010)

73. Hoyt JR, Cheng TL, Langwig KE, Hee MM, Frick WF. 2015 Bacteria isolated from bats inhibit the growth of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome. PLoS ONE 10, e0121329. (doi:10.1371/journal.pone. 0121329)

74. Canessa S et al. 2018 Decision making for mitigating wildlife diseases: from theory to practice for an emerging fungal pathogen of amphibians. J. Appl. Ecol. 55, 1987–1996. (doi:10.1111/1365-2664.13089)

Annex 10: Proof of concept that the application of this measure protects a salamander community in an experimental setup

Integral chain management of wildlife diseases

An Martel[1*], Mireia Vila-Escale[2], Daniel Fernández-Giberteau[3], Albert Martinez-Silvestre[4], Stefano Canessa[1], Sarah Van Praet[1], Pep Pannon[2], Koen Chiers[1], Albert Ferran[2], Moria Kelly[1], Mariona Picart[2], Dolors Piulats[5], Zhimin Li[1], Viviana Pagone[5], Laia Pérez-Sorribes[3], Carolina Molina[3], Aïda Tarragó-Guarro[6], Roser Velarde-Nieto[7], Francesc Carbonell[8], Elena Obon[8], Diego Martínez-Martínez[9], Daniel Guinart[2], Ricard Casanovas[6], Salvador Carranza[5], Frank Pasmans[1].

Abstract

The chytrid fungus *Batrachochytrium dendrobatidis* has caused the most prominent loss of vertebrate diversity ever recorded, which peaked in the 1980's. Recent incursion by its sister species *B. salamandrivorans* in Europe raised the alarm for a new wave of declines and extinctions in western Palearctic urodeles.The European Commission has responded by restricting amphibian trade. However, private amphibian collections, the main end consumers, were exempted from the European legislation. Here, we report how invasion by a released, exotic newt coincided with *B. salamandrivorans* invasion at over 1000 km from the nearest natural outbreak site, causing mass mortality in indigenous marbled newts (*Triturus marmoratus*), and posing an acute threat to the survival of nearby populations of the most critically endangered European newt species (Montseny brook newt, *Calotriton arnoldi*). Disease management was initiated shortly after detection in a close collaboration between policy and science and included drastic on site measures and intensive disease surveillance. Despite these efforts, the disease is considered temporarily contained but not eradicated and continued efforts will be necessary to minimize the probability of further pathogen dispersal. This precedent demonstrates the importance of tackling wildlife diseases at an early stage using an integrated approach, involving all stakeholders and closing loopholes in existing regulations.

1. Introduction

Counteracting drivers of biodiversity loss is a major challenge for global change science and policy (IPBES, 2019). Globalization has precipitated multiple introductions of devastating wildlife and plant fungal diseases such as Dutch elm blight, sudden oak death, American chestnut blight, White nose syndrome in bats and chytridiomycosis in amphibians (Fisher et al., 2012). Of all known pathogens, *Batrachochytrium dendrobatidis* has caused the most prominent loss of vertebrate diversity ever recorded, with extinctions or declines in 500 amphibian species in Australia and the Americas (Scheele et al., 2019). The recent emergence of its sister species *B. salamandrivorans* (Martel et al., 2013) raised the alarm for a possible new wave of declines and extinctions similar to that caused by *B. dendrobatidis* (Martel et al., 2014, Yap et al., 2015). The European Commission took action to restrict amphibian trade

(EU2018/320), include *B. salamandrivorans* in EU-wide regulations on transmissible animal diseases (EU2018/1882), and deploy a European wide early warning system with disease emergency teams and a network of diagnostic centers (ENV.B.3/SER/2016/0028). Trade in live amphibians is a prominent source of invasive alien species and pathogen pollution, serving as the most probable vehicle for *B. salamandrivorans* introductions (Fitzpatrick et al., 2018). The temporary trade restriction law (EU2018/320) lists health protection measures for commercial animal movements between EU member states and for introduction of salamanders from a third country. Unfortunately, the main end consumers of this trade, private amphibian collections, are exempted from existing European legal frameworks. Noncommercial animal movements among private collectors are not governed by legislation that can be used as a basis for controlling pathogen outbreaks.

We report how *B. salamandrivorans* invaded and caused mortalities in a Spanish amphibian community, likely through spill-over from introduced alien pet amphibians, in a region home to the most critically endangered European newt species. The combination of early detection, intensive management, and close collaboration between policy and science succeeded in temporary disease containment but not eradication. This precedent demonstrates the importance of tackling wildlife diseases at an early stage using an integrated approach, involving all stakeholders and closing loopholes in existing regulations.

2. Drastic response to disease outbreak

In March 2018, *B. salamandrivorans* was detected in a small reservoir in the Montnegre i el Corredor Natural Park in Catalonia (NE Spain), approximately 1,000 km from its nearest known occurrence in northern Europe (Figure 1). Infection was discovered during a campaign to eradicate invasive exotic newts (*Triturus anatolicus* (Anatolian crested newt) and *Ichthyosaura alpestris* (alpine newt)). Initial detection of *B. salamandrivorans* in two healthy Anatolian crested newts during an opportunistic disease screening was followed by a mortality event in native marbled newts (*T. marmoratus*) in May 2018 (Figure 1). The inclusion of *B. salamandrivorans* in regulatory frameworks, awareness of its threat to biodiversity and close proximity to the range of the critically endangered Montseny brook newt (*Calotriton arnoldi*) (Carranza & Martinez-Solano, 2009) stimulated decision making by local and regional authorities and their response to the detected outbreak, in close collaboration with scientists. Such a combination of policy, science and action on the ground is common against epidemics of livestock diseases, but rarely applied to wildlife disease (OIE, 2018). Absence of efficient protocols to curb chytridiomycosis-driven loss of biodiversity (Garner et al., 2016) prompted authorities to implement broad-spectrum precautionary actions. Disease control included implementation of biosecurity, habitat management and disinfection, host removal and disease surveillance throughout the park (Figure 1, Supporting information Materials and Methods and Table S1) and was based on a combination of a successful mitigation action of *B. dendrobatitidis* in Mallorcan midwife toads (Bosch et al., 2015) and by epidemiological models suggesting that removal of the host community is currently the only possible response to eliminate a *B. salamandrivorans* outbreak (Canessa et al., 2018, 2019; Thomas et al., 2019).

In total, 690 urodeles and 184 anurans were tested for *B. salamandrivorans* infection during the period March 2018 – May 2019 (Table S1). Streamlined decision processes, including permit

issuing and contracting, allowed deployment of resources from six weeks after first detection of *B. salamandrivorans* onwards (Figure 1).

One year after detection, analysis of the removal data suggests a large proportion of the indigenous *T. marmoratus* population has been removed (mean estimate: 0.82, 95% C.I. 0.75-0.89; Table S1; methods in Supplementary Material). Estimates for the invasive Anatolian newts are highly uncertain, but the species has not been resighted since May 2019 (Figure 1, Table S1). Several screening surveys of all waterbodies in the park did not return any positive result beyond the outbreak site; therefore, we currently consider *B. salamandrivorans* to be at least temporarily contained, albeit not eradicated at the outbreak site (Table S1).

Our experience with *B. salamandrivorans* field management – to our knowledge, the first such attempt ever made – highlights several useful lessons for future analogous efforts. The analysis of the results indicates the largely passive trapping strategy achieved very low removal rates (e.g. a mean rate of 3% for indigenous newts), whereas epidemiological studies suggest eliminating *B. salamandrivorans* requires an intensive effort, with >90% removal within a very short time frame (Canessa et al. 2018, 2019). Moreover, juvenile stages without reproductive activity might escape traps located near waterbodies. In our case, large numbers of infected juveniles were found outside the fenced perimeter a year after detection (Figure 1). We recommend actively targeting those terrestrial life stages; soil sanitation might also be considered. In general, in a future attempt we would seek greater integration of quantitative data collection and analysis (e.g. epidemiological and removal modelling) into management planning from the beginning and during the outbreak, and not simply for post-hoc analysis. Such an "outbreak science" framework is increasingly recommended for mitigation of human and livestock diseases (Polonsky et al. 2019). Increasing likelihood of pathogen eradication could be effectuated by increasing the probability of early disease detection and minimizing response time. An efficient early warning system combined with the availability of specific, evidence-based emergency action plans would facilitate an immediate response. Such plans should provide a strong decision support framework for potentially controversial measures such as the removal of protected species.

3. Tracing threats to endangered wildlife

In parallel with the emergency precautionary responses, laboratory experiments (see infection trial section Supplementary Materials) were carried out to assess the risk for the indigenous urodele species and the suitability of the invasive newts as pathogen vectors. Experimental exposure of the endangered Montseny brook newts and indigenous fire salamanders (*Salamandra salamandra*) and marbled newts to the local *B. salamandrivorans* isolate (Figure 1) resulted in lethal infections. In contrast, the invasive Anatolian crested newts developed chronic, non-lethal infections, with latency periods of undetectable infection and subsequent flare ups that allowed spillover of infection to marbled newts (Figure S1). These experimental findings are highly consistent with the disease dynamics observed in the field, confirm the threat to native wildlife, and corroborate the likelihood of the invasive newts as disease vectors and reservoirs. The experimental evidence, however, is circumstantial, and does not pinpoint the source of invasive newts and pathogen. We presume exotic newts have been released to the site by a private collector since at least 2016. This assumption is reinforced by the remarkable local

diversity of alien invasive newts, known past introductions by the suspected collector in the region (e.g. introduction of Turkish *Ommatotriton ophryticus*; Fontelles et al., 2011), and experimental evidence that the invasive Anatolian newts can be long term carriers and disease reservoirs. Moreover, the distance to the nearest known outbreaks (over 1000 km), poor dispersal ability (Spitzen et al., 2018) and the known sensitivity of *B. salamandrivorans* to environmental factors (Blooi et al., 2015; Stegen et al., 2017) reduce the likelihood of passive transport. However, existing regulations do not allow access to private collections. Private amphibian keepers are not subject to sanitary regulations, hampering epidemiological tracing and disease eradication, which leaves the invasion hazard undetermined and unmitigated. Although we here link *B. salamandrivorans* invasion to pet release, alternative routes of pathogen introduction on passive vectors such as fomites should be considered. As a precautionary principle the application of biosecurity measures during activities in amphibian habitats is likely to minimize opportunities for human mediated pathogen introductions and further dispersal.

4. Integral chain management of wildlife diseases

Prevention of wildlife diseases along the entire invasion pathway is a priority that cannot be further delayed. Pathogen invasions in wildlife are mostly addressed when threatening livestock and/or public health. However, attempts to mitigate the impact of infectious threats should be considered integral components of biodiversity protection legislation, in this case the EU Habitat's Directive. Decision making should clearly define objectives, then risk and choose whether and how to respond. Here, the emergence of an acute, invasive and human-mediated threat to the survival of a critically endangered species prompted decision-makers to act rapidly and drastically in order to contain and eradicate disease. The inability to eradicate disease in our case, even following detection and coordinated response using best practice, demonstrates the necessity of intercepting wildlife diseases at an early stage, before the invasion of natural systems. Failure to do so has resulted in the emergence of a World Organization for Animal Health (OIE) listed wildlife disease (Aquatic OIE, 2017) 1000 km from the nearest outbreak, directly threatening Europe's most endangered newt and requiring ongoing intensive mitigation efforts. To avoid similar scenarios, we propose an integral chain management of trade-associated wildlife diseases, aimed at minimizing the probability of disease introduction using principles such as Hazard Analysis of Critical Control Points (Codex Alimentarius, 1997), as is commonplace in disease mitigation in humans and livestock. We envisage three links to this chain: the animal trade, the domestic host population, and hosts/susceptible species in the wild. Regulation of the wildlife trade is slowly improving; response to disease outbreaks in the wild, although challenging, can be made easier by early warning systems, science support and streamlined decision processes as evidenced by the Catalan case.

Current evidence points to the role of the captive *B. salamandrivorans* reservoir combined with amphibian movements (in a broad sense, including traffic of animals between hobbyists) as likely vehicle for further *B. salamandrivorans* introductions in naïve regions (Fitzpatrick et al., 2018; this report). Elimination of this reservoir requires extensive screening and treatments. While current legislation regulates commercial trade, hobbyists (pet keepers) are exempted from European legislation, yet allegedly play a key role in *B. salamandrivorans* epidemiology

(Fitzpatrick et al., 2018; this report). In the absence of legislation, disease control in amphibians is largely based on stakeholders' voluntary participation, stressing the need for increased awareness and voluntary compliance of the private sector with the clean trade principle. Since the domestic host population presents the weak link, initiatives to reduce the probability of pathogen pollution by supporting amphibian pathogen-free collections of pet keepers would be a valuable addition to the existing pan European policy initiatives (EU2018/1882, ENV.B.3/SER/2016/0028) and OIE (OIE, 2017).

The current, voluntary participation of hobbyists in *B. salamandrivorans* disease control may be encouraged by the distinct advantage of improved health of a negative collection. Hobbyist societies should raise awareness and encourage their members to subscribe to the clean trade principle. Absence of *B. salamandrivorans* (and other amphibian pathogens) from the commercial trade would benefit from a code of conduct subscribed by professional organizations. The European Commission is advised to implement the temporary directive EU2018/1882, ENV.B.3/SER/2016/0028 in the upcoming Animal Health Law and to extend this legislation to include the private sector. The principle to eradicate the *B. salamandrivorans* disease reservoir from the live amphibian trade chain could be expanded to include other trade related and OIE listed amphibian pathogens (ranaviruses, *B. dendrobatidis*) and amphibians (anurans, caecilians). Finally, the EU and EU member states should be encouraged to adopt and maintain early warning systems and emergency action plans that can be deployed immediately upon pathogen detection. Wide implementation of biosecurity protocols for activities in amphibian habitats is encouraged.

Acknowledgements

References

Blooi, M., Martel, A., Haesebrouck, F., Vercammen, F., Bonte, D. & Pasmans, F. (2015) Treatment of urodelans based on temperature dependent infection dynamics of *Batrachochytrium salamandrivorans. Scientific Reports, 5,* 8037.

Bosch, J., Sanchez-Tomé, E., Fernandez-Loras, A., Oliver, J.A., Fisher, M.C., & Garnter, T.W.J. (2015). Successful elimination of a lethal wildlife infectious disease in nature. *Biology Letters, 11,* 20150874.

Canessa, S., Bozzuto, C., Grant, E.H.C. , Cruickshank, S.S., Fisher, M.C., Koella, J.C., …Schmidt B.R. (2018). Decision-making for mitigating wildlife diseases: from theory to practice for an emerging fungal pathogen of amphibians. *Journal of Applied Ecology, 55,* 1987-1996.

Canessa, S., Bozzuto, C., Pasmans, F., & Martel, A. (2019). Quantifying the burden of managing wildlife diseases in multiple host species. *Conservation Biology, 33(5),* 1131-1140.

Carranza, S., & Martínez-Solano, I. (2019). *Calotriton arnoldi*. The IUCN Red List of Threatened Species 2009:e.T136131A4246722.

Codex Alimentarius Commission. Codex Alimentarius (1997). Annex to CAC/RCP 1-1969, Rev. 3.EU 2018/320. Commission Implementing Decision (EU) 2018/320 of February 2018 on certain animal health protection measures for intra-Union trade in salamanders and the introduction into the Union of such animals in relation to the fungus *Batrachochytrium salamandrivorans*. *Official Journal of the European Union L 62*, 5.3.2018, pp.18-33.

EU 2018/1882. Commission Implementing Regulation (EU) 2018/1882 on the 'Animal Health Law' and Commission Delegated Regulation (EU) 2018/1629. *Official Journal of the European Union, L308/21.*

ENV.B.3/SER/2016/0028. European Commission Tender. Mitigating a new infectious Disease in Salamanders to counteract the loss of European biodiversity.

Fisher, M.C., Henk, D.A., Briggs, C.J, Brownstein, J.S., Madoff, L.C., McCraw, S.L.,& Gurr, S.J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature, 484*, 186–194.

Fitzpatrick, L.D., Pasmans, F., Martel, A., & Cunningham, A.A. (2018). Epidemiological tracing of *Batrachochytrium salamandrivorans* identifies widespread infection and associated mortalities in private amphibian collections. *Scientific Reports, 8*, 13845.

Fontelles, F., Guixé, D., Martínez-Silvestre, A., Soler, J., &Villero, D. (2011). Hallada población introducida de *Ommatotriton ophryticus* en el Prepirineo catalán. *Boletín de la Asociación Herpetológica Española, 22,* 153-156.

Garner, T.W.J., Schmidt, B.R., Martel, A., Pasmans, F., Muths, E., Cunningham, A.A., … Bosch, J. (2016). Mitigating amphibian chytridiomycoses in nature. *Philosophical Transactions of the Royal Society B, 371*, 20160207.

IPBES Global Assessment Report on Biodiversity and Ecosystem Services (2019).

Martel, A., Spitzen-van der Sluijs, A., Blooi, M., Bert, W., Ducatelle, R., Fisher, M.C., ...Pasmans, F. (2013). *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proceedings of the National Academy of Sciences of the United States of America, 110*, 15325-15329.

Martel, A., Blooi, M., Adriaensen, C., Van Rooij, P., Beukema, W., Fisher, M.C., … Pasmans F. (2014). Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science, 346*, 630-631.

OIE World Organisation for Animal Health. (2018) Terrestrial Animal health Code.

OIE World Organisation for Animal Health (2017). Aquatic Animal Health Code. Available online:http://www.oie.int/en/international-standard-setting/aquatic-code/access-online/.

Polonsky, J.A., Baidjoe, A., Kamvar, Z.N., Cori, A., Durski, K., Edmunds, …& Keating, P. (2019). Outbreak analytics: a developing data science for informing the response to emerging pathogens. *Philosophical Transactions of the Royal Society B, 374(1776),* 20180276.

Scheele, B., Pasmans, F., Skerratt, L., Berger, L., Martel, A., Beukema, W., …Canessa, S. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science, 363*, 1459-1463.

Spitzen-van der Sluijs, A., Stegen, G., Bogaerts, S., Canessa, S., Steinfartz, S., Janssen, N., …& Martel, A. (2018). Post-epizootic salamander persistence in a disease-free refugium suggests poor dispersal ability of *Batrachochytrium salamandrivorans*. *Scientific Reports, 8,* 3800.

Stegen, G., Pasmans, F., Schmidt, B., Rouffaer, L.O., Van Praet, S., Schaub, M., & Martel, A. (2017). Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans. Nature, 544,* 353–356. *....*

Thomas, V., Wang, Y., Van Rooij, P., Verbrugghe, E., Balaz, V., Bosch, J.,…Pasmans, F. (2019). Mitigating *Batrachochytrium salamandrivorans* in Europe. *Amphibia-Reptilia, 40,* 265-290.

Yap, T.A., Koo, M.S., Ambrose, R.F., Wake, D.B., Vredenburg, V.T. (2015). Averting a North American biodiversity crisis. *Science, 349*, 481-482.

Supporting information:

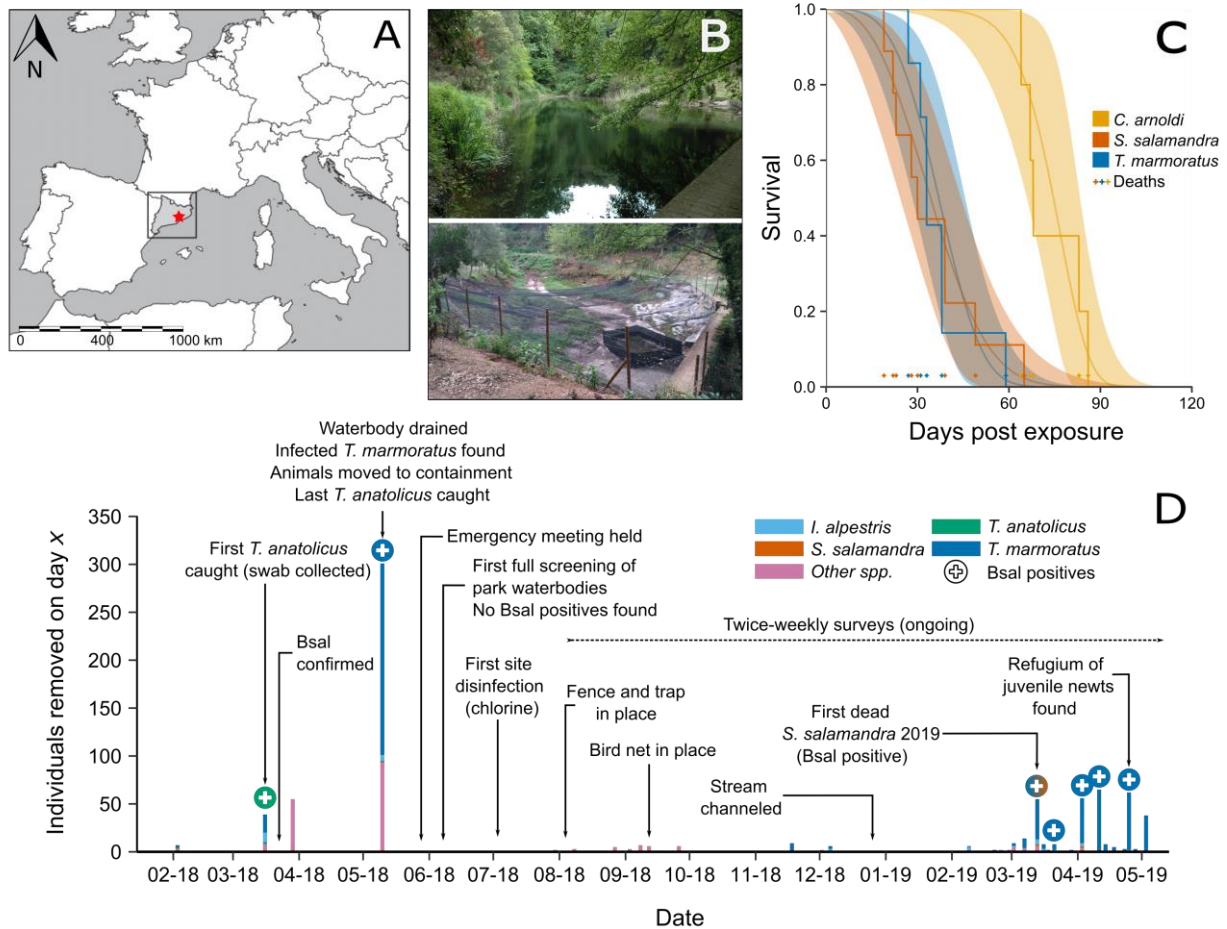Materials and Methods Figures S1 Tables S1-S2 External Databases S1 References (19-25)

Figure 1. Overview *B. salamandrivorans* detection and subsequent actions. (**A**) Location of the Montnegre i el Corredor Natural Park in Catalonia, Spain and Europe; (**B**) the outbreak site before and after mitigation interventions; (**C**) survival of *T. marmoratus*, *S. salamandra* and the Critically Endangered Montseny brook newt *C. arnoldi* after experimental infection with *B. salamandrivorans*; (**D**) Timeline of management actions and removal of amphibian hosts at the outbreak site.