Mitigating *Batrachochytrium salamandrivorans* in Europe

Batrachochytrium salamandrivorans Action Plan for European urodeles

European Commission

Colophon

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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.

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*.

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 50 × 50 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

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.

Family	Species/subspecies	Estimated susceptibility to Bsal			
		Laboratory trial	Field out-break	Captivity	Inferred from phylogeny
Hynobiidae	*Salamandrella keyserlingii*	Low[1]	NA	NA	NA
Plethodontidae	*Speleomantes ambrosii*	NA	NA	NA	High
Plethodontidae	*Speleomantes ambrosii ambrosii*	NA	NA	NA	High
Plethodontidae	*Speleomantes ambrosii bianchii*	NA	NA	NA	High
Plethodontidae	*Speleomantes flavus*	NA	NA	NA	High
Plethodontidae	*Speleomantes genei*	High[2]	NA	NA	NA
Plethodontidae	*Speleomantes imperialis*	Low[2]	NA	NA	NA
Plethodontidae	*Speleomantes italicus*	NA	NA	NA	High
Plethodontidae	*Speleomantes sarrabusensis*	NA	NA	NA	High
Plethodontidae	*Speleomantes strinatii*	High[1]	NA	NA	NA
Plethodontidae	*Speleomantes supramontis*	NA	NA	NA	High
Proteidae	*Proteus anguinus*	Low[2]	NA	NA	NA
Proteidae	*Proteus anguinus anguinus*	Low[2]	NA	NA	NA
Proteidae	*Proteus anguinus parkelj*	NA	NA	NA	Low
Salamandridae	*Calotriton arnoldi*	High[3]	NA	NA	NA
Salamandridae	*Calotriton asper*	Low[4]	NA	NA	NA
Salamandridae	*Chioglossa lusitanica*	High[2]	NA	Yes[5]	NA
Salamandridae	*Chioglossa lusitanica longipes*	High[2]	NA	NA	NA
Salamandridae	*Chioglossa lusitanica lusitanica*	NA	NA	NA	High
Salamandridae	*Euproctus montanus*	NA	NA	NA	High
Salamandridae	*Euproctus platycephalus*	High[1]	NA	Yes[6]	NA
Salamandridae	*Ichthyosaura alpestris*	Moderate[1,7]	No[8,9]	NA	NA
Salamandridae	*Ichthyosaura alpestris alpestris*	Moderate[1,7]	No[8,9]	NA	NA
Salamandridae	*Ichthyosaura alpestris apuana*	NA	NA	NA	Moderate
Salamandridae	*Ichthyosaura alpestris cyreni*	NA	NA	NA	Moderate
Salamandridae	*Ichthyosaura alpestris montenegrina*	NA	NA	NA	Moderate
Salamandridae	*Ichthyosaura alpestris reiseri*	NA	NA	NA	Moderate
Salamandridae	*Ichthyosaura alpestris veluchiensis*	NA	NA	NA	Moderate
Salamandridae	*Lissotriton boscai*	Moderate	NA	Yes[6]	NA
Salamandridae	*Lissotriton graecus*	NA	NA	NA	Moderate
Salamandridae	*Lissotriton helveticus*	Low[1]	No[8]	NA	NA
Salamandridae	*Lissotriton italicus*	High[1]	NA	NA	NA
Salamandridae	*Lissotriton montandoni*	NA	NA	NA	Moderate
Salamandridae	*Lissotriton schmidtleri*	NA	NA	NA	Moderate
Salamandridae	*Lissotriton vulgaris*	Moderate[10]	No[8,9]	NA	NA
Salamandridae	*Lissotriton vulgaris ampelensis*	NA	NA	NA	Moderate
Salamandridae	*Lissotriton vulgaris meridionalis*	NA	NA	NA	Moderate

Population level risk of extinction	Confidence	Taxon level risk of extinction			Range size*	IUCN Red List Category**	Listed in Annex IV Habitats Directive
		10 years	100 years	Confidence			
Low	Low	Low	Low	Low	>25	LC	No
High	Low	High	High	Low	1-5	NT	Yes
High	Low	High	High	Low	1-5	NA	Yes
High	Low	High	High	Low	1-5	NA	Yes
High	Low	High	High	Low	1-5	VU	Yes
High	Low	High	High	Low	1-5	VU	Yes
Low	Low	Low	Medium	Low	1-5	NT	Yes
High	Low	Medium	High	Low	6-25	NT	Yes
High	Low	High	High	Low	1-5	VU	Yes
High	Low	Medium	High	Low	6-25	NT	Yes
High	Low	High	High	Low	1-5	EN	Yes
Low	Low	Low	Low	Low	6-25	VU	Yes
Low	Low	Low	Low	Low	6-25	NA	Yes
Low	Low	Low	Low	Low	1-5	NA	Yes
High	Low	High	High	Low	1-5	CR	Yes
Low	Low	Low	Medium	Low	>25	NT	Yes
High	High	Low	Medium	High	>25	VU	Yes
High	Low	Low	Medium	Low	>25	NA	Yes
High	Low	Medium	High	Low	6-25	NA	Yes
High	Low	Medium	High	Low	6-25	LC	Yes
High	Low	Medium	High	Low	6-25	EN	Yes
Medium	High	Low	Low	High	>25	LC	No
Medium	High	Low	Low	High	>25	NA	No
Medium	Low	Low	Medium	Low	6-25	NA	No
Medium	Low	Low	Medium	Low	6-25	NA	No
Medium	Low	Medium	Medium	Low	1-5	NA	No
Medium	Low	Low	Low	Low	>25	NA	No
Medium	Low	Low	Medium	Low	6-25	NA	No
High	High	Low	Medium	High	>25	LC	No
Medium	Low	Low	Low	Low	>25	NE	No
Low	High	Low	Low	High	>25	LC	No
High	Low	Low	Medium	Low	>25	LC	Yes
Medium	Low	Low	Low	Low	>25	LC	Yes
Medium	Low	Low	Low	Low	>25	NE	No
Medium	High	Low	Low	High	>25	LC	No
Medium	Low	Low	Medium	Low	6-25	NA	Yes
Medium	Low	Low	Low	Low	>25	NA	No

Family	Species/subspecies	Estimated susceptibility to Bsal			
		Laboratory trial	Field out-break	Captivity	Inferred from phylogeny
Salamandridae	*Lissotriton vulgaris vulgaris*	Moderate[10]	No[8,9]	NA	NA
Salamandridae	*Lyciasalamandra helverseni*	High[2]	NA	NA	NA
Salamandridae	*Lyciasalamandra luschani****	NA	NA	NA	High
Salamandridae	*Lyciasalamandra luschani basoglui*	NA	NA	NA	High
Salamandridae	*Pleurodeles waltl*	High[1,3]	NA	NA	NA
Salamandridae	*Salamandra atra*	NA	NA	Yes[6]	NA
Salamandridae	*Salamandra atra atra*	NA	NA	NA	High
Salamandridae	*Salamandra atra aurorae*	NA	NA	NA	High
Salamandridae	*Salamandra atra pasubiensis*	NA	NA	NA	High
Salamandridae	*Salamandra atra prenjensis*	NA	NA	NA	High
Salamandridae	*Salamandra corsica*	NA	NA	Yes[6,11]	NA
Salamandridae	*Salamandra lanzai*	NA	NA	NA	High
Salamandridae	*Salamandra salamandra*	High[1,12]	Yes[8,9,12]	Yes[6,11]	NA
Salamandridae	*Salamandra salamandra almanzoris*	NA	NA	Yes[11]	NA
Salamandridae	*Salamandra salamandra bejarae*	NA	NA	NA	High
Salamandridae	*Salamandra salamandra bernardezi*	NA	NA	Yes[11]	NA
Salamandridae	*Salamandra salamandra crespoi*	NA	NA	NA	High
Salamandridae	*Salamandra salamandra fastuosa*	NA	NA	Yes[11]	NA
Salamandridae	*Salamandra salamandra gallaica*	NA	NA	Yes[11]	NA
Salamandridae	*Salamandra salamandra gigliolii*	NA	NA	Yes[11]	NA
Salamandridae	*Salamandra salamandra longirostris*	NA	NA	NA	High
Salamandridae	*Salamandra salamandra morenica*	NA	NA	NA	High
Salamandridae	*Salamandra salamandra salamandra*	NA	NA	Yes[11]	NA
Salamandridae	*Salamandra salamandra terrestris*	High[12]	Yes[9,12]	Yes[11]	NA
Salamandridae	*Salamandrina perspicillata*	High[1]	NA	NA	NA
Salamandridae	*Salamandrina terdigitata*	NA	NA	NA	High
Salamandridae	*Triturus carnifex*	NA	NA	Yes[2,6]	NA
Salamandridae	*Triturus cristatus*	High[1,10]	Yes[8]	NA	NA
Salamandridae	*Triturus dobrogicus*	NA	NA	Yes[6]	NA
Salamandridae	*Triturus ivanbureschi*	NA	NA	Yes[6]	NA
Salamandridae	*Triturus karelinii*	NA	NA	Yes[2,6]	NA
Salamandridae	*Triturus macedonicus*	NA	NA	Yes[6]	NA
Salamandridae	*Triturus marmoratus*	High	Yes[3]	Yes[6]	NA
Salamandridae	*Triturus pygmaeus*	NA	NA	Yes[2,6]	NA

* *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.*

Population level risk of extinction	Confidence	Taxon level risk of extinction			Range size*	IUCN Red List Category**	Listed in Annex IV Habitats Directive
		10 years	100 years	Confidence			
Medium	High	Low	Low	High	>25	NA	No
High	Low	High	High	Low	1-5	VU	Yes
High	Low	High	High	Low	1-5	VU	Yes
High	Low	High	High	Low	1-5	NA	Yes
High	Low	Low	Medium	Low	>25	NT	No
High	Low	Low	Medium	Low	>25	LC	Yes
High	Low	Low	Medium	Low	>25	NA	Yes
High	Low	High	High	Low	1-5	NA	Yes
High	Low	High	High	Low	1-5	NA	Yes
High	Low	Medium	High	Low	6-25	NA	Yes
High	Low	Medium	High	Low	6-25	LC	No
High	Low	High	High	Low	1-5	VU	Yes
High	High	Low	Medium	High	>25	LC	No
High	Low	High	High	Low	1-5	NA	No
High	Low	Low	Medium	Low	>25	NA	No
High	Low	Medium	High	Low	6-25	NA	No
High	Low	Medium	High	Low	6-25	NA	No
High	Low	Medium	High	Low	6-25	NA	No
High	Low	Low	Medium	Low	>25	NA	No
High	Low	Low	Medium	Low	>25	NA	No
High	Low	Medium	High	Low	6-25	NA	No
High	Low	Low	Medium	Low	>25	NA	No
High	Low	Low	Medium	Low	>25	NA	No
High	High	Low	Medium	High	>25	NA	No
High	Low	Low	Medium	Low	>25	LC	Yes
High	Low	Medium	High	Low	6-25	LC	Yes
High	Low	Low	Medium	Low	>25	LC	Yes
High	High	Low	Medium	High	>25	LC	Yes
High	Low	Low	Medium	Low	>25	NT	No
High	Low	Low	Medium	Low	>25	NE	Yes
High	Low	High	High	Low	1-5	LC	Yes
High	Low	Low	Medium	Low	>25	NE	Yes
High	High	Low	Medium	High	>25	LC	Yes
High	Low	Low	Medium	Low	>25	NT	Yes

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).

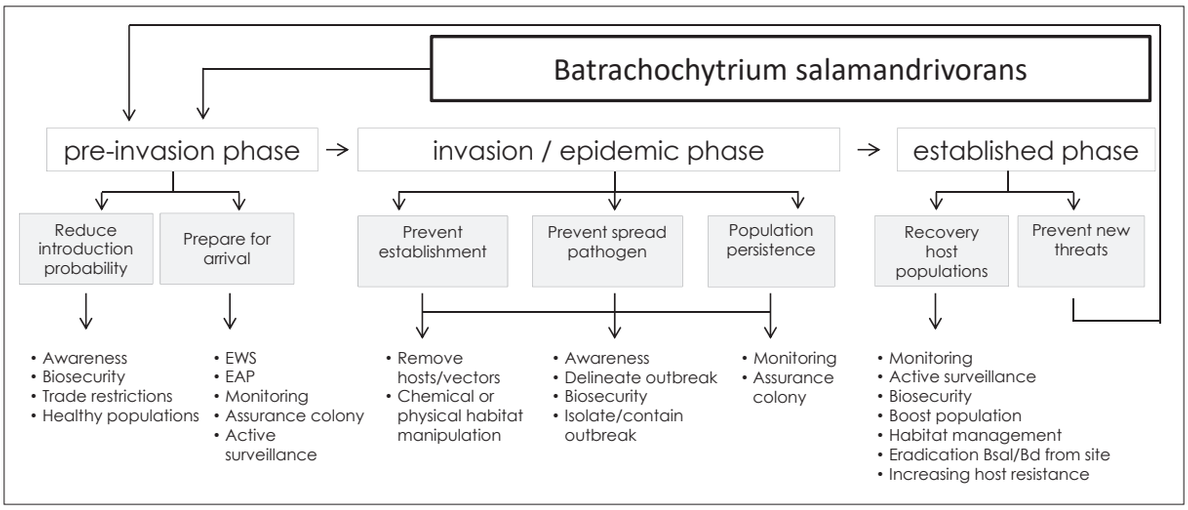

Figure 2. Illustration of the three invasive phases: pre-invasion phase (the fungus has not yet invaded the considered country or population), invasion (epidemic) phase and the established phase, in which a conservation unit might go extinct due to Bsal, or the situation could become endemic in which 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
  - Monitor the site and its surroundings (set perimeter)
  - Identify the entire network of potentially affected locations (and demarcate the perimeter)
  - 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.:
  - Fencing off an area
  - Prohibiting the public to enter
  - The potential use of chemicals in the environment
  - The complete removal of hosts and vectors (vertebrate and invertebrate)
- List the agreements made on financial responsibility
  - Sufficient budget should be allocated
  - It should be possible to immediately have access to this budget
  - 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 spec-

imens 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
    - 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 spill-over 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;

Family	Species	Bsal risk	Annex IV*	AD	AL	AT	BA	BE	BG	BY	CH	CY	CZ	DE	DK	EE	ES	FI	FO	FR
Hynobiidae	*Salamandrella keyserlingii*	Low																		
Plethodontidae	*Speleomantes ambrosii*	High	1																	
Plethodontidae	*Speleomantes flavus*	High	1																	
Plethodontidae	*Speleomantes genei*	High	1																	
Plethodontidae	*Speleomantes imperialis*	Low	1																	
Plethodontidae	*Speleomantes italicus*	High	1																	
Plethodontidae	*Speleomantes sarrabusensis*	High	1																	
Plethodontidae	*Speleomantes strinatii*	High	1																	1
Plethodontidae	*Speleomantes supramontis*	High	1																	
Proteidae	*Proteus anguinus*	Low	1				1													
Salamandridae	*Calotriton arnoldi*	High	1														1			
Salamandridae	*Calotriton asper*	Low	1	1													1			1
Salamandridae	*Chioglossa lusitanica*	High	1														1			
Salamandridae	*Euproctus montanus*	High	1																	1
Salamandridae	*Euproctus platycephalus*	High	1																	
Salamandridae	*Ichthyosaura alpestris*	Medium			1	1	1	1	1		1		1	1	1		1			1
Salamandridae	*Lissotriton boscai*	High															1			
Salamandridae	*Lissotriton graecus*	Medium			1		1		1											
Salamandridae	*Lissotriton helveticus*	Low		1				1			1		1	1			1			1
Salamandridae	*Lissotriton italicus*	High	1																	
Salamandridae	*Lissotriton montandoni*	Medium	1										1							
Salamandridae	*Lissotriton schmidtleri*	Medium							1											
Salamandridae	*Lissotriton vulgaris*	Medium				1	1	1	1	1	1		1	1	1	1		1		1
Salamandridae	*Lyciasalamandra helverseni*	High	1																	
Salamandridae	*Lyciasalamandra luschani*	High	1																	
Salamandridae	*Pleurodeles waltl*	High															1			
Salamandridae	*Salamandra atra*	High	1		1	1	1				1									1
Salamandridae	*Salamandra corsica*	High																		1
Salamandridae	*Salamandra lanzai*	High	1																	1
Salamandridae	*Salamandra salamandra*	High		1	1	1	1	1	1		1		1	1			1			1
Salamandridae	*Salamandrina perspicillata*	High	1																	
Salamandridae	*Salamandrina terdigitata*	High	1																	
Salamandridae	*Triturus carnifex*	High	1			1	1				1		1							
Salamandridae	*Triturus cristatus*	High	1			1		1	1	1	1		1	1	1	1		1		1
Salamandridae	*Triturus dobrogicus*	High					1		1											
Salamandridae	*Triturus ivanbureschi*	High	1						1											
Salamandridae	*Triturus karelinii*	High	1																	
Salamandridae	*Triturus macedonicus*	High	1		1		1													
Salamandridae	*Triturus marmoratus*	High	1														1			1
Salamandridae	*Triturus pygmaeus*	High	1														1			
Total			**29**	**3**	**5**	**6**	**9**	**5**	**8**	**2**	**7**	**0**	**7**	**5**	**3**	**2**	**10**	**2**	**0**	**12**

** Species listed in Annex IV Habitats Directive.*

*** Presence based on environmental DNA (Gorički et al. 2017).*

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.

	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
																									1									**1**
									1																									**1**
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			1	1												1	1																	**7**
	1											1							1			1												**11**
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																					1		1					1			1			**5**
			1																											1				**3**
	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**
			1																															**1**
			1																															**1**
																						1												**2**
				1					1	1						1								1			1							**11**
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									1																									**2**
			1	1	1				1			1				1	1		1		1	1	1	1			1	1	1	1	1		1	**29**
									1																									**1**
									1																									**1**
				1	1																						1		1					**8**
	1									1	1	1	1		1				1	1	1		1	1	1	1		1			1			**26**
				1	1										1								1	1				1			1			**9**
			1														1							1						1				**5**
																									1						1			**2**
			1													1	1							1									1	**7**
																						1												**3**
																						1												**2**
	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**

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

European distribution of *Salamandrella keyserlingii*.

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

References

Bannikov, A. G. et al. 1977. Opredelitel Zemnovodnykh i Presmykayushchikhsya Fauny SSSR. Moscow: Prosveshchenie Publ. 414 p. (in Russian,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).

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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.

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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*.

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.

European distribution of *Speleomantes ambrosii*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low

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.

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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

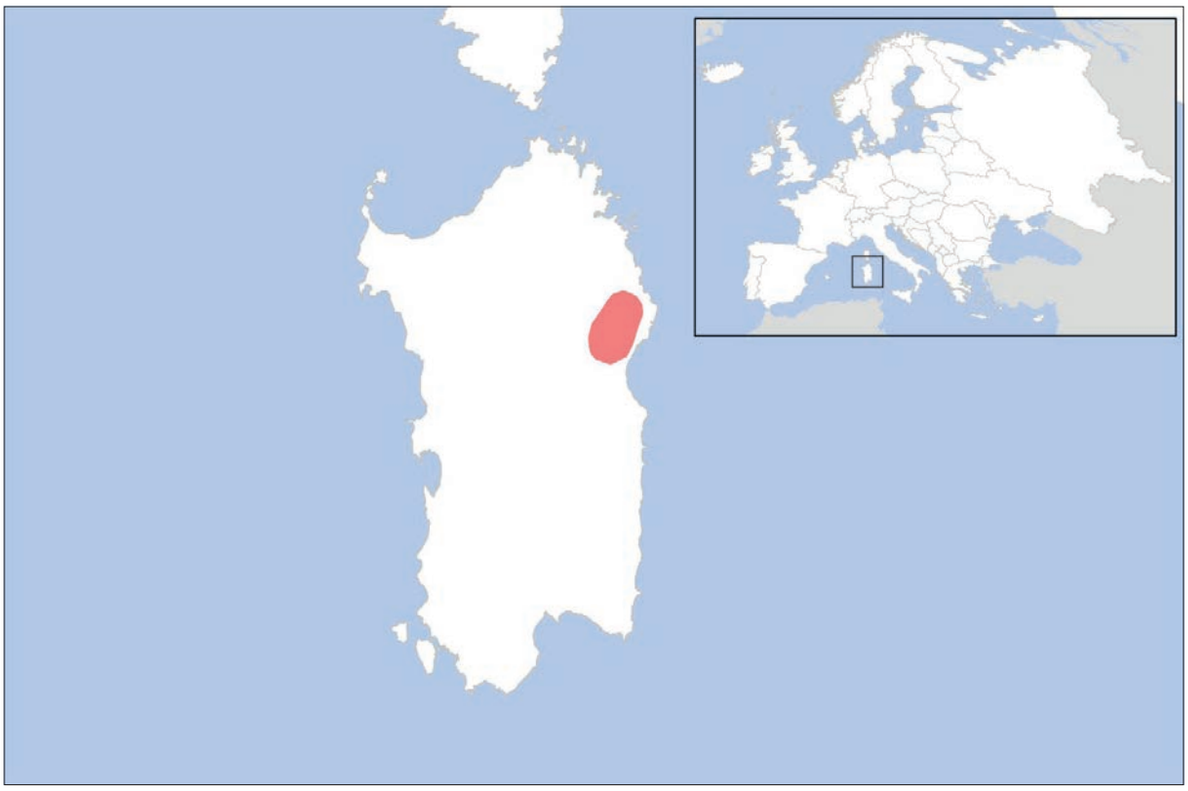
The level of intraspecific genetic isolation and variation is high, with two major lineages based on mitochondrial DNA analyses, which can be considered as conservation units. Endemic to northeastern Sardinia.

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.



European distribution of *Speleomantes flavus*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low

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.

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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

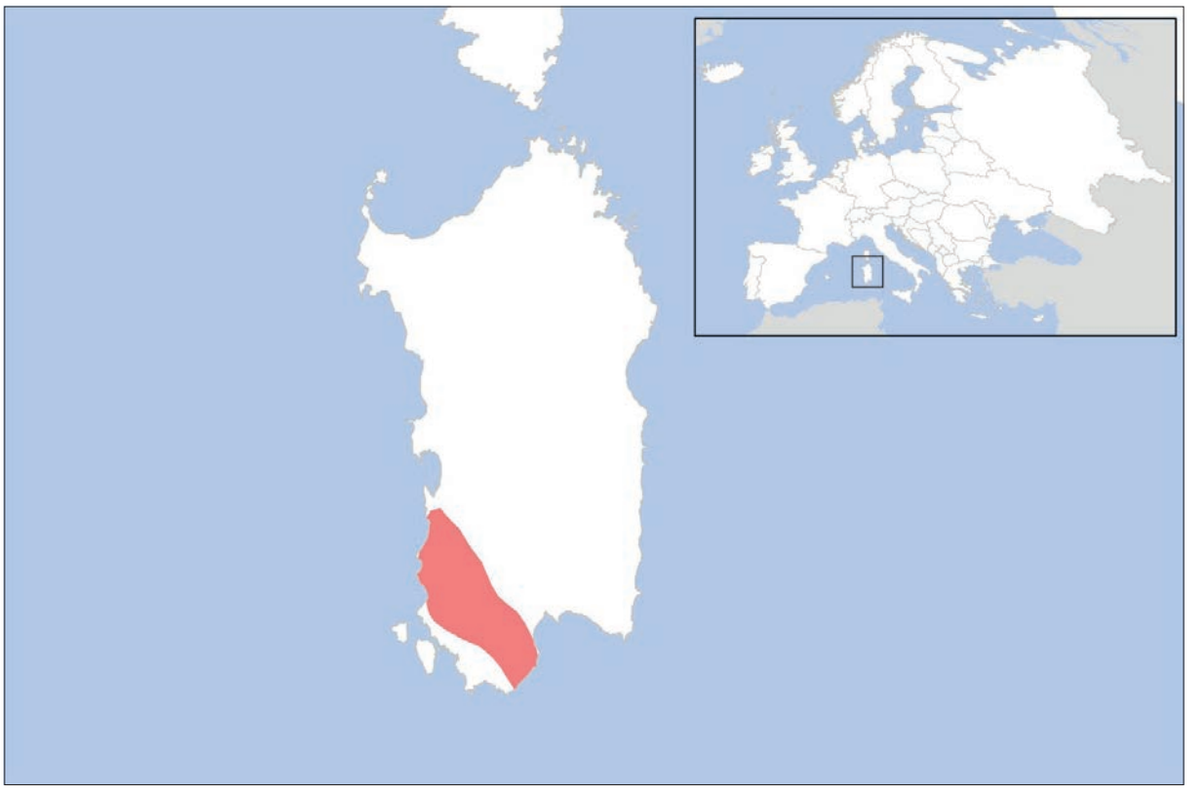
NA

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.

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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

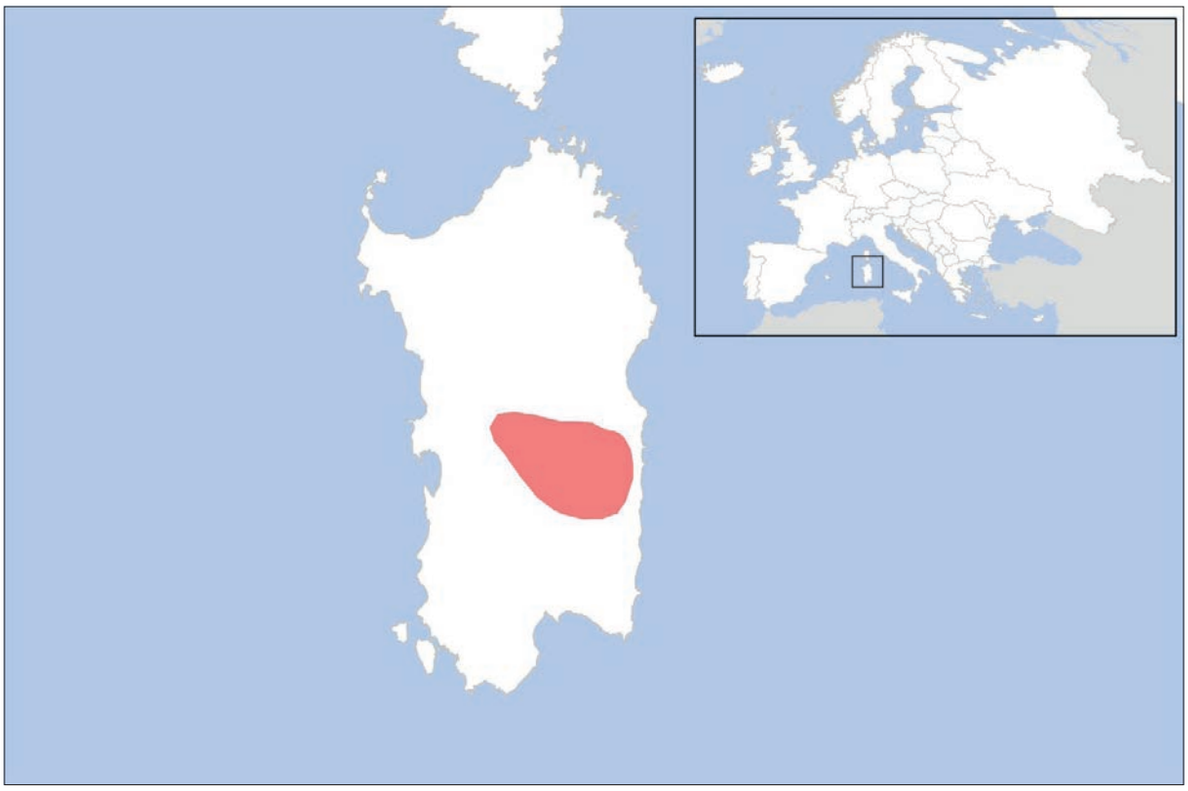
Species-specific actions

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

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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.

Currently recognized subspecies

NA

European distribution of *Speleomantes italicus*.

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

References

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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

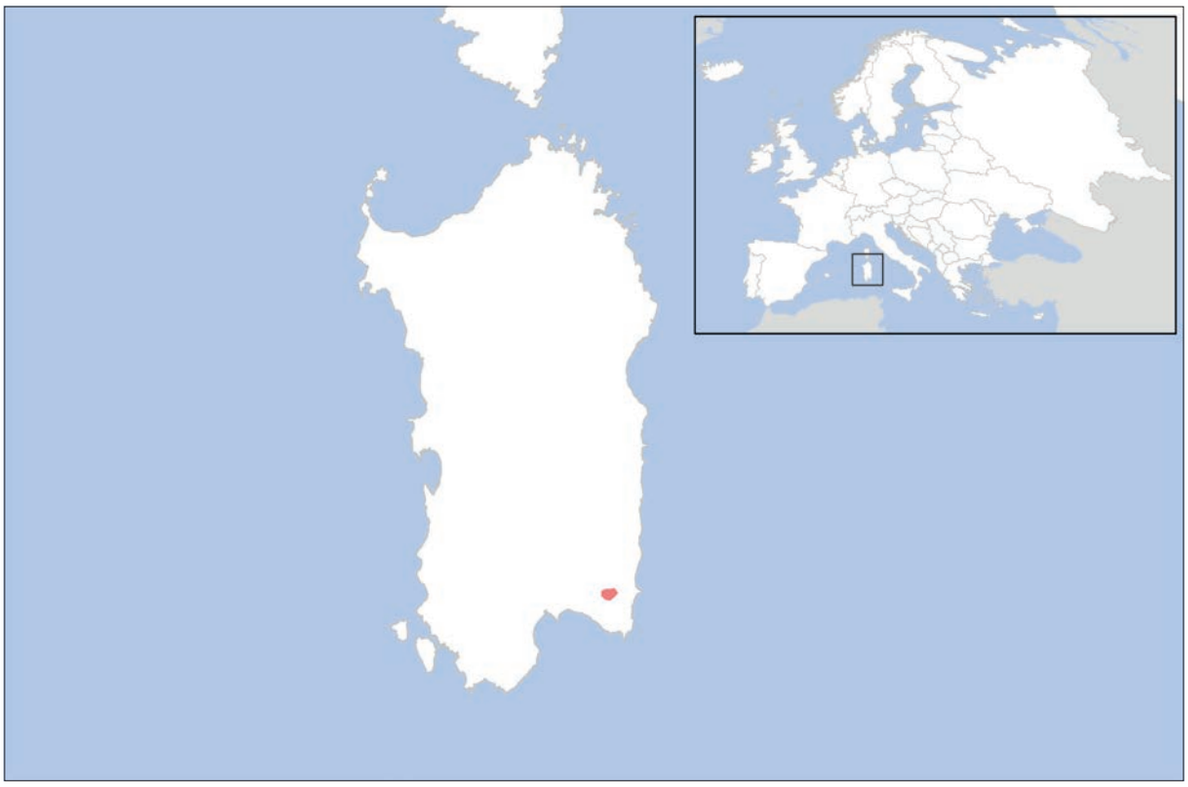
Euproctus platycephalus.

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.



European distribution of *Speleomantes sarrabusensis*.

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

References

Chiari, Y., et al. 2012. Phylogeography of Sardinian cave salamanders (Genus Hydromantes) is mainly determined by geomorphology. PLoS ONE 7(3): e32332.
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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.

European distribution of *Speleomantes strinatii*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Difficult	Low

References

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Salvidio, S. 1998. Estimating abundance and biomass of a *Speleomantes strinatii* (Caudata, Plethodontidae) population by temporary removal sampling. Amphibia-Reptilia **19**(2): 113-124.

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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

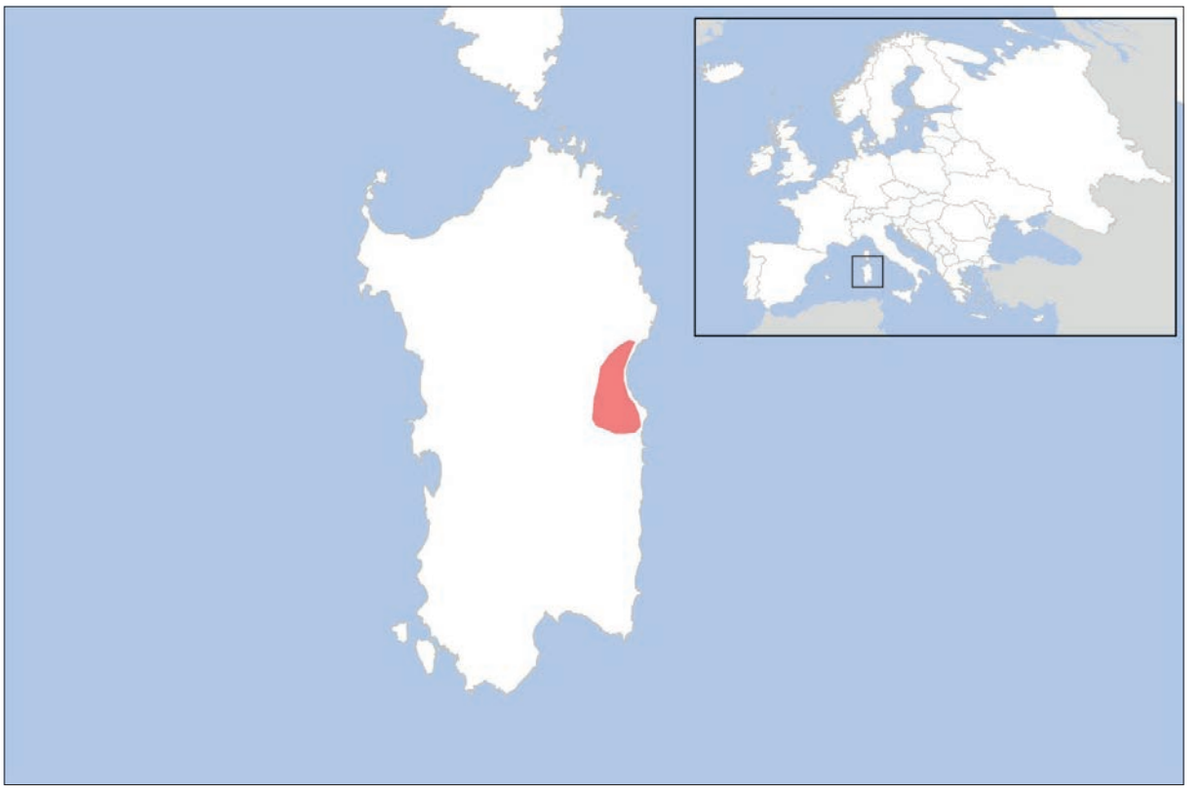
Bsal susceptibility has not been tested for this species, likely lethal based on close relationship to the Bsal susceptible species *Speleomantes strinatii*. Restricted range, high impact when Bsal when is introduced in its distribution range.

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



European distribution of *Speleomantes supramontis*.

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

References

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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.

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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.

European distribution of *Proteus anguinus*.

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

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.

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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

European distribution of *Calotriton arnoldi*.

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

References

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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.

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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).

European distribution of *Calotriton asper*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Moderate	Moderate	High

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.

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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

European distribution of Golden-striped salamander (*Chioglossa lusitanica*).

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

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.

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Salamandridae *Euproctus montanus* Corsican brook newt

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

European distribution of *Euproctus montanus*.

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

References

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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.

European distribution of *Euproctus platycephalus*.

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

References

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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/m^2 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 apuana
Ichthyosaura alpestris cyreni
Ichthyosaura alpestris montenegrina
Ichthyosaura alpestris reiseri
Ichthyosaura alpestris veluchiensis

European distribution of *Ichthyosaura alpestris*.

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

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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).

European distribution of *Lissotriton boscai*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

References

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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).

European distribution of *Lissotriton graecus*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

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.

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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

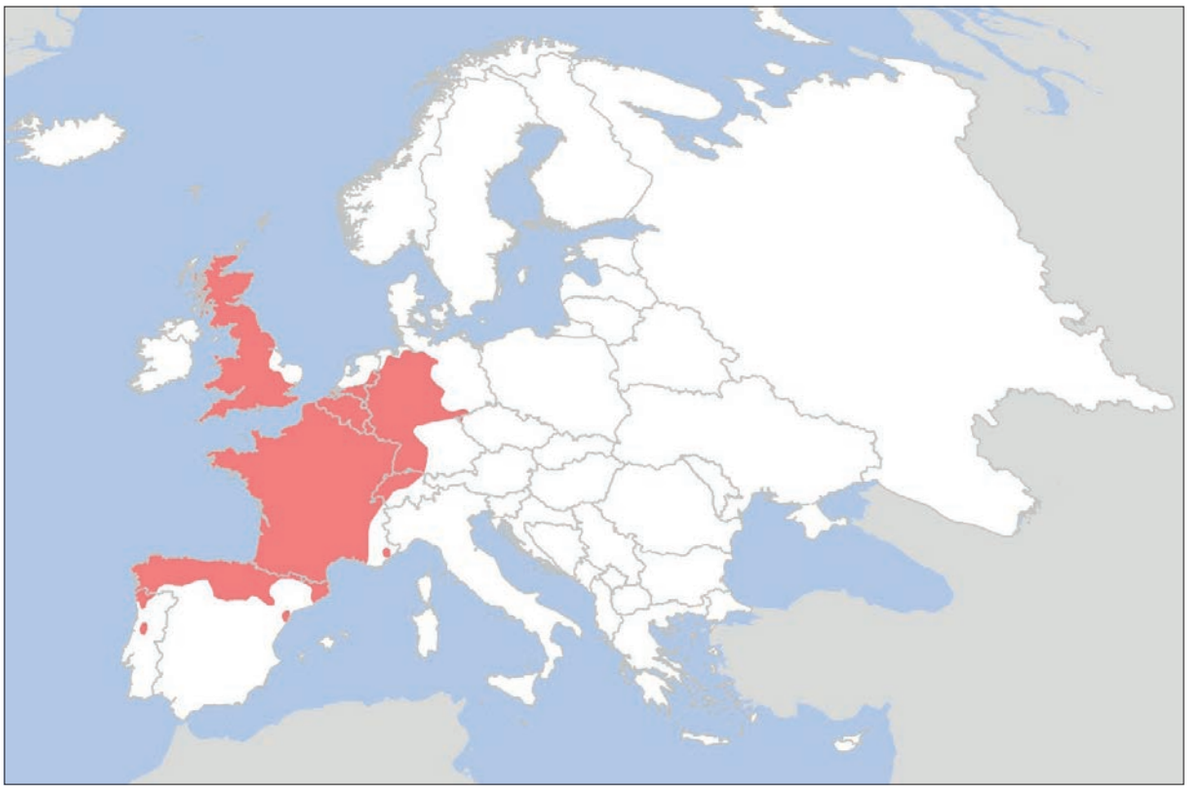
The level of intraspecific genetic isolation and variation appears to be low compared to other *Lissotriton* species, with four different mitochondrial haplotypes on the Iberian Peninsula. Nuclear genes were not geographically structured, suggesting gene flow and incomplete lineage sorting. Populations 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.

Currently recognized subspecies

NA

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).



European distribution of *Lissotriton helveticus*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

References

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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).

European distribution of *Lissotriton italicus*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

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.

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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

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*.

Species-specific actions

There are no specific *in situ* or *ex situ* conservation actions required (see § 4).

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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).

European distribution of *Lissotriton schmidtleri*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

References

Kuzmin, S.L. 1999. The amphibians of the former Soviet Union. Pensoft, Sofia, Moscow.

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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

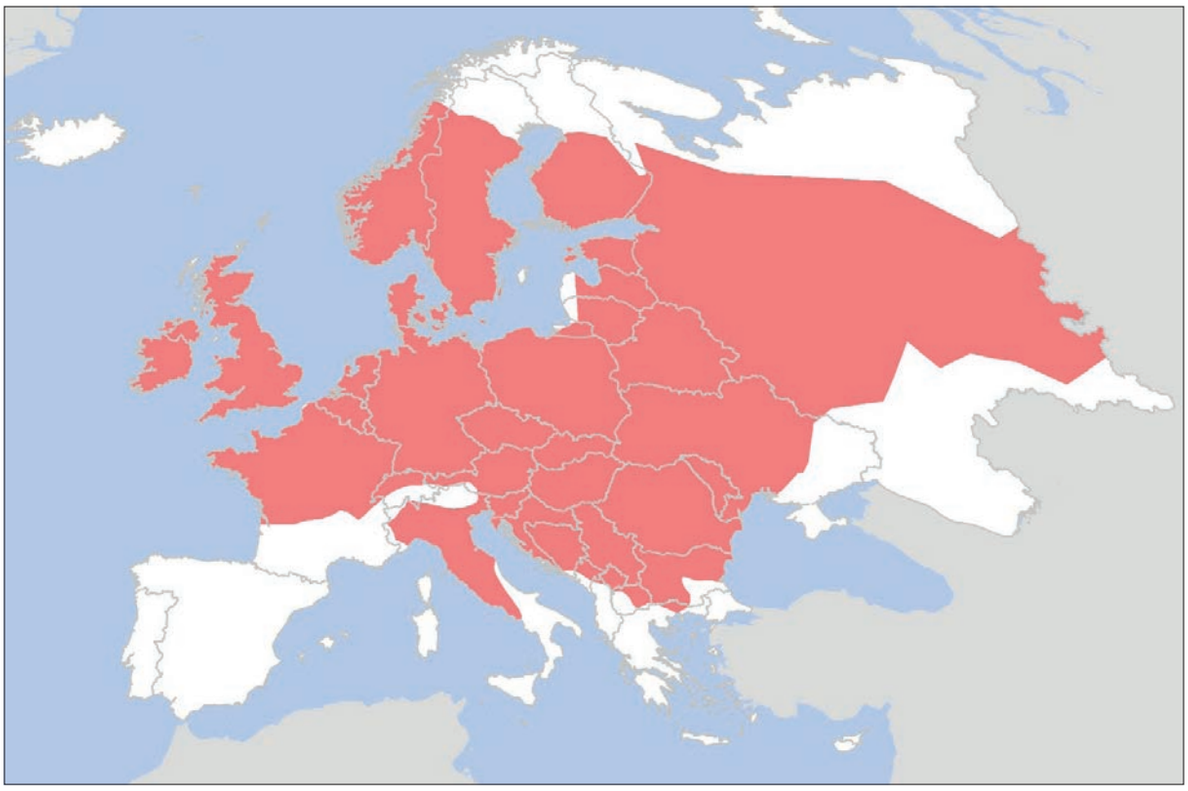
Salamandra salamandra, S. atra, *Salamandrina perspicillata*, *S. terdigitata*, *Lissotriton graecus, L. helveticus, L. montandoni, L. italicus, Ichthyosaura alpestris*, *Triturus cristatus*, *T. marmoratus* and *Salamandrella keyserlingii*.

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.



European distribution of *Lissotriton vulgaris* sensu stricto.

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

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.

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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

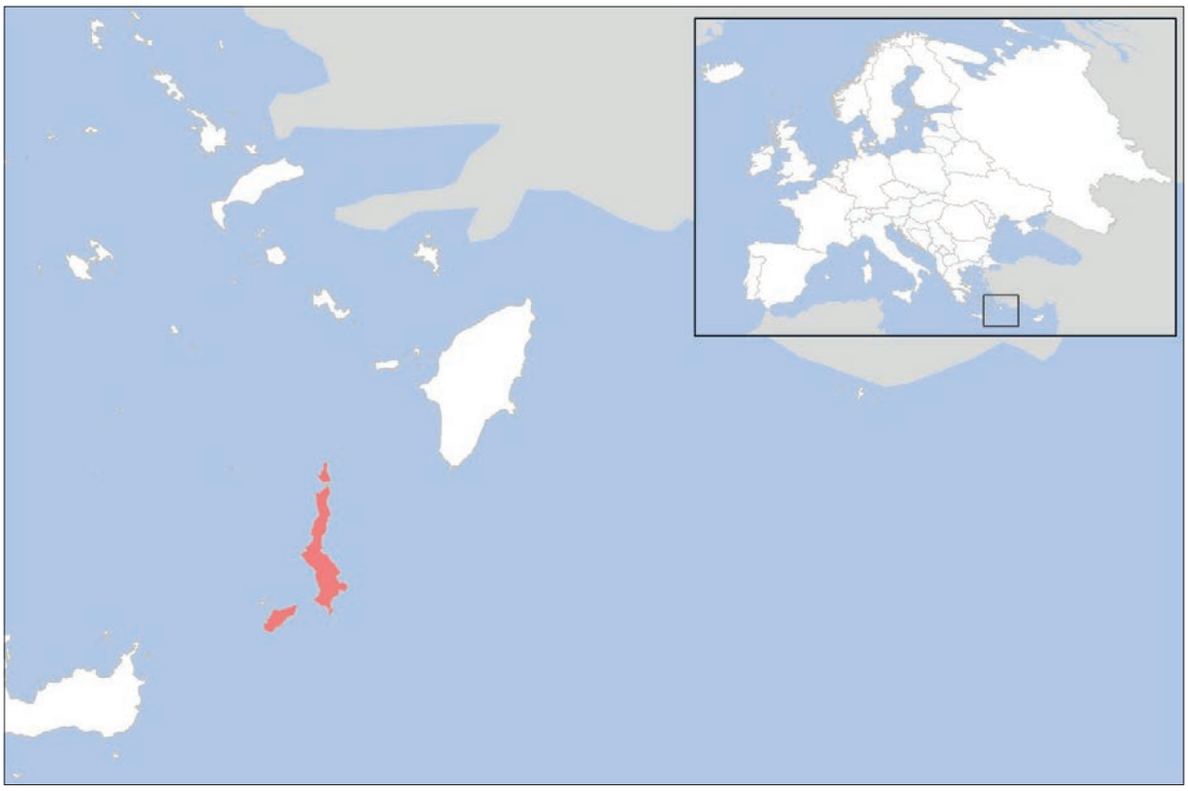
Consistent within this genus is the occurrence of small to very small range lineages, with little overlap even at short distances, suggesting very limited gene flow between populations. Marked differentiation was shown to occur both on the islands of Karpathos and Kasos, with two major lineages on separate 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.



European distribution of *Lyciasalamandra helverseni*.

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

References

Eleftherakos, K., et al. 2007. Conservation units in the insular endemic salamander *Lyciasalamandra helverseni* (Urodela, Salamandridae). Ann. Zool. Fennici 44: 387-399.

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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

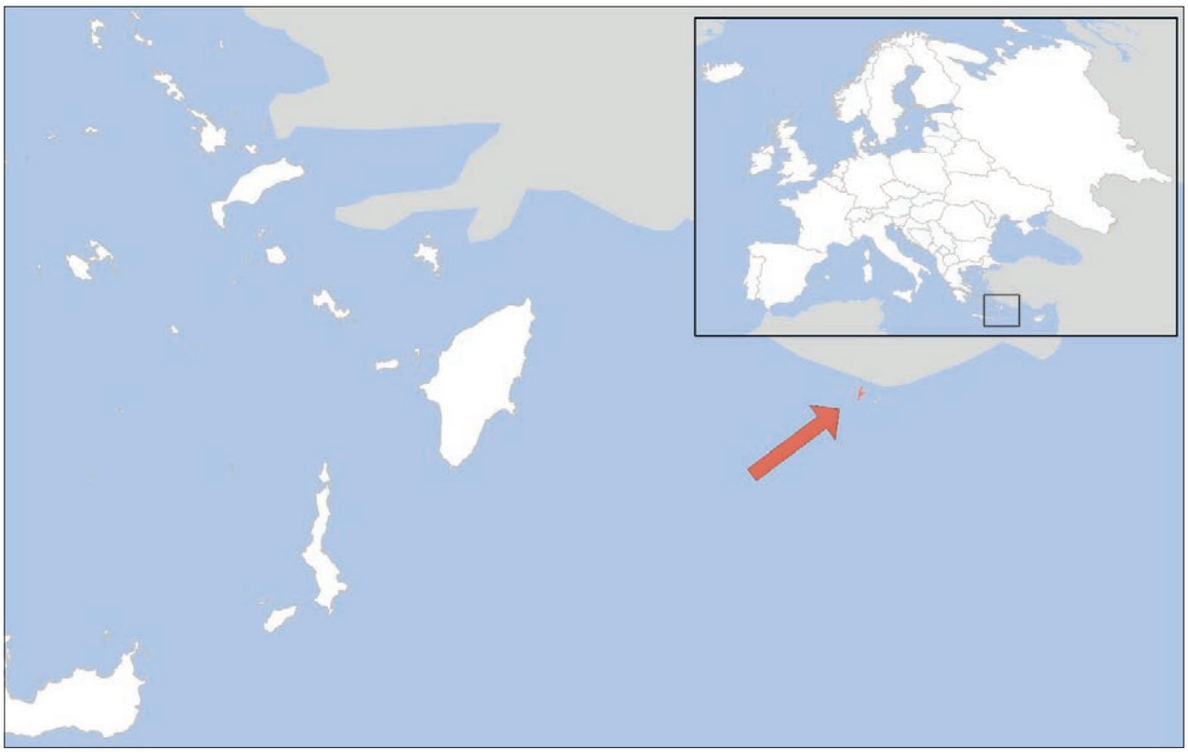
High risk is based on lethal susceptibility of the sister species *L. helverseni* to Bsal and its restricted range. Human-mediated introduction can have high impact on this species. The high susceptibility is also based on its close relationship to the Bsal susceptible *Salamandra* genus.

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.



European distribution of *Lyciasalamandra luschani*.

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

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.

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Salamandridae *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).

European distribution of *Pleurodeles waltl*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

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.

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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.

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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.

European distribution of *Salamandra atra*.

Currently recognized subspecies

Salamandra atra atra
*Salamandra atra aurorae**
Salamandra atra pasubiensis
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

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.

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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).

European distribution of *Salamandra corsica*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Moderate	High

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.

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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.

European distribution of *Salamandra lanzai*.

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.*

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.

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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 m^2). 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

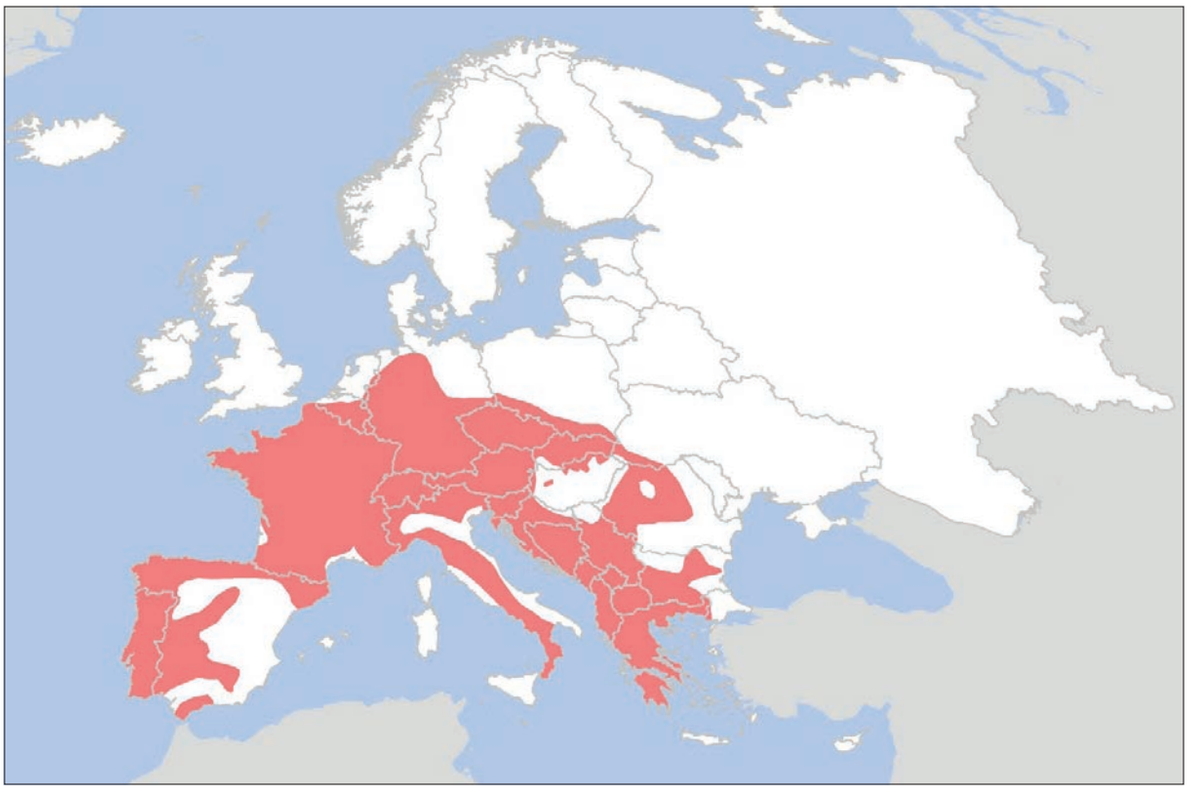
High risk is based on confirmed lethal susceptibility to Bsal, well-documented persistent significant population declines and presence of potential Bsal reservoir species within its range (i.e. *Ichthyosaura alpestris*). Bsal has been shown lethal for this species, both after experimental inoculation 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*	



European distribution of *Salamandra salamandra*.

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.

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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).

European distribution of *Salamandrina perspicillata*.

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.

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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).

European distribution of *Salamandrina terdigitata*.

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.

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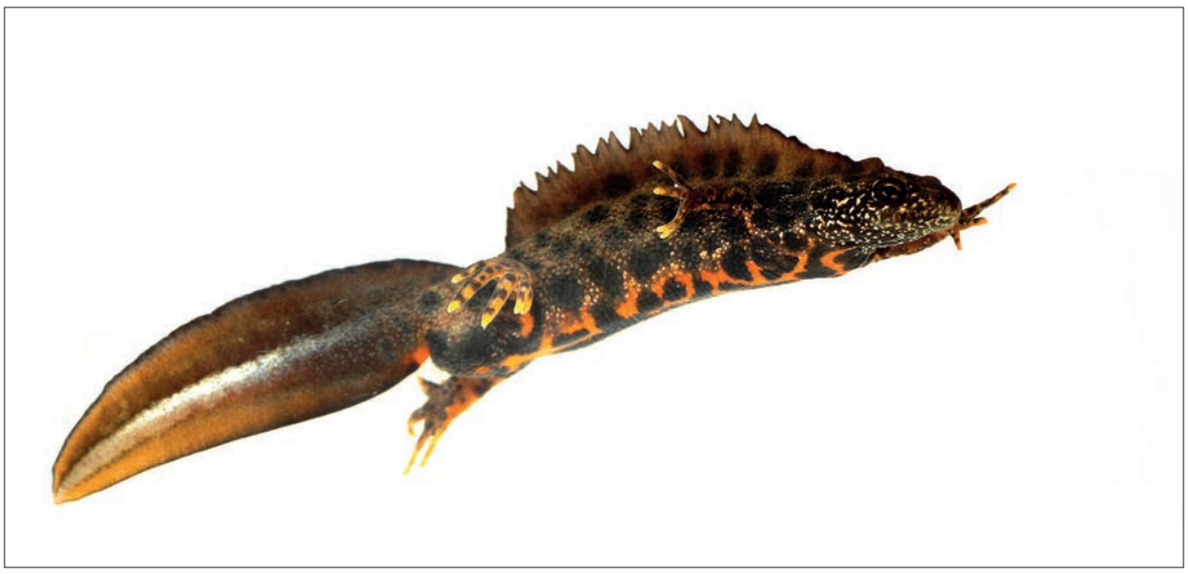
Salamandridae *Triturus carnifex* Italian newt

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).

European distribution of *Triturus carnifex*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

References

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Salamandridae *Triturus cristatus* Great 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	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 mdian 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

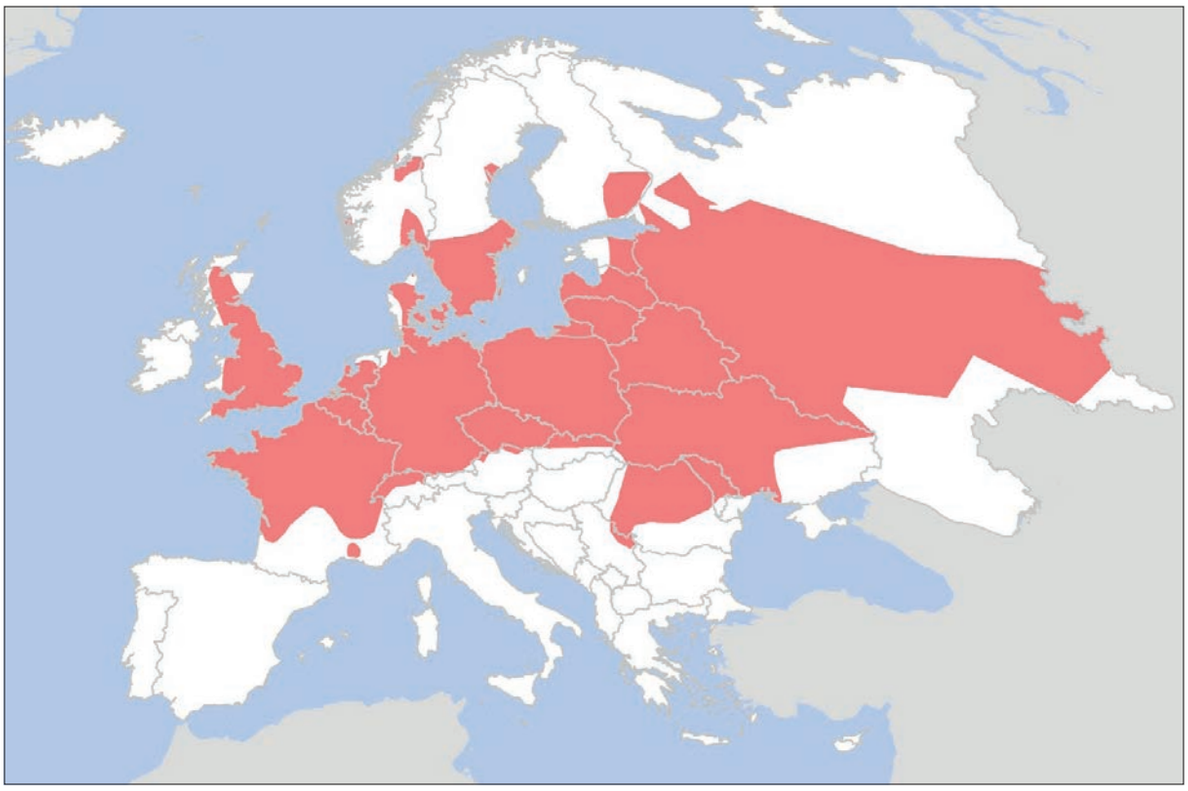
High risk based on high susceptibility to Bsal (laboratory experiments) and suspected Bsal-related declines in nature. The species has a large range, but co-occurrence with Bsal vectoring species and high susceptibility warrant caution.

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



European distribution of *Triturus cristatus*.

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

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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).

European distribution of *Triturus dobrogicus*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

References

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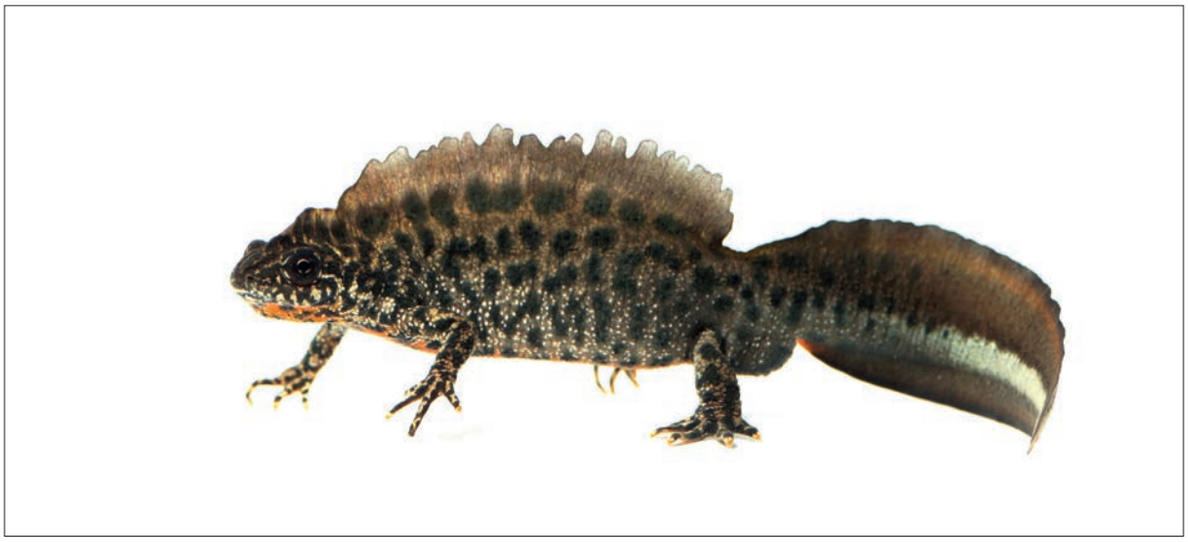
Salamandridae *Triturus ivanbureschi* Buresch's crested newt

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).

European distribution of *Triturus ivanbureschi*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

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.

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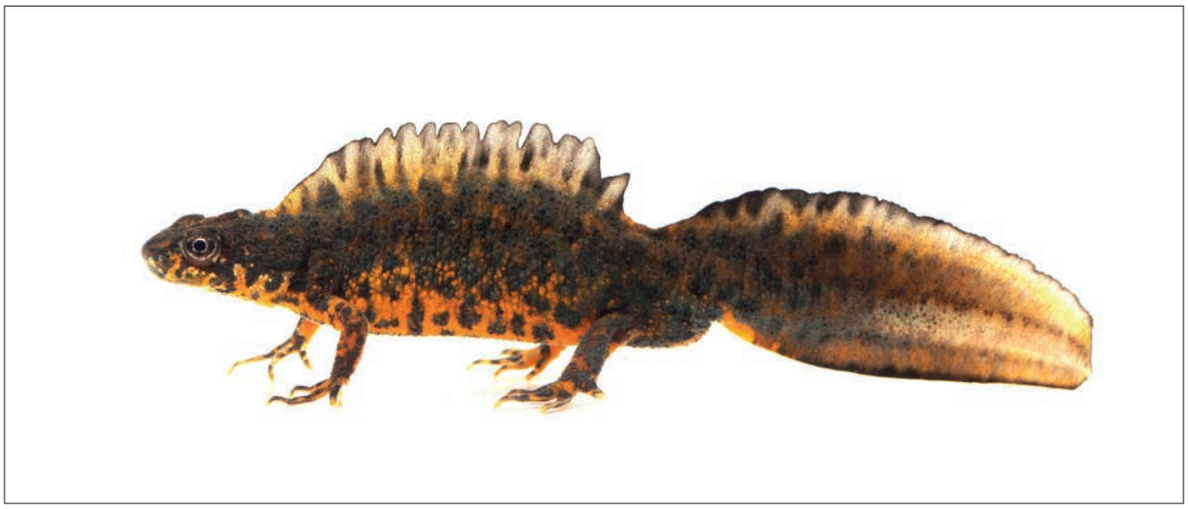
Salamandridae *Triturus karelinii* Karelin's crested newt

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).

European distribution of *Triturus karelinii*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

References

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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

European distribution of *Triturus macedonicus*.

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

References

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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).

European distribution of *Triturus marmoratus*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

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.

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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).

European distribution of *Triturus pygmaeus*.

Ex situ management

Ease of keeping	Ease of breeding	Reproductive potential in captivity
Easy	Easy	High

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Appendices

Appendix 1 - 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.

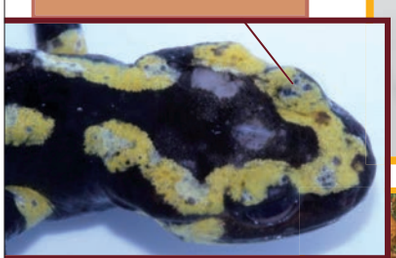
Metamorphosed salamanders and newts often show multifocal superficial erosions (holes in the skin) and extensive epidermal ulcerations (ulcers on the skin) all over the body. The animal may also suffer from anorexia (stop eating) and ataxia (muscle spasms) and show excessive shedding of the skin. Ultimately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

This fire salamander suffers extensive shedding of the skin

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

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

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



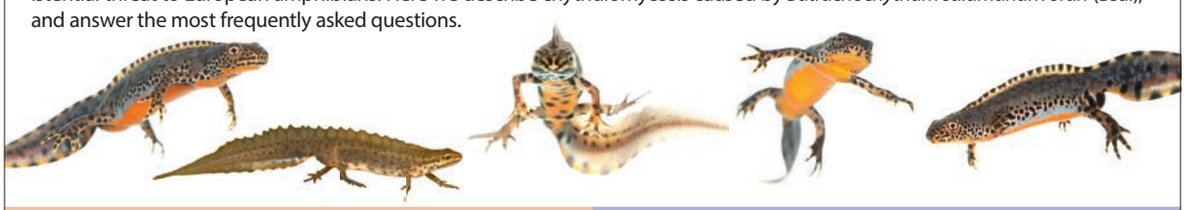
The erosion of the skin is obvious in this fire salamander

A suspicious mass mortality event of fire salamanders in a forest

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.

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!

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.

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!

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Photo credits: Jelger Herder, Rolf van Leeningen

Appendix 2 - Bsal recognition leaflet veterinarians

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°C 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)

Appendix 3 - Disinfection protocol fieldwork

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.

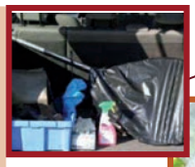
Many emerging infectious diseases, among which the chytrid fungi *Batrachochytrium salamandrivorans* and *B. dendrobatidis*, but also ranavirosis, currently pose a significant threat to amphibians in Europe. Anthropogenic spread of pathogens has been identified as a considerable threat to amphibian health. 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.

Disinfect your field material

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.



Handle dead amphibians with disposable gloves.

Help to prevent mass mortality.

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

Photo credits: 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.

Avoid direct contact

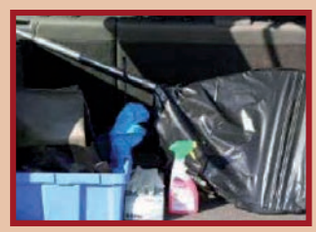
The disinfection solutions described in this document may be harmful for humans but also for amphibians, fish and other organisms. Use carefully.

Dispose of disinfectant solution

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

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

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

Appendix 4 - Disinfection protocol heavy machinery

Disinfection protocol heavy machinery

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

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

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

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 main-

tained 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.