



## The amphibian pathogen *Batrachochytrium salamandrivorans* in the hotspot of its European invasive range: past – present – future

STEFAN LÖTTERS<sup>1\*</sup>, NORMAN WAGNER<sup>1\*</sup>, GONZALO ALBALADEJO<sup>2,3</sup>, PHILIPP BÖNING<sup>1</sup>, LUTZ DALBECK<sup>4</sup>,  
HEIDRUN DÜSSEL<sup>4</sup>, STEPHAN FELDMEIER<sup>1</sup>, MAIKE GUSCHAL<sup>5</sup>, KAI KIRST<sup>5</sup>, DAGMAR OHLHOFF<sup>4</sup>,  
KATHLEEN PREISSLER<sup>6</sup>, TIMM REINHARDT<sup>7</sup>, MARTIN SCHLÜPMANN<sup>8</sup>, ULRICH SCHULTE<sup>1,9</sup>, VANESSA SCHULZ<sup>6,10</sup>,  
SEBASTIAN STEINFARTZ<sup>6</sup>, SÖNKE TWIETMEYER<sup>11</sup>, MICHAEL VEITH<sup>1</sup>, MIGUEL VENCES<sup>10</sup> & JOSEF WEGGE<sup>5</sup>

<sup>1)</sup> Universität Trier, Biogeographie, Universitätsring 15, 54296 Trier, Germany

<sup>2)</sup> Centre for Biodiversity and Environment Research, Department of Genetics, Evolution and Environment,  
University College London, London, U.K.

<sup>3)</sup> Institute of Zoology, Zoological Society of London, Regent's Park, London, U.K.

<sup>4)</sup> Biologische Station im Kreis Düren e.V., Zerkaller Str. 5, 52385 Nideggen, Germany

<sup>5)</sup> Biologische Station StädteRegion Aachen, Zweifaller Str. 162, 52224 Stolberg/Rheinland, Germany

<sup>6)</sup> Universität Leipzig, Institut für Molekulare Evolution und Systematik der Tiere, Talstr. 33, 04103 Leipzig, Germany

<sup>7)</sup> Bundesamt für Naturschutz, Zoologischer Artenschutz, Konstantinstr. 110, 53179 Bonn, Germany

<sup>8)</sup> Biologische Station Westliches Ruhrgebiet, Ripshorster Str. 306, 46117 Oberhausen, Germany

<sup>9)</sup> Büro für Faunistische Gutachten, Kaiserstr. 2, 33829 Borgholzhausen, Germany

<sup>10)</sup> Technische Universität Braunschweig, Zoologisches Institut, Mendelssohnstr. 4, 38106 Braunschweig, Germany

<sup>11)</sup> Nationalparkverwaltung Eifel, Fachgebiet Forschung und Dokumentation, Urftseestr. 34, 53937 Schleiden-Gemünd, Germany

\* These authors contributed equally to this work.

Corresponding author: STEFAN LÖTTERS, e-mail: loetters@uni-trier.de

Manuscript received: 9 April 2020

Accepted: 18 May 2020 by JÖRN KÖHLER

**Abstract.** The salamander plague, caused by the amphibian chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*), is one of the most devastating amphibian diseases, currently threatening the entire Western Palearctic caudate diversity with extinction. Apparently of Asian origin and recently introduced into Europe, *Bsal* is known from currently ca. 80 sites in the wild in four European countries. Germany is the *Bsal* 'hotspot', with more than half (N = 50) of all known European records to date. We here present data based on > 8,500 caudate specimens sampled for *Bsal* mainly via skin swabbing and quantitative real time PCR (> 3,300 since 2019). Within regions of *Bsal* occurrence ~ 6–7% of the studied caudates were *Bsal*-positive. The oldest known European record of the pathogen is from this country (2004), but a massive *Bsal* dispersal has only been recognized within the last five years with 17 new *Bsal* sites since 2019 alone. Currently, *Bsal* is spreading within the northern and the southern Eifel and – since 2017 – the Ruhr District. Most recently, the pathogen was for the first time detected in southern Germany (Bavaria) and a further range expansion is expected. A new species distribution model (SDM) of *Bsal* based on > 100 native and invasive records predicts suitable areas in most parts of Germany. *Bsal* affects all five caudate species known from these regions and has catastrophic effects on the European fire salamander (*Salamandra salamandra*). All affected populations in Germany (as well as Belgium and The Netherlands) have dramatically declined. While some may have become extinct due to the salamander plague, in most *Bsal*-positive sites European fire salamanders can still be recorded at low numbers (at least via systematic larval surveys), and at least one population seems to have recovered as currently *Bsal* detection remains negative. Little is known about the effect of *Bsal* on newts, and both prevalence and individual infection load can vary greatly over time, even within one population. However, the situation of the northern crested newt (*Triturus cristatus*) is alarming, as this species also undergoes declines due to *Bsal* invasions at some sites. Although some anurans are suggested as potential *Bsal* reservoirs and transmitters, we detected *Bsal* in only one individual of the common frog (*Rana temporaria*) out of 365 anurans of various species tested. Co-infection of *Bsal* with the related chytrid taxon *Batrachochytrium dendrobatidis* is known from three taxa (*S. salamandra*, *T. cristatus*, *Ichthyosaura alpestris*) and at four sites. The alarming data from *Bsal* in Germany call for immediate conservation action at all levels, including ex situ conservation. We therefore strongly support the establishment and implementation of a national *Bsal* Action Plan.

Key words. Amphibia, Caudata, *Bd/Bsal* co-infection, *Bsal*, chytridiomycosis, EID, Germany, monitoring, national action plan, salamander plague, *Salamandra salamandra*, *Triturus cristatus*, species distribution model.

**Zusammenfassung.** Die Salamanderpest, verursacht durch den Amphibien-Chytrid-Pilz *Batrachochytrium salamandrivorans* (*Bsal*), ist eine der weltweit gefährlichsten Amphibienkrankheiten. Sie bedroht derzeit die gesamte Diversität westpaläarktischer Schwanzlurche. Offenbar asiatischen Ursprungs und erst kürzlich nach Europa eingeschleppt, wurde *Bsal* im Freiland an bislang ca. 80 Standorten in vier Ländern nachgewiesen. Deutschland ist derzeit mit etwa der Hälfte ( $N = 50$ ) aller bekannten europäischen Nachweise ein *Bsal*-„Hotspot“. Unsere Daten basieren auf > 8.500 Schwanzlurch-Proben, die hauptsächlich durch Hautabstriche und quantitative PCR auf *Bsal* untersucht wurden (> 3.300 davon neu seit 2019). Innerhalb von *Bsal*-Regionen waren ~ 6-7% der untersuchten Schwanzlurche *Bsal*-positiv. Der bislang älteste bekannte europäische Nachweis des Pathogens stammt zwar aus Deutschland (2004), eine massive Ausbreitung von *Bsal* ist aber erst in den letzten fünf Jahren mit 17 neuen *Bsal*-Standorten allein seit 2019 beobachtet worden. Derzeit breitet sich *Bsal* offenbar in der Nord- und der Südeifel und – seit 2017 – im Ruhrgebiet aus. Neuerdings ist das Pathogen auch aus Süddeutschland (Bayern) bekannt und eine weitere Ausbreitung ist zu erwarten. Ein neues Artverbreitungsmodell (SDM) für *Bsal*, das auf > 100 nativen und invasiven Fundorten des Pathogens basiert, sagt großflächig geeignete Gebiete in den meisten Teilen Deutschlands voraus. *Bsal* infiziert alle fünf aus den betroffenen Regionen bekannten Schwanzlurcharten und hat nachweislich katastrophale Auswirkungen auf den Europäischen Feuersalamander (*Salamandra salamandra*). Alle Populationen in Deutschland (sowie die in Belgien und den Niederlanden) sind im Zusammenhang mit *Bsal*-Infektionen dramatisch zurückgegangen. Einige sind möglicherweise sogar durch die Salamanderpest ausgestorben. Dennoch kann an den meisten *Bsal*-positiven Standorten noch immer eine geringe Anzahl Europäischer Feuersalamander (zumindest durch systematische Larvenerehebungen) erfasst werden. Mindestens eine Population scheint sich sogar erholt zu haben und ist derzeit offensichtlich *Bsal*-frei. Über die Auswirkungen von *Bsal* auf Molche ist wenig bekannt, und sowohl die Prävalenzen als auch die individuellen Infektionsbelastungen können über die Zeit stark variieren, selbst innerhalb einer Population. Die Situation des Nördlichen Kammmolchs (*Triturus cristatus*) ist jedoch alarmierend, da auch diese Art, offenbar aufgrund von *Bsal*, an einigen Standorten einen Rückgang erfährt. Obwohl einige Anuren als potentielle *Bsal*-Reservoirs und Überträger diskutiert werden, haben wir nur bei einem von 365 untersuchten Froschlurchen *Bsal* entdeckt (ein Grasfrosch, *Rana temporaria*). Eine Ko-Infektion von *Bsal* mit dem verwandten Chytridpilz *Batrachochytrium dendrobatidis* ist bei insgesamt drei Arten (*S. salamandra*, *T. cristatus*, *Ichthyosaura alpestris*) und vier Standorten nachgewiesen worden. Die alarmierenden *Bsal*-Daten aus Deutschland erfordern sofortige Schutzmaßnahmen auf allen Ebenen, einschließlich der ex situ-Erhaltung. Wir unterstützen daher nachdrücklich die Erstellung und Umsetzung eines nationalen *Bsal*-Aktionsplans.

## Introduction

In our rapidly changing world, emerging infectious diseases (EIDs) of fungal origin have become central drivers of global biodiversity loss (DASZAK et al. 2000, FISHER et al. 2012, SIKES et al. 2018). Amphibians are a famous example, mostly due to two parasitic chytrid fungi that cause massive skin damage (chytridiomycosis) in these animals. Of Asian origin, these chytrids have dramatically spread, now affecting numerous naïve amphibian hosts around the world. Accordingly, chytridiomycosis has been identified as a major cause of the ongoing global amphibian decline (WOODHAMS et al. 2011, VAN ROOIJ et al. 2015, O'HANLON et al. 2018, SCHEELE et al. 2019).

*Batrachochytrium dendrobatidis* (*Bd*) is known on all continents where amphibians occur. It parasitizes species from all three extant amphibian orders (VAN ROOIJ et al. 2015). *Bd*-related population declines and extinctions have been observed in amphibians around the world (e.g. LA MARCA et al. 2005, SCHEELE et al. 2019). At the same time, certain amphibian populations persist, with some being resistant to infection (sensu VAN ROOIJ et al. 2015). Other populations survive infection and thus may become reservoirs and transmitters of the pathogen ('tolerant' species and/or populations sensu VAN ROOIJ et al. 2015). The pet trade and other human activities contribute greatly to *Bd* dispersal (FISHER et al. 2012, GARNER et al. 2016). Since its discovery in the 1990s, our knowledge on *Bd* has become fairly

advanced; this includes the recognition of various within-species lineages of the pathogen. One of them (*Bd*-GPL, the globally-dispersed pandemic lineage) is hypervirulent and widely distributed, while others are more local and less aggressive (e.g. VAN ROOIJ et al. 2015, O'HANLON et al. 2018).

The second identified amphibian chytrid species, *Batrachochytrium salamandrivorans* (*Bsal*), is known from Asia and Europe. Only caudate amphibians are known to be negatively affected, though the pathogen is known to survive on a few anuran taxa (MARTEL et al. 2014, NGUYEN et al. 2017, STEGEN et al. 2017). In its native range, i.e. in Vietnam, *Bsal* is found in the wild alongside salamanders that are resistant or that can tolerate *Bsal* and are able to clear infection (MARTEL et al. 2014, LAKING et al. 2017). This is in sharp contrast to field observations made in Europe. Here, *Bsal* disproportionately affects the European fire salamander, *Salamandra salamandra*, and its congeners, causing significant mortality (MARTEL et al. 2014, SABINO-PINTO et al. 2015, SPITZEN-VAN DER SLUIJS et al. 2016, STEGEN et al. 2017, DALBECK et al. 2018). Accordingly, *Bsal* chytridiomycosis has been dubbed the 'salamander plague' (STOKSTAD 2014). As *Bsal* is relatively new to science (MARTEL et al. 2013), many questions regarding its biology and epidemiology – as well as host responses – are presently unaddressed.

By feeding on keratin, *Bsal* zoosporangia destroy skin cells of adult salamanders and produce signs such as lesions, ulcerations, and other tissue damage (MARTEL et al.

2013). *Bsal*-induced skin damage is well recognizable via histopathology. However, since visible signs do not occur at an early stage of infection – or can occasionally be completely absent (MARTEL et al. 2013, 2014) – the pathogen is best detected at any stage by quantitative PCR (qPCR) (MARTEL et al. 2013, BLOOI et al. 2013, 2016), though there are other methods, such as microscopic observation of skin scrapings. THOMAS et al. (2018) state that the most reliable diagnosis – in accordance with guidelines by the World Organization for Animal Health (OIE) – always entails the combination of different diagnostic techniques. This is especially recommended for apparently new *Bsal* localities in areas far from known outbreaks (further discussed below).

In members of the genus *Salamandra*, advanced *Bsal* chytridiomycosis is linked to secondary bacterial infection that can lead to death within two weeks after the first contact with *Bsal*. While immune responses apparently fail completely in European fire salamanders, some urodelan species can clear *Bsal* (MARTEL et al. 2014). Among the European Caudata, the alpine newt (*Ichthyosaura alpestris*) is known to be able to clear infection when parasite load is comparatively low (STEGEN et al. 2017).

*Bsal* produces two types of infectious zoospores: motile ones with a flagellum, and encysted ones. The former actively approach host specimens, while the latter are more robust, can float on the water surface, and are understood as permanent or dispersal stages; together they manifest an increased environmental resilience of this pathogen (STEGEN et al. 2017). The salamander plague is a serious EID with an alarming increase of cases (e.g. SPITZEN-VAN DER SLUIJS et al. 2016, DALBECK et al. 2018, THOMAS et al. 2019). Because *Bsal* is highly infectious and pathogenic, it represents a serious threat to the entire community of Western Palearctic salamanders and newts, and at present, measures for *in situ* mitigation are limited (MARTEL et al. 2014, SPITZEN-VAN DER SLUIJS et al. 2016, BEUKEMA et al. 2018, THOMAS et al. 2019).

*Bsal* was assessed and listed within the framework of the European Animal Health Law (Regulation (EU) No 2016/429) (EFSA AHAW et al. 2017). Furthermore, the risk of pathogen survival, establishment, and spread in the European Union (EU) has been officially assessed (EFSA et al. 2017, EFSA AHAW et al. 2018). Accordingly, the EU Commission implemented the decision 2018/320 (notified under document C[2018] 1208) on 28 February 2018 regarding ‘certain animal health protection measures for intra-Union trade in salamanders and the introduction into the Union of such animals in relation to the fungus *Batrachochytrium salamandrivorans*’. This decision, so far valid until April 2021, includes at least temporary trade restrictions and quarantine measures for introductions of salamanders into and within the EU. However, exchange between private pet owners is not efficiently restricted, and anurans and gymnophiones are not part of this decision. This is despite the fact that high *Bsal* prevalence has been observed in some private collections (e.g. FITZPATRICK et al. 2018, SABINO-PINTO et al. 2015, 2018), and that traded anurans can carry *Bsal* (NGUYEN et al. 2017).

Within the last decade, more than 50 *Bsal* sites have been recorded in the pathogen’s invasive European range. Most of them are in Germany (Fig. 1), with a dramatic increase of new outbreaks since 2015, making this country the current ‘hotspot’ of the pathogen’s invasive distribution. The situation in Germany has already been reported and discussed by DALBECK et al. (2018), LÖTTERS et al. (2018), SCHULZ et al. (2018) and WAGNER et al. (2019a, b). However, the ongoing expansion of the salamander plague makes an update necessary.

The purpose of this paper is (1) to summarize the published knowledge on the *Bsal* invasion in Europe with an emphasis on Germany, (2) to report new data from this country and, (3) to assess the ongoing *Bsal* spread within this hotspot.

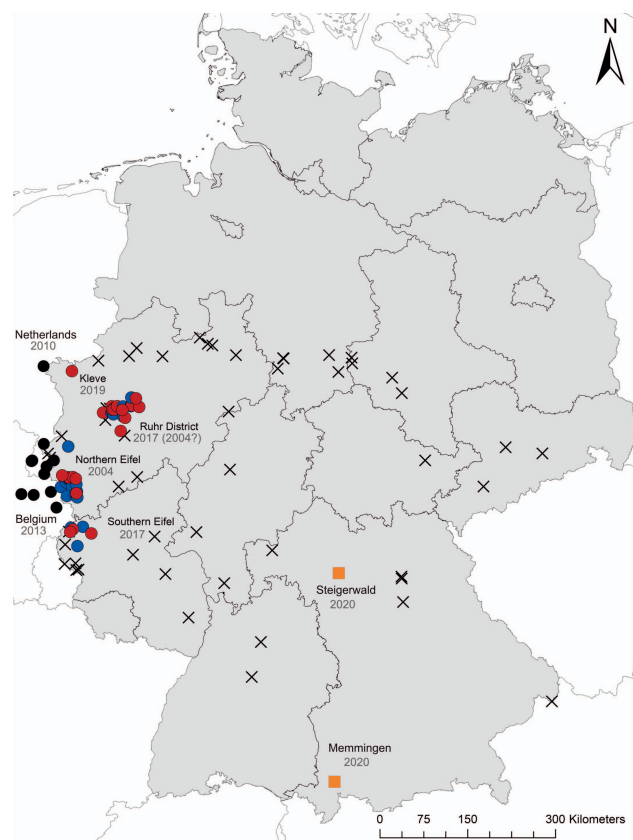


Figure 1. A map of Germany with its federal states showing sites tested positive for *Bsal* until 2018 (blue circles) and since 2019 (red circles); in part these are new records (Supplementary document 3). The two sites in southern Germany (orange squares) are new records reported by SCHMELLER et al. (2020 in this issue) and THEIN et al. (2020 in this issue). *Bsal* sites outside Germany are indicated by black circles. Sites where the pathogen could not be detected in Germany are indicated by cross-marks (note that in addition there are a few sites in Supplementary documents 2 and 4 for which no coordinates are available). Years in which the pathogen was first recorded in each region are given in grey font. For detailed maps of the northern and the southern Eifel see Figures 2 and 4, respectively; for a detailed map of the Ruhr District see SCHULZ et al. (2020 in this issue).



## Summary of the published knowledge about the *Bsal* invasion in Europe

### General

Outside China, Japan, Taiwan, Thailand, and Vietnam (MARTEL et al. 2014, LAKING et al. 2017, BEUKEMA et al. 2018, YUAN et al. 2018), *Bsal* is known from wild populations in Belgium, Germany, The Netherlands, and Spain (SPITZEN-VAN DER SLUIJS et al. 2016, STEGEN et al. 2017, DALBECK et al. 2018, LASTRA GONZÁLEZ et al. 2019, THOMAS et al. 2019, MARTEL et al. 2020). However, it should be mentioned that to date not all of these *Bsal* records have been confirmed by the recommended combination of different diagnostic techniques – e.g. by qPCR and histopathology (cf. THOMAS et al. 2018) – so some European records should be considered ‘suspect cases’ so far. This applies especially to the interesting discovery of *Bsal* in Cantabria and Asturias, Spain, as these reports are from remote areas not associated with dense human populations (LASTRA GONZÁLEZ et al. 2019), but were detected by qPCR only, and in newts with no obvious signs of chytridiomycosis.

In addition to the findings from the wild, *Bsal* was also confirmed in captive amphibian collections in Germany (DE), The Netherlands (NL), Spain (ES), Sweden, and the United Kingdom (UK) (CUNNINGHAM et al. 2015, SABINO-PINTO et al. 2015, 2018, FITZPATRICK et al. 2018). While many of the suspect cases almost certainly refer to *Bsal* (confirmed for various captive cases by histopathology; see SABINO-PINTO et al. 2015), in others, false positives or matching of qPCR probes to other, unknown chytrid fungi rather than *Bsal*, cannot be excluded (see discussion in SABINO-PINTO et al. 2018).

Non-systematic field surveys did not detect *Bsal* in Austria, Croatia, Czech Republic, France, Italy, Montenegro, Poland, Slovakia, Slovenia, and Switzerland, suggesting that it is likely absent in these countries (PARROTT et al. 2017, BALÁŽ et al. 2018, EFSA AHAW et al. 2018, GRASSELLI et al. 2019, LASTRA GONZÁLEZ et al. 2019, THOMAS et al. 2019). Similarly, large-scale screening in the UK did not detect *Bsal* in wild caudate populations despite its occurrence in private collections (CUNNINGHAM et al. 2019).

*Bsal* was discovered for the first time in the European fire salamander population at ‘Bunderbos’ in The Netherlands, where from 2010 onwards > 95% mortality led to a population collapse (SPITZEN-VAN DER SLUIJS et al. 2013, 2016). From 2014 on, additional declines in this species and the detection of *Bsal* were reported from Belgium (BE) and Germany, respectively (MARTEL et al. 2014, SPITZEN-VAN DER SLUIJS et al. 2016). By that time, most of these records were concentrated in an area encompassing the Ardennes (BE), the northern Eifel (DE), and the Southern Limburg Province (NL). However, spatial outliers were noted at a distance of > 50 km from this ‘core’ area; thus, in early 2016 the entire area of *Bsal* occurrences comprised about 10,000 km<sup>2</sup> (SPITZEN-VAN DER SLUIJS et al. 2016). Since 2016, only three new records from Belgium have been reported. In contrast, numerous additional *Bsal* outbreaks have been noted in Germany, partly within the

core area (northern Eifel), but also in the southern Eifel and the Ruhr District of Western Germany (DALBECK et al. 2018, LÖTTERS et al. 2018, SCHULZ et al. 2018, THOMAS et al. 2019, WAGNER et al. 2019a, b). The situation in this country is reviewed in detail below. Most recently, *Bsal* reports from Spain have become available (LASTRA GONZÁLEZ et al. 2019, MARTEL et al. 2020), however, some of these have to be considered as suspect cases (see above).

To date, *Bsal* is known in the wild from the following European salamandrid taxa and countries: European fire salamander, *Salamandra salamandra terrestris* (BE, DE, ES, NL); alpine newt, *Ichthyosaura alpestris alpestris* (BE, DE, NL); palmate newt, *Lissotriton helveticus helveticus* (DE, ES – the latter are suspect cases, see above); smooth newt, *L. vulgaris vulgaris* (DE, NL); Anatolian crested newt, *Triturus anatolicus* (ES, where this is a non-native species); northern crested newt, *T. cristatus* (DE); marbled newt, *T. marmoratus* (ES) (DALBECK et al. 2018, LASTRA GONZÁLEZ et al. 2019, WAGNER et al. 2019a, b, MARTEL et al. 2020).

As European fire salamanders are particularly vulnerable to the salamander plague, the incursion of *Bsal* into a population typically leads to a sharp decline through mass mortality (e.g. SPITZEN-VAN DER SLUIJS et al. 2013, STEGEN et al. 2017, CANESSA et al. 2018, SCHULZ et al. 2018, DALBECK et al. 2018). Limited survival and low population densities post-infection have been observed over some years, and full extirpations have not yet been found (SPITZEN-VAN DER SLUIJS et al. 2018, DALBECK et al. 2018). However, European fire salamanders are not known to become *Bsal*-resistant or tolerant, nor is the pathogen known to entirely disappear allowing population recovery (but see below: Vichtbach, DE). *Bsal* can persist in infection sites in Europe even when host population is low (cf. MARTEL et al. 2020). Some caudate and apparently anuran amphibians can cope with *Bsal* and serve as reservoirs and vectors; furthermore, infectivity of *Bsal* via soil was shown for at least 48 hrs (STEGEN et al. 2017). In line with this, MARTEL et al. (2020) found that *T. anatolicus*, introduced in Spain, can develop chronic, non-lethal *Bsal* infection, allowing repeated spill-over to syntopic native *T. marmoratus* to which *Bsal* is mostly lethal. While *Bsal* can survive at a site for a longer period, it is not necessarily always transmitted to neighbouring populations. Recently, a *Bsal*-free salamander population was discovered next to (about 1 km distant) from a Dutch index site (SPITZEN-VAN DER SLUIJS et al. 2018).

### Germany

We are aware of at least one *Bsal* outbreak in Germany at the Vichtbach (Fig. 2) that dates back to 2004, suggesting that this pathogen had arrived in Europe at least one decade before it was discovered and formally described scientifically. The accompanying mass mortality in the 2004 event, observed by a member of the public, remained unrecognized by scientists and conservationist at that time (DALBECK et al. 2018, LÖTTERS et al. 2020 in this issue). Therefore, it is likely that we have missed additional ‘silent’ outbreaks of the

salamander plague in Germany prior to 2015 (cf. SCHULZ et al. 2020 in this issue), the year when *Bsal* was first detected in a wild European fire salamander population in the northern Eifel (SPITZEN-VAN DER SLUIJS et al. 2016).

As a consequence of the pathogen detection in Germany, the Research and Development (R+D) project ‘Monitoring und Entwicklung von Vorsorgemaßnahmen zum Schutz vor der Ausbreitung des Chytridpilzes *Batrachochytrium salamandrivorans* (“*Bsal*”) im Freiland’ (Monitoring and development of prevention and mitigation measures against the spread of the chytrid fungus *Batrachochytrium salamandrivorans* (“*Bsal*”) in the wild) – funded by the German Federal Agency for Nature Conservation – was established (January 2018 to February 2021). As a result of this project, *Bsal* was recorded in numerous other sites in the northern Eifel by the end of 2018 (DALBECK et al. 2018, WAGNER et al. 2019a; Supplementary document 1).

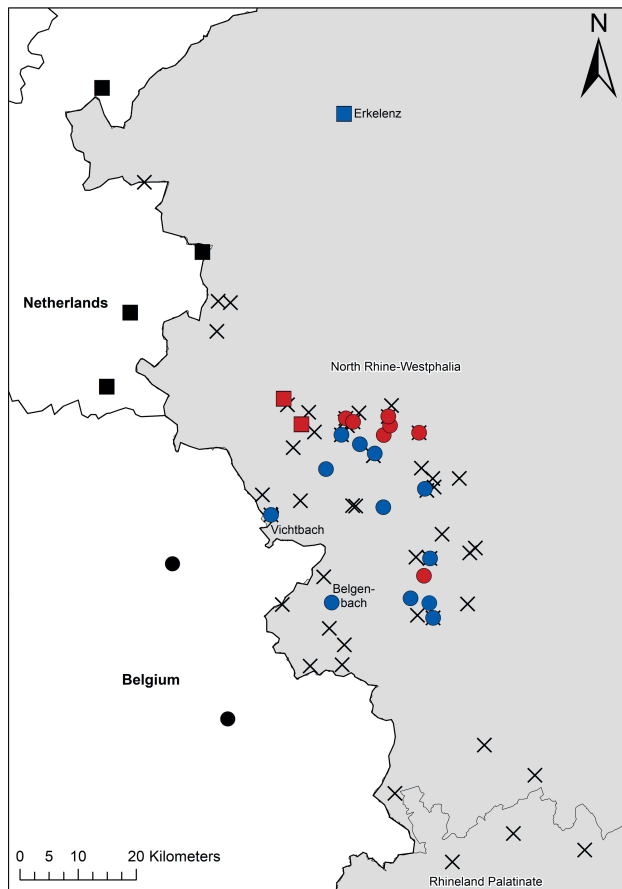


Figure 2. Schematic map of the northern Eifel (see Fig. 1) and the surrounding areas with sites that tested positive for *Bsal* until 2018 (blue symbols) and since 2019 (red symbols); in part these are new records (Supplementary document 3). Known *Bsal* sites outside Germany are indicated by black symbols. Circles: *Bsal*-positive sites with European fire salamanders and newts; squares: *Bsal*-positive sites with newts only (mostly outside the geographic range of the European fire salamander). Names of particular sites referred to in the text are indicated. Sites where *Bsal* could not be detected in any of the study years are indicated by cross-marks.

Prior to the first *Bsal* detection, European fire salamander populations were considerably larger in several locations in this area (although rigorous quantitative pre-decline data are missing), indicating the potential for massive population declines. However, as aforementioned, none of the northern Eifel European fire salamander populations has become extinct so far. At least in some cases, the continued presence of the salamander was corroborated by freshly deposited larvae, indicating reproductive activity (DALBECK et al. 2018, WAGNER et al. 2020a in this issue). This is even true at the Belgenbach site, where in 2015 a mass mortality was witnessed. In the northern Eifel, *Bsal*-positive sites were recorded in the counties of StädteRegion Aachen and Düren, as well as in the Eifel National Park, all in the federal state of North Rhine-Westphalia (Supplementary document 1, Fig. 2). Infection loads and prevalence rates varied considerably, and in some sites, *Bsal* could not be confirmed in any year since its first recognition (DALBECK et al. 2018; Supplementary document 1, Fig. 3). An example is the Vichtbach, where the European fire salamander has an established and solid population, and *Bsal* was not documented after its apparent presence in 2004. In 2018, 29 *Bsal*-negative specimens were found at this site and successful reproduction was documented (Supplementary document 1). Moreover – similar to the observations by SPITZEN-VAN DER SLUIJS et al. (2018) in The Netherlands – we found that *Bsal* does not necessarily disperse by steady range expansion (i.e. modes of diffusion), since the pathogen can apparently remain absent from some amphibian populations in close proximity to *Bsal* outbreaks (DALBECK et al. 2018, WAGNER et al. 2019a).

*Bsal*-positive newts of all native species – i.e. alpine, northern crested, palmate and smooth newt – were found in habitats both with and without European fire salamanders (Fig. 2). In 2018, we made a noteworthy observation at the Brockenberg, northern Eifel (this is a former quarry outside the local distribution of European fire salamander populations). Only a single dead northern crested newt was found and alpine newts were completely absent, while the other two taxa remained relatively common. The years before, all four species – but all *Bsal*-infected – were found (cf. SPITZEN-VAN DER SLUIJS et al. 2016, WAGNER et al. 2019a). Similarly, 14 of the 15 specimens in 2017, and all 11 northern crested newts found in the Helingsbach area – Eifel National Park (i.e. within the local distribution of the European fire salamander; Fig. 2) – in 2018 were *Bsal*-positive with high infection loads. In contrast, prior to 2017, ten northern crested newts and 18 alpine and smooth newts were found to be *Bsal*-negative at this site (Supplementary document 1). The dead northern crested newt from 2018 was also *Bd*-positive (discussed below).

The European fire salamander was reported from the southern Eifel, federal state of Rhineland-Palatinate, in the 1990s. However, monitoring efforts across the region in 2015 were unsuccessful in finding the species. In 2018, the occurrence of *Bsal* in the southern Eifel was shown for the first time by analysing swabs collected in 2017. Alpine and palmate newts from various sites in the counties

of Eifelkreis Bitburg-Prüm and Vulkaneifel tested positive (Supplementary document 1, Fig. 4) (WAGNER et al. 2019a). In some localities, European fire salamander presence could not be confirmed by a standardized monitoring protocol aimed at finding larval stages that are relatively 'easy' to detect (WAGNER et al. 2019b, 2020b in this issue, SANDVOß et al. 2020 in this issue). The current presence of *Bsal* in the southern Eifel, including former European fire salamander sites, suggests that *Bsal*-induced silent European fire salamander declines may have taken place in the past and have led to local extinctions (WAGNER et al. 2019b, SANDVOß et al. 2020 in this issue). Much like in the northern Eifel, *Bsal* could not be detected in all studied sites (Fig. 4). This suggests a patchy pathogen distribution, similar to the northern Eifel (DALBECK et al. 2018, WAGNER et al. 2019a), and indicates that perhaps *Bsal* range expansion is not proceeding at a steady pace.

In 2017, *Bsal* was detected in the Ruhr District – federal state of North Rhine-Westphalia – followed by an apparent spread within the counties of Essen, Bochum, Mülheim an der Ruhr, and Heiligenhaus (SCHULZ et al. 2018, WAGNER et al. 2019a; Supplementary document 1, Fig. 4). These outbreaks far from the Eifel region suggest either long-dis-

tance dispersal or independent *Bsal* introductions. European fire salamanders and alpine newts have been affected, including two mass mortality events of salamanders (as of the end of 2018): Ruthertal in 2016 and Essen-Fulerum in 2018 (DALBECK et al. 2018, LÖTTERS et al. 2018, SCHULZ et al. 2018, WAGNER et al. 2019a). For detailed information on *Bsal* in the Ruhr District see SCHULZ et al. (2020 in this issue).

Amongst the suspected *Bsal* cases in 2018 from Erkelenz (administrative county of Heinsberg, federal state of North Rhine-Westphalia), every European fire salamander (from an outdoor enclosure in which all specimens died within a short period) and smooth newt (from a nearby garden pond) tested *Bsal*-positive (Supplementary document 1). The site is adjacent to the northern Eifel (Fig. 2). DALBECK et al. (2018) referred to additional sites with *Bsal*-positive newts from the Heinsberg county. Although these suspected cases from the Heinsberg area had very low infection loads (between 0.5 and 1 GE = genomic equivalents in qPCR; cf. BLOOM et al. 2013) and thus do not count as 'positives' under the elevated threshold of 1 GE proposed by THOMAS et al. (2018), it is entirely possible that they do represent genuine infections. The presence of one con-

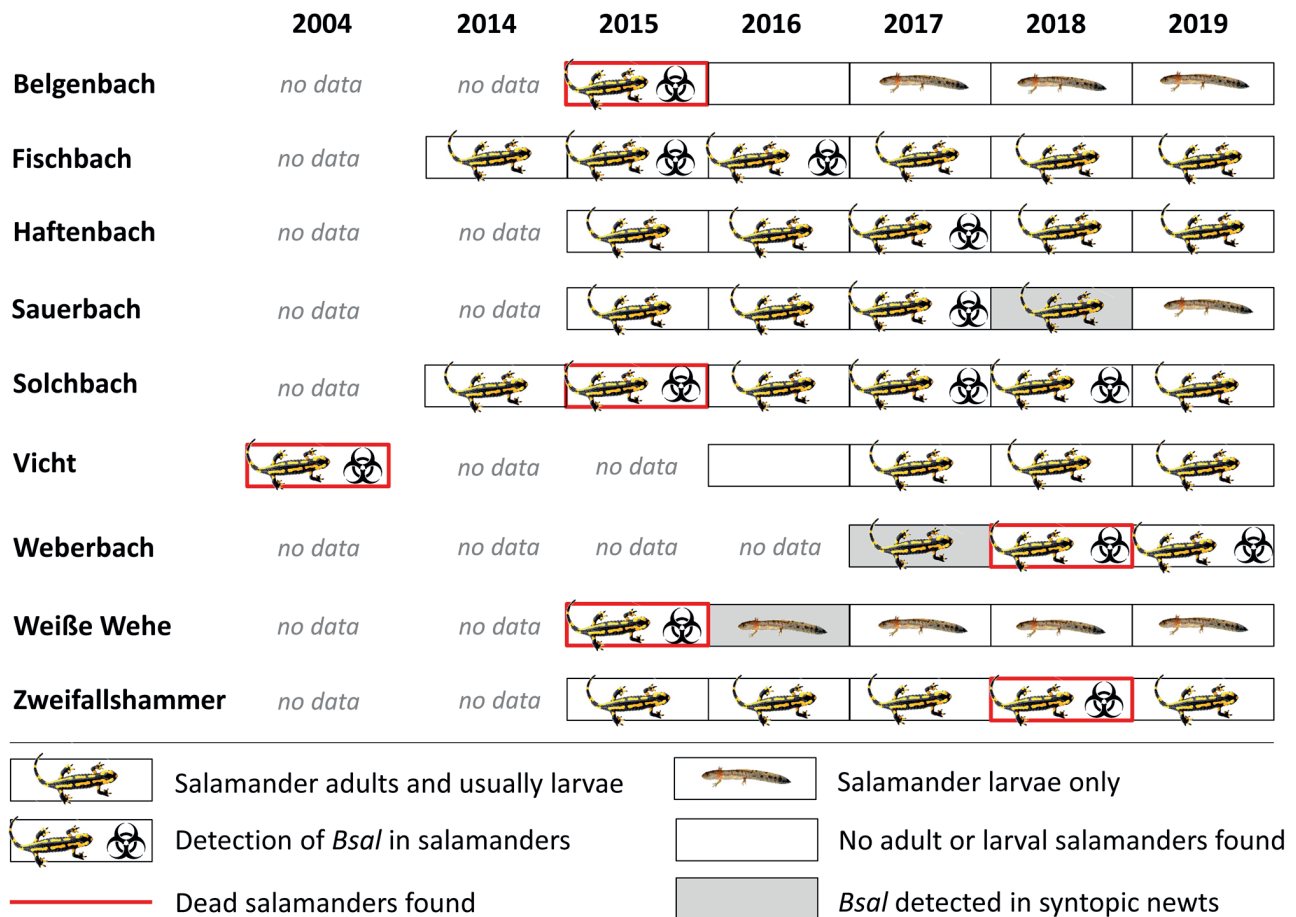


Figure 3. Graph showing European fire salamander and *Bsal* detection history at selected northern Eifel study sites (cf. Supplementary documents 1–4).

firmed positive smoot newt provides clear evidence for the presence of *Bsal* in this county, and the extent of its distribution in the area requires reassessment. In this regard, it is noteworthy that some samples of DALBECK et al. (2018) were only just below the new detection limit.

Taken together, *Bsal* was confirmed at 33 German sites in two federal states by 2018 (Supplementary document 1). These were North Rhine-Westphalia (northern Eifel 15, Ruhr District 12, Heinsberg county 1) and Rhineland-Palatinate (southern Eifel 5). From these *Bsal* regions, a total of 3,447 specimens were tested by applying qPCR methodology to swab samples, and in 188 samples (~ 6%) *Bsal* was confirmed (Supplementary document 1, Figs 1–2, 4). It is important to note here that we perform double-testing by taking two swabs (A and B samples) and analyse them independently in different labs (cf. DALBECK et al. 2018). In 13 of these sites, only European fire salamanders were found

to be infected. In nine sites, infection of both salamanders and newts occurred, and in eleven sites European fire salamanders were absent, and only newts tested positive. Up to the end of 2018, *Bsal* was not found in 176 screened caudate populations (3,454 screened individuals, mainly European fire salamanders) from sites across Germany (northern Eifel 38, southern Eifel 49, Ruhr District 16, other sites in Germany 63; Supplementary document 2, Figs 1–2, 4). Some of these screened populations were reported to us as localities where *Bsal* was suspected, while others were screened as part of randomized sampling within the geographic range of the European fire salamander in Germany within the framework of a project funded by the European Commission (Tender ENV.B.3/SER/2016/0028, ‘Mitigating a new infectious disease in salamanders to counteract the loss of biodiversity’). In addition, JUNG et al. (2020 in this issue) were unable to detect *Bsal* in various salamandrid and ambystomid taxa during a survey in 2019 among nine captive collections in the federal state of Hesse (Germany).

In sampling conducted through 2018, *Bsal* was not detected in a total of 156 anuran specimens (common frog, *Rana temporaria*; common toad, *Bufo bufo*; common midwife toad, *Alytes obstetricans*; yellow-bellied toad, *Bombina variegata*; WAGNER et al. 2019a) from 27 sites within the currently known German regions of *Bsal* presence, including ponds in which *Bsal*-positive newts occurred (Supplementary document 5).

In the 2015 (Fig. 2) *Bsal*-induced mass mortality event at the Belgenbach (mentioned above), 21 European fire salamanders were *Bsal*-positive and 16 of these also tested positive for *Bd*. Infection loads varied considerably for *Bsal* and were low for *Bd*, 0.13–167.1 GE and 0.1–0.4 GE, respectively (LÖTTTERS et al. 2018). This was the first report worldwide of host co-infection with the two pathogenic amphibian chytrid fungi, which is further discussed below.

#### New data from Germany

In 2019 and early 2020, 3,535 additional swabs from caudate and anuran amphibians from the northern and southern Eifel, the Ruhr District, and from suspected localities across Germany were tested for *Bsal* (Supplementary documents 3–5). Sampling methods were the same as in previous years (cf. DALBECK et al. 2018), with *Bsal* only considered ‘confirmed’ when, in two independent labs, A and B samples had > 1 GE (cf. THOMAS et al. 2018). We set the following classification for infection loads: ‘low’ = 1–10 GE, ‘medium’ = > 10–100 GE and ‘high’ = > 100 GE. For each locality, 95% Bayesian credible intervals (CI) for *Bsal* prevalence were calculated following the procedure described in LÖTTTERS et al. (2012). Several dead European fire salamanders from the Eifel area and one specimen from the Ruhr District were further submitted to histopathological analysis, and *Bsal* chytridiomycosis was confirmed (A. MARTEL and F. PASMANS pers. comm.). The presence of the pathogen in both areas was thereby confirmed by an independent, non-DNA based line of evidence.

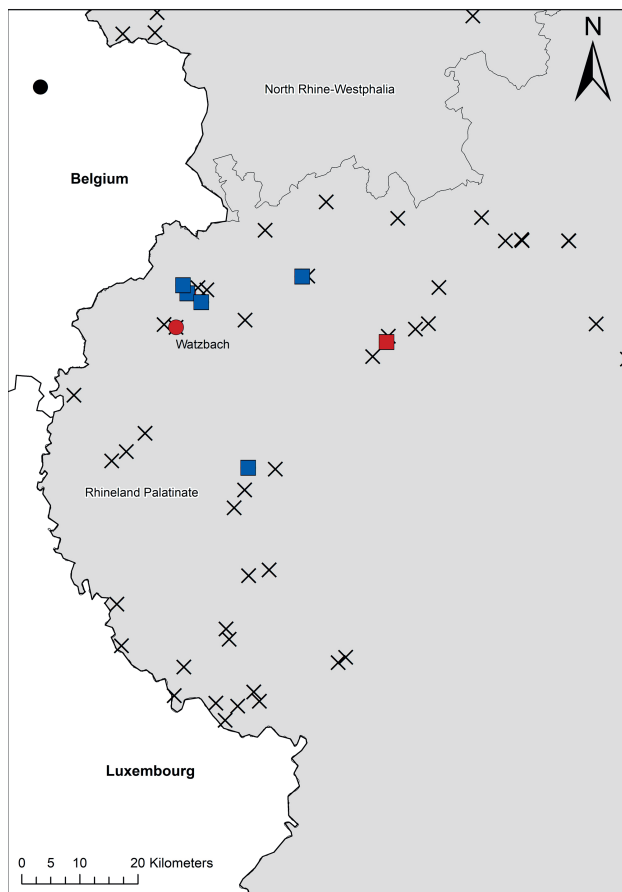


Figure 4. Schematic map of the southern Eifel (see Fig. 1) and the surrounding areas with sites that tested positive for *Bsal* until 2018 (blue symbols) and since 2019 (red symbols); in part these are new records (Supplementary document 3). Known *Bsal* sites outside Germany are indicated by black symbols. Circles: *Bsal*-positive sites with European fire salamanders only; squares: *Bsal*-positive sites with newts (European fire salamanders absent or not found). The Watzbach site, referred to in the text, is indicated. Sites where *Bsal* could not be detected in any of the study years are indicated by cross-marks.



*Bsal* distribution and dispersal

In 2019 and early 2020, we detected the pathogen in 29 sites. Fifteen of these were new localities, within the northern and southern Eifel, the Ruhr District, and the county of Kleve (close to the Dutch border) (Supplementary document 3, Figs 1–2, 4). This included sites which had previously been studied for the presence of *Bsal*, but with no positive result; this strongly supports recent *Bsal* incursions (Fig. 3). By early 2020, this elevated the number of *Bsal*-positive sites in Germany to 48 (Supplementary documents 1, 3), most of which are sites with infected and apparently declining populations of European fire salamanders. Among the latter are one and 12 populations in the southern Eifel and the Ruhr District, respectively (Supplementary document 3, Figs 1, 4).

The *Bsal* outbreak in European fire salamanders in the southern Eifel (Watzbach, county of Bitburg-Prüm; Fig. 4) is noteworthy. According to our surveys from 2016 to 2018, this population was apparently pathogen-free (Supplementary document 2). However, in an area < 1 km away, *Bsal*-positive newts were recorded repeatedly since 2018 (WAGNER et al. 2019a, b). This indicates that whilst *Bsal* can spill-over to neighbouring populations, it does not always do so (cf. SPITZEN-VAN DER SLUIJS et al. 2018). In addition to the detection of *Bsal* in southern Eifel European fire salamanders, we have also become aware (via a report from the public) of several dead specimens found in spring 2019 near Gerolstein in the county of Vulkaneifel. In a subsequent survey, we did not find salamanders there, but confirmed *Bsal* in alpine newts (Supplementary document 3), making a previous *Bsal*-induced European fire salamander extirpation likely.

The recent *Bsal* outbreaks in the Ruhr District, accompanied by two new observed mass mortality events (Hattingen and Witten, Supplementary document 3), mark a pathogen range expansion. The maximum known distance between known *Bsal* records in Germany is about 300 km (Fig. 1). The alarming situation in the Ruhr District is described in detail by SCHULZ et al. (2020 in this issue).

As in previous years, infection loads and prevalence rates during the 2019 survey were mostly low in infected newt and European fire salamander populations (Supplementary documents 1, 3). This seems to be a paradox, considering the observed high mortality or the remarkable population declines. An explanation could be that since European fire salamanders are highly susceptible to *Bsal* and die soon after infection (MARTEL et al. 2014, STEGEN et al. 2017), local *Bsal* prevalence can rapidly increase when individuals aggregate, followed by a loss of infected individuals which are then not sampled; therefore, the estimation of *Bsal* prevalence in European fire salamanders may be strongly affected by the timing of sampling. European fire salamanders are known to aggregate during hibernation (cf. FELDMANN 1967, LEEB et al. 2013) – the perfect time for *Bsal* growth (MARTEL et al. 2013) – which could be generating cryptic mass mortality events.

Since 2019, the pathogen was found in 210 (~ 7%) of 3,186 studied caudate amphibians (Supplementary documents 3 + 4) from Germany's *Bsal* regions (Fig. 1). Only a limited number of specimens from outside the known German *Bsal* regions were examined, i.e. 141 (Fig. 1), these were mainly suspicious cases, i.e. reports from the public. Here, the pathogen was not detected (Supplementary document 4). The same largely applies to all 209 anurans that we studied (Supplementary document 5). However, it is noteworthy that one common frog from the Ruhr District tested positive for *Bsal* in both the A and B samples. Although this is only a single individual, it is the first indication of an anuran *Bsal* carrier belonging to the Neobatrachia (cf. NGUYEN et al. 2017, STEGEN et al. 2017), and perhaps this species represents a so far overlooked potential *Bsal* transmitter, which needs further attention. For more details on this common frog sample see SCHULZ et al. (2020 in this issue).

Among the 28 *Bsal*-positive specimens from the northern and the southern Eifel (cf. Supplementary document 3), two alpine newts from distinct sites (Omerbach, Helingsbach) tested positive for *Bd/Bsal* co-infection. In both, the individual infection loads were high for *Bsal* (> 2,000 GE) and low for *Bd* (1 GE). In addition to that, ongoing laboratory testing revealed that the *Bsal*-positive northern crested newt from Brockenberg found dead in 2018 (mentioned above) was also *Bd*-positive. The infection load was remarkably high in *Bsal* (> 10,000 GE) and low in *Bd* (3 GE), confirmed using singleplex qPCR.

After the present manuscript was nearly completed, we became aware of two additional records of *Bsal* from Bavaria, southern Germany, detected in 2020 (see SCHMELLER et al. 2020 in this issue, THEIN et al. 2020 in this issue) and elevating the number of new records since 2019 to 17.

*Bsal* in wild newts

Our current understanding indicates that *Bsal* can be present in alpine and palmate newts without the dramatic effects that are observed in European fire salamanders (e.g. SPITZEN-VAN DER SLUIJS et al. 2016, DALBECK et al. 2018). Alpine newts were even suggested as *Bsal* reservoirs and transmitters by STEGEN et al. (2017). According to our previous results, prevalence rates in both newt taxa can be low (Supplementary document 1), and 'cryptic' *Bsal* presence could promote the pathogen's long-term survival in a habitat. In 2019, we therefore studied *Bsal* prevalence in newt populations from the northern Eifel.

In three northern Eifel sites with beaver ponds – accommodating large newt populations – swabs from more than 600 alpine and palmate newts were analysed by qPCR. At the Thönbach, among the 132 specimens sampled *Bsal* prevalence was only 4% (Supplementary document 3), and at the Rote Wehe (where *Bsal* was confirmed in 2017; DALBECK et al. 2018), *Bsal* was not found in any of the 320 individuals studied (Supplementary document 4). Conversely, in 2019, newts at the Teufelspütz (*Bsal* detected in 2018 at



very low prevalence; Supplementary document 1) showed a high prevalence, with 21% of the 171 swabbed specimens being infected (Supplementary document 3). Of the remaining studied sites (with smaller sample sizes), high infection prevalence (29%) in alpine and palmate newts was found in 2019 at the Omerbach (Supplementary document 3), though sampling here during the previous year had not confirmed any *Bsal* infection in the 29 newts sampled (Supplementary document 2). These results demonstrate that *Bsal* prevalence is highly variable in northern Eifel alpine and palmate newts, and that perhaps the pathogen can either entirely disappear rapidly or remain highly inconspicuous. Data on infection rates in smooth and

northern crested newts are scarce because alpine and palmate newts predominantly occur in the regions currently affected by *Bsal*.

Although present data are limited (DALBECK et al. 2018; Supplementary document 1), the observations of *Bsal*-infected northern crested newt populations from 2017 and 2018 are nonetheless worrying. They suggest recent and dramatic *Bsal*-related declines of this species in two sites (Brockenberg, Helingsbach area). Survey efforts at the Brockenberg in the northern Eifel – where *Bd/Bsal* co-infection was demonstrated in this species – were increased in 2019; however, only four northern crested newt specimens were found. Three of them were swabbed, and one was

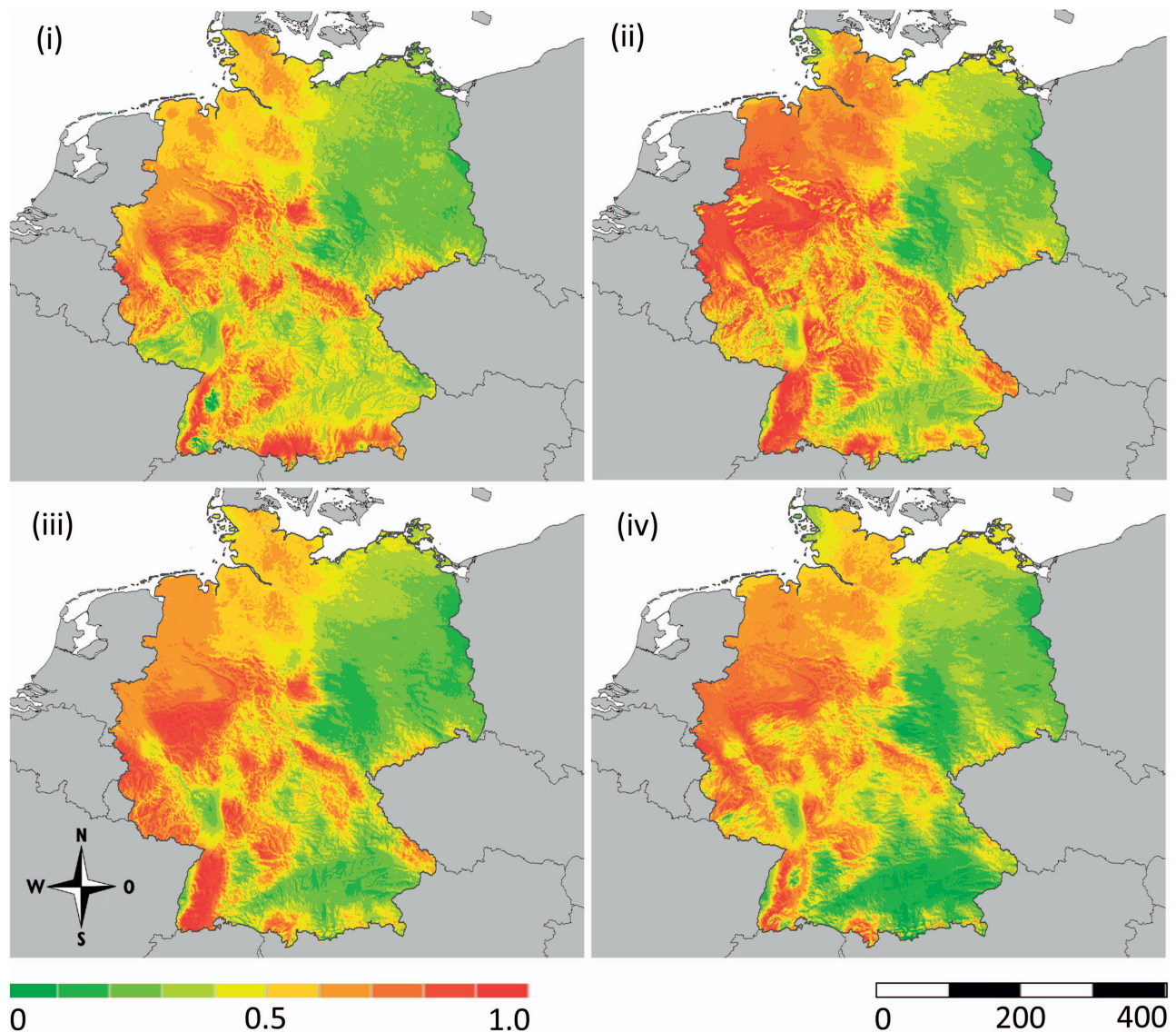


Figure 5. Mapped output of four Maxent species distribution models for *Bsal* in Germany, built with > 100 native and invasive records and different combinations of CHELSA bioclim variables. Warmer colours indicate higher suitability (ranging 0–1). Note that while these models provide insight into particularly suitable areas for the pathogen (i.e. largely in the West), it cannot be excluded that *Bsal* may also expand into areas with low predicted suitability. A 400 km scale bar is shown. For details of data processing see Supplementary data 6.

found to be *Bsal*- (but not *Bd*-) positive (Supplementary document 3). Despite increased efforts in the Helingsbach area, Eifel National Park, we found no northern crested newts since 2019. Since other explanations for these apparent rapid declines are lacking, these findings imply that – just like the European fire salamanders – the northern crested newt may be highly sensitive to *Bsal*. In addition to these three northern crested newt populations, a second known population in the Eifel National Park (Winkelenberg) and one from Kleve county tested *Bsal*-positive in 2019, and therefore, further observations regarding their population status is crucial (Supplementary document 3).

### Assessing the ongoing *Bsal* invasion in Germany *Bsal* range expansion

Our data show that *Bsal* is widely distributed and is expanding its range within the three aforementioned regions (Figs 1, 4–5). The salamander plague is known to be present since at least 2004 in the northern Eifel (Vichtbach; DALBECK et al. 2018; LÖTTERS et al. 2020 in this issue), and was apparently present in the southern Eifel before it was scientifically detected there (WAGNER et al. 2019b, 2020b in this issue, SANDVOß et al. 2020 in this issue). *Bsal* emergence in the Ruhr District may be a more recent occurrence, which would suggest a remarkable extant range expansion. However, SCHULZ et al. (2020 in this issue) – who discuss the salamander plague in the Ruhr District – consider the possibility that *Bsal* was present within the area since at least 2004, based on unconfirmed but photographed specimens demonstrating typical symptoms of *Bsal*-related chytridiomycosis. Independent of this, our data provide evidence of outbreaks in European fire salamander populations in all three *Bsal* regions within the last five years that were previously *Bsal*-free (Supplementary documents 1–4). The apparently recent extension of the pathogen's distribution to sites in southern Germany (SCHMELLER et al. 2020 in this issue, THEIN et al. 2020 in this issue), geographically close to the Alps, further exacerbates the situation dramatically, as it brings *Bsal* into the vicinity of threatened species and subspecies of *Salamandra* such as *S. atra* (with subspecies *aurorae* and *pasubiensis*) and *S. lanzai*.

Apparently, long-distance dispersal is a key driver for the ongoing *Bsal* range expansion in its invasive range (SPITZEN-VAN DER SLUIJS et al. 2016, THOMAS et al. 2019, MARTEL et al. 2020). According to STEGEN et al. (2017), the encysted type of infectious zoospores is a dispersal stage that can float on the water surface. Once *Bsal* is carried into a stream (e.g. when female European fire salamanders release their larvae in spring), long-distance drift is very likely. The authors also showed that water fowl should not be ruled out as *Bsal* carriers over larger distances, but this requires further study, and evidence from the field is lacking. Further, it is expected that – as with *Bd* – *Bsal* can be widely distributed via anthropogenic activities (GARNER et al. 2016, THOMAS et al. 2019, MARTEL et al. 2020). If the incursion of *Bsal* into the Ruhr District is very recent, it

could have occurred by this mode. One explanation for its presence in the Ruhr District could be an independent introduction into the wild. With regard to this, beside possible *Bsal* introductions via asymptomatic amphibians from the pathogen's native Asian range (i.e. pet trade), European captive *Bsal* reservoirs (e.g. FITZPATRICK et al. 2018, SABINO-PINTO et al. 2018) are a source that should not be neglected (THOMAS et al. 2019). Such reservoirs actually do exist in various European countries, including those where the pathogen is currently unknown in the wild. *Bsal* is clearly present in captive collections in Germany (SABINO-PINTO et al. 2015, 2018). The elimination of this latent reservoir of *Bsal* must be a key objective of measures to reduce further infection of natural populations of naïve amphibians. When considering independent releases of *Bsal* into the wild, we must recognize the possibility that different within-species lineages ('strains') may have a different virulence – as is the case with *Bd* (e.g. VAN ROOIJ et al. 2015, O'HANLON et al. 2018).

Both long-distance dispersal as well as independent introductions make regional range expansions of *Bsal* in Germany likely (plus elsewhere in Europe; cf. THOMAS et al. 2019, MARTEL et al. 2020), but highly unpredictable. This is especially true in Germany, where most of the landscape is suitable for *Bsal* emergence according to species distribution models (SDMs) (FELDMERER et al. 2016, BEUKEMA et al. 2018). The second mentioned aspect deserves some insight. In a species which is not in equilibrium with its environment – like *Bsal* in its invasive European range – such predictive models generally entail a high degree of uncertainty (FRANKLIN 2010). However, this uncertainty is perhaps negligible in Germany, due to the many *Bsal* records here. We therefore suggest that SDMs based on all global records – including those from Germany – mirror *Bsal*'s niche here reasonably well, so that we expect that SDMs provide a supportive tool when predicting the potential *Bsal* range expansions in this country. Figure 5 shows updated SDM results based on > 100 global records (for methods used see Supplementary document 5). Note that while our models provide the insight that some areas are of lower *Bsal* suitability (in the more eastern portions of Germany), we observe that *Bsal* expansion into these regions is still possible.

Although long-distance dispersal occurs in amphibians, it is likely a 'rare' phenomenon (MARIN DA FONTE et al. 2019) and therefore probably of minor significance on the time scale in which *Bsal* dispersal occurs in its invasive range. Amphibian hosts apparently play a more important role for the range expansion of the salamander plague via modes of 'normal' or short-distance host dispersal. That is, pathogen spill-over via the expected movement of host organisms within continuous tracts of suitable habitat or between patches of suitable habitat that are close together (cf. DE QUEIROZ 2014). In this way, *Bsal* outbreaks that have been observed in the vicinity of known outbreaks can be explained, e.g. the Watzbach in 2019 in the southern Eifel (Supplementary document 3). However, in a more focused study, SPITZEN-VAN DER SLUIJS et al. (2018) demonstrated that amphibian populations next to long-known *Bsal*

emergences can remain *Bsal*-free, at least for several years. As a result, in an area of *Bsal* presence, patchy occurrence patterns can often be observed (cf. Figs 2, 5). It is currently hard to predict if *Bsal* may locally disappear before an entire region is continuously infected, so that its distribution remains patchy (see below). In our study, the pathogen could be detected in the year(s) subsequent to an outbreak (Supplementary documents 1–4) in some instances.

As a last aspect, it is important to emphasize from all main regions with *Bsal* records in Germany (northern and southern Eifel, Ruhr District, Kleve, Memmingen, Steigerwald), the pathogen has been confirmed by concordant results of different diagnostic techniques as recommended by THOMAS et al. (2018) in accordance with the OIE guidelines (see above). However, within these regions, we accepted new sites (i.e., new streams) being *Bsal*-positive based on qPCR only (from two different swabs extracted and analysed in different labs, however). Given the fast spread of *Bsal* that unfortunately is to be expected in Germany and neighbouring European countries in the next years, it is likely that an independent confirmation with a second method (e.g. histology) will not be possible for all new records. We here suggest to define new records that require confirmation by two independent techniques (e.g. qPCR and histology) as those that fulfil at least one of the following criteria: (i) being in a new region at the landscape scale (i.e. macrochore), especially when potential geographical barriers have been crossed (we suggest a distance of at least ~ 200 km as a guiding value for Western Europe), (ii) recorded from a host species, subspecies or evolutionary significant unit (ESU) previously unknown for this pathogen, (iii) within the geographical range of a new salamander host species, subspecies or ESU likely to be affected by its presence.

## Effects on species and populations

The European fire salamander is highly susceptible to *Bsal* and accordingly suffers drastic population declines (e.g. SPITZEN-VAN DER SLUIJS et al. 2016, STEGEN et al. 2017, DALBECK et al. 2018). However, this does not necessarily mean complete extinction, such as it has almost happened in the Dutch Bunderbos, where the salamander plague led to a population collapse around ten years ago. In some cases, European fire salamanders do still exist in markedly low densities (SPITZEN-VAN DER SLUIJS et al. 2018). In most European fire salamander populations we studied, adult, juvenile, or larval specimens can still be found at low numbers even many years after a *Bsal*-associated population crash occurred (DALBECK et al. 2018, WAGNER et al. 2019a; Supplementary document 1, Fig. 3). This also applies to mass mortality sites, such as the Belgenbach in the northern Eifel, where in 2015 a fire salamander population collapsed. Although no adult salamanders could be found at this site after 2015, a few larvae were detected every year (WAGNER et al. 2020a in this issue). It can be assumed that this population strongly declined, but did not (yet) go completely extinct. There are several former southern Eifel European fire salamander populations, however, where the species is now absent but where *Bsal* can be found in newts. This suggests that local *Bsal*-related salamander extinctions indeed occurred (WAGNER et al. 2019b, SANDVOß et al. 2020 in this issue). Such a scenario is also supported by epidemiological models (CANESSA et al. 2018). Extinction scenarios are contrasted – but not contradicted – by the aforementioned observation made at Vichtbach, where an apparently healthy European fire salamander population persists despite a *Bsal* outbreak in 2004 (LÖTTERS et al. 2020 in this issue; Supplementary documents 1, 4).

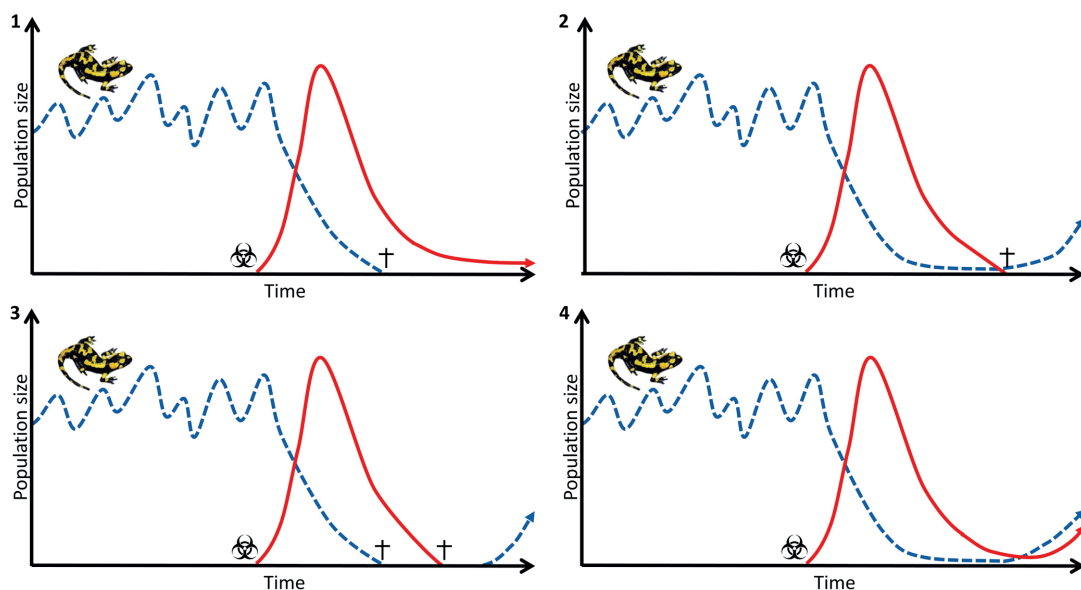


Figure 6. Graphs showing four possible, hypothetical schematic scenarios of host-pathogen relationship in the case of *Bsal* (red) entering a European fire salamander population (blue). A cross represents an extirpation of either the host or the pathogen.



These observations underscore the fact that the impact of *Bsal* on host populations is not yet fully understood. We propose four possible host-pathogen scenarios which could happen in the short- to mid-term after *Bsal* enters a European fire salamander population (Fig. 6):

- (1) host extinction and pathogen decline followed by pathogen survival (e.g. in reservoirs or as encysted zoospores in soil);
- (2) host decline and pathogen extinction followed by host recovery;
- (3) host and pathogen extinction followed by host re-colonisation;
- (4) host and pathogen decline followed by host and pathogen recovery.

Resistance or tolerance (sensu VAN ROOIJ et al. 2015) to *Bsal* has not been observed in infection experiments (MARTEL et al. 2013, 2014, STEGEN et al. 2017). Therefore, we do not consider the possibility of a positive immune response allowing European fire salamander survival under *Bsal*. However, it is worth mentioning that SABINO-PINTO et al. (2018) found high yet non-lethal *Bsal* infection loads in asymptomatic captive European fire salamanders, and BLETZ et al. (2017) reported prolonged survival of some salamander individuals after experimental manipulation of their cutaneous microbiome. This at least emphasizes that there remain unanswered questions with regard to a host's resistance to *Bsal*. Specifically, it remains to be tested whether the conditions of microclimate, microhabitat, or microbiome-induced immunity that possibly confer resistance under captive conditions can also in exceptional circumstances be met in the wild.

To date, almost nothing is known about the effects of *Bsal* incursions into newt populations. The limited field data, the lack of systematic population monitoring, and the variability in observed prevalence data make any conclusion highly speculative. However, our data on northern crested newts (Supplementary documents 1, 4) at least suggest that this species might be considered at 'high risk'. This is especially alarming given that the northern crested newt is under strict protection in the European Union, as it is listed in the Habitats Directive Annex II and Annex IV (Council Directive 92/43/EEC).

#### *Bd/Bsal* co-infection

We consider co-infection with chytrid species on the same host specimen to be a potential threat to amphibians. So far, little attention has been given to *Bd/Bsal* co-infection. LÖTTERS et al. (2018) and our recent data show that both pathogens can co-exist in the wild on the same host individual in at least three taxa. We found co-infection in the European fire salamander, the alpine newt and the northern crested newt at a total of four sites. It is noteworthy that individual *Bd* loads were always low and those of *Bsal* high. In principle, coinfection can induce an interaction among the pathogens, which can then lead to a higher host sus-

ceptibility and an increased mortality (VAUMOURIN et al. 2015). Recently, this has been demonstrated for *Bd* and *Bsal* under experimental conditions in a Nearctic newt (LONGO et al. 2019): (i) specimens simultaneously infected with both fungi cleared *Bd* and largely died of an apparently ongoing *Bsal* infection, and (ii) strong exposition with *Bd* to *Bd*-resistant specimens led to an increased susceptibility to *Bsal*. Moreover, McDONALD et al. (in press) showed that *Bd/Bsal* co-infection might compromise the host's immune response active against *Bsal* alone.

In addition, we consider horizontal gene transfer as a potential risk of co-infection, resulting in a hypothetical '*Bdal*' that could be more dangerous than either *Bd* or *Bsal* alone. It is already known that *Bd* has acquired virulence effector genes from bacteria and oomycete pathogens via horizontal gene transfer (SUN et al. 2011).

While *Bsal* is currently known from comparatively few sites only (Figs 1–2, 4), *Bd* is widely distributed all over Germany (OHST et al. 2011) and elsewhere in the world (e.g. SCHEELE et al. 2019); therefore, in the future, observing more cases of co-occurrence of the two pathogens is likely.

#### Mitigation

EIDs pose a severe challenge to the safeguarding of biodiversity in the Anthropocene. Combating pathogen spread remains a crucial task in biodiversity conservation around the globe (e.g. MENDELSON et al. 2006, WOODHAMS et al. 2011, GARNER et al. 2016). We are far from understanding *Bsal* as such, because efforts to make effective predictions and mitigation strategies are severely hampered. Our observations in the wild indicate complex courses of *Bsal* emergence and infection that are difficult to assess. There remain numerous unsolved key aspects including: long-term effects on host populations, intrinsic and human-mediated long-distance dispersal, mechanisms of short-distance dispersal (spill-over), and identification of *Bsal* transmitters and reservoirs. Accordingly, ongoing field studies – including monitoring of hosts and the pathogen – constitute indispensable research and conservation activities (e.g. SPITZEN-VAN DER SLUIJS et al. 2016, DALBECK et al. 2018, LÖTTERS et al. 2018, SCHULZ et al. 2018, WAGNER et al. 2019a, b).

The alarming data from *Bsal* research in Europe – and the resulting threat to the entire Western Palearctic amphibian diversity – suggest that beyond research, conservation action is urgently needed (MARTEL et al. 2014, GARNER et al. 2016, THOMAS et al. 2019). This is especially true for Germany, which emerges as the current hotspot of the salamander plague. THOMAS et al. (2019) suggest potential mitigation methods and discuss their possible advantages and limitations. According to them, long-term, context-dependent, multi-faceted approaches are needed, ideally initiated prior to the arrival of *Bsal*. Further, ex situ assurance colonies are recommended, in order to preserve affected populations and the affected species' genetic vari-



ability for potential post-*Bsal* reintroductions. As in ex situ conservation, preventive measures which aim to improve biosecurity – such as limiting amphibian trade – may be implemented quickly. However, the biggest challenges ahead are the containment and mitigation of the salamander plague in situ, as well as increasing public awareness for EIDs in non-domestic organisms.

Much of what was advocated by THOMAS et al. (2019) has been implemented in a recently published European *Bsal* Action Plan (GILBERT et al. 2020). However, in addition to this milestone in *Bsal* mitigation, strategies need to be adapted to national levels, and we strongly support the establishment and implementation of a German *Bsal* Action Plan. In accordance with the European plan, we should endeavour to:

- establish an early warning system for early and rapid identification of *Bsal* in the wild, based on a combination of active (targeted) and passive surveillance.
- institute long-term monitoring for high-risk species and/or conservation units (some of which may need to be identified) in order to prepare for their in situ and ex situ conservation.
- build capacity for a rapid response to *Bsal* incursions with (i) elimination, (ii) prevention of establishment, (iii) prevention of spread, and (iv) subsequent monitoring and evaluation to minimize ecological damage and future financial costs.
- strengthen biosecurity measures in the amphibian trade.
- increase *Bsal* awareness in amateur pet keepers and provide affordable screening kits.
- implement a protocol to immediately and effectively remove non-native amphibians (potential transmitters and reservoirs) when novel introductions of these are detected in the wild.
- convey scientific findings on *Bsal* mitigation to the relevant authorities, policy makers and stakeholders to assist in the development of regulations and guidelines for conservation management and monitoring as well as awareness strategies.
- educate nature enthusiasts and the broad public on the problem, on biosecurity in general and other preventive measures.
- promote research to fill knowledge gaps regarding the prevention or mitigation of *Bsal* incursion.
- exchange data and results amongst scientific and conservation authorities and policy makers regarding *Bsal* mitigation.

In addition to complying with Germany's legal obligation to protect species under Annex II of the European Habitats Directive (Council Directive 92/43/EEC) and the Federal Nature Conservation Act (Bundesnaturschutzgesetz – BNatSchG), the implementation of the proposed mitigation measures will also enable us to meet our ethical and societal obligation to halt a further loss of biodiversity. These actions may be our only hope to prevent the loss of iconic species, such as the European fire salamander.

## Acknowledgments

We are grateful to colleagues and collaborators (in alphabetical order), PAUL BACHHAUSEN, NIKLAS BANOWSKI, FREDERIK BARTSCH, CHRISTOPHER BAUSE, JANA BECKERT, SVEN BODINGBAUER, LEONARD BOLTE, STEPHAN BÜRGER, NINJA DYCZMONS, JULIAN ENSS, CHRISTOPHER FALKE, CHARLOTTE FAUL, SAM FIELINGHAUS, KARIN FISCHER, PETER GAUSMANN, ARNO GEIGER, BENEDIKT GLINGENER, JULIAN GLOS, MARC GOSE, FABIAN GÖSSER, MAREIKE HANSEN, ANDREAS HERTZ, JENS HOBERG, THOMAS HÖRREN, KATHARINA HUND, SVEN KARTIK, ALEXANDER KERRES, ANDREAS KOCH, HENRIKE KÖRBER, MATHIAS KRISCH, LOUISA KRON, CHRISTIANE KURAU, HANNAH KURAU, SVENJA LUTHER, AMY MACLEOD, LISA MARGOWSKI, AN MARTEL, LINA MARTIN, MARTIN MASCHKA, PAUL MAUSBACH, CHRISTOPHER MERTES, MARLA MÜLLER, LENA NEUHARDT, VERENA NIEHUIS, CAROLIN NIESTER, SUAN LEE ONG, FRANK PASMANS, CHRISTINA RASCH, HANNAH RAU, TOBIAS RAUTENBERG, PHILIP REHSEN, MAURICE RÖMHILD, LOUISA ROTHE, YUL ROTTMANN, MAXIMILIAN SCHWEINSBERG, JOANA SABINO-PINTO, BENEDIKT R. SCHMIDT, HEIDI SELHEIM, LARISSA SEUFER, PETER SOUND, DANIELA SPECHT, MORITZ SPECHT, RAINER STAWIKOWSKI, ANNEMARIEKE SPITZEN-VAN DER SLUIJS, LISA STEGMEYER, DANIEL STEINORT, JONAS VIEBAHN, JONAS VIRGO and THOMAS ZIEGLER, as well as all 'Föjler', students and volunteer contributors. Funding was kindly made available through the German Federal Agency for Nature Conservation (Bundesamt für Naturschutz, BfN) in the R+D project 'Monitoring und Entwicklung von Vorsorgemaßnahmen zum Schutz vor der Ausbreitung des Chytridpilzes *Batrachochytrium salamandrivorans* („*Bsal*“) im Freiland' (3517821000), European Commission (Tender ENV.B.3/SER/2016/0028, 'Mitigating a new infectious disease in salamanders to counteract the loss of biodiversity'), Deutsche Bundesstiftung Umwelt (DBU), Bezirksregierung Köln, the Landesamt für Natur-, Umwelt und Verbraucherschutz NRW, Nikolaus-Koch-Stiftung, DGHT Wilhelm-Peters-Fonds and Zoo Landau. We are particularly grateful to Eva KAROLINE HESS† (Federal Ministry the Environment, Nature Conservation and Nuclear Safety) for promoting governmental involvement in fighting the salamander plague. Permissions to carry out fieldwork were kindly issued by Untere Naturschutzbehörde StädteRegion Aachen (NRW), Untere Naturschutzbehörde Bochum, Untere Naturschutzbehörde Düren (NRW), Untere Naturschutzbehörde Ennepe-Ruhr-Kreis, Untere Naturschutzbehörde Essen, Untere Naturschutzbehörde Mülheim an der Ruhr, Untere Naturschutzbehörde Oberhausen, Untere Naturschutzbehörde Wuppertal and Struktur- und Genehmigungsdirektion Nord (RLP).

## References

- BALÁŽ, V., M. SOLSKÝ, D. LASTRA GONZÁLEZ, B. HAVLÍKOVÁ, J. G. ZAMORANO, C. G. SEVILLEJA, L. TORRENT & J. VOJAR (2018): First survey of the pathogenic fungus *Batrachochytrium salamandrivorans* in wild and captive amphibians in the Czech Republic. – *Salamandra*, **54**: 87–91.
- BEUKEMA, W., A. MARTEL, T. T. NGUYEN, K. GOKA, D. S. SCHMELER, Z. YUAN, A. E. LAKING, T.-Q. NGUYEN, C.-F. LIN, J. SHELTON, A. LOYAU & F. PASMANS (2018): Environmental context and differences between native and invasive observed niches of *Batrachochytrium salamandrivorans* affect invasion risk assessments in the Western Palearctic. – *Diversity & Distributions*, **24**: 1788–1801.
- BLETZ, M. C., M. KELLY, J. SABINO-PINTO, E. BALES, S. VAN PRAET, W. BERT, F. BOYEN, M. VENCES, S. STEINFARTZ, F. PASMANS &

- A. MARTEL (2018): Disruption of skin microbiota contributes to salamander disease. – Proceedings of the Royal Society B, **285**: 20180758.
- BLOOI, M., F. PASMANS, J. E. LONGCORE, A. SPITZEN-VAN DER SLUIJS, F. VERCAMMEN & A. MARTEL (2013): Duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in amphibian samples. – Journal of Clinical Microbiology, **51**: 4173–4177.
- BLOOI, M., F. PASMANS, J. E. LONGCORE, A. SPITZEN-VAN DER SLUIJS, F. VERCAMMEN & A. MARTEL (2016): Correction for BLOOI et al., duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in amphibian samples. – Journal of Clinical Microbiology, **54**: 246.
- CANESSA, S., C. BOZZUTO, E. H. CAMPBELL GRANT, S. S. CRUICKSHANK, M. C. FISHER, J. C. KOELLA, S. LÖTTERS, A. MARTEL, F. PASMANS, B. C. SCHEELE, A. SPITZEN-VAN DER SLUIJS, S. STEINFARTZ & B. R. SCHMIDT (2018): Decision making for mitigating emerging wildlife diseases: from theory to practice. – Journal of Applied Ecology, **55**: 1987–1996.
- CUNNINGHAM, A. A., K. BECKMANN, M. PERKINS, L. D. FITZPATRICK, R. CROMIE, J. REDBOND, M. F. O'BRIEN, P. GHOSH, J. SHELTON & M. C. FISHER (2015): Emerging disease in UK amphibians. – Veterinary Record, **176**: 468.
- CUNNINGHAM, A. A., F. SMITH, T. J. MCKINLEY, M. W. PERKINS, L. D. FITZPATRICK, O. N. WRIGHT & B. LAWSON (2019): Apparent absence of *Batrachochytrium salamandrivorans* in wild urodeles in the United Kingdom. – Scientific Reports, **9**: 2831.
- DALBECK, L., H. DÜSSEL-SIEBERT, A. KERRES, K. KIRST, A. KOCH, S. LÖTTERS, D. OHLHOFF, J. SABINO-PINTO, K. PREISSLER, U. SCHULTE, V. SCHULZ, S. STEINFARTZ, M. VEITH, M. VENCES, N. WAGNER & J. WEGGE (2018): Die Salamanderpest und ihr Erreger *Batrachochytrium salamandrivorans* (Bsal): aktueller Stand in Deutschland. – Zeitschrift für Feldherpetologie, **25**: 1–22.
- DASZAK P., A. A. CUNNINGHAM & A. D. HYATT (2000): Emerging infectious diseases of wildlife – threats to biodiversity and human health. – Science, **287**: 443–449.
- DE QUEIROZ, A. (2014) The monkey's voyage: how improbable journeys shaped the history of life. – Basic Books: New York.
- EFSA (European Food Safety Authority), V. BALÁŽ, C. G. SCHMIDT, K. MURRAY, E. CARNESECCHI, A. GARCIA, A. GERVELMEYER, L. MARTINO, I. MUNOZ-GUAJARDO, F. VERDONCK, G. ZANCANARO & C. FABRIS (2017): Scientific and technical assistance concerning the survival, establishment and spread of *Batrachochytrium salamandrivorans* (Bsal) in the EU. – EFSA Journal, **15**: e04739.
- EFSA AHAW (European Food Safety Authority Panel on Animal Health and Welfare), S. MORE, A. BÖTNER, A. BUTTERWORTH, P. CALISTRI, K. DEPNER, S. EDWARDS, B. GARIN-BASTUJI, M. GOOD, C. G. SCHMIDT, V. MICHEL, M. A. MIRANDA, S. S. NIELSEN, M. RAJ, L. SIHVONEN, H. SPOOLDER, J. A. STEGEMAN, H.-H. THULKE, A. VELARDE, P. WILLEBERG, C. WINCKLER, F. BALDINELLI, A. BROGLIA, D. CANDIANI, C. FABRIS, M. GEORGIADIS et al. (2017): Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): *Batrachochytrium salamandrivorans* (Bsal). – EFSA Journal, **15**: e05071.
- EFSA AHAW (European Food Safety Authority Panel on Animal Health and Welfare), S. MORE, M. A. MIRANDA, D. BICOUT, A. BÖTNER, A. BUTTERWORTH, P. CALISTRI, K. DEPNER, S. EDWARDS, B. GARIN-BASTUJI, M. GOOD, V. MICHEL, M. RAJ, S. S. NIELSEN, L. SIHVONEN, H. SPOOLDER, J. A. STEGEMAN, H.-H. THULKE, A. VELARDE, P. WILLEBERG, C. WINCKLER, V. BALÁŽ, M. MURRAY, C. FABRIS et al. (2018): Risk of survival, establishment and spread of *Batrachochytrium salamandrivorans* (Bsal) in the EU. – EFSA Journal, **16**: e05259.
- FELDMANN, R. (1967): Winterquartiere des Feuersalamanders, *Salmandra salamandra terrestris*, in Bergwerkstollen des südlichen Westfalen. – Salamandra, **3**: 1–3.
- FELDMEIER, S., L. SCHEFCZYK, N. WAGNER, G. HEINEMANN, M. VEITH & S. LÖTTERS (2016): Present and future high risk zones for the spreading lethal salamander chytrid fungus in its invasive range in Europe using bioclimate and weather extremes. – PLoS ONE, **11**: e0165682.
- FISHER, M. C., D. A. HENK, C. J. BRIGGS, J. S. BROWNSTEIN, L. C. MADOFF, S. L. MCCRAW & S. J. GURR (2012): Emerging fungal threats to animal, plant and ecosystem health. – Nature, **282**: 186–194.
- FITZPATRICK, L. D., F. PASMANS, A. MARTEL & A. A. CUNNINGHAM (2018): Epidemiological tracing of *Batrachochytrium salamandrivorans* identifies widespread infection and associated mortalities in private amphibian collections. – Scientific Reports, **8**: 13845.
- FRANKLIN J. (2010): Mapping species distributions. Spatial inference and prediction. – Cambridge University Press, Cambridge and New York.
- GARNER, T. W., B. R. SCHMIDT, A. MARTEL, F. PASMANS, E. MUTHS, A. A. CUNNINGHAM, C. WELDON, M. C. FISHER & J. BOSCH (2016): Mitigating amphibian chytridiomycoses in nature. – Philosophical Transactions of the Royal Society London B, **5**: 371.
- GILBERT, M. J., A. M. SPITZEN-VAN DER SLUIJS, S. CANESSA, J. BOSCH, A. CUNNINGHAM, E. GRASSELLI, A. LAUDELOUT, S. LÖTTERS, C. MIAUD, S. SALVIDIO, M. VEITH, A. MARTEL & F. PASMANS (2020): Mitigating *Batrachochytrium salamandrivorans* in Europe. *Batrachochytrium salamandrivorans* emergency action plan for European urodeles. – European Commission, Directorate-General Environment, Directorate B – Natural Capital Unit B.3 – Nature, Nijmegen.
- JUNG, L., J. DUSEK, T. LÜDDECKE, V. SCHULZ, K. MAIER-SAM, L. HABICH, A. MOSEBACH, M. LIERZ & H.-P. ZIEMEK (2020): Epidemiological screening of captive salamanders reveals current absence of *Batrachochytrium salamandrivorans* in private collections throughout the federal state of Hesse (Germany). – Salamandra, **56**: 233–238 [this issue].
- GRASSELLI, E., G. BIANCHI, L. DONDERO, V. MARCHIANÒ, M. CARAFA, M. PERRONE & S. SALVIDIO (2019): First screening for *Batrachochytrium salamandrivorans* (Bsal) in wild and captive salamanders from Italy. – Salamandra, **55**: 124–126.
- LA MARCA, E., K. R. LIPS, S. LÖTTERS, R. PUSCHENDORF, R. IBÁÑEZ, J. V. RUEDA-ALMONACID, R. SCHULTE, C. MARTY, F. CASTRO, J. MANZANILLA-PUPPO, J. E. GARCÍA-PÉREZ, F. BOLAÑOS, G. CHAVES, J. A. POUNDS, E. TORAL & B. E. YOUNG (2015): Catastrophic population declines and extinctions in Neotropical harlequin frogs (Bufonidae: *Atelopus*). – Biotropica, **37**: 190–201.
- LAKING, A. E., H. N. NGO, F. PASMANS, A. MARTEL & T. T. NGUYEN (2017): *Batrachochytrium salamandrivorans* is the predominant chytrid fungus in Vietnamese salamanders. – Scientific Reports, **7**: 44443.
- LASTRA GONZÁLEZ, D., V. BALÁŽ, M. SOLSKÝ, B. THUMSOVÁ, K. KOLENDÁ, A. NAJBAR, B. NAJBAR, M. KAUTMAN, P. CHAJMA, M. BALOGOVÁ & J. VOJAR (2019): Recent findings of potentially

- lethal salamander fungus *Batrachochytrium salamandrivorans*. – *Emerging Infectious Diseases*, **25**: 1416–1418.
- LEEB, C., W. HÖDL & M. RINGLER (2013): A high-quality, self-assembled camera trapping system for the study of terrestrial poikilotherms tested on the fire salamander. – *Herpetozoa*, **2**: 164–171.
- LONGO, A. V., R. C. FLEISCHER & K. R. LIPS (2019): Double trouble: co-infections of chytrid fungi will severely impact widely distributed newts. – *Biological Invasions*, **21**: 2233–2245.
- LÖTTTERS, S., J. KIELGAST, M. SZTATECSNY, N. WAGNER, U. SCHULTE, P. WERNER, D. RÖDDER, J. DAMBACH, T. REISSNER, A. HOCHKIRCH & B. R. SCHMIDT (2012): Absence of infection with the amphibian chytrid fungus in the terrestrial Alpine salamander, *Salamandra atra*. – *Salamandra*, **48**: 58–62.
- LÖTTTERS, S., N. WAGNER, A. KERRES, M. VENCES, S. STEINFARTZ, J. SABINO-PINTO, L. SEUFER, K. PREISSLER, V. SCHULZ & M. VEITH (2018): First report of host co-infection of parasitic amphibian chytrid fungi. – *Salamandra*, **54**: 287–290.
- LÖTTTERS, S., M. VEITH, N. WAGNER, A. MARTEL & F. PASMANS (2020): *Bsal*-driven salamander mortality pre-dates the European index outbreak. – *Salamandra*, **56**: 239–242 [this issue].
- MARIN DA FONTE, L. F., M. MAYER & S. LÖTTTERS (2019): Long-distance dispersal in amphibians. – *Frontiers of Biogeography*, **11.4**: e44577.
- MARTEL, A., A. SPITZEN-VAN DER SLUIJS, M. BLOOI, W. BERT, R. DUCATELLE & M. C. FISHER (2013): *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. – *Proceedings of the National Academy of Sciences of the USA*, **110**: 15325–15329.
- MARTEL, A., M. BLOOI, C. ADRIAENSEN, P. VAN ROOIJ, W. M. BEUKEMA, M. C. FISHER, R. A. FARRER, B. R. SCHMIDT, U. TOBLER, K. GOKA, K. R. LIPS, C. MULETZ, K. R. ZAMUDIO, J. BOSCH, S. LÖTTTERS, E. WOMBWELL, T. W. J. GARNER, A. A. CUNNINGHAM, A. SPITZEN-VAN DER SLUIJS, S. SALVIDIO, R. DUCATELLE, K. NISHIKAWA, T. T. NGUYEN, J. E. KOLBY, I. VAN BOCKLAER et al. (2014): Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. – *Science*, **346**: 630–631.
- MARTEL, A., M. VILA-ESCALÉ, D. FERNÁNDEZ-GIBERTEAU, A. MARTINEZ-SILVESTRE, S. CANESSA, S. VAN PRAET, P. PANNON, K. CHIERI, A. FERRAN, M. KELLY, M. PICART, D. PIULATS, Z. LI, V. PAGONE, L. PÉREZ-SORRIBES, C. MOLINA, A. TARRAGÓ-GUARRO, R. VELARDE-NIETO, F. CARBONELL, E. OBON, D. MARTÍNEZ-MARTÍNEZ, D. GUINART, R. CASANOVAS, S. CARRANZA & F. PASMANS (2020): Integral chain management of wildlife diseases. – *Conservation Letters*: e12707.
- MCDONALD, C. A., A. V. LONGO, K. R. LIPS & K. R. ZAMUDIO (in press): Incapacitating effects of fungal coinfection in a novel pathogen system. – *Molecular Ecology*. <https://doi.org/10.1111/mec.15452>
- MENDELSON, J. R., K. R. LIPS, R. W. GAGLIARDO, G. B. RABB, J. P. COLLINS, J. E. DIFFENDORFER, P. DASZAK, R. IBÁÑEZ D., K. C. ZIPPEN, D. P. LAWSON, K. M. WRIGHT, S. N. STUART, C. GASCON, H. R. DA SILVA, P. A. BURROWES, R. L. JOGLAR, E. LA MARCA, S. LÖTTTERS, L. H. DU PREEZ, C. WELDON, A. HYATT, J. V. RODRIGUEZ-MAHECHA, S. HUNT, H. ROBERTSON, B. LOCK et al. (2006): Confronting amphibian declines and extinctions. – *Science*, **313**: 48.
- NGUYEN, T. T., T. V. NGUYEN, T. ZIEGLER, F. PASMANS & A. MARTEL (2017): Trade in wild anurans vectors the urodelan pathogen *Batrachochytrium salamandrivorans* into Europe. – *Amphibia-Reptilia*, **38**: 554–556.
- O'HANLON, S. J., A. RIEUX, R. A. FARRER, G. M. ROSA, B. WALDMAN, A. BATAILLE, T. A. KOSCH, K. A. MURRAY, B. BRANKOVICS, M. FUMAGALLI, M. D. MARTIN, N. WALES, M. ALVARADO-RYBAK, K. A. BATES, L. BERGER, S. BÖLL, L. BROOKES, F. CLARE, E. A. COURTOIS, A. A. CUNNINGHAM, T. M. DOHERTY-BONE, P. GHOSH, D. J. GOWER, W. E. HINTZ, J. HÖGLUND et al. (2018): Recent Asian origin of chytrid fungi causing global amphibian declines. – *Science*, **360**: 621–627.
- OHST, T., Y. GRÄSER, F. MUTSCHMANN & J. PLÖTNER (2011): Neue Erkenntnisse zur Gefährdung europäischer Amphibien durch den Hauptpilz *Batrachochytrium dendrobatidis*. – *Zeitschrift für Feldherpetologie*, **18**: 1–17.
- PARROTT, J. C., A. SHEPACK, D. BURKART, B. LA BUMBARD, P. SCIMÈ, E. BARUCH & A. CATENAZZI (2017): Survey of pathogenic chytrid fungi (*Batrachochytrium dendrobatidis* and *B. salamandrivorans*) in salamanders from three mountain ranges in Europe and the Americas. – *EcoHealth*, **14**: 296–302.
- SABINO-PINTO, J., M. BLETZ, R. HENDRIX, R. G. B. PERL, A. MARTEL, F. PASMANS, S. LÖTTTERS, F. MUTSCHMANN, D. S. SCHMELLER, B. R. SCHMIDT, M. VEITH, N. WAGNER, M. VENCES & S. STEINFARTZ (2015): First detection of the emerging fungal pathogen *Batrachochytrium salamandrivorans* in Germany. – *Amphibia-Reptilia*, **36**: 411–416.
- SABINO-PINTO, J., M. VEITH, M. VENCES & S. STEINFARTZ (2018): Asymptomatic infection of the fungal pathogen *Batrachochytrium salamandrivorans* in captivity. – *Scientific Reports*, **8**: 11767.
- SANDVOß, M., N. WAGNER, S. LÖTTTERS, S. FELDMEIER, V. SCHULZ, S. STEINFARTZ & M. VEITH (2020): Spread of the pathogen *Batrachochytrium salamandrivorans* and large-scale absence of larvae suggest unnoticed declines of the European fire salamander in the southern Eifel Mountains. – *Salamandra*, **56**: 215–226 [this issue].
- SCHÉELE, B. C., F. PASMANS, L. F. SKERRATT, L. BERGER, A. MARTEL, W. BEUKEMA, A. A. ACEVEDO, P. A. BURROWES, T. CARVALHO, A. CATENAZZI, I. DE LA RIVA, M. C. FISHER, S. V. FLECHAS, C. N. FOSTER, P. FRÍAS-ÁLVAREZ, T. W. J. GARNER, B. GRATWICKE, J. M. GUAYASAMIN, M. HIRSCHFELD, J. E. KOLBY, T. A. KOSCH, E. LA MARCA, D. B. LINDENMAYER, K. R. LIPS, A. V. LONGO, R. MANEYRO et al. (2019): Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. – *Science*, **363**: 1459–1463.
- SCHMELLER, D. S., R. UTZEL, F. PASMANS & A. MARTEL (2020): *Batrachochytrium salamandrivorans* kills alpine newts (*Ichthyosaura alpestris*) in southernmost Germany. – *Salamandra*, **56**: 230–232 [this issue].
- SCHULZ, V., S. STEINFARTZ, A. GEIGER, K. PREISSLER, J. SABINO-PINTO, M. KRISCH, N. WAGNER & M. SCHLÜPMANN (2018): Ausbreitung der Salamanderpest in Nordrhein-Westfalen. – *Natur in NRW*, **2018**: 26–30.
- SCHULZ, V., A. SCHULZ, M. KLAMKE, K. PREISSLER, J. SABINO-PINTO, M. MÜSKEN, M. SCHLÜPMANN, L. HELDT, F. KAMPRAD, J. ENSS, M. SCHWEINSBERG, J. VIRGO, H. RAU, M. VEITH, S. LÖTTTERS, N. WAGNER, S. STEINFARTZ & M. VENCES (2020): *Batrachochytrium salamandrivorans* in the Ruhr District, Germany: history, distribution, decline dynamics and disease symptoms of the salamander plague. – *Salamandra*, **56**: 189–214 [this issue].
- SIKES, B. A., J. L. BUFFORD, P. E. HULME, J. A. COOPER, P. R. JOHNSTON & R. P. DUNCAN (2018): Import volumes and biosecurity interventions shape the arrival rate of fungal pathogens. – *PLoS BIOLOGY*, **16**: e2006025.



- SPITZEN-VAN DER SLUIJS, A., F. SPIKMANS, W. BOSMAN, M. DE ZEEUW, T. VAN DER MEIJ, E. GOVERSE, M. J. L. KIK, F. PASMANS & A. MARTEL (2013): Rapid enigmatic decline drives the fire salamander (*Salamandra salamandra*) to the edge of extinction in the Netherlands. – *Amphibia-Reptilia*, **34**: 233–239.
- SPITZEN-VAN DER SLUIJS, A., A. MARTEL, J. ASSELBERGHS, E. K. BALES, W. BEUKEMA, M. C. BLETZ, L. DALBECK, E. GOVERSE, A. KERRES, T. KINET, K. KIRST, A. LAUDELOUT, L. F. MARIN DA FONTE, A. NÖLLERT, D. OHLHOFF, J. SABINO-PINTO, B. R. SCHMIDT, J. SPEYBROECK, F. SPIKMANS, S. STEINFARTZ, M. VEITH, M. VENCES, N. WAGNER, F. PASMANS & S. LÖTTERS (2016): Expanding distribution of lethal amphibian fungus *Batrachochytrium salamandrivorans* in Europe. – *Emerging Infectious Diseases*, **22**: 1286–1288.
- SPITZEN-VAN DER SLUIJS, A., G. STEGEN, S. BOGAERTS, S. CANESSA, S. STEINFARTZ, N. JANSSEN, W. BOSMAN, F. PASMANS & A. MARTEL (2018): Post-epizootic salamander persistence in a disease-free refugium suggests poor dispersal ability of *Batrachochytrium salamandrivorans*. – *Scientific Reports*, **8**: 3800.
- STEGEN, G., F. PASMANS, B. R. SCHMIDT, L. O. ROUFFAER, S. VAN PRAET, M. SCHAUB, S. CANESSA, A. LAUDELOUT, T. KINET, C. ADRIAENSEN, F. HAESBROUCK, W. BERT, F. BOSSUYT & A. MARTEL (2017): Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. – *Nature*, **544**: 353–356.
- STOKSTAD, E. (2014): The coming salamander plague. – *Science*, **346**: 530–531.
- SUN, G., Z. YANG, T. KOSCH, K. SUMMERS & J. HUANG (2011): Evidence for acquisition of virulence effectors in pathogenic chytrids. – *BMC Evolutionary Biology*, **11**: 195.
- THEIN, J., U. RECK, C. DITTRICH, A. MARTEL, V. SCHULZ & G. HANSBAUER (2020): Preliminary report on the occurrence of *Batrachochytrium salamandrivorans* in the Steigerwald, Bavaria, Germany. – *Salamandra*, **56**: 227–229 [this issue].
- THIESMEIER, B. & R. GÜNTHER (1996): Feuersalamander – *Salamandra salamandra* (Linnaeus, 1758). – pp. 82–104 in: GÜNTHER, R. (ed.): Die Amphibien und Reptilien Deutschlands. – Spektrum Akademischer Verlag, Heidelberg.
- THOMAS, V., M. BLOOI, P. VAN ROOIJ, S. VAN PRAET, E. VERBRUGGHE, E. GRASSELLI, M. LUKAC, S. SMITH, F. PASMANS & A. MARTEL (2018): Recommendations on diagnostic tools for *Batrachochytrium salamandrivorans*. – *Transboundary and Emerging Diseases*, **65**: e478–e488.
- THOMAS, V., Y. WANG, P. VAN ROOIJ, E. VERBRUGGHE, V. BALÁZ, J. BOSCH, A. A. CUNNINGHAM, M. C. FISCHER, T. W. J. GARNER, M. J. GILBERT, E. GRASSELLI, T. KINET, A. LAUDELOUT, S. LÖTTERS, A. LOYAU, C. MIAUD, S. SALVIDIO, D. S. SCHMELLER, B. R. SCHMIDT, A. SPITZEN-VAN DER SLUIJS, S. STEINFARTZ, M. VEITH, M. VENCES, N. WAGNER, S. CANESSA, A. MARTEL & F. PASMANS (2019) Mitigating *Batrachochytrium salamandrivorans* in Europe. – *Amphibia-Reptilia*, **40**: 265–290.
- VAUMOURIN, E., G. VOURC'H, P. GASQUI & M. VAYSSIER-TAUSSAT (2015): The importance of multiparasitism: examining the consequences of co-infections for human and animal health. – *Parasites & Vectors*, **8**: 545.
- VAN ROOIJ, P., A. MARTEL, J. NERZ, S. VOITEL, F. VAN IMMERSEEL, F. HAESBROUCK & F. PASMANS (2011): Detection of *Batrachochytrium dendrobatidis* in Mexican bolitoglossine salamanders using an optimal sampling protocol. – *EcoHealth*, **8**: 237–243.
- VAN ROOIJ, P., A. MARTEL, F. HAESBROUCK & F. PASMANS (2015): Amphibian chytridiomycosis: a review with focus on fungus-host interactions. – *Veterinary Research*, **46**: 137.
- VAN ROOIJ, P., F. PASMANS, Y. COEN & A. MARTEL (2017): Efficacy of chemical disinfectants for the containment of the salamander chytrid fungus *Batrachochytrium salamandrivorans*. – *PLoS ONE*, **12**: e0186269.
- WAGNER, N., V. SCHULZ, S. STEINFARTZ, T. REINHARDT, M. VENCES, S. LÖTTERS, L. DALBECK, H. DÜSSEL-SIEBERT, M. GUSCHAL, K. KIRST, D. OHLHOFF, J. WEGGE & M. VEITH (2019a): Aktuelle Erkenntnisse zum Status der Salamanderpest in Deutschland. – *Natur und Landschaft*, **94**: 463–471.
- WAGNER, N., S. LÖTTERS, S. FELDMEIER, J. BENINDE, K. BREDIMUS, D. C. CHRISTIANSEN, J. EWEN, L. FEILER, G. FICHERA, B. FONTAINE, C. KOLWELTER, W. HARMS, F. HILDEBRANDT, F. KELTSCH, L. F. MARIN DA FONTE, A. MARTENS, S. L. ONG, L. SCHMITZ, U. SCHULTE, V. SCHULZ, S. STEINFARTZ, M. VENCES, J. VIEBAHN, M. WAGNER, K. WALLRICH & M. VEITH (2019b): Aktueller Kenntnisstand zur Verbreitung des Erregers der Salamanderpest (*Batrachochytrium salamandrivorans*) in Rheinland-Pfalz. – *Dendrocopos*, **46**: 35–66.
- WAGNER, N., S. LÖTTERS, L. DALBECK, H. DÜSSEL, M. GUSCHAL, K. KIRST, D. OHLHOFF, J. WEGGE, T. REINHARDT & M. VEITH (2020a): Long-term monitoring of European fire salamander populations (*Salamandra salamandra*) in the Eifel Mountains (Germany): five years of removal sampling of larvae. – *Salamandra*, **56**: 243–253 [this issue].
- WAGNER, N., W. HARMS, F. HILDEBRANDT, A. MARTENS, S. L. ONG, K. WALLRICH, S. LÖTTERS & M. VEITH (2020b): Do habitat preferences of European fire salamander (*Salamandra salamandra*) larvae differ among landscapes? A case study from Western Germany. – *Salamandra*, **56**: 254–264 [this issue].
- WOODHAMS, D. C., J. BOSCH, C. J. BRIGGS, S. CASHINS, L. R. DAVIS, A. LAUER, E. MUTHS, R. PUSCHENDORF, B. R. SCHMIDT, B. SHEAFOR & J. VOYLES (2011): Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. – *Frontiers in Zoology*, **8**: 8.
- YUAN, Z., A. MARTEL, J. WU, S. VAN PRAET, S. CANESSA & F. PASMANS (2018): Widespread occurrence of an emerging fungal pathogen in heavily traded Chinese urodelan species. – *Conservation Letters*, **11**: e12436.

### Supplementary data

The following data are available online:

- Supplementary document 1. Published site records (populations) of caudate species from Germany in which *Bsal* was detected until 2018.
- Supplementary document 2. Published site records (populations) of caudate species from Germany in which *Bsal* was not detected until 2018.
- Supplementary document 3. *Bsal*-positive sites (populations) of caudate species from Germany from 2019 and early 2020.
- Supplementary document 4. *Bsal*-negative sites (populations) of caudate species from Germany from 2019 and early 2020.
- Supplementary document 5. Details of anurans tested for *Bsal*.
- Supplementary document 6. Methods used to build *Bsal* species distribution models.