SALAMANDRA

German Journal of Herpetology

Published by
Deutsche Gesellschaft für Herpetologie und Terrarienkunde e.V.
Mannheim, Germany

VOLUME 56 · NUMBER 3

15 AUGUST 2020

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Salamandra salamandra from the Solling, Lower Saxony, Germany. Photo: Miguel Vences.

ISSN 0036-3375

E-mail: salamandra@dght.de.

Layout: Birgit Rödder, Kop Nück 3, 53902 Bad Münstereifel, Germany. Delivery to book dealers: DGHT-Geschäftsstelle, Vogelsang 27, 31020 Salzhemmendorf, Germany.

DGHT
Deutsche Gesellschaft für
Herpetologie und Terrarienkunde

SALAMANDRA **56**(3): 169–171
15 August 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

The salamander plague in Europe – a German perspective

The devastating effects of the 'salamander plague', caused by the invasive fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*) on native caudate amphibians in Europe, have been intensively documented in the Netherlands (Spitzen-van der Sluijs et al. 2013). The decline of a Dutch population of the European fire salamander, *Salamandra salamandra,* indeed led to the discovery and taxonomic description of this fungus (Martel et al. 2013). Despite an enormous global concern about the emergence of this new pathogen (e.g. Yap et al. 2015) and intensive research on its origins and impact on European salamanders and newts (e.g. Martel et al. 2014, Stegen et al. 2017, Beukema et al. 2018, Canessa et al. 2018, O'Hanlon et al. 2018), there is only limited awareness of the catastrophic salamander declines that the pathogen is currently causing in Germany – a country that we can consider, without exaggerating, as the current *Bsal* 'hotspot' in Europe.

The presence of *Bsal* in the wild in Germany was first detected by Spitzen-van der Sluijs et al. (2016), while Sabino-Pinto et al. (2015) provided evidence for the presence of the pathogen in captive salamander collections in the country. This triggered an intensification of survey and monitoring activities, funded primarily by the Bundesamt für Naturschutz (BfN) and the Deutsche Bundesstiftung Umwelt (DBU), involving massive fieldwork efforts and the analysis, overall, of more than 8,000 swabs of wild amphibians and more than 1,000 swabs of captive specimens via quantitative PCR, in a comprehensive and collaborative approach led by a team of university researchers and professional conservation biologists. Updates of this research activity were released mostly in German language by Dalbeck et al. (2018), Schulz et al. (2018) and Wagner et al. (2019), and the captive collection results were published by Sabino-Pinto et al. (2018). Given the many affected salamander populations in Germany, our observation of declines in real time, and indications for prolonged presence of the pathogen for at least 16 years in the country, the assembled data give important insights into the impact of this novel pathogen. We therefore aimed at making the entire knowledge available to the international scientific and conservation community without further delay.

No journal could be better suited than SALAMANDRA, published by the German Herpetological Society, DGHT, when it comes to documenting these dramatic disease-related population declines in German populations of *S. salamandra.* The collection of articles in this issue of the journal provides a detailed summary of our knowledge on the impact of *Bsal* in the epicentre of its invasive range that Germany sadly has become. This includes accounts on the known distribution of the pathogen across geography and hosts, observed mass mortality and hypothesized population collapses, documentation of externally visible symptoms, co-infection with *Batrachochytrium dendrobatidis* (*Bd*) and presence of other pathogens at some sites, methodological development as well as species distribution models.

The collection of articles in this issue first presents an overview of the occurrence and impact of *Bsal* in wild amphibian populations in Germany (Lötters et al. 2020a), followed by more detailed accounts on the situation in the Ruhr District (Schulz et al. 2020) and the southern Eifel region (Sandvoß et al. 2020). Schmeller et al. (2020) and Thein et al. (2020) report two very recent *Bsal* outbreaks from southern Germany. Wagner et al. (2020a, b) report on conclusions on European fire salamander habitat requirements and hypothesized extinctions derived from in-depth surveys of their larvae. Jung et al. (2020) add further information on the (non-)occurrence of *Bsal* in several captive collections. Lötters et al. (2020b) provide evidence for the earliest occurrence of *Bsal* in Europe from histopathological evidence, and Sachs et al. (2020) summarize the results of a pathogen screening on amphibians in the city of Cologne, not far from the known *Bsal* outbreaks, where widespread *Bd* infection as well as other potentially harmful microorganisms were detected. Finally, Wagner et al. (2020c) contribute to methodological improvements of salamander monitoring at the *Bsal* expansion front by providing comparative data on different methods that are available to estimate larval population sizes of European fire salamanders.

Many of the papers in this issue include, on purpose, a large amount of 'anecdotal' information which will require additional scrutiny from future studies, but may help to inform and guide these. This applies to the apparent disappearance of European fire salamanders in the southern Eifel region – we are aware that absence of a species is always hard to demonstrate, and even more so in elusive salamanders. It also includes the apparent infection of an anuran host, *Rana temporaria*, with *Bsal* – although confirmed by independent analyses in two laboratories, this single case will require confirmation from additional research. We also find that *Bsal* has been present in Germany since at least 2004: confirmed for the Eifel by histopathology, and possibly – and thus in need of confirmation – also in the Ruhr District as indicated by photos.

By making all these data and results available, we hope to draw the attention to the threats that salamander and newt populations are facing in Germany by the salamander plague, and we are confident that having all this information available will stimulate future research and proactive conservation management. In the next future our goal must go beyond documenting declines towards understanding spatio-temporal disease dynamics and the factors influencing the spread and impact of *Bsal* in different situations. The data we assembled over the past years and which we summarized in this issue of SALAMANDRA are reason for both despair and hope. On one hand, drastic and extremely fast population declines of European fire salamanders caused by *Bsal* are a reality, and probably the pathogen has led to the regional extinction of the species in large parts of the southern Eifel region. On the other hand, even in the strongly affected European fire salamander sites in the Ruhr District, a few salamanders can still be seen 2–3 years after the disease struck, and in the northern Eifel, populations where the pathogen was detected still thrive – although often at low numbers – several years later. And, apparently, at least some Central European species of newts can survive in considerable population sizes despite the presence of the pathogen, suggesting that not all European caudate species will be fatally affected by *Bsal*. However, there is reason to be seriously concerned about the northern crested newt (*Triturus cristatus*) which appears to be affected by population declines when *Bsal* appears.

Ultimately, we need to scientifically inform conservation managers on habitat management strategies advisable to ensure survival of European fire salamander and other caudate populations in face of *Bsal*. Should we embrace – in the short term – the isolation of small salamander populations to avoid the spread of the pathogen into them? Or do metapopulations offer better opportunities for decline-recovery dynamics allowing for coexistence of pathogen and host? Will increased temperature and drought – in the context of climate change, or from active measures such as partial forest clearance – affect the pathogen's long-term survival, or rather be an additional threat for the salamander populations? Which management units are worthy of ex situ preservation, and which re-introduction strategies should be followed after ex situ breeding?

As claimed by Lötters et al. (2020a) in this issue, a national *Bsal* Action Plan is needed to coordinate and prioritize the next steps in research, but especially in conservation of salamanders and newts in Germany. A long-term monitoring plan is necessary to understand not only the spread of the pathogen but also possible recovery of populations that have been affected by declines. An ex situ emergency strategy is needed to preserve genetic Management Units of especially the European fire salamander if local extinctions cannot be halted. And for all this, large-scale diagnostic capacities are necessary to allow for quick testing of suspicious cases of salamander mortality, as well as for continued testing of long-term monitored populations. We hope this collection of articles constitutes a first step towards such a German *Bsal* Action Plan.

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SALAMANDRA **56**(3): 173–188
15 August 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

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

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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 Larvenerhebungen) 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 congenerics, 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.

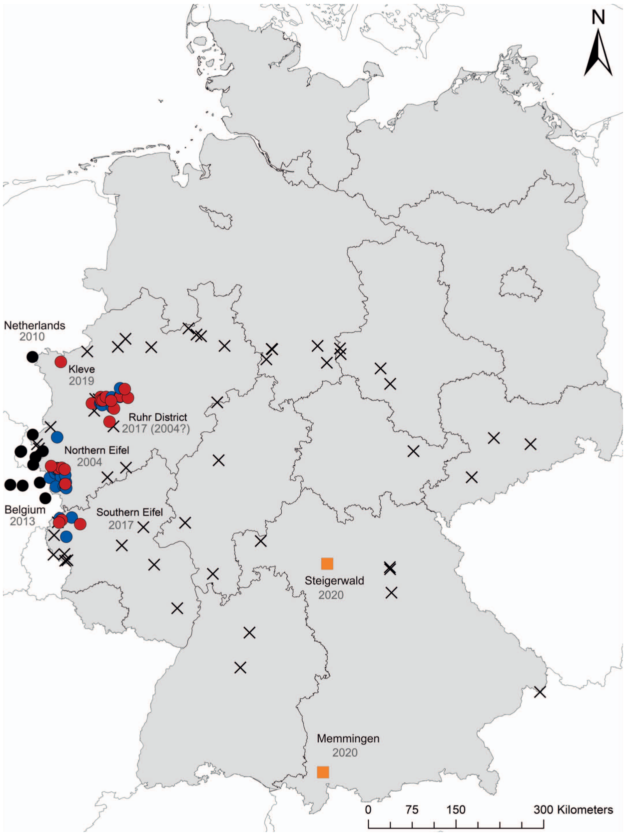

Figure 1. A map of Germany with its federal states showing sites tested positive for *Bsal* until 2018 (blue circles) and since 2019 (red circles); in part these are new records (Supplementary document 3). The two sites in southern Germany (orange squares) are new records reported by Schmeller et al. (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 lead 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^2 (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).

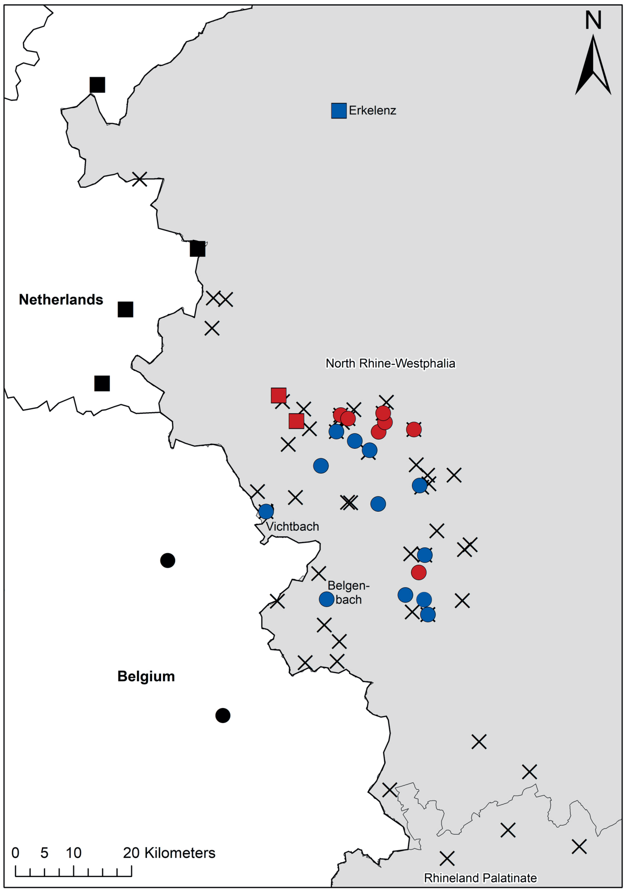

Figure 2. Schematic map of the northern Eifel (see Fig. 1) and the surrounding areas with sites that tested positive for *Bsal* until 2018 (blue symbols) and since 2019 (red symbols); in part these are new records (Supplementary document 3). Known *Bsal* sites outside Germany are indicated by black symbols. 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-distance 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. Blooi 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-

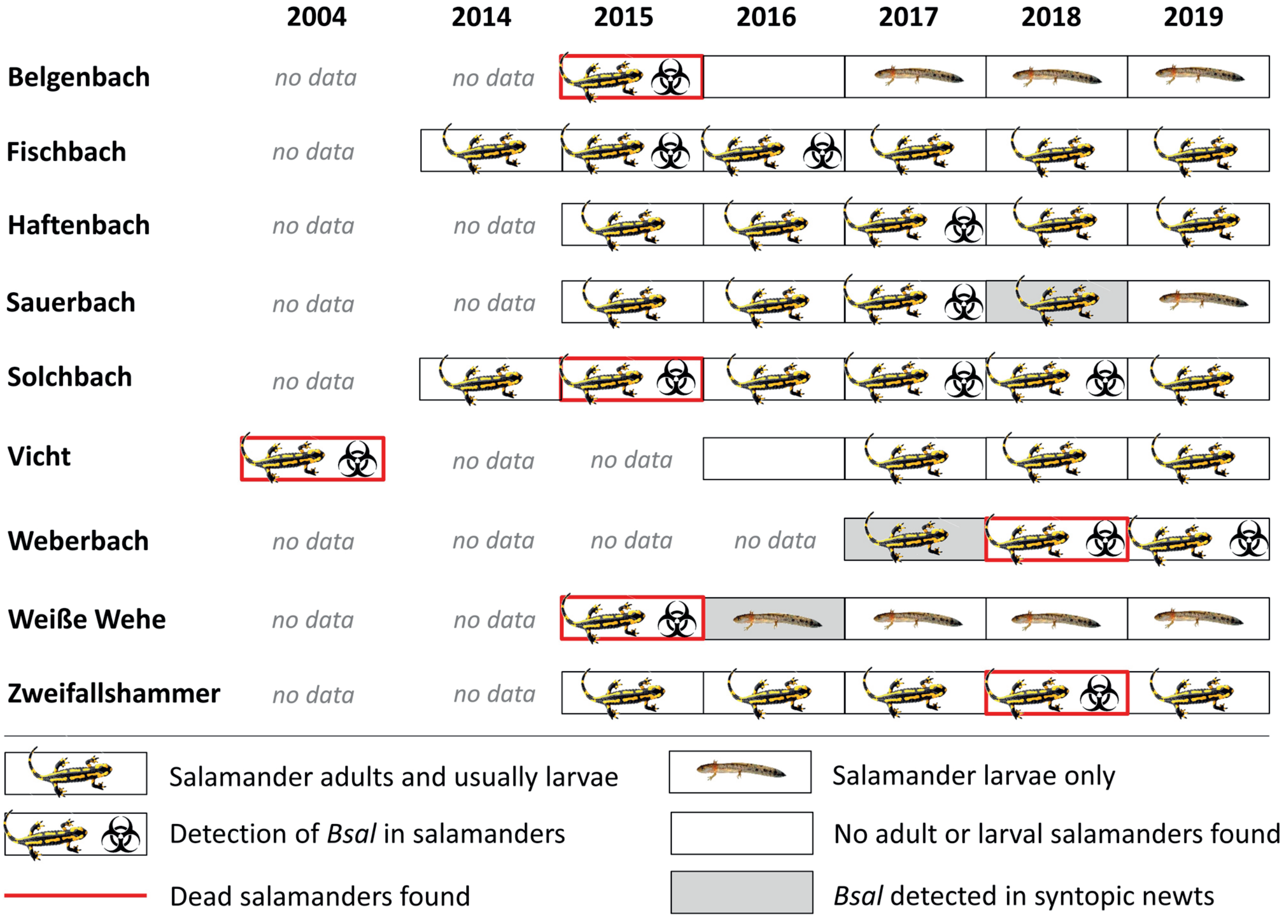

Figure 3. Graph showing European fire salamander and *Bsal* detection history at selected northern Eifel study sites (cf. Supplementary documents 1–4).

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).

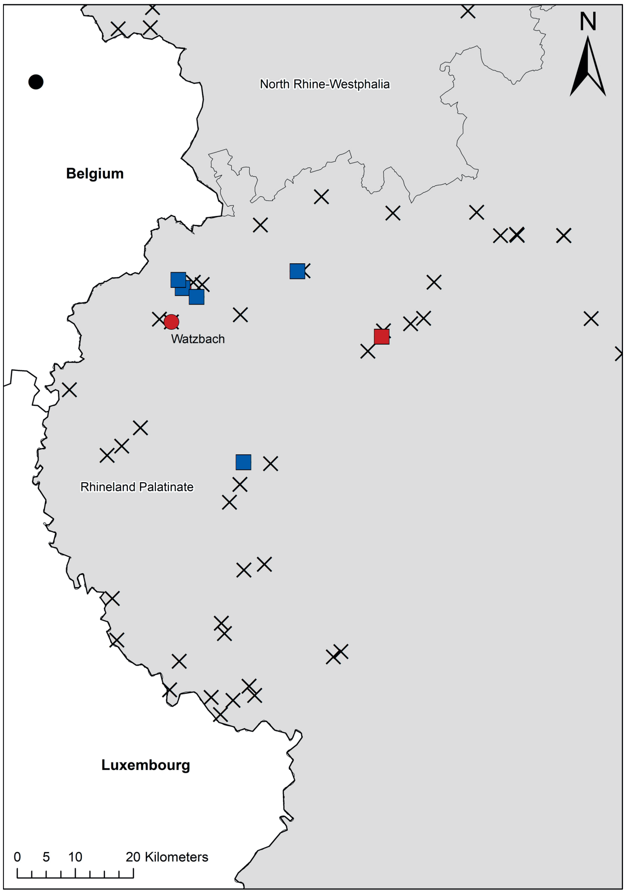

Figure 4. Schematic map of the southern Eifel (see Fig. 1) and the surrounding areas with sites that tested positive for *Bsal* until 2018 (blue symbols) and since 2019 (red symbols); in part these are new records (Supplementary document 3). Known *Bsal* sites outside Germany are indicated by black symbols. 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.

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 (Lötters 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 Lötters 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.

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

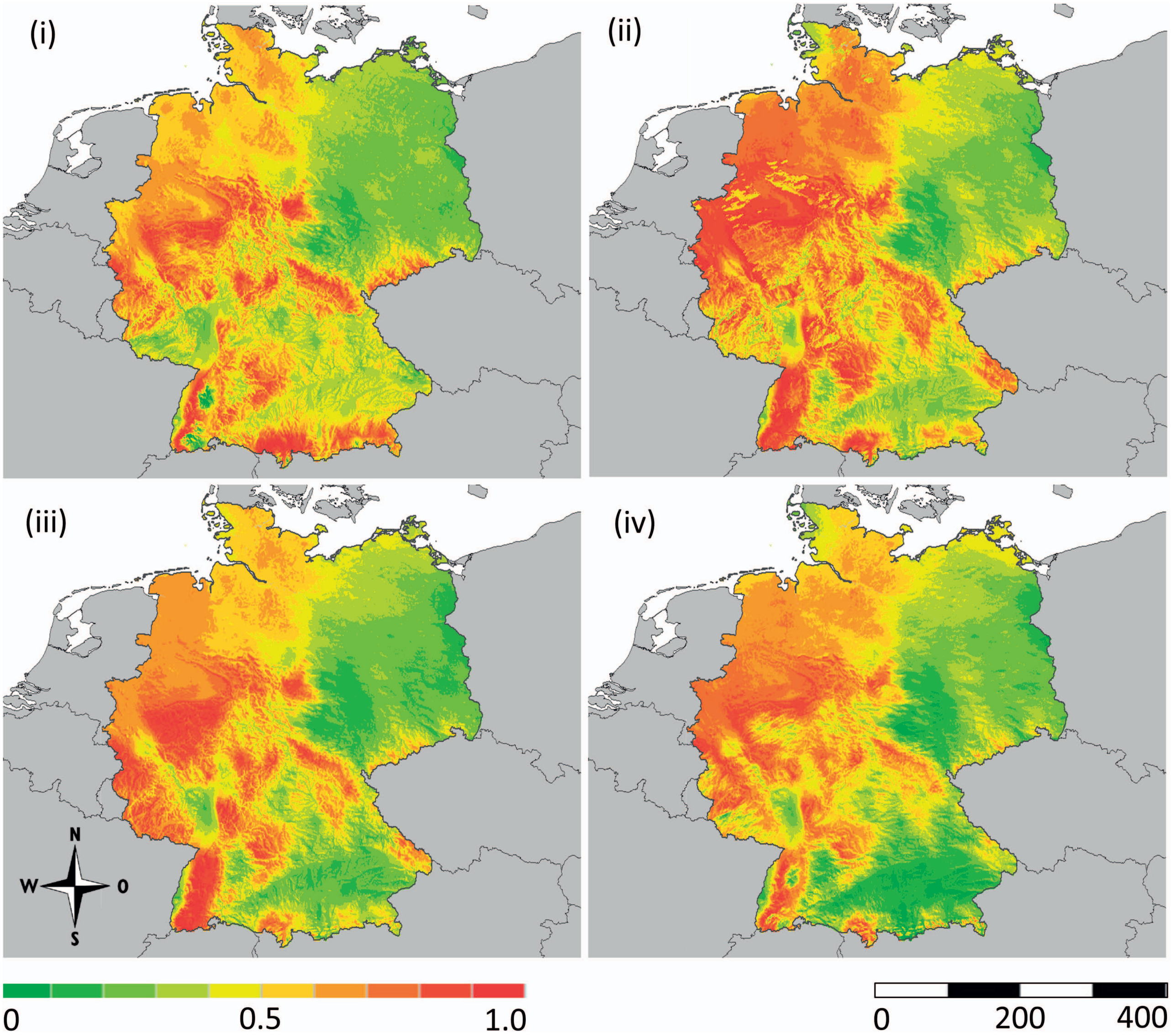

Figure 5. Mapped output of four Maxent species distribution models for *Bsal* in Germany, built with > 100 native and invasive records and different combinations of CHELSA bioclim variables. Warmer colours indicate higher suitability (ranging 0–1). Note that while these models provide insight into particularly 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) (Feldmeier 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).

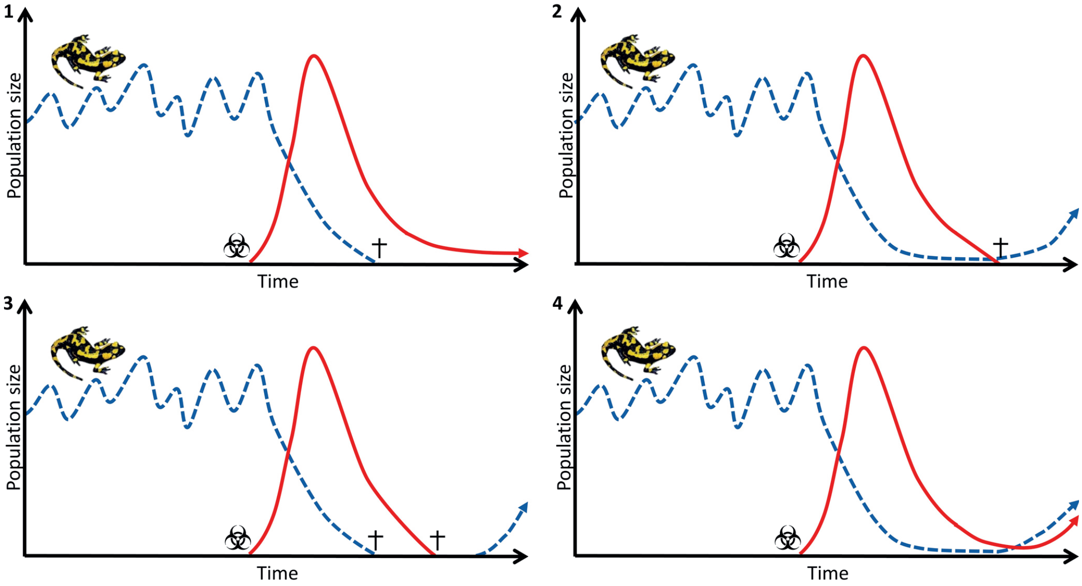

Figure 6. Graphs showing four possible, hypothetical schematic scenarios of host-pathogen relationship in the case of *Bsal* (red) entering a European fire salamander population (blue). A cross represents an extirpation of either the host or the pathogen.

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 susceptibility 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 variability 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).

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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.

Supplementary document 1. Published site records (populations) of caudate species from Germany in which *Bsal* was detected until 2018. Data mostly summarized from Spitzen-van der Sluijs et al. (2016), Dalbeck et al. (2018), Lötters et al. (2018), Schulz et al. (2018) and Wagner et al. (2019a). In addition, new findings from the ongoing laboratory testing (especially quality assurance) of samples collected in same time frame were also included, so that some entries differ from those in the mentioned articles. Specimens tested positive for *Bd/Bsal* and negative for only *Bd* are indicated under remarks. Legend: † = dead specimen(s); + = ‘low’ infection load (1–10 GE); ++ = ‘medium’ infection load (> 10–100 GE); +++ = ‘high’ infection load (> 100 GE); CI = credible interval per year.

Site	District	Coordinates (latitude, longitude)	Species	Year	N samples	N samples tested Bsal-positive	Infection loads	Prevalence per year	95% Bayesian CI	Remarks
Northern Eifel										
North Rhine-Westphalia, Belgenbach	StädteRegion Aachen	50.578169, 6.278448	Fire salamander, *Salamandra salamandra*	2015	22 (of which 16 †)	21 (of which 16 †)		96%	79–99%	mass mortality, 8 of 16 specimens had *Bd/Bsal* co-infections
			Fire salamander, *Salamandra salamandra*	2017	12 larvae	0	0	0%	0–26%	
North Rhine-Westphalia, Brockenberg	StädteRegion Aachen	50.746724, 6.232078	Northern crested newt, *Triturus cristatus*	2015	2	0	0	0%	0–70%	
			Northern crested newt, *Triturus cristatus*	2016	3	0	0	6%	2–24%	
			Smooth newt, *Lissotriton vulgaris*		15	1	++’			
			Northern crested newt, *Triturus cristatus*	2017	3	3	+++’	93%	83–97%	
			Smooth newt, *Lissotriton vulgaris*		43	40	+’ to ‘++’			
			Palmate newt, *Lissotriton helveticus*		7	6	+’ to ‘++’			
			Northern crested newt, *Triturus cristatus*	2018	1 (of which 1 †)	1 (of which 1 †)	+++’	6%	2–38%	*Bd/Bsal* co-infection, no other specimens than one dead-found
			Smooth newt, *Lissotriton vulgaris*		16	0	0			
North Rhine-Westphalia, Fischbach	StädteRegion Aachen	50.735368, 6.294149	Fire salamander, *Salamandra salamandra*	2014	26	0	0	0%	0–13%	
			Fire salamander, *Salamandra salamandra*	2015	59 (of which 1 †)	1 (of which 1 †)	+++’	2%	1–9%	
			Fire salamander, *Salamandra salamandra*	2016	30 (of which 2 †)	4 (of which 2 †)	+’ to ‘+++’	13%	1–30%	both dead-found specimens with medium infection loads, but both already decayed, all *Bd*-negative
			Fire salamander, *Salamandra salamandra*	2017	23	0	0	0%	0–14%	
			Fire salamander, *Salamandra salamandra*	2018	43	0	0	0%	0–8%	22 swabbed in spring, 21 in autumn
			Palmate newt, *Lissotriton helveticus*	2018	1	0	0			
North Rhine-Westphalia, Solchbach	StädteRegion Aachen	50.70178, 6.270098	Fire salamander, *Salamandra salamandra*	2014	26	0		0%	0–13%	
			Fire salamander, *Salamandra salamandra*	2015	20 (of which 1 †)	1 (of which 1 †)	+’	5%	1–25%	
			Fire salamander, *Salamandra salamandra*	2016	5	0		0%	0–31%	
			Fire salamander, *Salamandra salamandra*	2017	45	2	+’	4%	2–15%	
			Fire salamander, *Salamandra salamandra*	2018	50	1	+++’	2%	1–11%	25 swabbed in spring, 25 in autumn
			Alpine newt, *Ichthyosaura alpestris*		1	0	0			
			Palmate newt, *Lissotriton helveticus*		1	0	0			
North Rhine-Westphalia, Vichtbach	StädteRegion Aachen	50.676448, 6.198452	Fire salamander, *Salamandra salamandra*	2017	16 adults, 15 larvae	0	0	0%	0–11%	mass mortality in 2004, *Bsal*-record in 2 formalin-fixed specimen (see text)
			Fire salamander, *Salamandra salamandra*	2018	29	0	0	0%	0–11%	23 swabbed in spring, 6 in autumn, all *Bd*-negative

Site	District	Coordinates (latitude, longitude)	Species	Year	N samples	N samples tested Bsal-positive	Infection loads	Prevalence per year	95% Bayesian CI	Remarks
North Rhine-Westphalia, Rote Wehe	Düren	50.726667, 6.323306	Alpine newt, *Ichthyosaura alpestris*	2017	30	1	+’	1%	0–6%	
			Palmate newt, *Lissotriton helveticus*		57	0	0			
			Alpine newt, *Ichthyosaura alpestris*	2018	38	0	0	0%	0–6%	
			Palmate newt, *Lissotriton helveticus*		20	0	0			
North Rhine-Westphalia, Simonskall	Düren	50.664329, 6.358932	Fire salamander, *Salamandra salamandra*	2016	1 (of which 1 †)	1 (of which 1 †)	++’	100%		all *Bd*-negative
North Rhine-Westphalia, Teufelspütz	Düren	50.747069, 6.366387	Alpine newt, *Ichthyosaura alpestris*	2016	23	0	0	0%	0–7%	
			Palmate newt, *Lissotriton helveticus*		24	0	0			
			Alpine newt, *Ichthyosaura alpestris*	2017	20	0	0	0%	0–9%	
			Palmate newt, *Lissotriton helveticus*		20	0	0			
			Smooth newt, *Lissotriton vulgaris*	2018	2	0	0	3%	1–11%	
			Alpine newt, *Ichthyosaura alpestris*		30	2	+’			
			Palmate newt, *Lissotriton helveticus*		30	0	0			
North Rhine-Westphalia, Weberbach	Düren	50.735089, 6.359651	Fire salamander, *Salamandra salamandra*	2017	23	0	0	21%	14–32%	
			Alpine newt, *Ichthyosaura alpestris*		17	13	+’ to ‘+++’			
			Palmate newt, *Lissotriton helveticus*		30	2	+’			
			Fire salamander, *Salamandra salamandra*	2018	32 (of which 1 †)	6 (of which 1 †)	+’ to ‘+++’	9%	5–17%	dead fire salamander highly infected, all *Bd*-negative
			Alpine newt, *Ichthyosaura alpestris*		31	3	+’			
			Palmate newt, *Lissotriton helveticus*		33	0	0			
North Rhine-Westphalia, Weiße Wehe	Düren	50.717116, 6.345695	Fire salamander, *Salamandra salamandra*	2015	11	2	+’	18%	5–50%	all *Bd*-negative
			Alpine newt, *Ichthyosaura alpestris*	2016	6	1	+’	30%	11–61%	
			Palmate newt, *Lissotriton helveticus*		4	2	+’			
			Alpine or palmate newt	2017	41	0	0	0%	0–8%	
			Alpine newt, *Ichthyosaura alpestris*	2018	15	0	0	0%	0–11%	
			Palmate newt, *Lissotriton helveticus*		15	0	0			
North Rhine-Westphalia, Zweifallshammer	Düren	50.684688, 6.412117	Fire salamander, *Salamandra salamandra*	2015	37	0	0	0%	0–9%	
			Fire salamander, *Salamandra salamandra*	2016	14	0	0	0%	0–20%	
			Fire salamander, *Salamandra salamandra*	2017	15	0	0	0%	0–19%	
			Fire salamander, *Salamandra salamandra*	2018	25 (of which 2 †)	2 (of which 1 †)	++’-’+++’	8%	2–25%	1 dead salamander from spring highly infected

Site	District	Coordinates (latitude, longitude)	Species	Year	N samples	N samples tested Bsal-positive	Infection loads	Prevalence per year	95% Bayesian CI	Remarks
North Rhine-Westphalia, Haftenbach	Nationalpark Eifel	50.613929, 6.431371	Fire salamander, *Salamandra salamandra*	2015	46	0	0	0%	0–8%	
			Fire salamander, *Salamandra salamandra*	2016	23	0	0	0%	0–14%	
			Fire salamander, *Salamandra salamandra*	2017	31	3	+’ and ‘+++’			all *Bd*-negative
			Fire salamander, *Salamandra salamandra*	2018	15	0	0	0%	0–11%	
			Palmate newt, *Lissotriton helveticus*		19	0	0			7 swabbed in spring, 12 in autumn
North Rhine-Westphalia, NP Helingsbach Amphibienteich	Nationalpark Eifel	50.570040, 6.430098	Smooth newt, *Lissotriton vulgaris*	2017	3	2	++’ to ‘+++’	40%	11–77%	
			Alpine newt, *Ichthyosaura alpestris*		2	0				
North Rhine-Westphalia, NP Helingsbach Artenschutz- und Quellteich	Nationalpark Eifel	50.555410, 6.436180	Northern rested newt, *Triturus cristatus*	2016	10	0	0	0%	0–8%	
			Smooth newt, *Lissotriton vulgaris*		11	0	0			
			Alpine newt, *Ichthyosaura alpestris*		10	0	0			
			Northern crested newt, *Triturus cristatus*	2017	15	14	+’ to ‘+++’	74%	52–88%	
			Smooth newt, *Lissotriton vulgaris*		3	0	0			
			Alpine newt, *Ichthyosaura alpestris*		1	0	0			
			Northern crested newt, *Triturus cristatus*	2018	11	11	+’ to ‘+++’	88%	64–96%	
			Smooth newt, *Lissotriton vulgaris*		2	1	+’			
			Alpine newt, *Ichthyosaura alpestris*		3	2	+’			
North Rhine-Westphalia, Sauerbach	Nationalpark Eifel	50.574681, 6.401072	Fire salamander, *Salamandra salamandra*	2015	23	0	0	0%	0–14%	
			Fire salamander, *Salamandra salamandra*	2016	24	0	0	0%	0–15%	
			Fire salamander, *Salamandra salamandra*	2017	9	2	+++’	22%	7–55%	all *Bd*-negative
			Northern crested newt, *Triturus cristatus*	2018	1	1	+++’	100%		
Southern Eifel										
Rhineland-Palatinate, Alfbach	Bitburg-Prüm	50.266170, 6.341554	Alpine newt, *Ichthyosaura alpestris*	2018	6	0	0	7%	2–29%	
			Palmate newt, *Lissotriton helveticus*		9	1	+’			
Rhineland-Palatinate, Plütscheid	Bitburg-Prüm	50.073109, 6.431563	Alpine newt, *Ichthyosaura alpestris*	2017	3	1	+++’	33%	8–82%	
Rhineland-Palatinate, Schwarzer Mann	Bitburg-Prüm	50.266283, 6.359239	Alpine newt, *Ichthyosaura alpestris*	2017	2	0	0	0%	0–70%	
			Alpine newt, *Ichthyosaura alpestris*	2018	5	1	+’	7%	2–29%	
			Palmate newt, *Lissotriton helveticus*		10	0	0			
Rhineland-Palatinate, Sellerich	Bitburg-Prüm	50.237990, 6.366980	Alpine newt, *Ichthyosaura alpestris*	2018	16	1	+’	4%	7–20%	
			Palmate newt, *Lissotriton helveticus*		8	0	0			

Site	District	Coordinates (latitude, longitude)	Species	Year	N samples	N samples tested Bsal-positive	Infection loads	Prevalence per year	95% Bayesian CI	Remarks
Rhineland-Palatinate, Dreisbach	Vulkaneifel	50.269520, 6.523619	Alpine newt, *Ichthyosaura alpestris*	2018	14	1	++’	5%	2–19%	
			Palmate newt, *Lissotriton helveticus*		23	1	+’			
Ruhr area and vicinities										
North Rhine-Westphalia, Dahlhausen	Bochum	51.435, 7.1385	Fire salamander, *Salamandra salamandra*	2018	1 (of which 1 †)	1 (of which 1 †)	+++’	100%		all *Bd*-negative
North Rhine-Westphalia, Hiltrop	Bochum	51.52021, 7.26652	Fire salamander, *Salamandra salamandra*	2018	1 (of which 1 †)	1 (of which 1 †)	+++’	100%		found in summer
North Rhine-Westphalia, Kalwes	Bochum	51.443396, 7.272145	Fire salamander, *Salamandra salamandra*	2018	2	1	+++’	50%	9–88%	all *Bd*-negative
North Rhine-Westphalia, Querenburg	Bochum	51.441398, 7.264886	Fire salamander, *Salamandra salamandra*	2018	22	1	+++’	5%	1–21%	infected juvenile, *Bd*-negative
North Rhine-Westphalia, Lottental	Bochum	51.437, 7.257333	Fire salamander, *Salamandra salamandra*	2018	6	1	++’	25%	5–71%	all *Bd*-negative
North Rhine-Westphalia, Fulerum Südwestfriedhof	Essen	51.431025, 6.968945	Fire salamander, *Salamandra salamandra*	2018	8	8	+’ to’+++’	100%		over 60 dead-found salamanders during mass mortality event (not sampled), all *Bd*-negative
North Rhine-Westphalia, Ruthertal	Essen	51.382679, 6.972078	Fire salamander, *Salamandra salamandra*	2016	10	0	0	0%	0–31%	strong decayed, observed mass mortality
			Fire salamander, *Salamandra salamandra*	2018	23	1	+++’	4%	1–22%	3 swabbed in spring, 20 in autumn, positive specimen from autumn
North Rhine-Westphalia, Essen Stadtwald	Essen	51.423306, 7.033296	Fire salamander, *Salamandra salamandra*	2017	97	8	++’ to ‘+++’	8%	1–15%	all *Bd*-negative
			Alpine newt, *Ichthyosaura alpestris*		1	0	0			
North Rhine-Westphalia, Stadtwald, Garten	Essen	51.423306, 7.033296	Alpine newt, *Ichthyosaura alpestris*	2018	27	1	+’ and ‘++’	4%	1–18%	22 swabbed in spring, 5 in autumn, positive specimen from spring
North Rhine-Westphalia, Rottbachtal	Mülheim an der Ruhr	51.375838, 6.833334	Fire salamander, *Salamandra salamandra*	2017	30	0	0	0%	0–11%	
			Fire salamander, *Salamandra salamandra*	2018	3	1	+++’	50%	9–88%	swabbed in autumn
			Alpine newt, *Ichthyosaura alpestris*		1	1	+’			swabbed in autumn
North Rhine-Westphalia, Heiligenhaus	Mettmann	51.358604, 6.990914	Fire salamander, *Salamandra salamandra*	2018	2 (of which 2 †)	2 (of which 2 †)	++’ and ‘+++’	100%	26–99%	found in summer
North Rhine-Westphalia, Velbert Deilbach	Mettmann	51.343183, 7.077412	Fire salamander, *Salamandra salamandra*	2018	2 (of which 2 †)	1 (of which 1 †)	++’	50%	9–88%	
Others										
North Rhine-Westphalia, Erkelenz	Heinsberg	51.049363, 6.298195	Fire salamander, *Salamandra salamandra*	2018	7 (of which 1 †)	1 (of which 1 †)	+’	25%	7–61%	from outdoor enclosures, dead positive salamander swabbed in spring, the rest in autumn
			Smooth newt, *Lissotriton vulgaris*		1	1	+’			from a garden pond
				Totally tested	1760	188				

Supplementary document 2. Published site records (populations) of caudate species from Germany in which *Bsal* was not detected until 2018. Data mostly summarized from Spitzen-van der Sluijs et al. (2016), Dalbeck et al. (2018), Lötters et al. (2018), Schulz et al. (2018) and Wagner et al. (2019a). In addition, new data are included. Specimens tested positive or negative for *Bd* are indicated under remarks. Legend as in Table 1.

Site	District	Coordinates (latitude, longitude)	Species	Year	N samples	95% Bayesian CI	Remarks
Northern Eifel							
North Rhine-Westphalia, Binsfeldhammer	StädteRegion Aachen	50.756521, 6.243033	Smooth newt, *Lissotriton vulgaris*	2018	3	0–61%	
North Rhine-Westphalia, Döppeskaul	StädteRegion Aachen	50.509209, 6.295323	Alpine newt, *Ichthyosaura alpestris*	2018	8	0–16%	
			Palmate newt, *Lissotriton helveticus*		12		
North Rhine-Westphalia, Himmelsleiter	StädteRegion Aachen	50.676496, 6.171786	Palmate newt, *Lissotriton helveticus*	2018	19	0–18%	
North Rhine-Westphalia, Holderbach	StädteRegion Aachen	50.548397, 6.292036	Fire salamander, *Salamandra salamandra*	2017	3	0–61%	
			Fire salamander, *Salamandra salamandra*	2018	2	0–70%	
North Rhine-Westphalia, Kallerbach	Düren	50.707151, 6.391554	Fire salamander, *Salamandra salamandra*	2015	24	0–15%	
			Fire salamander, *Salamandra salamandra*	2016	1 adult, 3 larvae	0–30%	adult *Bd*-negative
			Alpine newt, *Ichthyosaura alpestris*		5		
			Fire salamander, *Salamandra salamandra*	2017	2	0–51%	
			Alpine newt, *Ichthyosaura alpestris*		2		
North Rhine-Westphalia, Kitzenhausen	StädteRegion Aachen	50.692644, 6.199071	Fire salamander, *Salamandra salamandra*	2017	8	0–31%	
North Rhine-Westphalia, Konzen	StädteRegion Aachen	50.595878, 6.266865	Smooth newt, *Lissotriton vulgaris*	2018	1	0–16%	
			Alpine newt, *Ichthyosaura alpestris*		8		
			Palmate newt, *Lissotriton helveticus*		11		
North Rhine-Westphalia, Lamersiefen	StädteRegion Aachen	50.756932, 6.321338	Fire salamander, *Salamandra salamandra*	2014	17	0–19%	
			Fire salamander, *Salamandra salamandra*	2015	32	0–11%	
			Fire salamander, *Salamandra salamandra*	2016	8	0–31%	
			Fire salamander, *Salamandra salamandra*	2017	26	0–13%	
			Fire salamander, *Salamandra salamandra*	2018	7	0–36%	all *Bd*-negative
North Rhine-Westphalia, Lensbach	StädteRegion Aachen	50.672648, 6.230209	Fire salamander, *Salamandra salamandra*	2017	3	0–61%	
			Fire salamander, *Salamandra salamandra*	2018	1		
North Rhine-Westphalia, NRW Stiftungsteich	StädteRegion Aachen	50.508012, 6.245711	Palmate newt, *Lissotriton helveticus*	2018	10	0–31%	
North Rhine-Westphalia, Omerbach	StädteRegion Aachen	50.754140, 6.307838	Fire salamander, *Salamandra salamandra*	2018	9	0–9%	*Bsal*-infected newts in 2019
			Alpine newt, *Ichthyosaura alpestris*		2		
			Palmate newt, *Lissotriton helveticus*		27		
North Rhine-Westphalia, Palsen Mützenich	StädteRegion Aachen	50.568565, 6.202638	Alpine newt, *Ichthyosaura alpestris*	2018	12	0–18%	
			Palmate newt, *Lissotriton helveticus*		6		
North Rhine Westphalia, Peterbach	StädteRegion Aachen	50.657733, 6.323633	Alpine newt, *Ichthyosaura alpestris*	2014	4	0–20%	
			Palmate newt, *Lissotriton helveticus*		12		
			Alpine newt, *Ichthyosaura alpestris*	2018	2	0–51%	
			Palmate newt, *Lissotriton helveticus*		2		

Site	District	Coordinates (latitude, longitude)	Species	Year	N samples	95% Bayesian CI	Remarks
North Rhine-Westphalia, Rothsiefen	StädteRegion Aachen	50.734514, 6.30931	Fire salamander, *Salamandra salamandra*	2017	25	0–13%	
North Rhine-Westphalia, Schlangenberg	StädteRegion Aachen	50.738106, 6.252416	Alpine newt, *Ichthyosaura alpestris*	2018	1	0–36%	
			Palmate newt, *Lissotriton helveticus*		6		
North Rhine-Westphalia, Asselbach	Düren	50.71486, 6.34355	Fire salamander, *Salamandra salamandra*	2017	3	0–61%	
North Rhine-Westphalia, Billerbach	Düren	50.619605, 6.494605	Fire salamander, *Salamandra salamandra*	2018	10	0–31%	
North Rhine-Westphalia, Brück	Düren	50.692652, 6.476640	Fire salamander, *Salamandra salamandra*	2018	2	0–70%	
North Rhine-Westphalia, Dresbach	Düren	50.702770, 6.417944	Fire salamander, *Salamandra salamandra*	2015	9	0–30%	
			Fire salamander, *Salamandra salamandra*	2016	2	0–51%	
			Fire salamander, *Salamandra salamandra*	2018	5	0–44%	
Norh Rhine-Westphalia, Drover Heide	Düren	50.722971, 6.533593	Northern crested newt, *Triturus cristatus*	2018	8	0–22%	
			Smooth newt, *Lissotriton vulgaris*		4		
			Palmate newt, *Lissotriton helveticus*		1		
North Rhine-Westphalia, Hasselbach	Düren	50.681882, 6.428362	Fire salamander, *Salamandra salamandra*	2016	6	0–43%	
			Fire salamander, *Salamandra salamandra*	2017	6	0–43%	
			Fire salamander, *Salamandra salamandra*	2018	5	0–44%	
North Rhine-Westphalia, Heimbach Waschbendchen	Düren	50.637806, 6.449917	Fire salamander, *Salamandra salamandra*	2018	1 (of which 1 †)		
North Rhine-Westphalia, Rosbach	Düren	50.692512, 6.435706	Fire salamander, *Salamandra salamandra*	2015	47	0–7%	
			Fire salamander, *Salamandra salamandra*	2016	1		
			Fire salamander, *Salamandra salamandra*	2017	24	0–15%	
			Fire salamander, *Salamandra salamandra*	2018	10	0–31%	all *Bd*-negative
North Rhine-Westphalia, Sandkaulsiefen	Düren	50.789973, 6.375219	Fire salamander, *Salamandra salamandra*	2018	4	0–51%	
North Rhine-Westphalia, Thönbach	Düren	50.747069, 6.366387	Fire salamander, *Salamandra salamandra*	2019	1	0–8%	
			Smooth newt, *Lissotriton vulgaris*		1		
			Alpine newt, *Ichthyosaura alpestris*		19		
			Palmate newt, *Lissotriton helveticus*		20		
North Rhine-Westphalia, Ursprungsbach	Düren	50.764144, 6.371654	Fire salamander, *Salamandra salamandra*	2018	20	0–16%	
Norh Rhine-Westphalia, Weg Kall	Düren	50.680758, 6.426492	Fire salamander, *Salamandra salamandra*	2018	12	0–26%	
North Rhine-Westphalia, Wollebach	Düren	50.737227, 6.413199	Fire salamander, *Salamandra salamandra*	2018	24	0–15%	*Bsal*-infected European fire salamander in 2019
North Rhine-Westphalia, Biberteiche Schafbach	Nationalpark Eifel	50.593152, 6.512388	Alpine newt, *Ichthyosaura alpestris*	2017	1	0–61%	
			Palmate newt, *Lissotriton helveticus*		2		
			Palmate newt, *Lissotriton helveticus*	2018	1		
North Rhine-Westphalia, Burg Vogelsang	Nationalpark Eifel	50.589495, 6.448526	Fire salamander, *Salamandra salamandra*	2018	3	0–61%	
North Rhine-Westphalia, Härtgessief	Nationalpark Eifel	50.550369, 6.358216	Fire salamander, *Salamandra salamandra*	2014	15	0–19%	since 2015, no more European fire salamanders found
North Rhine-Westphalia, Helingsbach Stauteich	Nationalpark Eifel	50.555043, 6.426633	Smooth newt, *Lissotriton vulgaris*	2018	1	0–51%	
			Alpine newt, *Ichthyosaura alpestris*		3		

Site	District	Coordinates (latitude, longitude)	Species	Year	N samples	95% Bayesian CI	Remarks
North Rhine-Westphalia, Neffesgesbach	Nationalpark Eifel	50.583735, 6.440269	Smooth newt, *Lissotriton vulgaris*	2016	1 (of which 1 †)		
North Rhine-Westphalia, Vogelsang Temporärgewässer	Nationalpark Eifel	50.575314, 6.440184	Alpine newt, *Ichthyosaura alpestris*	2016	11	0–18%	
			Palmate newt, *Lissotriton helveticus*		7		
North Rhine-Westphalia, Walbigbach	Nationalpark Eifel	50.624289,6.502341	Fire salamander, *Salamandra salamandra*	2018	12	0–26%	
North Rhine-Westphalia, Bad Münstereifel	Euskirchen	50.525597, 6.787115	Fire salamander, *Salamandra salamandra*	2018	20	0–16%	
North Rhine-Westphalia, NSG Nonnenbachtal	Euskirchen	50.429958, 6.515382	Northern crested newt, *Triturus cristatus*	2018	2	0–70%	
North Rhine-Westphalia, Oberschömbach	Euskirchen	50.400182, 6.593637	Alpine newt, *Ichthyosaura alpestris*	2018	3	0–44%	
			Palmate newt, *Lissotriton helveticus*		2		
Southern Eifel							
Rhineland-Palatinate, Arzfeld	Bitburg-Prüm	50.095484, 6.251196	Fire salamander, *Salamandra salamandra*	2016	3	0–43%	
			Alpine newt, *Ichthyosaura alpestris*		3		
			Palmate newt, *Lissotriton helveticus*	2017	2	0–70%	
Rhineland-Palatinate, Bitburg	Bitburg-Prüm	49.976417, 6.474156	Alpine newt, *Ichthyosaura alpestris*	2017	16	0–12%	
			Palmate newt, *Lissotriton helveticus*		12		
			Alpine newt, *Ichthyosaura alpestris*	2018	15	0–14%	
			Palmate newt, *Lissotriton helveticus*		8		
Rhineland-Palatinate, Bollendorf	Bitburg-Prüm	49.849103, 6.326625	Alpine newt, *Ichthyosaura alpestris*	2017	29	0–6%	
			Palmate newt, *Lissotriton helveticus*		30		
			Alpine newt, *Ichthyosaura alpestris*	2018	3	0–43%	
			Palmate newt, *Lissotriton helveticus*		3 (of which 1 †)		
Rhineland-Palatinate, Daleiden	Bitburg-Prüm	50.073971, 6.163864	Alpine newt, *Ichthyosaura alpestris*	2017	1	0–44%	
			Palmate newt, *Lissotriton helveticus*		4		
Rhineland-Palatinate, Ernzen Gutenbach	Bitburg-Prüm	49.828069, 6.403899	Fire salamander, *Salamandra salamandra*	2015	4	0–51%	
			Fire salamander, *Salamandra salamandra*	2016	16	0–20%	
			Fire salamander, *Salamandra salamandra*	2017	6	0–43%	
			Fire salamander, *Salamandra salamandra*	2018	1		
Rhineland-Palatinate, Fleissbach	Bitburg-Prüm	49.880861, 6.338427	Fire salamander, *Salamandra salamandra*	2017	4	0–51%	
			Fire salamander, *Salamandra salamandra*	2018	5	0–44%	
Rhineland-Palatinate, Fuhrbach	Bitburg-Prüm	50.039648, 6.417938	Alpine newt, *Ichthyosaura alpestris*	2017	2	0–51%	strong decline of European fire salamander larvae 2015-2019, no more adults found in 2019
			Palmate newt, *Lissotriton helveticus*		2		
Rhineland-Palatinate, Gentingen	Bitburg-Prüm	49.902448, 6.243511	Fire salamander, *Salamandra salamandra*	2016	6	0–43%	
			Fire salamander, *Salamandra salamandra*	2017	3	0–61%	

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Rhineland-Palatinate, Großkampenberg	Bitburg-Prüm	50.166200, 6.191891	Alpine newt, *Ichthyosaura alpestris*	2015	4	0–44%	
			Palmate newt, *Lissotriton helveticus*		1		
			Alpine newt, *Ichthyosaura alpestris*	2017	4	0–31%	
			Palmate newt, *Lissotriton helveticus*		4		
Rhineland-Palatinate, Habscheid	Bitburg-Prüm	50.193284, 6.306951	Alpine newt, *Ichthyosaura alpestris*	2017	1		
Rhineland-Palatinate, Hauchenbach	Bitburg-Prüm	49.919317, 6.405269	Fire salamander, *Salamandra salamandra*	2016	22	0–15%	
Rhineland-Palatinate, Heisdorf	Bitburg-Prüm	50.129688, 6.429261	Alpine newt, *Ichthyosaura alpestris*	2017	3	0–43%	
			Palmate newt, *Lissotriton helveticus*		3		
Rhineland-Palatinate, Holsthum	Bitburg-Prüm	49.884984, 6.409135	Palmate newt, *Lissotriton helveticus*	2017	1		
Rhineland-Palatinate, Hütten	Bitburg-Prüm	50.011122, 6.244127	Alpine newt, *Ichthyosaura alpestris*	2017	2	0–51%	
			Palmate newt, *Lissotriton helveticus*		2		
Rhineland-Palatinate, Idenheim	Bitburg-Prüm	49.890914, 6.590448	Alpine newt, *Ichthyosaura alpestris*	2017	12	0–16%	
			Palmate newt, *Lissotriton helveticus*		4		
Rhineland-Palatinate, Ingendorf	Bitburg-Prüm	49.920602, 6.435246	Alpine newt, *Ichthyosaura alpestris*	2017	1		
Rhineland-Palatinate, Irrel	Bitburg-Prüm	49.856404, 6.448138	Alpine newt, *Ichthyosaura alpestris*	2015	1		
			Fire salamander, *Salamandra salamandra*	2016	16	0–20%	
			Fire salamander, *Salamandra salamandra*	2017	15	0–19%	
			Palmate newt, *Lissotriton helveticus*		2		
			Fire salamander, *Salamandra salamandra*	2018			
Rhineland-Palatinate, Körperich	Bitburg-Prüm	49.925429, 6.284984	Alpine newt, *Ichthyosaura alpestris*	2017	16	0–18%	
			Palmate newt, *Lissotriton helveticus*		2		
Rhineland-Palatinate, Läuskopfbach	Bitburg-Prüm	50.057222, 6.434027	Alpine newt, *Ichthyosaura alpestris*	2017	7	0–14%	strong decline of European fire salamander larvae 2015-2019, no more adults found in 2019
			Palmate newt, *Lissotriton helveticus*		16		
			Alpine newt, *Ichthyosaura alpestris*	2018	4	0–16%	
			Palmate newt, *Lissotriton helveticus*		17		
Rhineland-Palatinate, Lichtenborn	Bitburg-Prüm	50.113526, 6.280058	Fire salamander, *Salamandra salamandra*	2016	1 (of which 1 †)	0–15%	
			Alpine newt, *Ichthyosaura alpestris*		10		
			Palmate newt, *Lissotriton helveticus*		11		
			Fire salamander, *Salamandra salamandra*	2018	31	0–6%	
			Alpine newt, *Ichthyosaura alpestris*		17		
			Palmate newt, *Lissotriton helveticus*		14		
Rhineland-Palatinate, Lindscheid	Bitburg-Prüm	50.278656, 6.292373	Alpine newt, *Ichthyosaura alpestris*	2017	1		
Rhineland-Palatinate, Lützkampen	Bitburg-Prüm	50.152251, 6.170181	Alpine newt, *Ichthyosaura alpestris*	2015	8	0–31%	
			Alpine newt, *Ichthyosaura alpestris*	2017	13	0–19%	
			Palmate newt, *Lissotriton helveticus*		2		

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Rhineland-Palatinate, Luppertsseifen	Bitburg-Prüm	50.035977, 6.372919	Alpine newt, *Ichthyosaura alpestris*	2017	15	0–16%	
			Palmate newt, *Lissotriton helveticus*		16		
Rhineland-Palatinate, Neuendorf	Bitburg-Prüm	50.296815, 6.462875	Alpine newt, *Ichthyosaura alpestris*	2017	10	0–16%	
			Palmate newt, *Lissotriton helveticus*		10		
Rhineland-Palatinate, Nimshuscheid	Bitburg-Prüm	50.081105, 6.474474	Alpine newt, *Ichthyosaura alpestris*	2017	8	0–26%	
			Palmate newt, *Lissotriton helveticus*		3		
Rhineland-Palatinate, Obermehlen	Bitburg-Prüm	50.239439, 6.396522	Alpine newt, *Ichthyosaura alpestris*	2017	3	0–44%	
			Palmate newt, *Lissotriton helveticus*		2		
Rhineland-Palatinate, Obersgegen	Bitburg-Prüm	49.943828, 6.236489	Palmate newt, *Lissotriton helveticus*	2017	9	0–30%	
			Palmate newt, *Lissotriton helveticus*	2018	1		
Rhineland-Palatinate, Olmscheid, Hof Klingendell	Bitburg-Prüm	50.053375, 6.213533	Fire salamander, *Salamandra salamandra*	2016	4	0–51%	
Rhineland-Palatinate, Prümzurlay	Bitburg-Prüm	49.880094, 6.426085	Alpine newt, *Ichthyosaura alpestris*	2017	26	0–11%	
			Palmate newt, *Lissotriton helveticus*		6		
Rhineland-Palatinate, Ralingen	Bitburg-Prüm	49.810117, 6.530386	Fire salamander, *Salamandra salamandra*	2016	3 (of which 1 †)	0–61%	
Rhineland-Palatinate, Reiff	Bitburg-Prüm	50.102032, 6.179619	Alpine newt, *Ichthyosaura alpestris*	2017	14	0–20%	
			Palmate newt, *Lissotriton helveticus*		2		
Rhineland-Palatinate, Sefferweich	Bitburg-Prüm	50.045353, 6.53039	Smooth newt, *Lissotriton vulgaris*	2017	3	0–44%	
			Alpine newt, *Ichthyosaura alpestris*		1		
			Palmate newt, *Lissotriton helveticus*		1		
Rhineland-Palatinate, Spielmannsholz	Bitburg-Prüm	50.14179, 6.284316	Alpine newt, *Ichthyosaura alpestris*	2017	4	0–43%	
			Palmate newt, *Lissotriton helveticus*		2		
Rhineland-Palatinate, Steinmehlen	Bitburg-Prüm	50.209456, 6.346912	Alpine newt, *Ichthyosaura alpestris*	2017	1		
Rhineland-Palatinate, Mauel, Steuenbach	Bitburg-Prüm	50.080676, 6.390217	Fire salamander, *Salamandra salamandra*	2016	7	0–36%	strong decline of European fire salamander larvae 2015-2019, no more adults found in 2019
Rhineland-Palatinate, Watzbach	Bitburg-Prüm	50.215607, 6.328830	Fire salamander, *Salamandra salamandra*	2016	2	0–44%	*Bsal*-infected European fire salamanders in 2019
			Alpine newt, *Ichthyosaura alpestris*		3		
			Fire salamander, *Salamandra salamandra*	2017	33	0–10%	
			Alpine newt, *Ichthyosaura alpestris*		2		
			Fire salamander, *Salamandra salamandra*	2018	27	0–32%	
			Alpine newt, *Ichthyosaura alpestris*		5		
Rhineland-Palatinate, Weilerbach	Bitburg-Prüm	49.845261, 6.389539	Alpine newt, *Ichthyosaura alpestris*	2015	2	0–31%	
			Palmate newt, *Lissotriton helveticus*		8		
			Alpine newt, *Ichthyosaura alpestris*	2017	6	0–26%	
			Palmate newt, *Lissotriton helveticus*		6		
			Palmate newt, *Lissotriton helveticus*	2018	4	0–51%	

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Rhineland-Palatinate, Wiesbaum	Bitburg-Prüm	50.326250, 6.670817	Alpine newt, *Ichthyosaura alpestris*	2018	5	0–44%	no European fire salamander larvae found in 2019
Rhineland-Palatinate, Winterscheid	Bitburg-Prüm	50.248808, 6.227066	Alpine newt, *Ichthyosaura alpestris*	2017	11	0–22%	
			Palmate newt, *Lissotriton helveticus*		2		
Rhineland-Palatinate, Winterspelt	Bitburg-Prüm	50.213135, 6.190452	Palmate newt, *Lissotriton helveticus*	2017	1		
Rhineland-Palatinate, Wolfsschlucht Prüm	Bitburg-Prüm	50.225602, 6.434867	Palmate newt, *Lissotriton helveticus*	2018	32	0–11%	
Rhineland-Palatinate, Bodenbach	Vulkaneifel	50.304250, 6.863183	Alpine newt, *Ichthyosaura alpestris*	2018	2	0–70%	no European fire salamander larvae found in 2019
Rhineland-Palatinate, Borler	Vulkaneifel	50.304050, 6.837533	Palmate newt, *Lissotriton helveticus*	2018	1		
Rhineland-Palatinate, Essingen	Vulkaneifel	50.257928, 6.734642	Alpine newt, *Ichthyosaura alpestris*	2018	12	0–20%	
			Palmate newt, *Lissotriton helveticus*		2		
Rhineland-Palatinate, Jünkerath	Vulkaneifel	50.342486, 6.560344	Alpine newt, *Ichthyosaura alpestris*	2018	28	0–11%	
			Palmate newt, *Lissotriton helveticus*		6		
Rhineland-Palatinate, Lindenquelle	Vulkaneifel	50.189603, 6.632222	Smooth newt, *Lissotriton vulgaris*	2018	5	0–44%	
Rhineland-Palatinate, Nohn	Vulkaneifel	50.327002, 6.800583	Palmate newt, *Lissotriton helveticus*	2018	6	0–43%	
Rhineland-Palatinate, Prümquelle	Vulkaneifel	50.3147510, 6.4617130	Alpine newt, *Ichthyosaura alpestris*	2017	16	0–13%	
			Palmate newt, *Lissotriton helveticus*		9		
			Alpine newt, *Ichthyosaura alpestris*	2018	8	0–15%	
			Palmate newt, *Lissotriton helveticus*		14		
Rhineland-Palatinate, Zermüllen	Vulkaneifel	50.303933, 6.935633	Alpine newt, *Ichthyosaura alpestris*	2018	2	0–70%	
Ruhr area and vicinities							
North Rhine-Westphalia, Siepental	Bochum	51.437833, 7.2525	Fire salamander, *Salamandra salamandra*	2018	9	0–30%	
North Rhine-Westphalia, Stiepel	Bochum	51.418056, 7.250944	Fire salamander, *Salamandra salamandra*	2018	2	0–70%	
North Rhine-Westphalia, Byfang	Essen	51.392093, 7.112520	Alpine newt, *Ichthyosaura alpestris*	2018	3	0–51%	
			Palmate newt, *Lissotriton helveticus*		1		
North Rhine-Westphalia, Bredeney	Essen	51.403281, 6.995988	Fire salamander, *Salamandra salamandra*	2018	30	0–11%	swabbed in summer and autumn
North Rhine-Westphalia, Fischlaken	Essen	51.378457, 7.057662	Fire salamander, *Salamandra salamandra*	2017	1 (of which 1 †)		
North Rhine-Westphalia, Schellenberg	Essen	51.413459, 7.043988	Fire salamander, *Salamandra salamandra*	2018	12	0–26%	swabbed in autumn
North Rhine-Westphalia, Rumbachtal	Mülheim an der Ruhr	51.418650, 6.930562	Fire salamander, *Salamandra salamandra*	2017	7	0–36%	
			Fire salamander, *Salamandra salamandra*	2018	2	0–70%	swabbed in summer
North Rhine-Westphalia, Hattingen Deilbach	Ennepe-Ruhr-Kreis	51.401582, 7.126877	Alpine newt, *Ichthyosaura alpestris*	2018	3 (of which 3 †)	0–51%	
			Palmate newt, *Lissotriton helveticus*		1		
North Rhine-Westphalia, Hagen	Hagen	51.387469, 7.463797	Alpine newt, *Ichthyosaura alpestris*	2018	2 (of which 2 †)	0–70%	
North Rhine-Westphalia, Vonderort	Oberhausen	51.505806, 6.902312	Fire salamander, *Salamandra salamandra*	2018	7	0–36%	swabbed in autumn
North Rhine-Westphalia, Ratingen	Mettmann	51.321740, 6.858843	Fire salamander, *Salamandra salamandra*	2017	22	0–15%	
			Fire salamander, *Salamandra salamandra*	2018	10	0–31%	3 swabbed in spring, 7 in autumn

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North Rhine-Westphalia, Velbert Bleibergquelle	Mettmann	51.343183, 7.077412	Fire salamander, *Salamandra salamandra*	2018	1 (of which 1 †)		
North Rhine-Westphalia, Selm Funne	Unna	51.666870, 7.551980	Fire salamander, *Salamandra salamandra*	2018	23	0–14%	
North Rhine-Westphalia, Selm Gerlingsbach	Unna	51.656975, 7.550057	Fire salamander, *Salamandra salamandra*	2018	3	0–51%	
			Alpine newt, *Ichthyosaura alpestris*		1		
North Rhine-Westphalia, Selm Paßbach	Unna	51.669187, 7.525273	Fire salamander, *Salamandra salamandra*	2018	23	0–14%	
North Rhine-Westphalia, Barmen	Wuppertal	51.247764, 7.168684	Fire salamander, *Salamandra salamandra*	2018	11	0–20%	1 specimen *Bd*-positive
			Alpine newt, *Ichthyosaura alpestris*		3		
Additional sites (various federal states)							
Baden-Wuerttemberg, Heidelberg	Heidelberg	not available	Fire salamander, *Salamandra salamandra*	2016	4	0–51%	
Baden-Wuerttemberg, Heilbronn	Heilbronn	49.126126, 9.255500	Fire salamander, *Salamandra salamandra*	2017	10	0–30%	
Baden-Wuerttemberg, Stuttgart	Stuttgart	48.772791, 9.115469	Fire salamander, *Salamandra salamandra*	2017	30	0–11%	
Bavaria, Pottenstein	Bayreuth	49.778186, 11.413166	Fire salamander, *Salamandra salamandra*	2017	40	0–9%	
Bavaria, Wiesthal	Main-Spessart	50.037548, 9.428539	Fire salamander, *Salamandra salamandra*	2017	10	0–30%	
Bavaria, Hersbrucker Alb	Nürnberger Land	49.525000, 11.437374	Fire salamander, *Salamandra salamandra*	2017	30	0–11%	
Bavaria, Obernzell Donauleiten	Passau	48.525530, 13.722739	Fire salamander, *Salamandra salamandra*	2017	30	0–11%	
Hesse, Felsenmeer, Lauertal	Bergstraße	49.71231, 8.692314	Fire salamander, *Salamandra salamandra*	2017	30	0–11%	
Hesse, Darmstadt	Darmstadt	49.869167, 8.678889	Fire salamander, *Salamandra salamandra*	2018	1 (of which 1 †)		
Hesse, Marburg	Marburg-Biedenkopf	50.826739, 8.781213	Fire salamander, *Salamandra salamandra*	2017	17	0–19%	
Hesse, Idstein	Rheingau-Taunus-Kreis	50.217173, 8.265894	Fire salamander, *Salamandra salamandra*	2017	30	0–11%	
Lower Saxony, Kleiwiesen	Braunschweig	52.32964, 10.58211	Northern crested newt, *Triturus cristatus*	2015	27	0–2%	
			Smooth newt, *Lissotriton vulgaris*		117		
			Alpine newt, *Ichthyosaura alpestris*		27		
Lower Saxony, Bad Harzburg	Goslar	51.898946, 10.646149	Fire salamander, *Salamandra salamandra*	2017	5	0–44%	
Lower Saxony, Clausthal-Zellerfeld	Goslar	51.762997, 10.445509	Fire salamander, *Salamandra salamandra*	2017	39	0–9%	
Lower Saxony, Wolfshagen	Goslar	51.923595, 10.303128	Fire salamander, *Salamandra salamandra*	2017	26	0–13%	
Lower Saxony, Lelm	Helmstedt	52.21327, 10.83035	Northern crested newt, *Triturus cristatus*	2015	29	0–3%	
			Smooth newt, *Lissotriton vulgaris*		16		
			Alpine newt, *Ichthyosaura alpestris*		57		
			Palmate newt, *Lissotriton helveticus*		6		
Lower Saxony, Mühlenberg	Holzminden	51.795175, 9.514919	Fire salamander, *Salamandra salamandra*	2017	50	0–7%	
Lower Saxony, Negenborn	Holzminden	51.894474, 9.595518	Fire salamander, *Salamandra salamandra*	2017	30	0–11%	
Lower Saxony, Shilo Ranch	Holzminden	51.891693, 9.604916	Fire salamander, *Salamandra salamandra*	2017	50	0–7%	
Lower Saxony, Kreis Lippe	Lippe	not available	Fire salamander, *Salamandra salamandra*	2016	2	0–70%	
Lower Saxony, Solling (various sub-sites)		51.80-51.89, 9.49-9.60	Fire salamander, *Salamandra salamandra*	2015	23	0–14%	all *Bd*-negative
				2017	45	0–8%	
North Rhine-Westphalia, Bielefeld Botanischer Garten	Bielefeld	52.014698, 8.511353	Fire salamander, *Salamandra salamandra*	2017	22	0–15%	

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North Rhine-Westphalia, Kottenforst (various sub-sites)	Bonn	50.65-50.69, 7.06-70.12	Fire salamander, *Salamandra salamandra*	2015	51	0–7%	all *Bd*-negative
			Fire salamander, *Salamandra salamandra*	2017	24	0–15%	
North Rhine-Westphalia, Rhede	Borken	51.870503, 6.761849	Fire salamander, *Salamandra salamandra*	2017	20	0–16%	
North Rhine-Westphalia, Baumberge	Coesfeld	51.990031, 7.350149	Fire salamander, *Salamandra salamandra*	2017	32	0–11%	
North Rhine-Westphalia, Billerbeck	Coesfeld	51.9860, 7.3330	Fire salamander, *Salamandra salamandra*	2017	4	0–51%	all *Bd*-negative
North Rhine-Westphalia, Coesfeld	Coesfeld	51.914888, 7.231555	Fire salamander, *Salamandra salamandra*	2017	34	0–11%	
North Rhine-Westphalia, Kirchdornberg	Dornberg	52.029909, 8.441465	Fire salamander, *Salamandra salamandra*	2017	25	0–13%	
North Rhine-Westphalia, Borgholzhausen	Gütersloh	52.088711, 8.317183	Fire salamander, *Salamandra salamandra*	2017	30	0–11%	
North Rhine-Westphalia, Blumenphul	Heinsberg	51.174364, 6.162144	Alpine newt, *Ichthyosaura alpestris*	2017	13	0–11%	
			Palmate newt, *Lissotriton helveticus*		19		
North Rhine-Westphalia, Effelder Wald	Heinsberg	51.141144, 6.150484	Smooth newt, *Lissotriton vulgaris*	2017	9	0–11%	
			Alpine newt, *Ichthyosaura alpestris*		2		
			Palmate newt, *Lissotriton helveticus*		19		
North Rhine-Westphalia, Dalheim	Heinsberg	51.148773, 6.197970	Smooth newt, *Lissotriton vulgaris*	2017	16		
			Alpine newt, *Ichthyosaura alpestris*		18		
			Palmate newt, *Lissotriton helveticus*		25		
North Rhine-Westphalia, Gangelter Bruch	Heinsberg	50.982539, 5.989102	Smooth newt, *Lissotriton vulgaris*	2017	6	0–18%	1 sample near detection limit (0.9 GE)
			Palmate newt, *Lissotriton helveticus*		13		
North Rhine-Westphalia, Teverener Heide	Heinsberg	50.947346, 6.027223	Northern crested newt, *Triturus cristatus*	2017	3	0–6%	
			Smooth newt, *Lissotriton vulgaris*		25		
			Alpine newt, *Ichthyosaura alpestris*		21		
			Palmate newt, *Lissotriton helveticus*		10		
North Rhine-Westphalia, Wassenberger Wald	Heinsberg	51.099426, 6.167827	Smooth newt, *Lissotriton vulgaris*	2017	4	0–11%	
			Alpine newt, *Ichthyosaura alpestris*		17		
			Palmate newt, *Lissotriton helveticus*		9		
North Rhine-Westphalia, Helminghausen	Hochsauerlandkreis	51.386061, 8.759663	Fire salamander, *Salamandra salamandra*	2017	30	0–11%	
North Rhine-Westphalia, Detmold	Lippe	51.923554, 8.871985	Fire salamander, *Salamandra salamandra*	2017	20	0–16%	
North Rhine-Westphalia, Wolbecker Tiergarten	Münster	51.910658, 7.745656	Fire salamander, *Salamandra salamandra*	2017	14	0–20%	
North Rhine-Westphalia, Remscheid	Remscheid	51.1541, 7.1763	Fire salamander, *Salamandra salamandra*	2016	5	0–44%	all *Bd*-negative
			Fire salamander, *Salamandra salamandra*	2017	30	0–11%	
North Rhine-Westphalia, Hennef	Rhein-Sieg-Kreis	50.755387, 7.357836	Fire salamander, *Salamandra salamandra*	2017	10	0–30%	
Rhineland-Palatinate, Niederhausen	Bad Kreuznach	49.803681, 7.789785	Fire salamander, *Salamandra salamandra*	2017	30	0–11%	
Rhineland-Palatinate, Wittlich	Bernkastel-Wittlich	50.009568, 6.888018	Fire salamander, *Salamandra salamandra*	2018	20	0–16%	swabbed in autumn
Rhineland-Palatinate, Altlayer Bachtal	Cochem-Zell	49.993891, 7.292991	Fire salamander, *Salamandra salamandra*	2017	29	0–11%	
Rhineland-Palatinate, Schmidtenhöhe	Koblenz	50.349104, 7.678652	Northern crested newt, *Triturus cristatus*	2015	1		

Site	District	Coordinates (latitude, longitude)	Species	Year	N samples	95% Bayesian CI	Remarks
Rhineland-Palatinate, Haardt	Neustadt a.d.W.	49.370101, 8.144377	Fire salamander, *Salamandra salamandra*	2017	7	0–36%	
Rhineland-Palatinate, Rheinbay	Rhein-Hunsrück-Kreis	50.173356, 7.62397	Fire salamander, *Salamandra salamandra*	2014	9	0–30%	
			Fire salamander, *Salamandra salamandra*	2017	30	0–11%	
Rhineland-Palatinate, Trier, Mattheiser Wald	Trier	49.719908, 6.652989	Northern crested newt, *Triturus cristatus*	2016	1		
Rhineland-Palatinate, Trier, Sirzenicher Bach	Trier	49.763169, 6.611662	Fire salamander, *Salamandra salamandra*	2014	3	0–61%	
Saxony, Ehrenberg, Kriebstein	Mittelsachsen	51.0375, 13.0167	Fire salamander, *Salamandra salamandra*	2017	25	0–13%	all *Bd*-negative
Saxony, Tharandt	Sächsische Schweiz-Osterzgebirge	50.9701, 13.5824	Fire salamander, *Salamandra salamandra*	2017	25	0–13%	all *Bd*-negative
Saxony, Hartenstein	Zwickau	50.661939, 12.671357	Fire salamander, *Salamandra salamandra*	2017	15	0–19%	
Saxony-Anhalt, Ilsenburg	Harz	51.8405, 10.6434	Fire salamander, *Salamandra salamandra*	2015	8	0–31%	all *Bd*-negative
			Fire salamander, *Salamandra salamandra*	2017	5	0–44%	
Saxony-Anhalt, Meisdorf	Harz	51.709798, 11.269965	Fire salamander, *Salamandra salamandra*	2017	31	0–11%	
Saxony-Anhalt, Siebigerode	Mansfeld-Südharz	51.562173, 11.418255	Fire salamander, *Salamandra salamandra*	2017	20	0–16%	
Thuringia, Closewitz	Jena	50.96, 11.57	Northern crested newt, *Triturus cristatus*	2015	23	0–14%	
Thuringia, Waldecker Schlossgrund	Saale-Holzland-Kreis	50.9139, 11.7769	Fire salamander, *Salamandra salamandra*	2015	22	0–15%	all *Bd*-negative
			Fire salamander, *Salamandra salamandra*	2017	15	0–19%	
Taunus	Wispertal	not available	Fire salamander, *Salamandra salamandra*	2017	14	0–20%	
Rhön		not available	Fire salamander, *Salamandra salamandra*	2017	22	0–15%	
Bergisches Land		not available	Fire salamander, *Salamandra salamandra*	2017	4	0–51%	
Bergisches Land		not available	Fire salamander, *Salamandra salamandra*	2017	9	0–30%	
Südschwarzwald	Schramberg	not available	Fire salamander, *Salamandra salamandra*	2017	16	0–20%	
Schwäbische Alb	Reutlingen	not available	Fire salamander, *Salamandra salamandra*	2017	10	0–30%	
Schwäbisch-Fränkischen Waldberge	Rudersberg	not available	Fire salamander, *Salamandra salamandra*	2017	12	0–26%	
				Totally tested	3454		

Supplementary document 3. *Bsal*-positive sites (populations) of caudate species from Germany from 2019 and early 2020. Specimens tested positive or negative for *Bd* are indicated. Legend as in Table 1.

Site	District	Coordinates (latitude, longitude)	Species	N samples	N samples Bsal-positive	Infection loads	Prevalence per year	95% Bayesian CI	N samples also Bd-positive (with GE)	Remarks
Northern Eifel										
North Rhine-Westphalia, Brockenberg	StädteRegion Aachen	50.746724, 6.232078	Northern crested newt, *Triturus cristatus*	3	1	+’	10%	2–43%	0	*Bsal*-infected northern crested newts 2017-2019
			Smooth newt, *Lissotriton vulgaris*	7	0	0			not tested	*Bsal*-infected smooth newts in 2016, 2017
North Rhine-Westphalia, Münsterbusch	StädteRegion Aachen	50.770778, 6.204713	Smooth newt, *Lissotriton vulgaris*	2	0		10%	2–43%	not tested	first *Bsal*-record in 2019
			Alpine newt, *Ichthyosaura alpestris*	8	1	++’ to ‘+++’	10%	2–43%	0	
North Rhine-Westphalia, Omerbach	StädteRegion Aachen	50.754140, 6.307838	Fire salamander, *Salamandra salamandra*	2	0	0	29%	19–42%	not tested	first *Bsal*-record in 2019
			Smooth newt, *Lissotriton vulgaris*	2	0	0			0	
			Alpine newt, *Ichthyosaura alpestris*	26	7	+’ to ‘+++’			1 (1 GE)	
			Palmate newt, *Lissotriton helveticus*	32	11	+’ to ‘+++’			0	
North Rhine-Westphalia, Thönbach	Düren	50.747069, 6.366387	Fire salamander, *Salamandra salamandra*	11	0	0	4%	2–8%	not tested	first *Bsal*record in 2019
			Alpine and palmate newts	132	5	+’ to ‘++’			0	
North Rhine-Westphalia, Teufelspütz	Düren	50.747069, 6.366387	Alpine and palmate newts	171	36	+’ to ‘+++’	21%	15–28%	0	*Bsal*-infected Alpine newts in 2018
North Rhine-Westphalia, Weberbach	Düren	50.735089, 6.359651	Fire salamander, *Salamandra salamandra*	10	2	+’ to ‘+++’	3%	1–9%	0	*Bsal*-infected fire salamanders in 2017
			Alpine newt, *Ichthyosaura alpestris*	33 (of which 2 †)	0	0			not tested	*Bsal*-infected alpine newts in 2017, 2018
			Palmate newt, *Lissotriton helveticus*	31	0	0			not tested	*Bsal*-infected palmate newts in 2017
North Rhine-Westphalia, Wollebach	Düren	50.737227, 6.413199	Fire salamander, *Salamandra salamandra*	26	1	+++	4%	1–19%	0	
North Rhine-Westphalia, Helingsbach Artenschutz- und Quellteich	NP Eifel	50.555410, 6.436180	Smooth newt, *Lissotriton vulgaris*	12	0		2%	1–11%	not tested	*Bsal*-infected northern crested newts 2017-2018
			Alpine newt, *Ichthyosaura alpestris*	29	1	+’			1 (3 GE)	*Bsal*-infected smooth and Aapine newts in 2018
			Palmate newt, *Lissotriton helveticus*	7	0				not tested	

Site	District	Coordinates (latitude, longitude)	Species	N samples	N samples Bsal-positive	Infection loads	Prevalence per year	95% Bayesian CI	N samples also Bd-positive (with GE)	Remarks
North Rhine-Westphalia, Winkelenberg	NP Eifel	50.596940, 6.420544	Northern crested newt, *Triturus cristatus*	40	3	+’ to ‘+++’	6%	2–15%	0	
			Smooth newt, *Lissotriton vulgaris*	3	0				not tested	
			Alpine newt, *Ichthyosaura alpestris*	6	0				not tested	
			Palmate newt, *Lissotriton helveticus*	5	0				not tested	
Southern Eifel										
Rhineland-Palatinate, Sellerich	Bitburg-Prüm	50.237990, 6.366980	Alpine newt, *Ichthyosaura alpestris*	24	2	+’ to ‘++’	3%	1–12%	0	
			Palmate newt, *Lissotriton helveticus*	34	0	0			not tested	
Rhineland-Palatinate, Watzbach	Bitburg-Prüm	50.215607, 6.328830	Fire salamander, *Salamandra salamandra*	63	11	++’ to ‘+++’	18%	10–28%	0	7 infected in spring, 4 in autumn, with typical skin lesions
			Alpine newt, *Ichthyosaura alpestris*	1	0	0			not tested	
Rhineland-Palatinate, Gerolstein	Vulkaneifel	50.209914, 6.658375	Alpine newt, *Ichthyosaura alpestris*	10	1	+’ to ‘++’	10%	2–43%	0	at least 5 dead-found fire salamanders reported in spring, not sampled
			Palmate newt, *Lissotriton helveticus*	1	0				not tested	
Ruhr area and vicinities										
North Rhine-Westphalia, Lottental	Bochum	51.437, 7.257333	Fire salamander, *Salamandra salamandra*	20	1	++’	5%	2–24%	not tested	infected from spring, 3 swabbed in autum,
			Alpine newt, *Ichthyosaura alpestris*	1					not tested	*Bsal*-infected European fire salamander in 2018
North Rhine-Westphalia, Dellwiger Bachtal	Dortmund	51.511440, 7.336399	Fire salamander, *Salamandra salamandra*	2 (of which 2†)	2 (of which 2†)	++’ to ‘+++’	100%	26–99%	not tested	
North Rhine-Westphalia, Solingen	Solingen	51.199496, 7.104182	Fire salamander, *Salamandra salamandra*	6 (of which 6†)	3 (of which 3†)	+’ to ‘+++’	50%	19–81%	not tested	mass mortality in more than 8 dead European fire salamanders
North Rhine-Westphalia, Bergerhausen	Essen	51.436599, 7.048501	Smooth newt, *Lissotriton vulgaris*	17	6	++’ to ‘+++’	33%	17–54%	not tested	
			Alpine newt, *Ichthyosaura alpestris*	5	2	++’ to ‘+++’			not tested	
			Palmate newt, *Lissotriton helveticus*	2	0				not tested	
North Rhine-Westphalia, Bredeney	Essen	51.403281, 6.995988	Fire salamander, *Salamandra salamandra*	331 (of which 8 †)	74 (of which 8 †)	+’ to ‘+++’	22%	18–27%	not tested	25 European fire salamanders had skin lesions
North Rhine-Westphalia, Fulerum Südwestfriedhof	Essen	51.431025, 6.968945	Fire salamander, *Salamandra salamandra*	4	1	+++’	2%	0.4–8%	not tested	reported mass mortality of European fire salamanders in 2018
			Smooth newt, *Lissotriton vulgaris*	10	0	0			not tested	
			Alpine newt, *Ichthyosaura alpestris*	21	0	0			not tested	
			Palmate newt, *Lissotriton helveticus*	5	0	0			not tested	

Site	District	Coordinates (latitude, longitude)	Species	N samples	N samples Bsal-positive	Infection loads	Prevalence per year	95% Bayesian CI	N samples also Bd-positive (with GE)	Remarks
North Rhine-Westphalia, Ruthertal	Essen	51.382679, 6.972078	Fire salamander, *Salamandra salamandra*	3 (of which 3 †)	3 (of which 3 †)	++’ to ‘+++’	100%	42–100%	not tested	
North Rhine-Westphalia, Essen Stadtwald	Essen	51.423306, 7.033296	Fire salamander, *Salamandra salamandra*	1	0	0	33%	4–57%	not tested	
			Alpine newt, *Ichthyosaura alpestris*	5	1	++’			not tested	
North Rhine-Westphalia, Stadtwald, Garten	Essen	51.423306, 7.033296	Alpine newt, *Ichthyosaura alpestris*	50	2	++’	4%	1–13%	not tested	
North Rhine-Westphalia, Wolfsbachtal	Essen	51.404382, 6.973143	Fire salamander, *Salamandra salamandra*	15 (of which 1 †)	6 (of which 1 †)	+’ to ‘+++’	44%	23–68%	not tested	
			Alpine newt, *Ichthyosaura alpestris*	1	1	+++’			not tested	
North Rhine-Westphalia, Rottbachtal	Mülheim an der Ruhr	51.375838, 6.833334	Fire salamander, *Salamandra salamandra*	13	3	+++’	24%	10–48%	not tested	infected common frog at this site
			Smooth newt, *Lissotriton vulgaris*	1	0	0			not tested	
			Alpine newt, *Ichthyosaura alpestris*	3	1	+++’			not tested	
North Rhine-Westphalia, Rottbachtal, Gartenteich	Mülheim an der Ruhr	51.401709, 6.856205	Smooth newt, *Lissotriton vulgaris*	17	0		4%	1–13%	not tested	decayed alpine newts in pond
			Alpine newt, *Ichthyosaura alpestris*	33	2	++’ to ‘+++’			not tested	
North Rhine-Westphalia, Hattingen Deilbach	Ennepe-Ruhr-Kreis	51.401582, 7.126877	Fire salamander, *Salamandra salamandra*	3 (of which 3 †)	2 (of which 2 †)	+’ to ‘+++’	67%	20–93%	not tested	
North Rhine-Westphalia, Hattingen Höhenweg	Ennepe-Ruhr-Kreis	51.326008, 7.171701	Fire salamander, *Salamandra salamandra*	8 (of which 8 †)	3 (of which 3 †)	+++’	38%	14–70%	not tested	
North Rhine-Westphalia, Witten	Ennepe-Ruhr-Kreis	51.429405, 7.387315	Fire salamander, *Salamandra salamandra*	13 (of which 13 †)	11 (of which 11 †)	++’ to ‘+++’	85%	57–95%	not tested	a total of 18 European fire salamanders that had left their wintering grounds and died, 13 sampled
North Rhine-Westphalia, Velbert Deilbach	Mettmann	51.337388, 7.142683	Fire salamander, *Salamandra salamandra*	2 (of which 2 †)	1 (of which 1 †)	++’	50%	9–88%	not tested	
Others										
North Rhine-Westphalia, Biener Altrhein	Kleve	51.771382, 6.354609	Northern crested newt, *Triturus cristatus*	1	1	+++’	17%	6–38%	not tested	
			Smooth newt, *Lissotriton vulgaris*	17	2	+’			not tested	
			Totally tested	1341	210				2	

Supplementary document 4. *Bsal*-negative sites (populations) of caudate species from Germany from 2019 and early 2020. Legend as in document 1.

Site (alphabetical order)	District	Coordinates (latitude, longitude)	Species	N samples	95% Bayesian CI	Remarks
Northern Eifel						
North Rhine-Westphalia, Binsfeldhammer	StädteRegion Aachen	50.756521, 6.243033	Smooth newt, *Lissotriton vulgaris*	2	0–51%	
			Alpine newt, *Ichthyosaura alpestris*	2		
North Rhine-Westphalia, Broichbachtal	StädteRegion Aachen	50.864910, 6.122293	Smooth newt, *Lissotriton vulgaris*	5	0–19%	
			Alpine newt, *Ichthyosaura alpestris*	12		
North Rhine-Westphalia, Döppeskaul	StädteRegion Aachen	50.509209, 6.295323	Alpine newt, *Ichthyosaura alpestris*	18	0–11%	
			Palmate newt, *Lissotriton helveticus*	13 (of which 1†)		
North Rhine-Westphalia, Fischbach	StädteRegion Aachen	50.735368, 6.294149	Fire salamander, *Salamandra salamandra*	26	0–12%	*Bsal*-infected European fire salamanders in 2015 and 2016
			Alpine newt, *Ichthyosaura alpestris*	2		
North Rhine-Westphalia, Geddautal	StädteRegion Aachen	50.764826, 6.210666	Alpine newt, *Ichthyosaura alpestris*	4	0–51%	
North Rhine-Westphalia, Herzogenrath Schwimmhalle	StädteRegion Aachen	50.866332, 6.103468	Smooth newt, *Lissotriton vulgaris*	5	0–44%	
North Rhine-Westphalia, Holderbach	StädteRegion Aachen	50.548397, 6.292036	Fire salamander, *Salamandra salamandra*	3	0–61%	
Norh Rhine-Westphalia, Holderbach-Fischteiche	StädteRegion Aachen	50.528889, 6.298801	Smooth newt, *Lissotriton vulgaris*	1	0–44%	
			Alpine newt, *Ichthyosaura alpestris*	2		
			Palmate newt, *Lissotriton helveticus*	2		
North Rhine-Westphalia, Konzen	StädteRegion Aachen	50.595878, 6.266865	Alpine newt, *Ichthyosaura alpestris*	13	0–8%	
			Palmate newt, *Lissotriton helveticus*	27		
North Rhine-Westphalia, Lamersiefen	StädteRegion Aachen	50.756932, 6.321338	Fire salamander, *Salamandra salamandra*	16	0–20%	
North Rhine-Westphalia, Lensbach	StädteRegion Aachen	50.672648, 6.230209	Fire salamander, *Salamandra salamandra*	23	0–13%	
			Palmate newt, *Lissotriton helveticus*	2		
North Rhine-Westphalia, Palsen Mützenich	StädteRegion Aachen	50.568479, 6.201584	Alpine newt, *Ichthyosaura alpestris*	1	0–31%	
			Palmate newt, *Lissotriton helveticus*	9		
North Rhine Westphalia, Peterbach	StädteRegion Aachen	50.657733, 6.323633	Fire salamander, *Salamandra salamandra*	1	0–6%	
			Alpine newt, *Ichthyosaura alpestris*	31		
			Palmate newt, *Lissotriton helveticus*	23		
North Rhine-Westphalia, Schlangenberg	StädteRegion Aachen	50.738106, 6.252416	Smooth newt, *Lissotriton vulgaris*	1	0–11%	
			Alpine newt, *Ichthyosaura alpestris*	7		
			Palmate newt, *Lissotriton helveticus*	22		
North Rhine-Westphalia, Solchbach	StädteRegion Aachen	50.70178, 6.270098	Fire salamander, *Salamandra salamandra*	39	0–9%	*Bsal*-infected European salamanders in 2015, 2017, 2018
			Alpine newt, *Ichthyosaura alpestris*	1		
North Rhine-Westphalia, Schomet	StädteRegion Aachen	50.722813, 6.219435	Smooth newt, *Lissotriton vulgaris*	10	0–16%	
			Alpine newt, *Ichthyosaura alpestris*	11		

Site (alphabetical order)	District	Coordinates (latitude, longitude)	Species	N samples	95% Bayesian CI	Remarks
North Rhine-Westphalia, Vygten	StädteRegion Aachen	50.771877, 6.289942	Smooth newt, *Lissotriton vulgaris*	18	0–15%	
			Palmate newt, *Lissotriton helveticus*	4		
North Rhine-Westphalia, Vichtbach	StädteRegion Aachen	50.676448, 6.198452	Fire salamander, *Salamandra salamandra*	34	0–10%	
			Alpine newt, *Ichthyosaura alpestris*	1		
North Rhine-Westphalia, Wurmtal Mühle Bardenberg	StädteRegion Aachen	50.836770, 6.101372	Palmate newt, *Lissotriton helveticus*	7	0–36%	
North Rhine-Westphalia, Asselbach	Düren	50.71486, 6.34355	Fire salamander, *Salamandra salamandra*	2	0–70%	
North Rhine-Westphalia, Ginnicker Bruch	Düren	50.694938, 6.579291	Northern crested newt, *Triturus cristatus*	0	0–5%	
			Smooth newt, *Lissotriton vulgaris*			
			Alpine newt, *Ichthyosaura alpestris*			
North Rhine-Westphalia, Hasselbach	Düren	50.681882, 6.428362	Fire salamander, *Salamandra salamandra*	9 (of which 1†)	0–30%	
North Rhine-Westphalia, Merkener Teiche	Düren	50.841698, 6.441830	Smooth newt, *Lissotriton vulgaris*	22	0–15%	
North Rhine-Westphalia, Merode Generalsweg	Düren	50.790850, 6.383657	Smooth newt, *Lissotriton vulgaris*	1	0–16%	
			Alpine newt, *Ichthyosaura alpestris*	11		
			Palmate newt, *Lissotriton helveticus*	99		
North Rhine-Westphalia, Pierer Wald	Düren	50.868203, 6.425354	Smooth newt, *Lissotriton vulgaris*	28	0–10%	
			Alpine newt, *Ichthyosaura alpestris*	7		
North Rhine-Westphalia, Quellteiche Linnich	Düren	50.969190, 6.277490	Smooth newt, *Lissotriton vulgaris*	24	0–15%	
North Rhine-Westphalia, Rosbach	Düren	50.692512, 6.435706	Fire salamander, *Salamandra salamandra*	29	0–11%	
North Rhine-Westphalia, Rote Wehe	Düren	50.726667, 6.323306	Alpine newt, *Ichthyosaura alpestris*	113	0–1%	
			Palmate newt, *Lissotriton helveticus*	207		
North Rhine-Westphalia, Sandkaulsiefen	Düren	50.789973, 6.375219	Fire salamander, *Salamandra salamandra*	6	0–43%	
North Rhine-Westphalia, Stetternich, temporärer Kleinweiher	Düren	50.916295, 6.413348	Smooth newt, *Lissotriton vulgaris*	7	0–18%	
			Alpine newt, *Ichthyosaura alpestris*	6		
			Palmate newt, *Lissotriton helveticus*	6		
North Rhine-Westphalia, Ursprungsbach	Düren	50.764144, 6.371654	Fire salamander, *Salamandra salamandra*	8	0–31%	
North Rhine-Westphalia, Weiße Wehe	Düren	50.717116, 6.345695	Alpine newt, *Ichthyosaura alpestris*	52	0–6%	*Bsal*-infected European fire salamanders in 2015
			Palmate newt, *Lissotriton helveticus*	8		*Bsal*-infected alpine and palmate newts in 2016
North Rhine-Westphalia, Zweifallshammer	Düren	50.684688, 6.412117	Fire salamander, *Salamandra salamandra*	1		*Bsal*-infected European fire salamanders in 2018
North Rhine-Westphalia, Billerbach	Nationalpark Eifel	50.619605, 6.494605	Fire salamander, *Salamandra salamandra*	16	0–20%	
North Rhine-Westphalia, Braubach	Nationalpark Eifel	50.569117, 6.489517	Fire salamander, *Salamandra salamandra*	16	0–20%	

Site (alphabetical order)	District	Coordinates (latitude, longitude)	Species	N samples	95% Bayesian CI	Remarks
North Rhine-Westphalia, Haftenbach	Nationalpark Eifel	50.613929, 6.431371	Fire salamander, *Salamandra salamandra*	6	0–26%	*Bsal*-infected European fires alamanders in 2017
			Alpine newt, *Ichthyosaura alpestris*	1		
			Palmate newt, *Lissotriton helveticus*	5		
North Rhine-Westphalia, Honigberg	Nationalpark Eifel	50.6152, 6.409683	Alpine newt, *Ichthyosaura alpestris*	8	0–13%	
			Palmate newt, *Lissotriton helveticus*	18		
North Rhine-Westphalia, Patersweiher	Nationalpark Eifel	50.544562, 6.428677	Smooth newt, *Lissotriton vulgaris*	1	0–51%	
			Palmate newt, *Lissotriton helveticus*	3		
North Rhine-Westphalia, Riffelsberg	Nationalpark Eifel	not available	Smooth newt, *Lissotriton vulgaris*	2	0–20%	
			Palmate newt, *Lissotriton helveticus*	12		
North Rhine-Westphalia, Vogelsang	Nationalpark Eifel	50.581426, 6.445964	Smooth newt, *Lissotriton vulgaris*	1	0–44%	
			Alpine newt, *Ichthyosaura alpestris*	4		
North Rhine-Westphalia, Walbigbach	Nationalpark Eifel	50.624289, 6.502341	Fire salamander, *Salamandra salamandra*	12	0–26%	
Southern Eifel						
Rhineland-Palatinate, Alfbach	Bitburg-Prüm	50.266170, 6.341554	Alpine newt, *Ichthyosaura alpestris*	6	0–15%	*Bsal*-infected palmate newts in 2018
			Palmate newt, *Lissotriton helveticus*	16		
Rhineland-Palatinate, Bitburg	Bitburg-Prüm	49.976417, 6.474156	Alpine newt, *Ichthyosaura alpestris*	35		
			Palmate newt, *Lissotriton helveticus*	16	0–7%	
Rhineland-Palatinate, Bollendorf	Bitburg-Prüm	49.849103, 6.326625	Alpine newt, *Ichthyosaura alpestris*	35		
			Palmate newt, *Lissotriton helveticus*	16	0–7%	
Rhineland-Palatinate, Fuhrbach	Bitburg-Prüm	50.039648, 6.417938	Alpine newt, *Ichthyosaura alpestris*	3	0–8%	
			Palmate newt, *Lissotriton helveticus*	39		
Rhineland-Palatinate, Hauchenbach	Bitburg-Prüm	49.919317, 6.405269	Fire salamander, *Salamandra salamandra*	10	0–31%	
Rhineland-Palatinate, Idenheim	Bitburg-Prüm	49.890914, 6.590448	Alpine newt, *Ichthyosaura alpestris*	6	0–11%	
			Palmate newt, *Lissotriton helveticus*	24		
Rhineland-Palatinate, Irrel	Bitburg-Prüm	49.856404, 6.448138	Fire salamander, *Salamandra salamandra*	9	0–30%	
Rhineland-Palatinate, Lichtenborn	Bitburg-Prüm	50.113526, 6.280058	Fire salamander, *Salamandra salamandra*	83	0–4%	
			Alpine newt, *Ichthyosaura alpestris*	2		
			Palmate newt, *Lissotriton helveticus*	7		
Rhineland-Palatinate, Läuskopfbach	Bitburg-Prüm	50.057222, 6.434027	Alpine newt, *Ichthyosaura alpestris*	11	0–16%	
			Palmate newt, *Lissotriton helveticus*	9		
Rhineland-Palatinate, Lützkampen	Bitburg-Prüm	50.152251, 6.170181	Alpine newt, *Ichthyosaura alpestris*	2	0–70%	
Rhineland-Palatinate, Nimshuscheid	Bitburg-Prüm	50.081105, 6.474474	Alpine newt, *Ichthyosaura alpestris*	2	0–31%	
			Palmate newt, *Lissotriton helveticus*	8		

Site (alphabetical order)	District	Coordinates (latitude, longitude)	Species	N samples	95% Bayesian CI	Remarks
Rhineland-Palatinate, Plütscheid	Bitburg-Prüm	50.073109, 6.431563	Alpine newt, *Ichthyosaura alpestris*	8	0–31%	*Bsal*-infected alpine newts in 2017
			Palmate newt, *Lissotriton helveticus*	2		
Rhineland-Palatinate, Schwarzer Mann	Bitburg-Prüm	50.266283, 6.359239	Alpine newt, *Ichthyosaura alpestris*	8	0–9%	*Bsal*-infected alpine newt in 2018
			Palmate newt, *Lissotriton helveticus*	29		
Rhineland-Palatinate, Weilerbach	Bitburg-Prüm	49.845261, 6.389539	Alpine newt, *Ichthyosaura alpestris*	6	0–14%	
			Palmate newt, *Lissotriton helveticus*	17		
Ruhr area and ist vicinities						
North Rhine-Westphalia, Berger Mühle	Bochum	51.511293, 7.235178	Fire salamander, *Salamandra salamandra*	43	0–8%	
North Rhine-Westphalia, Dahlhausen	Bochum	51.435, 7.1385	Fire salamander, *Salamandra salamandra*	3	0–36%	*Bsal*-infected European fire salamanders in 2018
			Smooth newt, *Lissotriton vulgaris*	2		
			Alpine newt, *Ichthyosaura alpestris*	2		
North Rhine-Westphalia, Hiltrop	Bochum	51.52021, 7.26652	Smooth newt, *Lissotriton vulgaris*	1		*Bsal*-infected European fire salamanders in 2018
North Rhine-Westphalia, Königsbüscher Wald	Bochum	51.448228, 7.279486	Fire salamander, *Salamandra salamandra*	4	0–51%	
North Rhine-Westphalia, Querenburg	Bochum	51.441398, 7.264886	Fire salamander, *Salamandra salamandra*	1		*Bsal*-infected European fire salamanders in 2018
North Rhine-Westphalia, Stiepel	Bochum	51.418056, 7.250944	Fire salamander, *Salamandra salamandra*	6	0–26%	
			Smooth newt, *Lissotriton vulgaris*	4		
			Alpine newt, *Ichthyosaura alpestris*	1		
North Rhine-Westphalia, Grevelsberger Stadtwald	Ennepe-Ruhr-Kreis	51.323936, 7.366966	Fire salamander, *Salamandra salamandra*	1 (of which 1 †)		
North Rhine-Westphalia, Rumbachtal	Mülheim an der Ruhr	51.418650, 6.930562	Fire salamander, *Salamandra salamandra*	1	0–51%	
			Alpine newt, *Ichthyosaura alpestris*	3		
North Rhine-Westphalia, Gysenberg	Herne	51.532070, 7.250724	Fire salamander, *Salamandra salamandra*	1		
North Rhine-Westphalia, Langeloh	Herne	51.532323, 7.289932	Fire salamander, *Salamandra salamandra*	18	0–18%	7 swabbed in summer
North Rhine-Westphalia, Hiesfelder Wald	Oberhausen	51.562273, 6.846767	Fire salamander, *Salamandra salamandra*	58	0–4%	
			Northern crested newt, *Triturus cristatus*	24		
			Alpine newt, *Ichthyosaura alpestris*	2		
			Palmate newt, *Lissotriton helveticus*	1		
North Rhine-Westphalia, Sterkrader Wald	Oberhausen	51.541882, 6.833255	Fire salamander, *Salamandra salamandra*	84	0–4%	
			Alpine newt, *Ichthyosaura alpestris*	2		
North Rhine-Westphalia, Ratingen, Baulofsbruch	Mettmann	51.312164, 6.861132	Fire salamander, *Salamandra salamandra*	24	0–13%	swabbed in autumn
			Smooth newt, *Lissotriton vulgaris*	1		
Others						
Baden-Wuerttemberg, Reicherthausen	Rhein-Neckar-Kreis	49.314267, 9.299841	Fire salamander, *Salamandra salamandra*	4 (of which 4 †)	0–51%	

Site (alphabetical order)	District	Coordinates (latitude, longitude)	Species	N samples	95% Bayesian CI	Remarks
Lower Saxony, Bonaforth	Göttingen	51.398075, 9.626154	Fire salamander, *Salamandra salamandra*	2 (of which 2 †)	0–70%	
Lower Saxony, Wolfshagen	Goslar	51.923595, 10.303128	Fire salamander, *Salamandra salamandra*	1 (1 †)		
North Rhine-Westphalia, Bielefeld	Bielefeld	not available	Fire salamander, *Salamandra salamandra*	14	0–20%	
North Rhine-Westphalia, Kottenforst (various sub-sites)	Bonn	50.65-50.69, 7.06-70.12	Fire salamander, *Salamandra salamandra*	1 (of which 1 †)		
North Rhine-Westphalia, Bad Driburg	Höxter	51.749378, 9.364750	Fire salamander, *Salamandra salamandra*	1 (of which 1 †)		
North Rhine-Westphalia, Biostation Höxter	Höxter	not available	Northern crested newt, *Triturus cristatus*	1	0–61%	
			Alpine newt, *Ichthyosaura alpestris*	1		
			Palmate newt, *Lissotriton helveticus*	1		
North Rhine-Westphalia, Teverener Heide	Heinsberg	50.947346, 6.027223	Northern crested newt, *Triturus cristatus*	3	0–16%	
			Smooth newt, *Lissotriton vulgaris*	6		
			Alpine newt, *Ichthyosaura alpestris*	12		
North Rhine-Westphalia, Kiesgrube	Kleve	51.759072, 6.440043	Northern crested newt, *Triturus cristatus*	2	0–19%	
			Smooth newt, *Lissotriton vulgaris*	13		
North Rhine-Westphalia, Schloss Hüth	Kleve	51.813430, 6.373628	Northern crested newt, *Triturus cristatus*	4	0–43%	
			Smooth newt, *Lissotriton vulgaris*	2		
North Rhine-Westphalia, Niepkuhlen	Krefeld	51.384235, 6.562701	Northern crested newt, *Triturus cristatus*	1	0–9%	
			Smooth newt, *Lissotriton vulgaris*	38		
North Rhine-Westphalia, Orbroich		51.390638, 6.513952	Northern crested newt, *Triturus cristatus*	1	0–43%	
			Smooth newt, *Lissotriton vulgaris*	5		
North Rhine-Westphalia, Mönchengladbach	Mönchengladbach	51.217208, 6.471023	Smooth newt, *Lissotriton vulgaris*	4	0–51%	
North Rhine-Westphalia, Mönchengladbach Entenweiher	Mönchengladbach	51.177977, 6.418822	Alpine newt, *Ichthyosaura alpestris*	10 (of which 10 †)	0–31%	
Rhineland-Palatinate, Butzweiler	Trier-Saarburg	49.816517, 6.614972	Alpine newt, *Ichthyosaura alpestris*	1 (of which 1 †)		
Rhineland-Palatinate, Ralingen	Trier-Saarburg	49.804369, 6.514113	Fire salamander, *Salamandra salamandra*	1		
Rhineland-Palatinate, Kiesgrube bei Landscheid	Bernkastel-Wittlich	50.004117, 6.775064	Northern crested newt, *Triturus cristatus*	3	0–31%	
			Alpine newt, *Ichthyosaura alpestris*	7		
Saarland, Gersheim	Saarpfalz-Kreis	49.158114, 7.180896	Fire salamander, *Salamandra salamandra*	1 (of which 1 †)		
			Totally tested	1985		

Supplementary document 6. Methods used to build *Bsal* species distribution models

Data

Grid-based correlative species distribution models, SDMs (Franklin 2010, Hui et al. 2018), were created to study the global suitability to *Bsal*. SDMs are based on ecological information at species presences and calculate an 'idealized' niche (in environmental space) which is then projected into geographic space. To catch as much as possible of *Bsal*'s niche, we used both invasive and native records (cf. Rödder & Lötters 2009). We employed most of the records known to date, in total 103 (30 of which native), accounting for the *Bsal* distribution in Belgium, China, Germany, Japan, the Netherlands, Spain, Taiwan, Thailand and Vietnam. Records were adopted from Feldmeier et al. (2016) Beukema et al. (2018), Dalbeck et al. (2018), Lötters et al. (2018), Martel et al. (2020), and, in addition, new records published in this paper were used:

The full list of records included the following coordinates (longitude, latitude in decimal degree): 99.05, 19.87; 105.05, 20.92; 105.04, 20.94; 106.73, 21.18; 106.66, 21.18; 105.72, 21.2; 105.65, 21.46; 104.35, 21.96; 104.26, 22; 105.81, 22.29; 105.73, 22.37; 104.42, 22.54; 114.2, 22.6; 105.87, 22.62; 104.87, 23.03; 104.85, 23.04; 121.28, 24.15; 121.29, 24.16; 121.31, 24.19; 127.77, 26.14; 127.8, 26.18; 128.03, 26.54; 128.24, 26.75; 119.61, 27.72; 129.3, 28.27; 129.5, 28.34; 135.43, 33.79; 139.39, 37.02; 144.41, 43.02; 144.28, 43.08; 6.41785, 50.039536; 6.420525, 50.045956; 6.439868, 50.079225; 4.89, 50.22; 6.36817, 50.241592; 6.344899, 50.252272; 6.338861, 50.259879; 6.522695, 50.268734; 6.1, 50.45; 6.279463, 50.570422; 6.400809, 50.574792; 5.59, 50.58; 6.431283, 50.614152; 6.09, 50.63; 6.185149, 50.65692; 6.358669, 50.664551; 6.423461, 50.682277; 6.27, 50.7; 6.270363, 50.702002; 6.345602, 50.717221; 6.32233, 50.726267; 6.361194, 50.736297; 6.301953, 50.739019; 6.36933, 50.744405; 6.232294, 50.745694; 5.95, 50.77; 5.97, 50.85; 5.75, 50.88; 6.08, 50.9; 5.74, 50.91; 6.02, 50.94; 5.99, 50.98; 6.298502, 51.049452; 5.92, 51.07; 4.51, 51.09; 7.05, 51.41; 6.97, 51.43; 7.26, 51.44; 7.27, 51.44; 7.14, 51.44; 5.92, 51.82; 2.499264, 41.554996; 7.266148, 51.442199; 7.139507, 51.435073; 7.270582, 51.442880; 7.256134, 51.440284; 7.019866, 51.423689; 6.968945, 51.431025; 6.423373, 50.682388; 6.232078, 50.746724; 6.204713, 50.770778; 6.307838, 50.754140; 6.366387, 50.747069; 6.366387, 50.747069; 6.359651, 50.735089; 6.413199, 50.737227; 6.366980, 50.237990; 6.328830, 50.215607; 6.658375, 50.209914; 7.257333, 51.437; 7.048501, 51.436599; 6.995988, 51.403281; 6.968945, 51.431025; 6.972078, 51.382679; 7.033296, 51.423306; 7.033296, 51.423306; 6.973143, 51.404382; 6.833334, 51.375838; 6.856205, 51.401709; 7.126877, 51.401582; 7.171701, 51.326008; 7.387315, 51.429405; 7.142683, 51.337388.

As ecological predictors we used current high resolution climate data for the period 1979-2013 at grid resolution 30 arc sec from the CHELSA version 1.2 database (Karger et al. 2017), data available at: http://chelsa-climate.org/). Although most *Bsal* records originate from the period 2010-2019, we decided to use CHELSA data, as we consider them the best available global predictors. CHELSA operates on monthly means and is based on a quasi-mechanistic statistical downscaling of the ERA interim global circulation model (GCM) with a GPCC bias correction (Karger et al. 2017). The CHELSA website provides 19 so called ‘bioclim’ variables (cf. Booth et al. 2014): Bio1 = Annual Mean Temperature, Bio2 = Mean Diurnal Range, Bio3 = Isothermality, Bio4 = Temperature Seasonality, Bio5 = Max Temperature of Warmest Month, Bio6 = Min Temperature of Coldest Month, Bio7 = Temperature Annual Range, Bio8 = Mean Temperature of Wettest Quarter, Bio9 = Mean Temperature of Driest Quarter, Bio10 = Mean Temperature of Warmest Quarter, Bio11 = Mean Temperature of Coldest Quarter, Bio12 = Annual Precipitation, Bio13 = Precipitation of Wettest Month, Bio14 = Precipitation of Driest Month, Bio15 = Precipitation Seasonality, Bio16 = Precipitation of Wettest Quarter, Bio17 = Precipitation of Driest Quarter, Bio18 = Precipitation of Warmest Quarter, Bio19 = Precipitation of Coldest Quarter.

These variables provide information independent of latitudinal variation and therefore are more useful in modelling than monthly values. As variable choice is one crucial aspect when generating SDMs, we computed SDMs with various bioclim variable combinations out of the 19 ones available: **(i)** Seven were selected via pair-wise Pearson correlation analyses to avoid effects of multicollinearity, which is important when projecting SDMs into new space (Dormann et al. 2013). Of highly correlated variables (|r| > 0.7), we excluded the less informative one, based on a priori assumptions on biological importance (cf. Martel et al. 2013) to our target organisms: Bio1, Bio2, Bio10, Bio13, Bio14, Bio18, Bio19. In accordance with previous published *Bsal* SDMs, we also ran predictor sets incorporating **(ii)** Bio8, Bio10, Bio11, Bio15, Bio16, Bio17 (cf. Feldmeier et al. 2016), **(iii)** Bio3, Bio4, Bio5, Bio6, Bio15, Bio16, Bio17 (cf. Beukema et al. 2018) and **(iv)** Bio2, Bio5, Bio7, Bio15, Bio18, Bio19 (cf. Basanta et al. 2018).

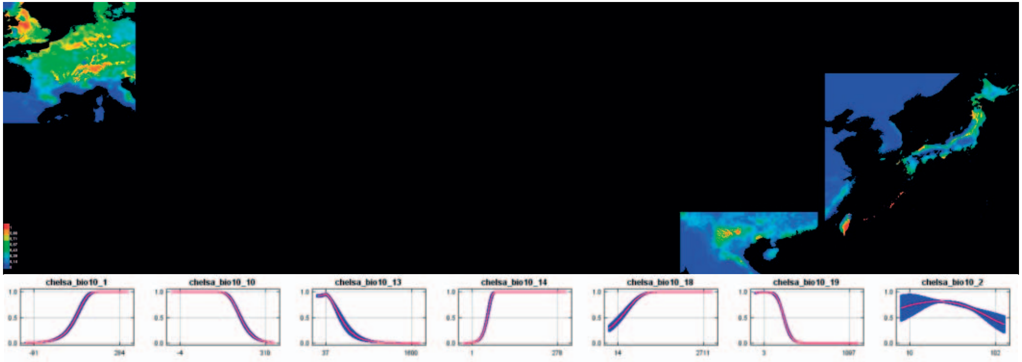
Modelling

Maxent 3.4.1 (https://biodiversityinformatics.amnh.org/open_source/maxent/) was used for SDM building (Phillips et al. 2006, 2017). This presence-only/background method operates with a machine-learning algorithm following the principle of maximum entropy. It makes predictions on the potential geographic range of a taxon by taking environmental (here: climatic) data from geo-referenced species records and random background data (Phillips et al. 2006, Yackulic et al. 2013). In this way, it contrasts the environmental conditions at species' presences against those at the background to fit a function to estimate the relative suitability to the species (Merow et al. 2013). Maxent is a widely used SDM tool and often performs better than other SDM methods (Elith et al. 2006, Phillips & Dudík 2008). It offers various settings for SDM building allowing fine-tuning (cf. Phillips et al. 2006, Phillips & Dudík 2008). This requires some caution, however, as with them, the output can be dramatically altered when uncritically used (Elith et al. 2010, 2011, Merow et al. 2013, Yackulic et al. 2013). Therefore, it is important to explore settings and to adapt them to the available data (Phillips & Dudík 2008, Warton & Aarts 2013, Phillips et al. 2017).

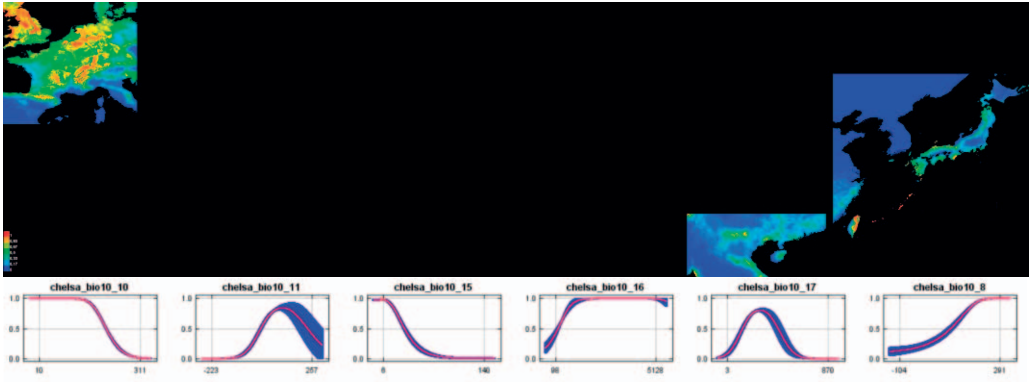
In our final model runs, we employed linear + quadratic + product (LQP) features under the subsample approach (100 replicates), with 25% of the records randomly set aside as test data. In response curve calculations, extrapolation was used and fading by clamping. Ample windows around *Bsal* presences in Asia and Europe were chosen as background (see below), while we projected models into Germany only. The number of background points was set to 100,000. All other Maxent settings were default, including the Cloglog output format (ranging 0-1) (Phillips & Dudík 2008, Shcheglovitova & Anderson 2013, Phillips et al. 2017).

Below we show average model performance as Cloglog heat maps (standard deviation was ≤ 0.17, not shown) and response curves (red, with the standard deviation each indicated in blue) for model runs (i) to (iv).

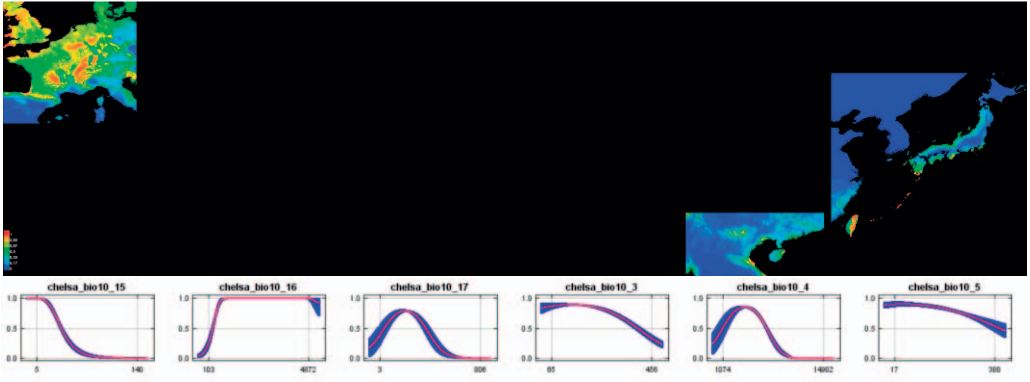
(i) Bio1, Bio2, Bio10, Bio13, Bio14, Bio18, Bio19



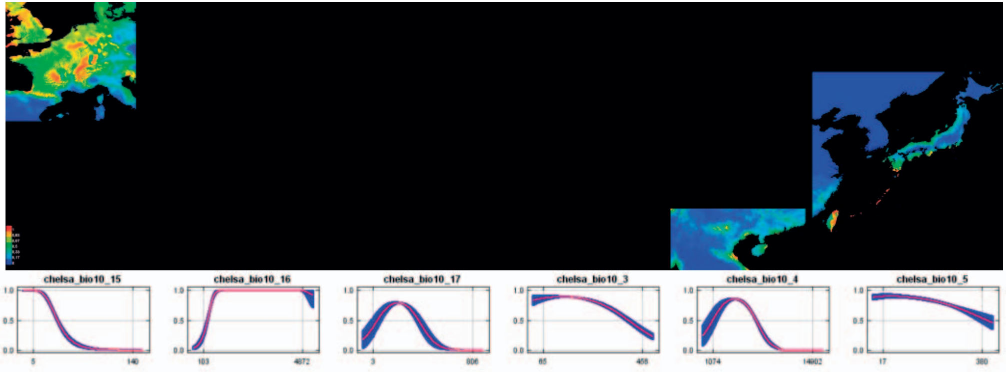
(ii) Bio8, Bio10, Bio11, Bio15, Bio16, Bio17



(iii) Bio3, Bio4, Bio5, Bio6, Bio15, Bio16, Bio17



(iv) Bio2, Bio5, Bio7, Bio15, Bio18, Bio19



Data processing and output maps

Maxent calculates the area under the receiver operating characteristic curve (AUC) as a measure of predictive accuracy (PHILLIPS et al. 2006). Following the classification of SWETS (1988), AUC values range between 0.5 for models with no predictive ability and 1.0 for models giving perfect predictions; values ≥ 0.91 describe 'high', ≥ 0.71 'moderate', ≥ 0.5 'low' model accuracy. Although criticized (e.g. YACKULIC et al. 2013), the AUC is informative as it mirrors the model's ability to distinguish between species records and background points, i.e. showing how general or restricted a distribution is along the range of the variables in the studied region (MEROW et al. 2013). The mean AUC values in our approaches were (i) 0.903, (ii) 0.862, (iii) 0.876 and (iv) 0.904, thus suggesting high accuracy in model performance.

For processing the resulting SDM maps (see Fig. 5), DIVA-GIS 7.5.0 (HIJMANS et al. 2001) was chosen. We did not set any threshold delimitating potential presence versus absence (cf. PHILLIPS et al. 2017).

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SALAMANDRA **56**(3): 189–214
15 August 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Batrachochytrium salamandrivorans in the Ruhr District, Germany: history, distribution, decline dynamics and disease symptoms of the salamander plague

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Manuscript received: 25 March 2020
Accepted: 11 May 2020 by Jörn Köhler

Abstract. The chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*), recently introduced from Asia to Europe, causes mortality in numerous species of salamanders and newts and has led to catastrophic declines and local extinctions of the European fire salamander (*Salamandra salamandra*) in the Netherlands, Belgium, and Germany. Due to the continuous spread of the pathogen, Germany can be considered as the current 'hotspot' of *Bsal*-driven salamander declines. The pathogen was detected in 2015 in the Eifel Mountains where it probably has been present at least since 2004. Moreover, *Bsal* was found in 2017 in the Ruhr District where it also might occur since 2004. The Ruhr District is a heavily urbanized and industrialized region in western Germany, which offers an unprecedented opportunity to monitor range expansion and infection dynamics of *Bsal* in an area affected by intense human activities. We here review the current knowledge on *Bsal* in the Ruhr District where the pathogen by now has been recorded based on qPCR data from 18 sites distributed over eight cities. Transect counts (adult salamanders) and larval removal-sampling at two sites where *Bsal* was recorded in 2017 and 2018, confirm that fire salamander populations at the affected sites have declined dramatically. However, single negative-tested individuals were still observed and reproduction could be ascertained. Moreover, we successfully detected *Bsal* by analysing environmental DNA (eDNA) from samples obtained from a standing water body as well as a stream. Detailed monitoring of a site in Essen (Kruppwald) from January to May 2019 provided data on infection and disease dynamics during an acute *Bsal*-outbreak in a population of European fire salamanders. After initial observation of single dead infected salamanders in January and February 2019, the maximum *Bsal* loads in the population ranged from 7.90E+03 ITS copies in early March to 2.29E+09 ITS copies at the end of March. Prevalence of infection ranged from 4% to 50% and significantly increased over time; prevalence of externally visible disease symptoms peaked on May 2 and May 8. Single dead salamanders were encountered throughout the monitoring period. Recaptures of two infected salamanders indicated an increase of *Bsal* load by about one order of magnitude within one week. Infected salamanders showed small-sized regular round ulcerations usually of 0.25–1 mm but sometimes up to 2.5 mm in diameter, which gave the impression of outward growth from the centre of each ulceration. Among salamander individuals monitored in the Kruppwald, such ulcerations were only found in infected salamanders, but we found no significant correlation between the intensity of the ulcerations and *Bsal* load. Heat treatment proved effective to cure even deep ulcerations when salamanders were kept for 10 days at 25–27°C or 14 days at 25°C, but infection persisted and ulcerations reappeared six weeks after the end of the treatment; only heat treatment at 25°C for 21 days proved effective to reliably clear the infection in three tested salamanders.

Key words. Amphibia, Caudata, *Salamandra salamandra*, European fire salamander, *Bsal*, chytridiomycosis, heat treatment, emerging infectious disease, amphibian disease, eDNA.

Introduction

Globalization, through increased mobility of people and goods, has opened the gates for invasive species, including pathogenic bacteria, viruses and fungi, to spread into new areas harbouring organisms vulnerable to these pathogens (Marano et al. 2007, Perrings et al. 2018). Especially areas with strong human footprint and activities, e.g. extended urbanization and dense networks of roads, are characterized by a high invasion intensity (e.g. Ehrenfeld 2008, Wichmann et al. 2009, Gallardo et al. 2015), whereas protected areas can offer refuge from invasive species (Gallardo et al. 2017).

In central Europe, one of the most urbanized areas is the Ruhr District ('Ruhrgebiet') in western Germany. Named after the river Ruhr, this large polycentric urban area in the federal state of North Rhine-Westphalia is characterized by a high human population density (~5 million residents) and numerous industrial agglomerations. The Ruhr District is geologically a 'Börde', i.e. a highly fertile lowland with loess substrate, located in a transition area from a low mountain range (Bergisches Land and Sauerland) to lowlands (Lower Rhineland and Münsterland) (Feldmann & Schlüpmann 2011a). As such, it has been largely deforested and was historically first used for agriculture, then industrialized with numerous heavy industries and associated urban areas built around intensive black coal mining. In this landscape mosaic, natural and agricultural elements were strongly reduced and fragmented, but remained locally preserved. For instance, numerous deciduous (beech) forest patches remain scattered among urban and industrial areas, often used as local recreation areas.

The Ruhr District is one of currently two areas in Germany where amphibian mass mortality caused by the invasive pathogenic fungus *Batrachochytrium salamandrivorans* (*Bsal*) have been observed in the wild. The outbreaks caused by this pathogen (also dubbed as 'salamander plague'; Dalbeck et al. 2018) have triggered a fast response by regional and national stakeholders who engaged in numerous monitoring and research activities, and our purpose is to herein provide an overview and summary of the data obtained so far. While the dense infrastructure in the Ruhr District presumably facilitates the spread of invasive species and pathogens, it also makes this area ideal for close monitoring of pathogen dispersal, prevalence, and host survival in a natural environment, as most sites are effortlessly accessible. Moreover, since the 1970s, the amphibian fauna of the Ruhr District has been intensively studied (Sell & Sell 1977, Feldmann 1981, Geiger & Niekisch 1983, Klewen 1988, Kordges et al. 1989, Hamann & Uthoff 1994, Kordges & Schlüpmann 2011), which provides an excellent baseline to estimate the impact of this pathogen.

Amphibians are among the vertebrates being most affected by infectious diseases caused by invasive pathogens (Blaustein et al. 2018). The chytrid fungi *B. dendrobatidis* (*Bd*) and *B. salamandrivorans* are native to Asia (Martel et al. 2014, Laking et al. 2017, Nguyen et al. 2017, Yuan et al. 2018, O'Hanlon et al. 2018) and have the potential to push species from all amphibian orders to the brink of extinction. While *Bd* infects and causes mortality in anurans, caecilians as well as caudates, *Bsal* appears to affect only caudates, with some anurans functioning as potential vectors and reservoirs (Martel et al. 2014, Stegen et al. 2017). Presumably, the global movement of goods and materials, including the pet trade, facilitated the spread of *Bd* into every continent inhabited by amphibians, causing massive die-offs in anurans, caudates and caecilians e.g. in the Neotropics (Scheele et al. 2019). The origin of *Bsal* in Europe is considered to be a recent introduction (Martel et al. 2014) with subsequent invasive range expansion (Spitzen-van der Sluijs et al. 2016, Thomas et al. 2019).

The most susceptible species to *Bsal* infection within the current range of the pathogen appears to be the European fire salamander, *Salamandra salamandra* (Stegen et al. 2017), a species with a substantial portion of its global population in Germany (Thiesmeier 2004); for simplicity hereafter mostly named "fire salamander". Within days upon infection, fire salamanders develop superficial skin lesions and deep epidermal ulcerations. After complete disruption of the important skin barrier, secondary bacterial infections will lead to septicaemia and death within less than a month (Martel et al. 2013, 2014, Stegen et al. 2017). Newt species can also develop and die from *Bsal*-driven chytridiomycosis as recently reported in marbled newts in Spain (Martel et al. 2020). However, large-scale population collapses have not been reported in other European newt species so far, suggesting they could also act as unpredictable reservoirs of *Bsal* (Martel et al. 2014, Wagner et al. 2019). The dispersal vectors are theoretically manifold (conspecifics, resistant amphibians, wildlife, humans, etc.), but practically unknown (Stegen et al. 2017). The only cure for chytridiomycosis to date is a temperature treatment at 25°C for 10 days and/or a fungicide (Blooi et al. 2015a, b), although intensive research is conducted regarding microbiome manipulation and vaccination/immunisation (McMahon et al. 2014, Bletz et al. 2018). The invasive distribution of *Bsal* currently encompasses the Netherlands, Belgium, Germany (Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018, Lötters et al. 2020a in this issue), and Spain (Martel et al. 2020). The first discovery of dead fire salamanders in Europe dates back to 2008 in the Netherlands (Spitzen-van der Sluijs et al. 2013), but it was not until 2013 that the mortality causing pathogen was identified and scientifically named (Martel et al. 2013). Subsequently, more infected populations were discovered in nearby areas in Belgium and Germany (Eifel) due to intensive monitoring (Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018), and a record from 2004 (Dalbeck et al. 2018, Lötters 2020b in this issue) suggests that the pathogen may have been present in the Eifel for much longer than originally assumed.

In the Ruhr District, sick or dead fire salamanders infected with *Bsal* were first recorded and confirmed by quantitative PCR (qPCR) in 2017 (Lötters et al. 2018, Schulz et al. 2018, Wagner et al. 2019). The species in this area inhabits a variety of habitats, from isolated forest remains to more extensive patches of beech forest, with abundances dropping rapidly towards the northern and western lowland areas. In the most recent Red List of endangered reptiles and amphibians of North Rhine-Westphalia (Schlüpmann et al. 2011b), the fire salamander was assessed as Least Concern for the entire state, but as Critically Endangered for the strongly urbanized part of the Ruhr District ('Ballungsraum Ruhrgebiet'; see map in Thimm & Weiss 2011) where only a few populations occur and are threatened by ongoing urban development (Schlüpmann et al. 2011b). The Ruhr District is part of a potentially wide contact zone between two subspecies that reached the area by postglacial recolonization, *S. s. salamandra* and *S. s. terrestris* (Weitere et al. 2004). Fire salamanders in the region usually show the striped pattern typical for the western subspecies *S. s. terrestris,* but carry some alleles typical for the eastern subspecies and may have evolved unique genetic characteristics through hybridization and adaptation (Weitere et al. 2004).

So far, 18 positive *Bsal* sites associated with eight distinct cities/counties were recorded in the Ruhr District (Wagner et al. 2019, Lötters et al. 2020a in this issue, V. Schulz unpubl. data) and the number is steadily increasing. As we report herein, the earliest unconfirmed indication of the possible presence of *Bsal* within this urban area, documented by a photo, dates back to 2004. The close collaboration between universities, biological stations, nature conservation authorities and volunteers facilitates an unprecedented opportunity to monitor infection dynamics in real-time. During 2017–2019, we repeatedly sampled fire salamanders and newts in infected as well as non-infected sites across the Ruhr District for *Bsal* infection by skin swabbing. Molecular analysis was conducted using qPCR. Every individual was photographed for recapture analysis which enabled us to compare infection loads over time and to search for possible resistant individuals that would be tested positive at first and negative at a later time. Furthermore, we conducted a removal sampling in streams to determine the abundance of fire salamander larvae as a proxy for the abundance of adults. Additionally, we conducted the first trials to detect *Bsal* by using environmental DNA. In this review, we aim to give an overview of the discovery and spread of *Bsal* in the Ruhr District, the observed population declines, and the observed disease symptoms. Moreover, we provide a report on our experiences in the treatment of infected fire salamanders and discuss future perspectives for their conservation management in the Ruhr District.

Materials and methods

Study area and distribution of European fire salamanders in the Ruhr District

From an administrative and political perspective, the Ruhr District (Fig. 1) is defined since 1900 as a conglomerate of many cities and counties, including Bergkamen, Bochum, Bottrop, Castrop-Rauxel, Dinslaken, Dortmund, Duisburg, Essen, Gelsenkirchen, Gladbeck, Hamm, Hattingen, Herdecke, Herne, Herten, Kamen, Lünen, Mülheim an der Ruhr, Oberhausen, Recklinghausen, Wetter and Witten. Also the city of Hagen, the entire Ennepe-Ruhr District (in the west Sauerland), the district of Wesel (predominantly Niederrhein), the district of Recklinghausen (in the north Münsterland) and the district of Unna (in the north Münsterland, in the south Sauerland) are politically linked to the region by the 'Regionalverband Ruhr'.

Geographically, the southern border of the current Ruhr area is bordered by the Ruhr valley and its ridges immediately to the south. The zone south of the river Ruhr is part of the natural area of the 'Süderbergland', bordering the 'Bergisches Land' in the west and the 'Sauerland' in the east. The Süderbergland is densely populated by the fire salamander (Schlüpmann et al. 2006, 2011a) which occurs in almost all of the countless V-shaped valleys, with spring streams in deciduous forest, as is well documented in Hagen (see Schlüpmann 2004, 2008).

North of the river Ruhr lies the ridge of the Ardey. It extends from Mülheim an der Ruhr in the west and the district of Unna in the east, with an overall mountainous and forested character in the south, on the mountains sloping towards the Ruhr, with many areas of beech forests with small streams flowing into the Ruhr, being characteristic habitats populated by fire salamanders (Kordges & Schlüpmann 2011). Towards the north, the terrain gradually slopes down to the Emscher valley which can be regarded as the core zone of the Ruhr District with the highest density of human settlements. The small and medium-sized brooks to the river Emscher had been used here since the beginning of the 20th century as open sewers, and only here some patches of forest can be found with clean source streams, such as in the mountains of Ardey near Witten and Dortmund. Fire salamanders were and are only found in a few places (Kordges & Schlüpmann 2011).

North of the Emscher, the metropolitan area gradually merges with the agricultural landscape, with some important extensions of beech forest that often host large fire salamander populations, such as the Sterkrader Wald, Hiesfelder Wald, Köllnischer Wald and Kirchheller Heide.

The fire salamander reaches the limit of its continuous distribution in North Rhine-Westphalia in the Ruhr area on the heights of the Ardey in the southern Ruhr District (Schlüpmann et al. 2006). To the north, only few isolated fire salamander populations inhabit the lowlands (e.g., the Hiesfelder Wald) and the Münsterland (e.g. Wollbecker Tiergarten or in the woodlands near Selm and Cappenberg). Throughout, these populations dwell in so-called old forest areas that have survived the medieval deforestation unscathed (Schlüpmann et al. 2006, Feldmann & Schlüpmann 2011b). West of the Rhine and in the northern lowlands the fire salamander is completely missing.

Besides the fire salamander, the Ruhr District is also inhabited by several newt species that are susceptible to *Bsal* (Martel et al. 2014). Alpine newt (*Ichthyosaura alpestris*) and smooth newt (*Lissotriton vulgaris*) are widespread, whereas the great crested newt (*Triturus cristatus*) is only locally represented (Kordges et al. 1989, Kordges & Schlüpmann 2011). The palmate newt (*L. helveticus*) reaches its northern distribution border in the Ruhr District; only at a few known sites does this species occur further north. To the west, in a locally limited area of the Duisburg-Mülheimer forest, it reaches the edge of the northern Rhine lowland. However, to the north there is an isolated occurrence of the palmate newt in the forests north of Oberhausen (Schlüpmann 2006) where it co-occurs with the fire salamander.

Bsal screening and detection

For the detection of *Bsal*, fire salamanders were caught by hand. Each individual was handled with clean nitrile gloves and its ventral surface was gently rubbed 10 times, simultaneously with two sterile rayon swabs representing A and B sample (MW113; Medical Wire & Equipment, Corsham, UK). Each swab was kept separately in a sterile 1.5 ml centrifuge tube and stored at -20°C until DNA extraction. One swab per sampled individual was analysed in the laboratory of the Technische Universität Braunschweig (Braunschweig, Germany). In case of a positive result, the B-sample was analysed in the laboratory of Trier University (Trier, Germany) for validation.

First, genomic DNA was extracted from the swabs using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's Animal Tissues protocol with pretreatment for gram-positive bacteria. Incubation for the initial enzymatic lysis was extended to 1 h, and the temperature of the proteinase K lysis was increased to 70°C to increase DNA yield. For the detection of the chytrid fungus *Bsal*, the region of the ITS (internal transcribed spacer) rRNA (120 bp) was amplified by quantitative PCR, following a standard protocol by Blooi et al. (2013) with the alteration of using a *Bsal*-specific FAM-labeled probe (Biomers, Ulm, Germany) and KlearKall Master Mix (LGC genomics, Middlesex, UK).

Quantitative polymerase chain reactions (qPCRs) were performed on a CFX96 Real-Time System (Bio-Rad Laboratories Inc., Hercules, USA) in Braunschweig, and on a StepOnePlus (Applied Biosystems, Foster City, USA) in Trier. Each sample was run in duplicate in Braunschweig. If contradictory results of the replicates occurred, a third

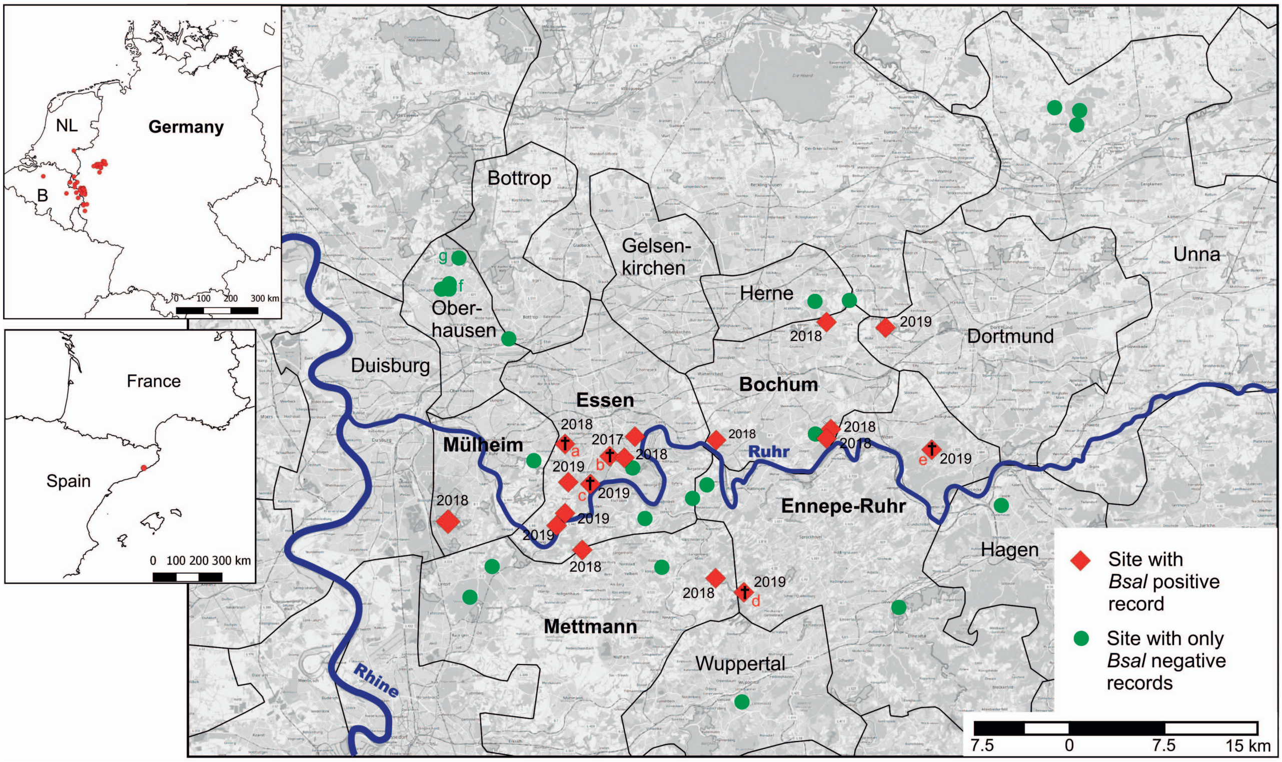

Figure 1. Map showing counties in the Ruhr District (Ruhrgebiet) in western Germany with populations surveyed for *Bsal*. For each positive site, the year of first *Bsal* detection is given. Counties with positive records are in boldface. Black crosses mark sites where mass mortality (> 5 dead salamanders) or drastic population crashes were detected: (a) Südwestfriedhof Essen, (b) Stadtwald Essen, (c) Kruppwald Essen, (d) Hattingen, (e) Witten-Annen. The two *Bsal*-negative sites used as reference monitoring sites in 2019 are (f) Sterkrader Wald and (g) Hiesfelder Wald. The map is based on Bundesamt für Kartographie und Geodäsie, Frankfurt am Main, 2011; note that county borders are only approximate. The main rivers in the area, Rhine and Ruhr, are indicated in blue. Small inset maps give overview of all *Bsal*-positive sites (red dots) in Europe known until 2019 (upper left: Germany, B: Belgium and NL: The Netherlands; below: Spain with one confirmed positive site).

replicate was run. Samples analysed at Trier University were run in triplicate. Each qPCR plate in Braunschweig contained two replicates of *Bsal* standards (10-fold dilution scale from 0.1–1,000 zoospores and 100–10,000,000 ITS copies) and two negative controls, while in Trier each plate had the *Bsal* standard (either zoospores or ITS copies) and the negative controls in triplicate. The threshold for the ITS copies was set to 100 ITS based on a comparison of Ct values between both standards (1 GE = 33 cycles/ 100 ITS copies = 35 cycles). Amplification signals were only considered as positive when the signal was between the highest and the lowest standard and when the amplification curve was logarithmic. Based on the extraction volume used, the estimated zoospore equivalents were converted to numbers of zoospores per swab (hereafter z/s) and ITS copies per swab (hereafter ITS/s).

The presence of *Bsal* at the site Kruppwald was verified for several individuals by independent qPCR at Trier University and at Ghent University (A. Martel, Ghent, Belgium), and furthermore by histology at Ghent University. However, analyses of *Bsal* prevalence and load over time, and of correlation with the presence of external ulcerations, are based on data from Braunschweig only.

Analysis of *Bsal* eDNA from water samples

This part of the study aimed to test the usability of DNA from environmental samples, so-called environmental DNA (eDNA), for *Bsal* detection. Eight streams and forty-five ponds were sampled between February and August 2019, comprising streams in the immediate vicinity of infected as well as non-infected fire salamander populations in Bochum (Botanical Garden of the Ruhr University Bochum/Kalwes, Dahlhausen, Lottental, Stiepel, Tippelsberg) and Essen (Fulerum, Kruppwald, Stadtwald). All samples were collected with clean nitrile gloves. In streams, four water samples of 500 ml each were taken from slow-moving areas of the main stream or side puddles. In ponds, four water samples were taken in a clockwise orientation using sterile 500 ml bottles that were either directly filtered at the same day or stored at 4°C overnight. Water samples were filtered using sterile 0.2 μm nitrocellulose membrane filter funnels (Thermo Fisher, Waltham, USA) and a water-jet vacuum pump. Each filter was kept separately in a sterile 1.5 ml Eppendorf tube and stored at -20°C until DNA extraction. Strict spatial separation of all eDNA related work was ensured during water filtering, DNA extraction and qPCR in different (sterile) laboratories to exclude DNA contamination. Filters were halved, shredded into small pieces and genomic DNA was extracted as already described for the swab extraction. For the detection of the chytrid fungus *Bsal*, a region of the ITS rRNA (120 bp) was amplified by quantitative PCR, following a standard protocol with the alteration of using SYBR Green GoTaq® qPCR Master (Promega, Fitchburg, USA). qPCRs were performed on Light Cycler® 96 System (Roche, Mannheim, Germany) and plates consisted of eight (stream) and four (pond) technical replicates for each sample, two replicates of synthetic *Bsal* standard (10-fold dilution scale from 100–10,000,000 ITS copies) and two negative controls. Amplification signals were only considered as positive when the signal was between the highest and the lowest standard and when the amplification curve was logarithmic. Positive samples were Sanger sequenced at the Faculty of Chemistry and Biochemistry at the Ruhr University Bochum to verify that the amplified DNA fragments corresponded to *Bsal*.

Visualization of *Bsal*-related ulcerations

High-resolution images of living salamanders and external symptoms of *Bsal* infection (ulcerations) were made with a Nikon D850 camera and a Sigma 105 mm macro lens. Amplified figures of ulcerations in a euthanized individual (in terminal stage of disease) were made with a Keyence VHX 900F digital microscope. For scanning electron microscopy, skin fragments were fixed with 5% formaldehyde and 2% glutaraldehyde overnight, washed twice with TE buffer and dehydrated in a graded series of acetone (10, 30, 50, 70 and 90% on ice, and twice in 100% acetone at room temperature) for 20 minutes each. Critical-point drying with liquid CO_2 was done with a Balzers CPD 30 and gold-palladium sputter coating with a Bal-Tec SCD500. The samples were examined with the field emission scanning electron microscope Merlin from Zeiss using both, the HESE2 Everhart Thornley SE detector and the in-lens SE detector and an acceleration voltage of 5 kV.

Heat treatment of *Bsal*-infected salamanders

A subset of fire salamanders with *Bsal*-related lesions and positive qPCR signal were subjected to heat treatment either (i) in a room with normal heaters and an additional electric heater, or (ii) in a climate chamber. As these treatments were primarily carried out to cure infected animals, they did not follow a rigorous experimental protocol (e.g. there were no controls). The animals were housed individually in plastic boxes on a moist tissue, with access to a hiding place as described in Blooi et al. (2015a). The tissues were humidified every day and renewed every second day. Fresh nitrile gloves were used for each box and animal. Earthworms and crickets powdered with vitamin supplement were provided as food. For minimizing the risk of spreading *Bsal* spores, only one person had access to the room / climate chamber, which was equipped with disinfection spray (1% Virkon S solution) in a bottle as well as filled in a disinfection mat placed in front of the door. After the treatment, the temperature was lowered slowly down to 15°C and animals were transferred into new plastic boxes which contained clay, wood and moss. All materials used in the rooms, including any substrate, plastic boxes, tissues or gloves were disinfected in a large tray filled with 1% Virkon S solution before disposal.

Capture-mark-recapture study

We used the natural individual dorsal colour pattern of fire salamanders for non-invasive individual recognition as implemented in the software AmphIdent, developed for automated amphibian photo analysis (http://www.amphident.de, Matthe et al. 2008, Drechsler et al. 2015). This software is based on a pattern recognition algorithm for the analysis of spotted dorsal patterns. We used photographs of the dorsal sides of each fire salamander, standardized in AmphIdent by adjusting the automatically generated body contour points during the process of uploading.

Larval monitoring

Larval monitoring was carried out following a removal sampling protocol suggested by Schmidt et al. (2015). A total of 75 m of a stream was divided into three transects of 25 m each. Each transect was searched for salamander larvae by one researcher during 15 min, using visual encounter and dip-netting, and temporarily removing all encountered larvae. Subsequently, researchers rotated and examined another 25 m transect, until all three transects were examined three times. The total number of larvae removed over the total of 75 m stream were counted and all larvae were released at the place of capture. The larval monitoring was always conducted at daytime to avoid a potential bias based on larval activity. Only at the *Bsal*-positive sites Stadtwald and Südwestfriedhof, larval monitoring was also conducted at night to confirm the decline.

Results and discussion

Bsal records in the Ruhr District

The first alert of suspected *Bsal*-infected fire salamanders in the Ruhr District was received in July 2017. A citizen found a fire salamander in the Stadtwald in Essen sitting exposed during daytime, obviously not in good shape as the body was covered by lesions and ulcerations characteristic for *Bsal*-infected animals. The animal was caught for treatment but died one day later. Analysis by qPCR in Braunschweig, Ghent and Trier confirmed that the animal was infected with *Bsal* and thus the pathogen's presence for the first time detected outside of the Eifel area in Germany. Swabs of 97 fire salamanders taken during two nights (August/September 2017) at this site yielded eight additional *Bsal*-positives by qPCR. Shortly afterwards, we received a report of several dead fire salamanders found on the graveyard in Essen-Fulerum (Südwestfriedhof) (Figs 2b, c), around 3.5 km from the Stadtwald. In late December 2017 and in January 2018, more than 60 dead fire salamanders (some already almost decomposed, several with *Bsal*-related ulcerations) had been observed on the paths (Fig. 2a), constituting the first documented mass mortality event in the Ruhr District. Our subsequent survey in the same period yielded eight fire salamanders, four of which were dead and all of which tested positive (qPCR results confirmed by duplicate analyses at Braunschweig and Trier Universities).

Subsequent surveillance was carried out in close collaboration with the local Nature Conservation authorities, the Ruhr University Bochum and the Biological Station of Western Ruhr (hereafter BSWR). In 2018, we screened a fire salamander population in Bochum-Querenburg and Stiepel; three out of 40 swabbed salamanders tested *Bsal*-positive, and one of these had a few of the typical *Bsal*-related skin ulcerations. Subsequent work led to the discovery of one dead *Bsal*-infected fire salamander each in Bochum-Hiltrop and Bochum-Dahlhausen, respectively.

A further *Bsal*-positive site in Essen was recorded in April 2018 close to the Stadtwald. In this case, only alpine newts were affected, with several dead individuals being observed. One living newt showed skin ulcerations characteristic for *Bsal* (Figure 3). In Essen-Bergerhausen, we also detected *Bsal* in alpine, smooth and palmate newts screened at a temporary amphibian drift fence consisting of pitfall traps to prevent road mortality events. At the same time, one dead, *Bsal*-positive, fire salamander was found in Velbert (Stumpsberg), approximately 5 km south of Essen. In June 2018, a citizen living at the border between Essen and Heiligenhaus discovered two dead fire salamanders showing obvious skin damage which we confirmed positive for *Bsal*. Some months later, we also located a *Bsal*-infected fire salamander in Mülheim an der Ruhr, west from Essen, simultaneously with the report of a citizen of a fire salamander with skin lesions and deep ulcerations in a garden, found during the day; in a previous screening in 2017, this population (N = 30) did not yield any *Bsal*-positives.

In January 2019, we received records from citizens in Essen of two dead fire salamanders indicating characteristic *Bsal*-lesions at Ruthertal (Essen-Kettwig and Essen-Werden), both of which we confirmed *Bsal*-positive. Just some days later, the first dead salamander with visual *Bsal*-symptoms was found at the site Essen-Kruppwald, where previous surveys in spring 2018 (N = 8) and autumn 2018 (N = 22) indicated no sign of *Bsal*. A second mass mortality event was then recorded in Witten, Borbach, by a citizen in February 2019. Here, 18 dead fire salamanders were lying close to a house next to a dry stone wall; in 13 we could still see ulcerations, and these were collected and tested *Bsal*-positive. Two months later, eight dead fire salamanders were observed in Hattingen (Deilbach); three in good condition for analysis and with signs of *Bsal*-ulcerations were collected and tested positive. Most recently, in December 2019 two dead fire salamanders were also found to the west of Dortmund, at the Dellwiger Bachtal. Both individuals tested *Bsal*-positive and had damaged skin parts with several ulcerations.

Although qPCR is currently the most reliable and widely used method for *Bsal* detection, a final confirmation of the pathogen's presence requires combination of qPCR with a second diagnostic technique (Thomas et al. 2018). In 2019, tissue samples of a dead infected fire salamander from the Kruppwald site were sent to the Central Diagnostic Laboratory for *Bsal* in Europe at the University of Ghent, Belgium (http://bsaleurope.com/laboratories/); here, qPCR from freshly taken swabs of this sample, as well as histology provided an official confirmation of *Bsal* in the Ruhr District (A. Martel & F. Pasmans pers. comm. May 2019).

In summary, at present the distribution of *Bsal* in the Ruhr District is covering an area of 800 km² including the cities Bochum, Dortmund, Essen, Hattingen, Heiligenhaus, Mülheim an der Ruhr, Velbert and Witten (Table 1, Fig. 1).

Earliest indication of *Bsal* presence in the Ruhr District

Bsal is known to be present in the Ruhr District since 2017 (Essen Stadtwald) proved by qPCR. By involving local media and other networks, we received several additional reports of fire salamanders with suspected *Bsal* infection. One of these indicated that in 2016 several dead fire sala-

Figure 2. Habitats of European fire salamanders (*Salamandra salamandra*) in the Ruhr District: (a) Dead salamander encountered next to a trail in the Südwestfriedhof graveyard area of Essen-Fulerum during a *Bsal*-related mass mortality event in early 2018; (b, c) stream valley adjacent to the Südwestfriedhof used for reproduction by the almost extinct population at this site; (d, e) stream and pond in the Sterkrader Wald, sites of larval development of fire salamanders; (f) fire salamander in the deciduous Kruppwald forest in Essen; (g, h) stream in the Kruppwald.

Table 1. Sites/districts (with decimal geographic coordinates) within the Ruhr District (Ruhrgebiet) in western Germany where different amphibians were screened for *Bsal* in the years 2017–2019. *Bsal*-positive sites are underlined. The amount of *Bsal*-positive individuals (in boldface) in relation to the amount of individuals sampled. *S.s. = Salamandra salamandra; I.a. = Ichthyosaura alpestris; L.h. = Lissotriton helveticus; L.v. = L. vulgaris; T.c. = Triturus cristatus; R.t. = Rana temporaria; B.b. = Bufo bufo*; † = dead individuals.

City / County	Site *(Coordinates given in decimal degrees)*	2017	2018	2019
Essen	Stadtwald (Forest) (51.423354, 7.020767)	*S.s.*: **8**/97	*I.a.*: **1**/2	*S.s.*: 0/1 *I.a.*: **1**/4 *B.b.*: 0/4 *R.t.*: 0/1
	Stadtwald (Garden) (51.423306, 7.033296)	No sampling	*I.a.*: **1**/25	*I.a.*: **2**/50
	Fulerum, Südwestfriedhof (51.431025, 6.968945)		*S.s.*: **8**/68 (64 †)	*I.a.*: 0/22*L.h.*: 0/5 *L.v.*: 0/10 *B.b.*: 0/32*S .s.*: **1**/5*R.t.*: 0/3
	Ruthertal (51.382679, 6.972078/ 51.376889, 6.959599)		*S.s.*: **1**/23	*S.s.*: **3**/3 (3 †)
	Kruppwald (51.403281, 6.995988)		*S.s.*: 0/30	*S.s.*: **73**/333 (8 †)
	Wolfsbachtal (51.404382, 6.973143)		No sampling	*S.s.*: **6**/15 *I.a.*: **1**/1
	Bergerhausen (amphibian fence) (51.436599, 7.048501)		No sampling	*I.a.*: **2**/5 *L.h.*: 0/2 *L.v.*: **6**/17
	Fischlaken (51.378457, 7.057662)	*S.s.* 0/1	No sampling	No sampling
	Byfang (51.392093, 7.112520)	No sampling	*R.t.* 0/2 *L.h.* 0/1 *I.a.* 0/3	No sampling
	Schellenberger Forest (51.413459, 7.043988)	*S.s.* 0/12	No sampling	No sampling
Bochum	Querenburg (several spots) (51.440654 7.254040/ 51.442381, 7.270643 / 51.448365, 7.280179)	No sampling	*S.s.*: **2**/25	*S.s.*: 0/5
	Stiepel (several spots) (51.418149, 7.251696/ 51.422972, 7.21072)		*S.s.*: **1**/15	*S.s.*: **1**/26 *I.a.*: 0/2 *L.v.*: 0/4
	Dahlhausen (51.435223, 7.139897)		*S.s.*: **1**/1 †	*S.s.*: 0/3 *I.a.*: 0/2 *L.v.*: 0/2
	Hiltroper Volkspark (51.52021, 7.26652)		*S.s.*: **1**/1 †	*L.v.*: 0/1 *B.b.*: 0/2
	NSG Tippelsberg/Berger Mühle (51.512009, 7.235154)		No sampling	*S.s.*: 0/43
Dortmund	Dellwiger Bachtal (51.511440, 7.336399)	No sampling	No sampling	*S.s*: **2**/2 (2 †)
Herne	Langeloh (51.532323, 7.289932)	No sampling	No sampling	*S.s.*: 0/18 *B.b.*: 0/1
	Gysenberg (51.532070, 7.250724)	No sampling	No sampling	*S.s.*: 0/1
Selm	Cappenberger Wald (51.669187, 7.525273/ 51.666870, 7.551980/51.656975, 7.550057)	No sampling	*S.s.*: 0/49 *I.a.*: 0/1	No sampling
Mülheim an der Ruhr	Rottbachtal (Forest/Garden) (51.377275, 6.839299)	*S.s.*: 0/30	*S.s.*: **1**/3 *I.a.*: **1**/1	*S.s.*: **3**/13 *R.t.*: **1**/1 *B.b.*: 0/1 *I.a.*: **4**/36 (0/3 †) *L.v.*: 0/18
	Rumbachtal (51.416847, 6.936570)	*S.s.*: 0/7	*S.s.*: 0/2	*S.s.*: 0/1 *I.a.*: 0/3 *R.t.*: 0/1 *B.b.*: 0/1
Wuppertal	Barmen, Hesselnberg (51.247764, 7.168684)	No sampling	*S.s.*: 0/11 *I.a.*: 0/3 *R.t.*: 0/1	No sampling
Kreis Mettmann	Ratingen (51.343460, 6.884168/51.321740, 6.858843)	*S.s.*: 0/22	*S.s.*: 0/10	*S.s.*: 0/24 *L.v.*: 0/1 *R.t.*: 0/2
	Heiligenhaus (51.358604, 6.990914)	No sampling	*S.s.*: **2**/2 †	No sampling
	Velbert (Bleibergquelle/Deilbach) (51.343183, 7.077412/ 51.337388, 7.142683)	No sampling	*S.s.*: **2**/3 (2†)	No sampling
Ennepe-Ruhr Kreis	Witten-Annen (51.429405, 7.387315)	No sampling	No sampling	*S.s.*: **13**/18 (18 †) R.t.: 0/2
	Hattingen - Deilbach (51.326008, 7.171701)	No sampling	No sampling	*S.s.*: **2**/8 (8 †)
	Hattingen – Niederwenigern (51.401187, 7.126893)	*S.s.* 0/1	No sampling	No sampling
	Ennepetal–Gevelsberg (51.315666, 7.346786)	No sampling	No sampling	*S.s.* 0/1
Hagen	Hagen-Nord (51.387469, 7.463797)	No sampling	*I.a.*: 0/2	No sampling
Oberhausen	Sterkrader Wald (51.542313, 6.833602)	No sampling	No sampling	*S.s.*: 0/109 *I.a.*: 0/2 *B.b.*: 0/1
	Hiesfelder Wald (51.562273, 6.846767/ 51.567875, 6.832103)	No sampling	No sampling	*S.s.*: 0/58 *I.a.*: 0/2 *L.h.*: 0/1 *T.c.*: 0/24
	Vonderort (51.505806, 6.902312)	No sampling	*S.s.*: 0/7	No sampling

manders had been observed in Essen-Ruthertal; qPCR of the preserved specimens did not yield *Bsal*-positive results, but this could be due to suboptimal conditions of preservation. Two other citizens reported having seen several dead fire salamanders in the winter of 2016 in their gardens or in the surroundings of their houses (Hattingen-Niederwenigern and Essen-Byfang, both sites at just 1.2 km from each other), and an absence or near-absence of fire salamanders at these sites since that time. Since no samples had been preserved, we could not confirm that the mortality at these two sites was due to chytridiomycosis. However, it seems possible that the pathogen was present already pre-2017 in the Ruhr District, especially when combining those reports with a photograph of a fire salamander in Bochum made in 2004 that shows typical *Bsal*-related lesions and ulcerations on the head (M. Maschka, pers. comm., March 2020; Fig. 3). No samples were taken of this salamander in 2004, and the population where it was photographed does not exist anymore; a confirmation that this salamander was indeed infected by *Bsal* therefore cannot be obtained. However, it is striking that the first evidence of *Bsal* in Germany, and in all of Europe, is also from 2004, referring to a preserved fire salamander from the Eifel Mountains where the pathogen was confirmed by histological examination at Ghent University (Dalbeck et al. 2018, Lötters et al. 2020b in this issue).

Quantifying *Bsal*-related declines

As reported above, for two sites in the Essen area, indications for fire salamander population crashes became available in 2017–2018 (Fig. 1a, b): In the Stadtwald, 97 individuals had been swabbed and analysed by us in 2017, eight of which turned out to be *Bsal*-positive and three had *Bsal*-characteristic external ulcerations; surveys in 2018 yielded no observations of infected fire salamanders. In the Südwestfriedhof graveyard, large numbers of dead individuals were observed at the end of 2017 and beginning of 2018, of which eight could be tested and resulted to be positive (Schulz et al. 2019, Wagner et al. 2019); afterwards, only two observations of a single fire salamander each were recorded by citizens during 2018 (Supplementary document 1). The Südwestfriedhof is highly frequented by promenaders, and many of these confirmed to us that in previous years fire salamanders were a common sight. However, given the secretive habits and not fully predictable activity pattern of fire salamanders, it is problematic to confidently assess the decline or disappearance of a population – a lack of encounters may simply be due to climate-induced inactivity or to a low overall activity in the respective night.

Figure 3. Photograph of a European fire salamander individual (*Salamandra salamandra*) taken in April 2004 in Bochum, Dahlhausen, by Martin Maschka. The animal shows possibly *Bsal*-related lesions and ulcerations especially on the head.

To obtain more reliable information on the status of declines/extinctions of the fire salamander populations at Südwestfriedhof and Stadtwald, we conducted an additional monitoring in spring 2019, along with standardized comparisons with two reference populations. As references, we chose two dense fire salamander populations without known *Bsal*-positives in the vicinity, Sterkrader Wald (Figs 1f, 2d, e) and Hiesfelder Wald in Oberhausen (Fig. 1g). At all four sites, transects of ca. 2 km were defined within suitable habitat, i.e. deciduous forest with mainly beech trees and close to small streams used by fire salamanders for reproduction. Transect walks took place during the night by a minimum of two observers, slowly walking along the transect and scanning the area left and right of the transect with electric torches for active salamanders and other amphibians. Because all encountered salamanders were photographed and swabbed, time per transect was not standardized.

At the two *Bsal*-positive decline sites, on five survey nights a total of only three salamanders could be recorded (Table 2): On April 2 and April 24, one individual each at the Südwestfriedhof site, on April 2, one individual at the Stadtwald site. In contrast, the two reference sites yielded 8–232 (Sterkrader Wald) and 14–28 (Hiesfelder Wald) salamander records per night (Table 2). On the two nights with highest numbers of active salamanders at the two reference sites (April 24 and May 2), surveys at all four sites were conducted simultaneously. Data suggest a heavy impact of the disease outbreak at the *Bsal*-positive sites, and considering the anecdotal observations from 2017 and 2018, it appears that the populations have drastically declined almost to complete extinction within only one or two years.

The single fire salamander found in 2019 at the Stadtwald was tested negative for *Bsal*; of other amphibians sampled at this site, two out of four alpine newts tested *Bsal*-positive, confirming the presence of the pathogen at this site. At the Südwestfriedhof, two fire salamanders were found during our five monitoring nights, and two additional individuals at other nights in 2019; one of these was found dead and tested *Bsal*-positive. Additionally, one living fire salamander without any visible sign of infection was recorded by a citizen during that time (Supplementary document 1). All of the 21 alpine newts, ten smooth newts

Table 2. Results of fire salamander surveys in the spring of 2019 at two *Bsal*-positive sites in Essen (Ruhr District) where declines were detected or assumed in 2017/2018 and at two *Bsal*-negative reference sites. During the first two surveys, not all sites could be assessed at the same day (* assessed on March 16; # assessed on April 3). NA = not assessed.

Site	March 14, 16	April 2, 3	April 24	May 2	May 21
Bsal-positive					
Südwestfriedhof	0	1	1	0	0
Stadtwald	0	1	0	0	0
Bsal-negative (Reference)					
Sterkrader Wald	8*	45#	232	118	NA
Hiesfelder Wald	NA	14	19	28	NA

Table 3. Results of monitoring of larvae of the European fire salamander (removal sampling) in streams in 2019, at two *Bsal*-positive sites in Essen (Ruhr District) where declines had been detected or assumed in 2017/2018, and at two *Bsal*-negative reference sites. Dates of surveys are listed with regard to the order of the sites. Numbers marked with (N) refer to nocturnal surveys.

Site	March 29/29/ 20/26	April 1	April 24/24/ 18/23	April 29	May 21/20/ 17/22	May 23/20
Bsal-positive						
Stadtwald	0	2 (N)	1	5 (N)	0	1 (N)
Südwestfriedhof	0	29 (N)	0	4 (N)	0	1 (N)
Bsal-negative (Reference)						
Sterkrader Wald	43	–	78	–	177	–
Hiesfelder Wald	237	–	463	–	364	–

and five palmate newts swabbed at the Südwestfriedhof in 2019 tested negative.

To obtain further quantitative data on population sizes in the *Bsal*-positive vs. reference sites, we performed monitoring of salamander larvae via removal sampling in all four sites. During diurnal sampling, we estimated numbers of 43–177 larvae (Sterkrader Wald) and 237–463 larvae (Hiesfelder Wald) per 75 m of stream in the reference sites, but only up to a maximum of one larva (Stadtwald) or no larvae (Südwestfriedhof) could be found at the *Bsal* positive sites (Table 3). To ascertain whether indeed at the decline sites larvae were absent, we sampled these sites three times at night, considering that fire salamander larvae are often more exposed and thus easier to spot in the streams during the night. At each sampling event, we obtained at least one larva at the *Bsal*-positive sites, with a maximum of up to 29 larvae on April 1 at Südwestfriedhof (Table 3).

Additionally, monitoring of the two *Bsal*-sites Stadtwald and Südwestfriedhof was conducted in October 2019 which resulted in no fire salamander observations over a period of five nights at the Stadtwald and one negatively tested individual over a period of seven nights at the Südwestfriedhof. In total, three negative and one positive tested fire salamanders were observed between March and October; only one per night out of 17 nights surveyed while only one non-infected fire salamander was found in one of 11 nights at the Stadtwald (Supplementary document 1).

Overall, these data confirm that the populations at the *Bsal*-positive sites have undergone severe declines, with only few females having deposited larvae in the respective streams in 2019. However, taking into account the results of further opportunistic searches for conservation genetic sampling, a total of 42 larvae from Stadtwald and 38 larvae from Südwestfriedhof were observed and sampled in 2019 (Schulz et al. 2019), and therefore we cannot consider the populations as being completely extinct yet.

Real-time history of a population crash

In late 2018, a large population of fire salamanders occurring in the Kruppwald (Figs 2f–h), northwest of the Baldeneysee, Essen-Bredeney, was selected for monitoring. Initially, this population was flagged as a *Bsal*-negative control site, based on the negative swabbing results of 30 fire salamanders (April 11, 2018: 8 individuals; September 21, 2018: 22 individuals). A transect was defined along a small stream serving salamanders for breeding, approximately between the geographical coordinates 51.4012N, 6.9973E and 51.4033N, 6.9957E.

In early 2019, two salamanders were found dead in this forest and reported to us by a citizen (one salamander each on January 3 and February 3). The site therefore offered the unique opportunity to observe the progress of a *Bsal* outbreak in real time. Nocturnal transect walks in the Kruppwald (transect length ca. 2 km) were carried out from February 28, 2019 to May 27, 2019 (plus three diurnal visits on March 21, 26 and 28), and altogether 278 salamanders were recorded during this time (including at least 20 individuals recaptured once or several times).

Table 4. Summary of individuals of European fire salamanders (*Salamandra salamandra*) from Kruppwald (Essen, Ruhr District) sampled in 2019 which were recaptured on at least one occasion during the study period. N and P refer to negative or positive (in boldface) results of qPCR tests of *Bsal* infection. Individual numbering as used in the AmphIdent project. * Infected salamander subsequently taken into the lab and cured by heat treatment. † Fire salamander found dead at the site.

Individual	Initial capture	First recapture	Second recapture	Third recapture
SA21	April 2 (N)	May 2 (N)	**September 23 (P)***	
SA61	April 2 (N)	May 21 (N)		
SA115	April 24 (N)	May 21 (N)		
SA118	April 24 (N)	May 8 (N)		
SA120	April 24 (N)	May 8 (N)		
SA122	April 24 (N)	May 21 (N)		
SA128	April 24 (N)	May 2 (N)	May 8 (N)	May 21 (N)
SA186	April 27 (N)	May 21 (N)		
SA217	May 2 (N)	May 21 (N)		
SA231	May 2 (N)	May 8 (N)		
SA238	May 2 (N)	May 8 (N)		
SA243	May 2 (N)	May 21 (N)		
SA246	May 2 (N)	May 21 (N)	**September 23 (P)**	
SA247	May 2 (N)	**May 8 (P)**		
SA265	**May 8 (P)**	**May 21 (P)**		
SA266	May 8 (N)	May 21 (N)	September 23 (N)	
SA272	May 8 (N)	September 23 (N)	October 4 (N)	
SA278	May 8 (N)	May 27 (N)		
SA289	May 8 (N)	May 17 (N)		
SA297	May 8 (N)	May 17 (N)		
SA323	May 21 (N)	May 27 (N)		
SA340	September 29 (N)	October 6 (N)		
SA343	October 4 (N)	October 6 (N)		
SA348	October 4 (N)	October 6 (N)		
SA352	September 23 (N)	September 29 (N)		
SA359	September 29 (N)	October 4 (N)		
SA466	March 1 (N)	April 2 (N)		
SA517	April 2 (N)	September 21 (N)		
SA534	**May 2 (P)**	**May 8 (P) †**		

Data are summarized in Table 4, with trends of prevalence and *Bsal* load over time given in Figure 4 / Table 5. Counting only the nights on which a regular transect walk of several hours was carried out, between 8 and 47 salamanders were observed, with maximum activities usually coinciding with rainfall. Although a general decrease in numbers of observed salamanders was observed during the last three survey days in May 2019, this cannot necessarily be interpreted as an indication of a major population decline because during these days the activity of salamanders decreased in general due to drier conditions, as also observed in *Bsal*-negative control populations (see below).

Maximum *Bsal* loads (in ITS/s) were relatively low until the beginning of March, (7.90E+03 to 5.66E+05), reached higher values at most sampling visits between the end of March to the end of April, with a maximum value of 2.29E+09 recorded on March 28, and reached moderate values in the last three visits in May, not exceeding a maximum value of 7.66E+06. The trend of mean *Bsal* loads of infected individuals followed a similar trend, as did prevalences which reached a maximum of 50% on the last sampling day, May 27, 2019 (Fig. 4). Both, *Bsal* loads and prevalences dropped towards the end of April 2019, with *Bsal* loads reaching its lowest post-March value on April 24 and prevalences dropping to 20% on April 24 and to 4% on April 27. Individuals with visible ulcerations scored from photographs peaked on May 2 and 8, 2019. Dead salamander individuals were observed on six occasions from January to March 2019, and one additional individual in May.

Using data from Winter 2018 and Spring 2019, and ordering all sampling days chronologically, a non-parametric Spearman correlation revealed a significant correlation between sampling day and prevalence (Spearman's R = 0.721; P = 0.019), and the relation between mean *Bsal* load and time was close to significance (Spearman's R = 0.486; P = 0.066). This suggests a probable overall trend of increasing prevalence of *Bsal* infection, and possibly an increasing *Bsal* load in infected individuals, in this population over

Table 5. Number of European fire salamander individuals (*Salamandra salamandra*) encountered during nocturnal transect walks at the Kruppwald (Essen, Ruhr District) in April and May 2019 (plus three salamanders encountered during diurnal visits in March 2019), and their *Bsal* load. Mean air temperature (Temp; °C) measured during the respective night or day. Note that the first two records in 2019 refer to dead salamanders encountered by citizen science observers. Prevalence values were only calculated for nights with > 2 individuals. *Bsal* loads were determined as numbers of ITS copies per swab, except in 2018 and early 2019 (zoospore genomic equivalents, abbreviated GE, per swab – transformed into ITS copies per swab by x 100). NA, not applicable/data not taken.

Date	Temp	Negatives	Positives (dead)	With lesions	Prevalence (%)	*Bsal* load mean	*Bsal* load mean positives	*Bsal* load maximum
April 4, 18	NA	8	0	NA	0	0	NA	NA
September 21, 18	NA	22	0	NA	0	0	NA	NA
January 3, 2019	NA	0	1 (1)	NA	NA	7.23E+04(723 GE)	7.23E+04 (723 GE)	7.23E+04(723 GE)
February 3, 2019	NA	0	1 (1)	NA	NA	3.00E+03(30 GE)	3.00E+03(30 GE)	3.00E+03(30 GE)
February 28, 2019	NA	19	1 (1)	NA	5.0	2.83E+04(283 GE)	5.66E+05(5660 GE)	5.66E+05(5660 GE)
March 1, 2019	NA	30	6 (1)	NA	16.7	7.79E+03	6.62E+04	1.37E+05
March 3, 2019	NA	0	1 (1)	NA	NA	7.90E+03	7.90E+03	7.90E+03
March 21, 2019	15	1	0	NA	NA	NA	NA	NA
March 26, 2019	9	0	1 (1)	NA	NA	1.58E+07	1.58E+07	1.58E+07
March 28, 2019	12	0	1	NA	NA	2.29E+09	2.29E+09	2.29E+09
April 2, 2019	12	27	15	0	35.7	3.12E+07	8.73E+07	1.16E+09
April 8, 2019	11.5	1	1	1	NA	1.10E+06	2.20E+06	2.20E+06
April 24, 2019	10	16	4	0	20.0	8.53E+03	4.27E+04	6.29E+04
April 27, 2019	8	22	1	1	4.3	NA	NA	NA
May 2, 2019	10.5	29	17	8	37.0	3.58E+07	9.68E+07	1.05E+09
May 8, 2019	10.5	27	20 (1)	12	42.6	1.13E+07	3.05E+07	3.80E+08
May 17, 2019	11	4	3	0	42.9	3.53E+05	8.24E+05	1.83E+06
May 21, 2019	11.5	16	7	2	30.4	2.03E+05	6.65E+05	1.28E+06
May 27, 2019	11	4	4	2	50.0	1.38E+07	2.75E+07	7.66E+06

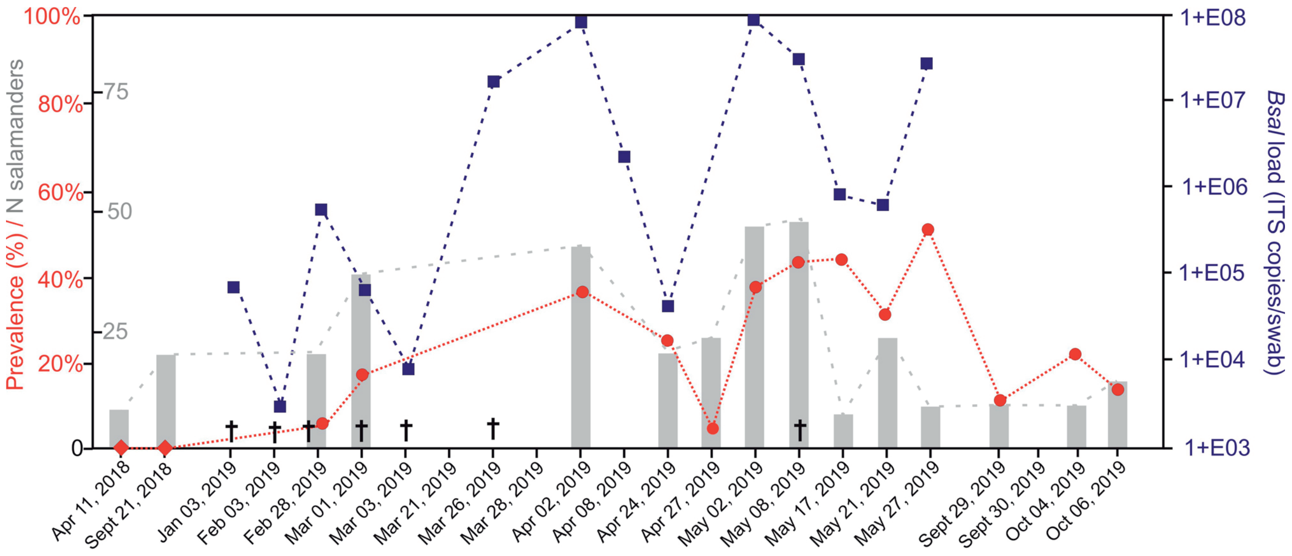

Figure 4. Graph showing number of European fire salamanders (*Salamandra salamandra*) encountered, *Bsal* infection prevalences (in percent of *Bsal*-positive individuals), average *Bsal* loads (ITS copies per swab) of positive individuals, and records of dead salamanders (crosses; on each marked day, a single dead individual was found) at various survey days in 2018 and 2019 at the Kruppwald site, along a ca. 2 km transect. Prevalences were calculated only for days with > 2 salamanders recorded; numbers of salamanders are only reported for nocturnal transect surveys with > 5 individuals. *Bsal* loads for some survey days were not calculated due to the use of different qPCR standards (see text).

the study period, despite the temporary drop of both variables at the end of April.

Additional surveys carried out in September 2019 revealed a comparatively low number of salamanders persisting in the population, many of which were subadults with relatively low infection prevalence (18%). A number of individual salamanders were recaptured on at least one occasion, as revealed by analysis of colour patterns in AmphIdent. In 24 out of 30 cases, recaptured individuals were tested *Bsal*-negative in the initial capture and all recaptures. Of the six recaptured individuals that were tested *Bsal*-positive on at least one occasion, three were already positive at first capture. In two of these, the data allowed comparing *Bsal* loads at two sampling dates: SA247 had a load of 1.37E+04 ITS/s on May 2 and 8.39E+05 on May 8; and SA265 had a load of 3.39E+04 ITS/s on 8 May and 2.12E+06 on 21 May. In SA247, *Bsal* load thus increased by one order of magnitude within a week; the individual had no visible external ulcerations at first capture but a few recognizable ulcerations at recapture. In SA265, the load increase was almost two orders of magnitude within 2 weeks; from the photos, a few ulcerations were recognizable at first capture but not at recapture, despite the higher infection loads. The other individual that was positive on the first sampling occasion (SA534) was observed on May 2 with a load of 1.05E+09 ITS/s, and was found dead on May 8.

Bsal loads and external symptoms

Bsal was first discovered in a declining population of fire salamanders in the Netherlands (Martel et al. 2013); external symptoms described consist of multifocal epidermal erosions and deep ulcerations on the entire body (Martel et al. 2013; see also van Rooij et al. 2015). Histologically, the periphery of these erosions is characterized by keratinocytes each containing one centrally located thallus, and the area of the lesions is often superficially colonized by bacteria (Martel et al. 2013). The increase in relative abundance of these opportunistic bacteria due to the destruction of the epidermis can contribute to septicaemic events, these bacteria thus acting as pathogens (Bletz et al. 2018).

At the Kruppwald site, we observed 84 *Bsal*-positive salamanders, of which 78 were alive. Many of these presented the typical ulcerations described previously for *Bsal*-infected fire salamanders (Figs 5–7), allowing for an

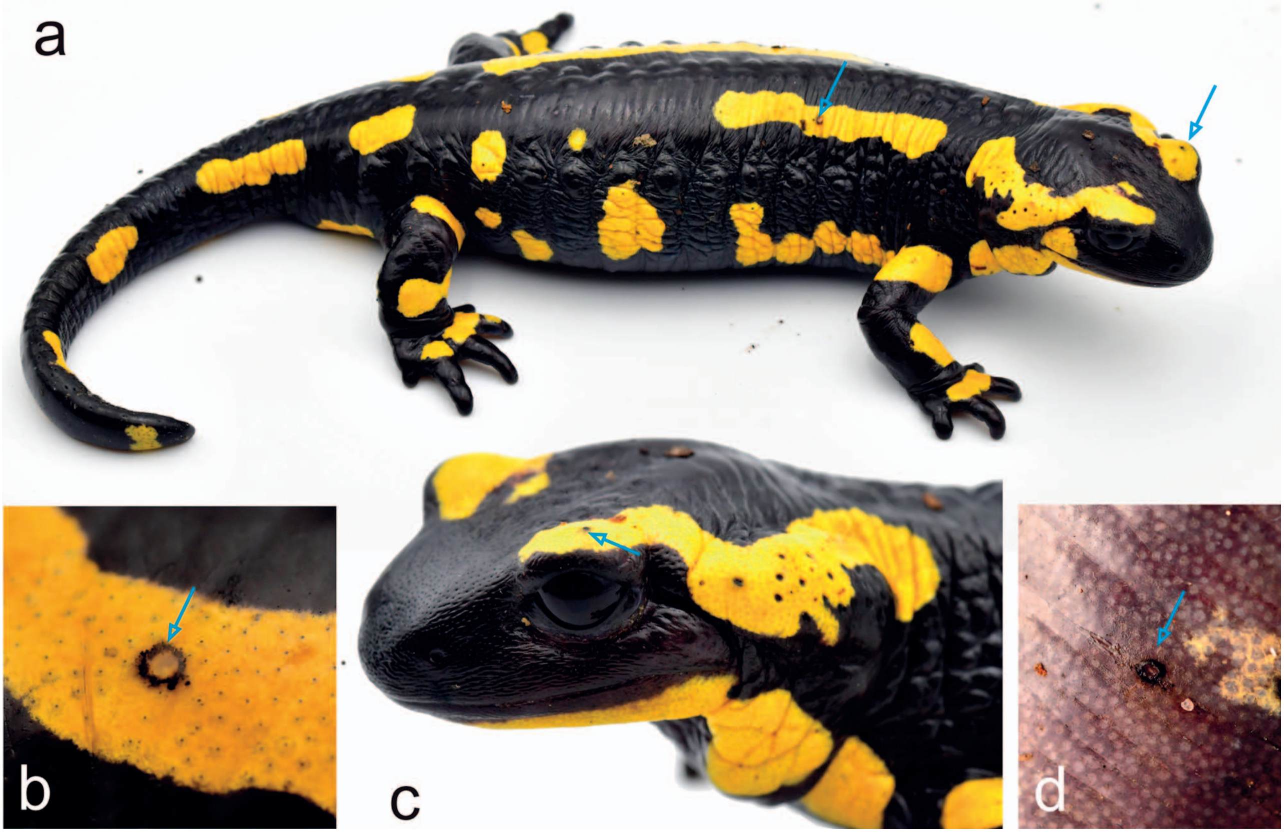

Figure 5. A *Bsal*-positive European fire salamander (*Salamandra salamandra*) female with a few external ulcerations on the dorsum (a, b), head (a, c) and venter (d) (indicated by blue arrows) detected at the Kruppwald site on April 2, 2019 (individual K0). This was the first salamander with external symptoms discovered at this site, and it survived for 39 days at the Technische Universität Braunschweig at a low temperature regime (ca. 15°C), with some of the ventral ulcerations and lesions healing, before it eventually died.

extended (macroscopic) description of these symptoms which may prove important for future initial detection of the disease, e.g. in the course of citizen science projects.

By far, most salamanders with visible signs of disease had small ulcerations which were best visible on the yellow skin patches but also exist on the black-coloured parts of the

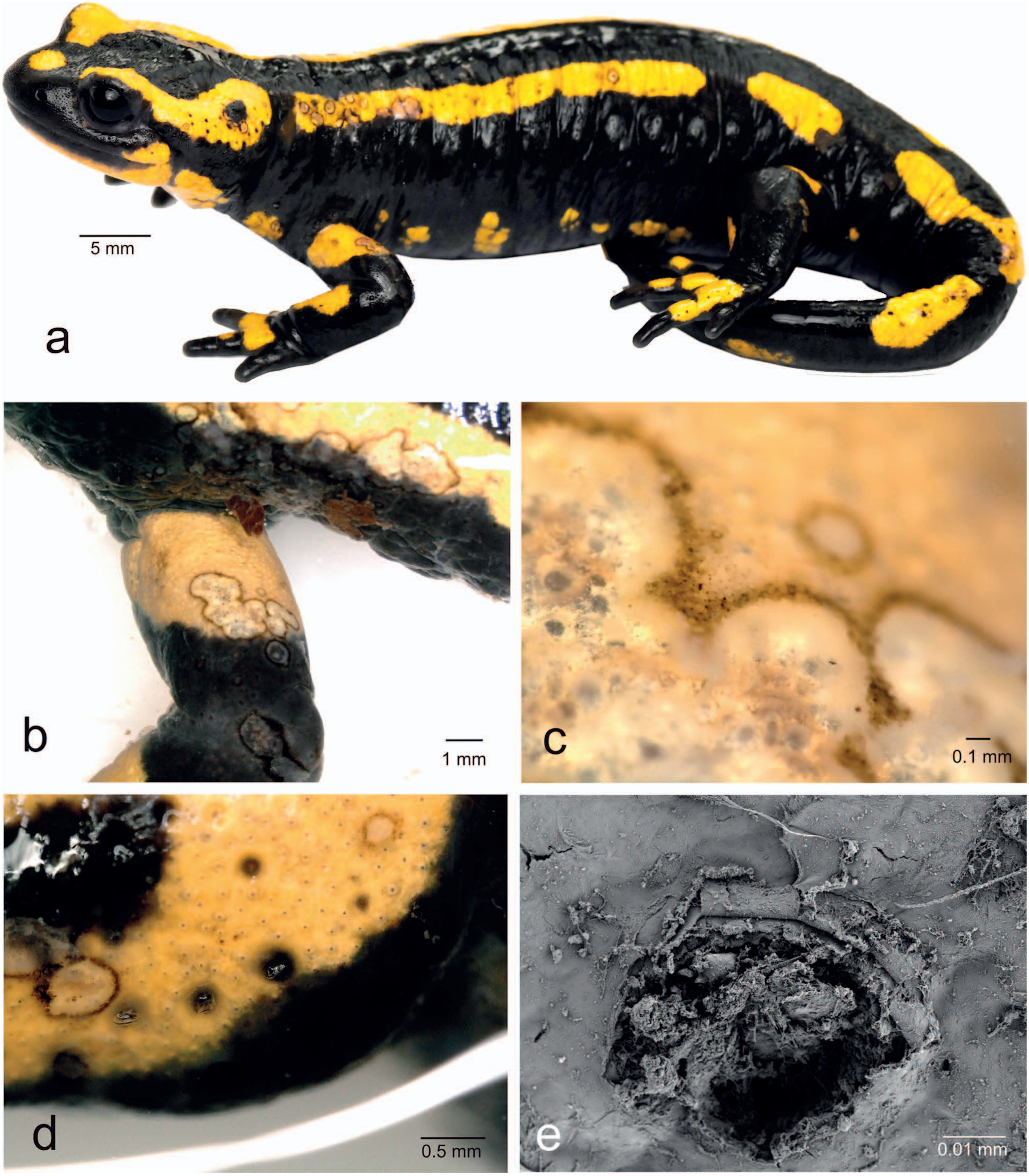

Figure 6. A European fire salamander (*Salamandra salamandra*) male from Kruppwald with a large number of partly large-sized ulcerations. (a) Dorsolateral view in life (snout–vent length 98.8 mm; voucher preserved under field number ZCMV 15514). This individual was sacrificed for close examination of the ulcerations under a light microscope. (b–d) Lesions visible in the living animal on the parotoid gland and forelimb. (e) Scanning electron microscope showing one of the crater-like ulcerations. The individual tested *Bsal*-positive.

Figure 7. Different individuals of European fire salamanders (*Salamandra salamandra*) from Kruppwald with externally visible *Bsal*-related ulcerations. All these individuals tested *Bsal*-positive.

body. Because in the Kruppwald population, salamanders have only rarely yellow colour on the ventral side, the ventral ulcerations are less readily detectable. In some individuals only single ulcerations were present, and sometimes it was not easy in such cases to macroscopically distinguish tiny dorsal ulcerations from poison gland duct openings (especially on the parotoid glands), small abnormal coloration phenomena (such as reddish pigment elements or small black dots on yellow patches), or small ventral injuries. Other individuals had multiple small ulcerations over the whole body (Figs 5, 6), and often these were present on the head, both in the region of snout and throat, and in some individuals also in high density on the parotoid glands (Figs 6d, 7g). In most cases, salamanders had a few or many small-sized regular round ulcerations of 0.25–1 mm in diameter, but in several individuals (Fig. 6) some ulcerations were larger (up to ca. 2.5 mm) and confluent with each other, giving the impression of outward growth from the centre of each ulceration, similar to a 'fairy circle'. The edge of the growth zone is brownish and in magnified view consists of bulbous structures probably corresponding to keratinocytes and maybe fungal thalli (Figs 6c, d). Examined with a scanning electron microscope, the ulcerations are revealed as deep crater-like formations in which the original skin layer is completely destroyed (Fig. 6e). In advanced stages of disease, these ulcerations become so deep that they form bleeding lesions, often in the region of the snout (Fig. 7e).

Because all recorded salamanders from April 2019 were photographed for individual recognition, it was possible to score the presence of these externally visible ulcerations and lesions for all individuals and relate the presence and intensity of these symptoms to *Bsal* presence and loads determined by qPCR. We classified individuals into four categories: (1) no recognizable ulcerations, (2) ulcerations visible according to field notes only, not visible in photos, (3) few/weak ulcerations, (4) moderate number of ulcerations, (5) many ulcerations. These data revealed that ulcerations were never found in *Bsal*-negative salamanders (Fig. 8a), suggesting that in regions with qPCR-confirmed *Bsal* outbreaks the presence of the typical lesions described here can be seen as a suitable indicator for individuals likely to be *Bsal*-infected. However, the majority of infected salamanders showed no visible ulcerations (47 of 74 scorable, *Bsal*-positive individuals), and only three and four individuals were observed in the highest categories of a moderate and high number of ulcerations, respectively (Fig. 8b). The median *Bsal* load for *Bsal*-positive individuals was lowest for individuals without lesions and highest for individuals with many ulcerations (Fig. 8c), but the differences among categories were not statistically significant (Kruskal-Wallis-ANOVA, P > 0.5), which could be either due to low sample sizes in the categories of moderate number and many ulcerations, or to differences in individuals regarding e.g. microbiome composition (Bataille et al. 2016), genetic variation (Horner et al. 2017), individual thermal preference (Sauer et al. 2018) or immunogenetic variation (Savage & Zamudio 2011).

Clearing *Bsal* infection with heat treatment

Disease dynamics are linked to the pathogen virulence, host factors and environmental determinants, and temperature seems to be one key environmental factor for both

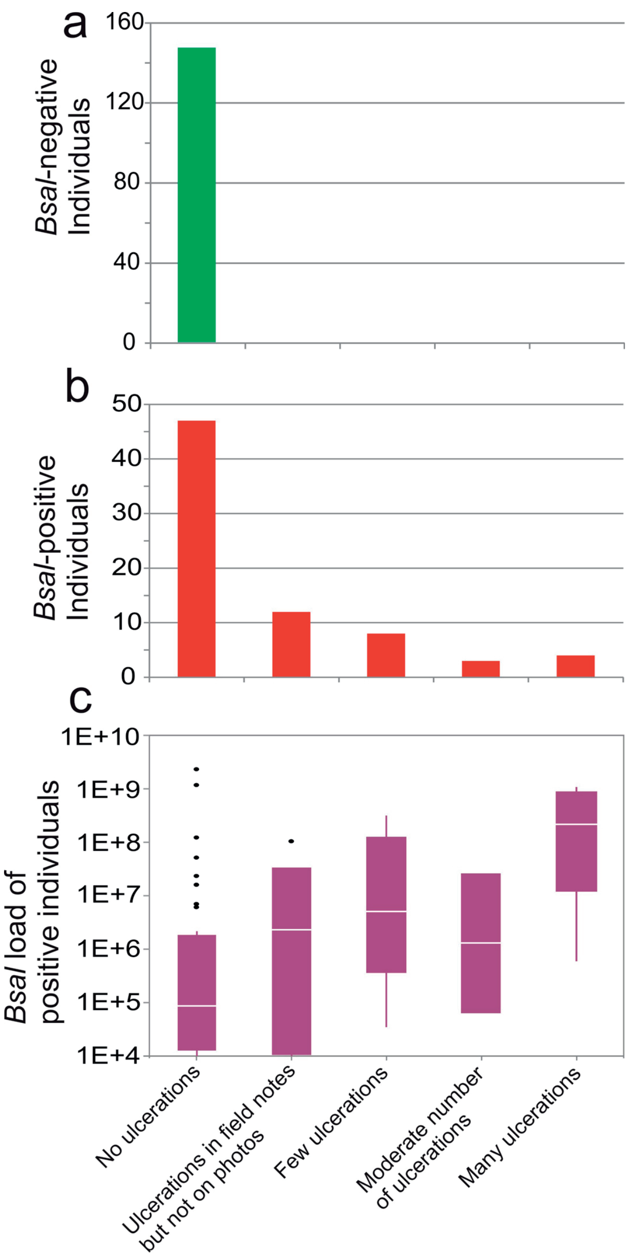

Figure 8. Occurrence of externally visible lesions and ulcerations in European fire salamanders (*Salamandra salamandra*) from Kruppwald, based on photographs and field notes of living individuals recorded in April and May 2019. Panels (a) and (b) show the distribution of categories of ulceration occurrence in salamanders that tested positive and negative for *Bsal* in qPCR assays, respectively (no ulcerations were visible in any *Bsal*-negative individual). Panel (c) shows boxplot of *Bsal* load (in ITS copy number per swab) for the four categories, based on data from *Bsal*-positive individuals only.

fungi, *Bd* and *Bsal* (Berger et al. 2004, Kriger & Hero 2007, Martel et al. 2013, Blooi et al. 2015a, b). Consequently, heat treatment protocols were published to clear infection in animals. However, since both fungi show different thermal growth characteristics most probably due to differences in their host spectrum (Olson et al. 2013, Martel et al. 2014), different protocols need to be followed for treating chytridiomycosis induced either by *Bd* or *Bsal* (Martel et al. 2011, Woodhams et al. 2012, Blooi et al. 2015a, b). Here, we used the heat treatment protocol published by Blooi et al. (2015a) to cure *Bsal*-infected fire salamanders.

First treatment in an irregularly heated room: Six *Bsal*-infected fire salamanders with few (Box C), several (Box K1) or many lesions (Box A, B) and two non-infected fire salamander (Box G, E) were transferred from the field to the lab at Braunschweig. They were kept individually in plastic boxes on a moist tissue in a room with normal heaters and an additional electric heater. When the animals arrived, the room temperature was about 15°C for two days. After two days, the heaters were turned up slowly until the temperature in the room reached 25°C after four days, fluctuating between 25–27°C. At these temperatures, all animals were intensively shedding their skin and ulcera-

Figure 9. Healing process during the heat treatment period 10–20 May 2019 at 25–27°C of the fire salamander that was kept in Box A (see Fig. 12) and had a high amount of small ulcerations at the dorsal and ventral sides, as well as deep wounds at the head. Skin recovered very fast during the treatment due to intensive skin shedding.

tions became smaller and darker every day, and had almost completely disappeared after seven days (Figs 9, 10). During the whole treatment period, the animals rejected food. After the suggested 10-day treatment (Blooi et al. 2015a) they were transferred to new plastic boxes with clay, wood and moss, first at 20°C, and three days later at 15°C. After the treatment, qPCR results revealed still very low amplification values for *Bsal* regarding the animals in Box A, B and C (below 100 ITS/s, mean Cq value 36.24 ± 0.51), i.e. below the suggested threshold of 1 GE (Thomas et al. 2018) which would correspond to an approximate Cq value around 33 (33.32 ± 0.62 with BSA; 32.99 ± 0.19 without BSA) (Blooi et al. 2013). These animals tested negative by the second laboratory. Approximately six weeks (40 days) after the end of the treatment (animals kept at 15°C during this time), the non-infected animals in Box G and E were still *Bsal*-negative. However, new skin ulcerations were observed in the other four animals (Boxes A, B, C and K1), and all of them

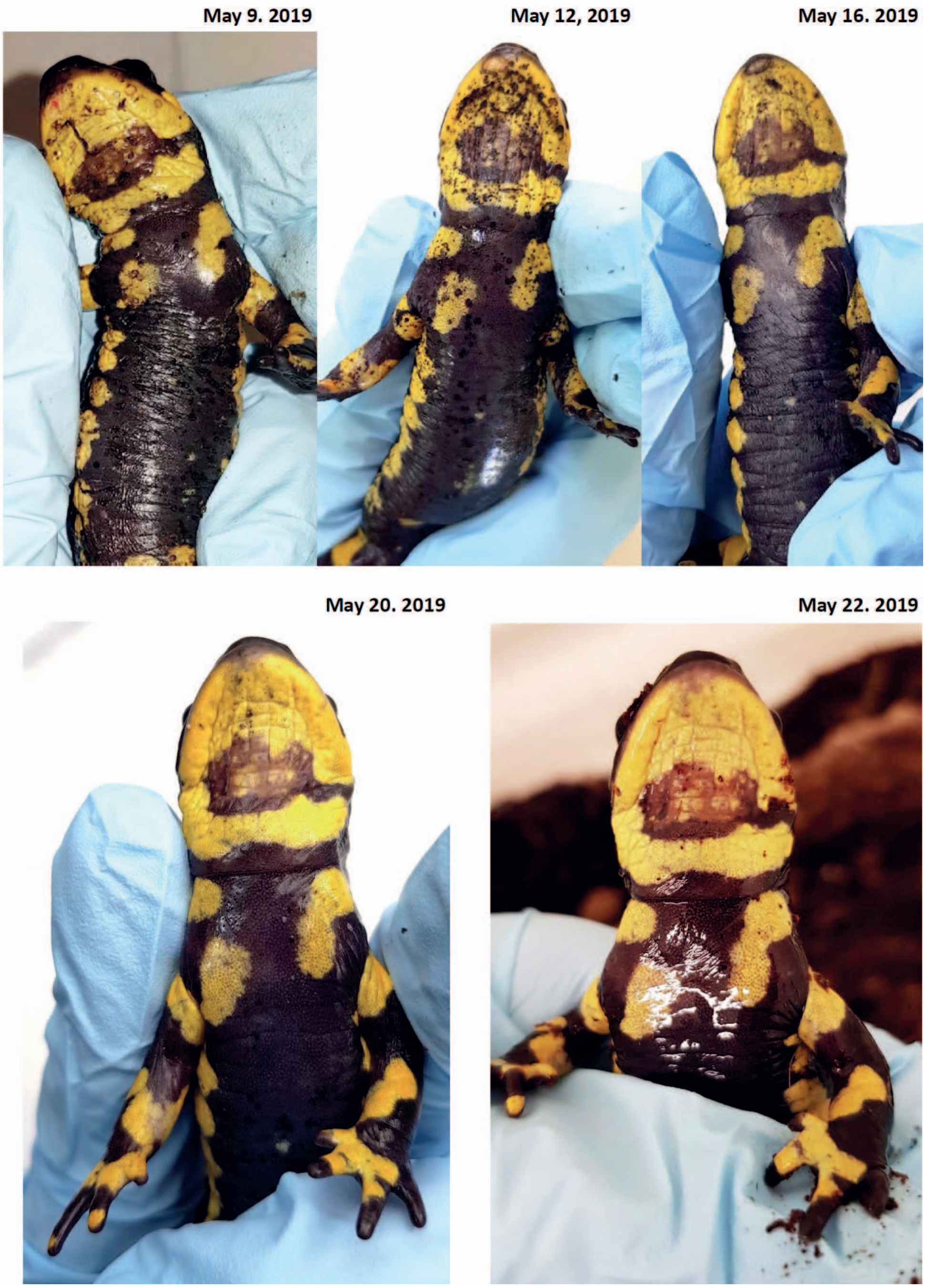

Figure 10. Healing process during the heat treatment period 10–20 May 2019 at 25–27°C in the fire salamander kept in Box B (see Fig. 12) which had some small ulcerations at the dorsal and a high amount at the ventral side, including a deep wound at the chin.

tested *Bsal*-positive again in qPCR. Although loads were similar for all four (mean load 2.16E + 07 ITS/s), the form and amount of ulcerations differed, suggesting an irregular expression of symptoms. The infected fire salamanders were transferred into new plastic boxes and placed into a climate chamber for a second heat treatment, one day at 20°C, one day at 23°C, and 21 days at 25°C (Fig. 11a). All three animals tested *Bsal*-negative directly after the end of the treatment, and again 19 days later (Fig. 12). It is of relevance to note that initially *Bsal*-negative fire salamanders in Box G and E had no indication of external symptoms of chytridiomycosis, and consistently tested *Bsal*-negative in all qPCRs. This confirms that transmission of *Bsal* spores among salamanders kept in separate boxes in the same room can be avoided if working under strict hygiene protocol.

Second treatment in a climate chamber under fully controlled conditions: An additional fire salamander collected in September 2019 that showed *Bsal*-lesions followed by a positive qPCR-result was directly transferred into a climate chamber where the temperature could be kept constant at 25°C after the third day of acclimation. Considering our previous experience we chose to treat the salamander for 14 days before lowering the temperature. Directly after the treatment, the animal tested negative, and was then transferred to a box with clay, moss and wood and kept at 15°C and below. Surprisingly, also this fire salamander again showed the first clinical signs of a *Bsal* infection after approximately 5 weeks (34 days) (Fig. 11b), confirmed by qPCR. After the animal was taken back into treatment (this time at 26°C), no immediate improvement could be

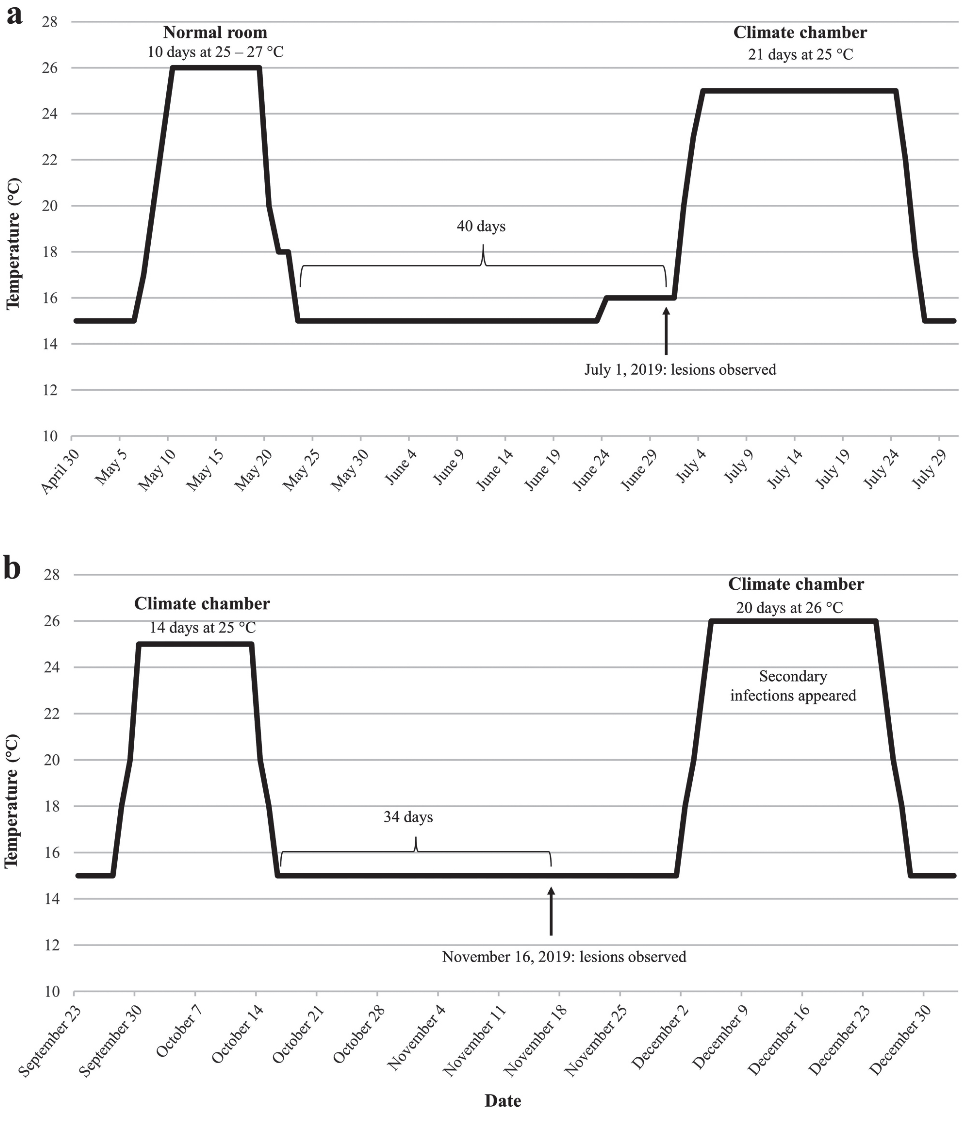

Figure 11. Graphs showing heat treatment time frames used to cure the infected European fire salamanders (*S. salamandra*) found in the wild in 2019. Panel a shows the ad-hoc treatment chosen for salamanders in boxes A, B, K1, C and E. Panel b shows the treatment chosen for box Z. Each box was used to house a single salamander during this time. See Fig. 12 for qPCR loads of salamanders subjected to these treatments.

noticed after 3 days as seen in previous treatments. In contrast, extended wounds emerged on the head where lesions were previously visible, almost certainly in concert with secondary infections caused by bacteria and other fungi. After 14 days of heat treatment (disinfecting wounds by dabbing them with a tissue dipped in Bactine every two days), *Bsal* could still be detected via qPCR (Fig. 12). The detected loads (mean value around 50 ITS/s) remained below the threshold, but positive curves were visible for more than five weeks. Eight weeks after the treatment the qPCR detection was again positive above the threshold, with recurrence of lesions and ulcerations especially at the head and back. This demonstrates the presence of viable *Bsal* when signals below the threshold are detected rather than just residual DNA from dead fungal remains in this individual. Since the wounds were still not healed and the animal obviously still infected by *Bsal*, we eventually decided to euthanize this salamander (by MS222 overdose).

In conclusion, our experiences with the treatment of *Bsal*-infected fire salamanders in principle agree with those of Blooi et al. (2015a): heat treatment can be an effective and low-cost option to heal *Bsal*-infected fire salamanders if well monitored. However, we stress that (i) in some fire salamander individuals infection may not be completely cleared after a 10 days / 25°C treatment, highlighting the importance of intensive monitoring during and after treatment, and the danger of missing post-treatment weak-intensity infections by qPCR (i.e. signals below threshold or even negative); and (ii) the possibility (as with the salamander in our second treatment) that in heavily infected animals with many external symptoms, the stressful heat treatment even for 14 days may be insufficient, and may even favour secondary infections.

This information might help private amphibian keepers conducting the treatment at home where a continuous monitoring of the infection status is not feasible. Animals might not feed properly or not at all during the treatment which needs to be considered. However, once the heat treatment is started we do not recommend to interrupt it (except if secondary infections occur that need specific treatment by a veterinary), to prevent reinfection and further stress for the fire salamander. Cured animals should be fed properly after treatment in combination with mineral and vitamin supplement to facilitate full recovery. We also highlight, once more that any material that came into contact with infected animals must be either immersed in disinfectant (e.g. 1% Virkon S solution or 80% ethanol), autoclaved or burnt.

Last but not least, we appeal to all amphibian keepers to test their individuals for detecting asymptomatic infections to prevent further passive spread of spores in captivity and into the wild. A few studies have already shown that the pathogen occurs in captive collections (Fitzpatrick et

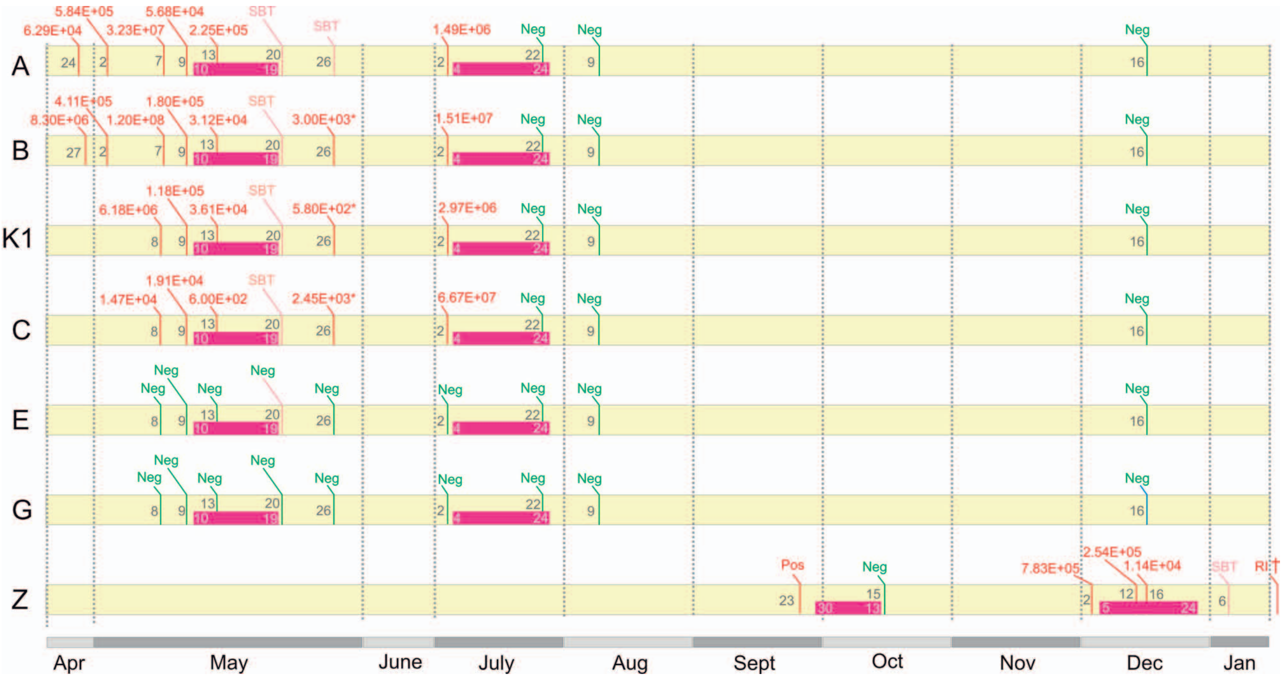

Figure 12. Chronology of results of qPCR testing for *Bsal* infection in European fire salamanders (*Salamandra salamandra*) collected from the wild in the Ruhr District and subjected to heat treatment to cure chytridiomycosis between April 2019 and January 2020. Each light yellow bar represents one salamander individual. Numbers within light yellow bars are dates of the respective month; heat treatment is indicated by magenta bars (see Fig. 11 for temperature regimes applied). Values above light yellow bars are qPCR results; red values give *Bsal* load for samples that tested positive; Neg in green font marks *Bsal*-negative test results, and pink SBT marks possibly positive samples with qPCR curves below the threshold. In order to graphically fit all values, May occupies a disproportionally wide part and June a disproportionally narrow part of the yellow bars. Salamander Z after January 2020 turned out to be reinfected (RI) and was euthanized.

al. 2018, Sabino-Pinto et al. 2018) although in other encouraging cases captive collections turned out to be apparently free of *Bsal* despite intensive testing (Jung et al. 2020 in this issue). Especially, reports about asymptomatic infections in several, non-native species are of concern (Sabino-Pinto et al. 2018), as are the recently documented chronic, non-lethal *Bsal* infections in Anatolian crested newts (*Triturus anatolicus*) invasive in Spain that may indicate latency periods of undetectable infection and subsequent flare-ups leading to spillover of infection to native species (Martel et al. 2020).

The role of newts as potential victims, reservoirs and vectors in the Ruhr District

Beside the fire salamander, at least three of the four native German newt species are also affected by *Bsal*: alpine newts, smooth newts and great crested newts died due to artificially induced *Bsal* infection (Martel et al. 2014); the fourth species, the palmate newt, could not be artificially infected, but *Bsal*-infected wild palmate newts in the field have been observed (Dalbeck et al. 2018, Lötters et al. 2020a in this issue). While in fire salamanders, *Bsal* infection is typically lethal, experimental infection of alpine newts at low zoospore doses resulted in frequent shedding for several months with fungal clearing and clinical cure (Stegen et al. 2017), suggesting newts may function as a pathogen reservoir and vectors for *Bsal*.

Only a few of the *Bsal*-positive sites in the Ruhr District are populated solely by newts. One affected alpine newt population (Stadtwald, garden) occurs at a garden pond located at a linear distance of only ca. 900 m from a *Bsal* site (Stadtwald, forest) populated by fire salamanders. In this pond, used by at least 50 alpine newts for reproduction, a citizen observed in April 2018 several dead newts and others with ulcerations (Fig. 13). In the same month, 20 individuals were swabbed of which one newt was unambiguously *Bsal*-positive while several others indicated very low loads (< 1 GE) in one of two samples. In September 2018, three tested alpine newts were *Bsal*-negative, and in May 2019, two out of 50 newts were *Bsal*-positive (mean load 5.00E + 03 ITS/s), and no decline of the newt population was apparent since the number of animals was still as high as in the previous year.

In Mülheim an der Ruhr, another garden pond has moved into focus after a *Bsal*-infected fire salamander was discovered in the garden in September 2018. The pond is used by both alpine and smooth newts for reproduction, and in April 2019, six out of 33 alpine newts and five out of 17 smooth newts tested *Bsal*-positive, while three dead alpine newts tested negative. A month before, in the forest surrounding this garden, we also detected three infected out of 13 tested fire salamanders, one infected alpine newt and, interestingly, one possibly *Bsal*-positive common frog (*Rana temporaria*); however, given that the latter observation refers to a single individual only, we consider the possibility of *Bsal* infection in this anuran species to be in need of confirmation by an independent method (e.g. histologically; see Thomas et al. 2018). However, it is worth noting that the possibility of a sample confusion can be excluded as from the same swab used for *Bsal* detection, we also sequenced a fragment of the mitochondrial 16S rRNA gene of the host which was confirmed as *R. temporaria* (Genbank accession number MT408024). In the Hiesfelder Wald in Oberhausen, a great crested newt population was also screened in spring 2019, but all animals were *Bsal*-negative.

The current data are insufficient to flag newts as unaffected reservoirs and vectors of *Bsal* in the Ruhr District, and we cannot exclude that they also undergo long-term population declines. Although newts are at high risk of infection when entering water bodies potentially infested by *Bsal* zoospores, they may in many cases also have an advantage over the almost purely terrestrial adult fire salamanders due

Figure 13. Garden pond in Essen-Stadtwald. The size of the pond (left) is 1 × 1.5 m and a depth of 30 cm. It represents the spawning water for an alpine newt population (*Ichthyosaura alpestris*) of > 50 individuals. Five out of 75 newts were tested *Bsal*-positive (April and September 2018 / May 2019). One individual showed ulcerations similar to those observed in infected fire salamanders (right).

to their semiaquatic life cycle, alternating between aquatic and terrestrial phases. In particular, in many populations living in stagnant water bodies, aquatic-phase newts may experience at least temporarily warm temperatures, potentially implying a periodic, passive heat treatment that may help them to clear *Bsal* infection. Additional factors may include the drastic changes in skin structure and cutaneous microbiome that newts undergo in their transition between aquatic and terrestrial phases (Perrotta et al. 2012, Sabino-Pinto et al. 2017). Clearly, a further close monitoring of the known newt sites, also including one pond in the Südwestfriedhof site, is warranted to understand the long-term impact of *Bsal* on newt populations. This in particular applies to the great crested newt, where in the Eifel Mountains two monitored populations disappeared within two years (Lötters et al. 2020a in this issue).

In areas of dense human population such as the Ruhr District, habitat fragmentation and road mortality (Fahrig et al. 1995, Hels & Buchwald 2001, Andrews et al. 2008) can impact amphibian populations, and temporary drift fences with pitfall traps are widely used to maintain connectivity between breeding sites and terrestrial habitats and to monitor population trends and breeding phenology (e.g. Houlahan et al. 2000, Bonari et al. 2011). As volunteers are often involved, an educational effect is often an added benefit (Schmidt & Zumbach 2008). However, the pitfall traps might also increase the risk of pathogen transmission among amphibians, and volunteers handling the animals may also contribute to the spread of the disease. Of 24 randomly screened newts caught at a temporary drift fence in Essen-Bergerhausen, about 2 km from the *Bsal*-positive site Essen Stadtwald (forest) in March 2019, two out of five alpine newts and six out of 17 smooth newts tested *Bsal*-positive. Despite this evidence for *Bsal*-positive newts at drift fences, we do not advocate abandoning the drift fence activities to rescue migrating amphibians given that (i) *Bsal* already seems to be widespread in the Ruhr District, (ii) it is likely that the pathogen is further spreading by multiple mechanisms, and (iii) anurans, which are predominantly caught in pitfall traps, are not affected by *Bsal*. However, next to the routine disinfection measures suggested for shoes, dip nets or newt traps, at least in areas with known *Bsal* occurrence it may be recommendable to disinfect pitfall buckets each time they are controlled (disinfection solution for effective exposure time: see van Rooij et al. 2017; then rinsing with water before next use); furthermore, gloves may be used, and changed for each bucket, to minimize the risk of transferring the pathogen. Ideally, the drift fence equipment should be used in only one area, and volunteers should not be in charge of multiple, geographically distant drift fences.

Use of water-borne environmental DNA for *Bsal* detection

The isolation and detection of amphibian pathogens via DNA from environmental samples, so-called environmental DNA (eDNA), is a promising tool for their early detection (e.g. Pierson & Horner 2016, Hall et al. 2016, Kamoroff & Goldberg 2017). In a pilot study, we here tested the potential of eDNA for the detection of *Bsal* in 52 water bodies located in the vicinity of either infected or non-infected fire salamander populations. *Bsal* was confirmed by eDNA analysis in two out of the 52 sample sites: from a stream at the site Essen-Kruppwald with a *Bsal* load of 2.23E + 03 to 4.48E + 03 ITS copies per 500 ml of water; and from a pond near the botanical Garden RUB/Kalwes, with amplification products in all four 500 ml water samples. The qPCR products of both sites were verified as *Bsal* by means of Sanger sequencing. Also, at both sites, several animals were confirmed as *Bsal*-positive by qPCR analysis from swabs, and many salamanders were observed along the stream at the Essen-Kruppwald site during water sampling. Therefore, it is possible that the detected *Bsal*-DNA originated directly from infected fire salamanders, potentially from females releasing larvae into the water.

Although only two of the water bodies showed positive results, our study points to the potential of detecting the pathogen *Bsal* based on DNA from environmental samples. More data on the reliability of this eDNA approach (rates of false positives/false negatives; sensitivity to low concentrations of *Bsal* DNA) are needed to understand which role it could play for studying and tracking the spread of the disease in the environment, and identifying potential reservoirs. Potentially, it could be used alongside simultaneous amphibian detection via eDNA in a landscape-wide approach (Thomas et al. 2019). However, little is known about the persistence, mobility, position and infection potential of the different stages of *Bsal* in water (Rosenblum et al. 2010, Gray et al. 2015, Stegen et al. 2017). Also, different sources of origin of the detected DNA have to be considered, as it can originate from infectious spores as well as from degenerated and non-infectious DNA. Therefore, further studies are needed to evaluate both the limitations and the benefits of using eDNA as a tool for *Bsal*-detection.

Future perspectives for salamander conservation management in the Ruhr District

This review is a testimony of the calamity that fire salamanders are experiencing in forests across the Ruhr District. It allows us a glance into a future with this once so abundant amphibian gone from this area if we do not take action. The fire salamander is a very charismatic species that engages the sympathy of citizens regardless of their understanding of nature. To halt the ongoing loss of salamanders due to *Bsal* infection, we aim to develop a broad conservation action plan.

The unpredictability of *Bsal* in terms of dispersal and reservoirs renders in situ conservation difficult (Stegen et al. 2017, Thomas et al. 2019), and the persistence of this highly infectious pathogen in a habitat is still uncertain. It is obvious, however, that with the decline and disappearance of every population the species can lose valuable genetic adaptations. Our data illustrate once more how fast *Bsal*-induced declines of salamander populations can proceed, thus demonstrating the urgency for conservation actions. At the short time, ex situ conservation (i.e, keeping animals outside their natural environment) appears to be the only viable solution to preserve the genetic diversity of the fire salamander from areas with *Bsal* outbreaks. This option is a last resort to save a species, subspecies or an important genetic lineage from extinction (Pritchard et al. 2012, McGowan et al 2017). Several amphibian species that can no longer survive in the wild due to habitat destruction, pollution or disease are currently bred in captivity to preserve them until the natural conditions improve (Valbuena-Ureña et al. 2017, Lewis et al. 2019).

During the massive *Bsal*-related population declines in the Netherlands, the remaining fire salamanders were rescued and are since then sheltered in a zoo (Spitzen-van der Sluijs et al. 2013). But an infectious pathogen like *Bsal* ignores political borders. Saving the fire salamander by means of ex situ breeding must be a joint effort of all affected countries which is currently represented by the 'Ex situ Salamandra Group' (ESG; Spitzen-van der Sluijs et al. 2018a). The ESG develops breeding guidelines, engages zoos and private keepers, and holds a consultative role, whereas the translation of ex situ conservation into practice should be a regional responsibility. By keeping fire salamanders, zoos in the Ruhr District can conserve an imperilled local species while simultaneously engage the public and conduct environmental education. As a further aspect, especially in a densely populated area like the Ruhr District, where citizens are directly witnessing the mass die-offs, it is crucial to also consider animal welfare aspects. Concepts for a temporary rescue centre for infected fire salamanders may therefore be necessary to be able to perform heat treatments of infected salamanders encountered by citizens.

As a long-term perspective, animals from zoos or rescue centres may be reintroduced to their natural habitats in a stepwise procedure, first to outdoor enclosures that allow a close monitoring of individuals and for periodically testing for infection, and eventually – hopefully – into the wild if the pathogen has disappeared. Only time can tell whether this strategy can be successful – but without immediate action we are at risk of losing the Ruhr District populations of the charismatic fire salamander without any option for return.

Acknowledgments

We are deeply grateful to a large number of volunteers, assistants and students who contributed to the current knowledge of the distribution of *Bsal* in the Ruhr District: Paul Bachhausen, Niklas Banowski, Frederik Bartsch, Christopher Bause, Jana Beckert, Sven Bodingbauer, Philipp Böning, Stephan Bürger, Ninja Dyczmons, Christopher Falke, Sam Fielinghaus, Peter Gausmann, Benedikt Glingener, Marc Gose, Fabian Gösser, Jens Hoberg, Thomas Hörren, Katharina Hund, Sven Kartik, Mathias Krisch, Louisa Kron, Christiane Kurau, Hannah Kurau, Lisa Margowski, Lina Martin, Martin Maschka, Paul Mausbach, Marla Müller, Verena Niehuis, Carolin Niester, Christina Rasch, Tobias Rautenberg, Philip Rehsen, Maurice Römhild, Louisa Rothe, Yul Rottmann, Andreas Schäfer, Larissa Seufer, Daniela Specht, Moritz Specht, Rainer Stawikowski, Lisa Stegmeyer, Daniel Steinort. We thank all citizens that reported conspicuous or dead fire salamanders and newts. We also acknowledge the help with laboratory procedures by Gabriele Keunecke, Meike Kondermann, Karin Fischer and Ina Schleicher. Molly Bletz, Salvador Carranza, An Martel, and Frank Pasmans contributed to fruitful discussions and provided advice and analyses. Permits were granted by Untere Naturschutzbehörde Essen, Mülheim an der Ruhr, Bochum, Ennepe-Ruhr-Kreis, Dortmund, Oberhausen; we are also grateful to the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen for ethics clearance of swabbing procedures (Az 1-02.05.40.18.039 from 13 April 2018). This work was supported by projects of the Bundesamt für Naturschutz (F+E project: 'Monitoring und Entwicklung von Vorsorgemaßnahmen zum Schutz vor der Ausbreitung des Chytridpilzes *Batrachochytrium salamandrivorans* "*Bsal*" im Freiland') and the Deutsche Bundesstiftung Umwelt.

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Supplementary data

The following data are available online:

Supplementary document 1. Overview of all reports and site visits with documented and tested fire salamanders from 2017–2019 at two *Bsal*-positive sites in Essen.

Supplementary document 1. Overview of all reports and site visits with documented and tested fire salamanders from 2017–2019 at two *Bsal*-positive sites in Essen. First proof of *Bsal* at the site in boldface. * Fire salamander documented by citizen. P: *Bsal*-positive. N: *Bsal*-negative. – No visit. NE: No examination. † Fire salamander found dead at the site.

Date	Südwestfriedhof	Stadtwald
	Number of fire salamander individuals	Number of fire salamander individuals
July 11, 2017	–	**1 (P)** †
August 15, 2017	–	35 (2 P)
September 29, 2017	–	62 (6 P)
January 5, 2018	**2 (2 P)**	–
January 6, 2018	4 (2 †) (4 P)	–
January 22, 2018	2 † (2 P)	
March 10, 2018	1* (NE)	–
March 31, 2018	–	–
April 24, 2018	1* (NE)	–
June 20, 2018	0	–
August 24, 2018	0	–
October 4, 2018	–	0
December 7, 2018	0	–
January 14, 2019	1* (NE)	–
March 14, 2019	0	0
March 25, 2019	1 (N)	–
March 26, 2019	0	–
March 28, 2019	0	0
April 2, 2019	1 (N)	1 (N)
April 9, 2019	1* (NE)	–
April 24, 2019	1 (N)	0
April 25, 2019	0	–
May 2, 2019	0	0
May 21, 2019	0	0
May 23, 2019	1*† (P)	–
October 1, 2019	0	–
October 3, 2019	–	0
October 9, 2019	0	–
October 10, 2019	0	0
October 11, 2019	0	–
October 15, 2019	1 (N)	–
October 16, 2019	–	0
October 17, 2019	0	–
October 25, 2019	–	0
October 26, 2019	0	–
October 28, 2019	–	0

SALAMANDRA **56**(3): 215–226
15 August 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

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

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Manuscript received: 29 March 2020
Accepted: 24 April 2020 by Miguel Vences

Abstract. Emerging infectious diseases are one of the main suggested reasons for global amphibian decline. Fungal agents play a key role. Since its introduction, the Asian pathogen *Batrachochytrium salamandrivorans* has driven the European fire salamander, *Salamandra salamandra*, to the edge of extinction in the Netherlands and caused severe population declines in Belgium and Germany. We screened 1,526 amphibians (1,431 urodelans and 95 anurans) from 50 sites in a 1,500 km^2 large area in Germany, south of the next known infected populations. Furthermore, we conducted a presence-absence mapping of larval salamanders in 88 randomly selected creeks and creeks where salamanders had been reported in the past using a standardized removal sampling approach. Our results revealed an expanded distribution of the pathogen in Western Germany and we could detect seven infected urodelan populations including the southernmost locality of the fungus in its exotic range. Larval salamanders were found in 54 out of 63 creeks south, but only in seven out of 25 creeks north of a highway that divides the study area. *Bsal* infection could mainly be detected in newts (*Ichthyosaura alpestris* and *Lissotriton helveticus*) because many previously known European fire salamander populations most likely disappeared in the affected forest regions, however, silently and without observed mass mortalities. The only detectable salamander population north of the highway was found to be *Bsal*-infected at high prevalence in 2019, but not from 2016 to 2018, suggesting a recent infection event. Overall, prevalence at the individual (2.6%) and population level (14%) was very low. Moreover, modelling habitat suitability in seemingly unaffected areas suggests that most reproduction creeks and surrounding land habitats in the seemingly affected area are still suitable for *S. salamandra*, supporting our suspicion that the absence of the species is disease-related rather than habitat related.

Key words. *Bsal,* chytridiomycosis, Germany, *Ichthyosaura alpestris, Lissotriton helveticus*, *Salamandra salamandra*.

Introduction

Emerging infectious diseases pose an increasing threat to global biodiversity (Daszak et al. 2000, 2003). They are often driven by fungal agents (Fisher et al. 2012). One of the most blatant such diseases for amphibians is chytridiomycosis (Fisher et al. 2009, van Rooij et al. 2015). The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has been suggested to play a key role in the sudden and rapid declines that we have started witnessing more than 30 years ago (Berger et al. 1998, La Marca et al. 2005, Skerratt et al. 2007). This pathogen is known from all continents where amphibians occur, and it has been recorded from all three amphibian orders (Fisher et al. 2009, van Rooij et al. 2015). The global pet trade has been suggested to have significantly disseminated *Bd* (Fisher et al. 2009, van Rooij et al. 2015, O'Hanlon et al. 2018). More recently, a second amphibian chytrid fungus (*Batrachochytrium salamandrivorans, Bsal*) has been identified as the cause of a mass dying event in a Dutch population of the European fire salamander, *Salamandra salamandra* (Spitzen-van der Sluijs et al. 2013, Martel et al. 2013).

Bsal is suggested to be native in Asia from where it has apparently been introduced into Western Europe via asymptomatic vectors, most probably via the pet trade (Martel et al. 2014, Laking et al. 2017). After its discovery in the Netherlands (Martel et al. 2013, 2014) and Belgium (Spitzen-van der Sluijs et al. 2016, Stegen et al. 2017), *Bsal* was detected in wild urodelan populations in Germany in 2015 (Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018). Its presence in Germany can be traced back to at least 2004 (Lötters et al. 2020a, b in this issue), and by now it is known from about 50 sites in the wild in Belgium, Germany, the Netherlands and Spain (for an overview see Lötters et al. 2020a in this issue). In addition, records of *Bsal* in captive amphibians add Sweden and the UK to the *Bsal*-positive countries outside its native range (Sabino-Pinto et al. 2015, 2018, Fitzpatrick et al. 2018). Different to *Bd*, *Bsal* affects urodelan amphibians only, while anurans can be infected without signs of disease; no data exist on gymnophiones (Martel et al. 2014, Stegen et al. 2017, Nguyen et al. 2017). So far, one case of *Bd* and *Bsal* co-infection in European fire salamanders, one case in great crested newt (*Triturus cristatus*) and two cases in alpine newts (*Ichthyosaura alpestris*) are known (Lötters et al. 2018, 2020a in this issue).

From the core area of *Bsal* in Europe (Belgium, Germany, the Netherlands), in addition to the European fire salamander, the following *Bsal* hosts have been confirmed in the wild: alpine newt, palmate newt (*Lissotriton helveticus*), smooth newt (*L. vulgaris*), great crested newt (Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018). In addition, two more *Triturus* species have recently been confirmed as *Bsal* hosts in Spain (Martel et al. 2020). While some newt species such as the alpine newt can carry *Bsal* (at low infection levels) without showing clinical symptoms and therefore serve as potential *Bsal* reservoirs and vectors, a *Bsal* infection is lethal for European fire salamanders (Stegen et al. 2017). As a result, all known Central European outbreaks in wild European fire salamanders led to massive population declines (Spitzen-van der Sluijs et al. 2013, 2016, Martel et al. 2014, Stegen et al. 2017, Dalbeck et al. 2018, Schulz et al. 2018, 2020 in this issue).

Approaches to model the suitable ranges for *Bsal* outside the current invasion range suggest that large areas of Western Europe might be suitable (Feldmeier et al. 2016, Beukema et al. 2018, Lötters et al. 2020a in this issue). Accordingly, both future and previously overlooked population collapses of *S. salamandra* are to be expected. Wagner et al. (2017) hypothesized that the apparent absence of the species in large portions of the northernmost Mountains ('Schneifel') in the Southern Eifel, an area close to known *Bsal* infection sites (cf. Spitzen-van der Sluijs et al. 2016, Stegen et al. 2017, Dalbeck et al. 2018) and where formerly European fire salamanders were widespread (Bitz et al. 1996), might be explained by overlooked *Bsal* outbreaks.

Apart from occasional detection of carcasses or living animals showing symptoms of salamander chytridiomycosis, also known as the 'salamander plague', or of *Bsal*-positive skin swabs taken from seemingly healthy specimens (Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018, Lötters et al. 2018, Schulz et al. 2018), indication of *Bsal*-related population declines or even extinctions can only be gained from a monitoring of European fire salamander populations. However, searching for terrestrial juvenile and adult salamanders is arduous and heavily depends on appropriate weather conditions (Thiesmeier 2004, Schmidt et al. 2015). Therefore, monitoring of larval populations has been suggested to control for potentially *Bsal*-induced population declines in European fire salamanders (Schmidt et al. 2015).

In Central Europe, female European fire salamanders desposit their larvae in spring, mainly in fish-free first and second order streams (Thiesmeier 2004), but occasionally also in stagnant water bodies (Weitere et al. 2004, Steinfartz et al. 2007). They develop on average within 120 days until metamorphosis (Thiesmeier 2004, Weitere et al. 2004). In principle, the purely aquatic larvae can easily be detected during the day by active searching, though detection is much easier during the night (Thiesmeier 2004, Schmidt et al. 2015). They show almost no weather-related activities, which makes standardised monitoring easier (Thiesmeier 2004). If during the larval season no larva is found in a creek, there are two explanations: either the creek may be unsuitable for reproduction, or no salamanders live in the respective terrestrial habitat. The latter may be due to unsuitability of the terrestrial habitat (or non-colonization of suitable habitats) or, especially if European fire salamanders have been present before, a severe population decline had occured, e.g. following a *Bsal* infection.

Detecting and documenting population declines is one of the most critical tasks in population ecology and conservation biology, but it is also one of the most difficult (Fournier et al. 2019). Usually, post-infection studies will lack solid information about the pre-infection status of populations, and a site-selection bias may mask real population trends in a focal study area (Fournier et al. 2019). On the other hand, identifying the ultimate cause of a decline once the decline has occurred is another important but difficult task, as this is the only way to find potential solutions to prevent similar declines in other regions. Therefore, post-decline evidence of disease-related decline can be obtained indirectly by applying a correlative rather than by a causative approach.

To test the assumption of Wagner et al. (2017) that the absence of European fire salamanders in the northernmost mountains of the Southern Eifel could be due to *Bsal* infection, we hypothesize that salamander-free habitats (especially where salamanders have been present before) in this region are nevertheless suitable for the species. We therefore built a habitat suitability model based on presence-absence of European fire salamander larvae from *Bsal*-unaffected regions (reference area) and projected this model to the potentially *Bsal*-affected parts of the Southern Eifel Mountains to test if these creeks are equally suitable for salamander larvae, plus their surrounding terrestrial habitat for juveniles/subadults and adults, as are those from the reference areas. If in fact *Bsal* had driven European fire salamanders almost to extinction in our focal area, we may still find the pathogen inhabiting few remaining specimens or other hosts which are less susceptible. We therefore conducted a *Bsal* screening of European fire salamanders and further amphibian species (mainly alpine and palmate newts) using skin swabs to get independent evidence for *Bsal* occurrence in the focal area.

Materials and methods

Study area

The study area is located in the Southern Eifel Mountains (Germany) and encompasses ca. 1,500 km² (Fig. 1). It extends from the south-western German border with Luxembourg and Belgium to the border between the German Federal States of Rhineland-Palatinate and North Rhine-Westphalia. Major parts of it are forested, mainly along river valleys and higher mountainous areas, while on plateaus with calcareous soils, agricultural land use dominates. European fire salamanders and alpine and palmate newts were reported to be quite common in the study area in the 1980s and 1990s (Bitz et al. 1996).

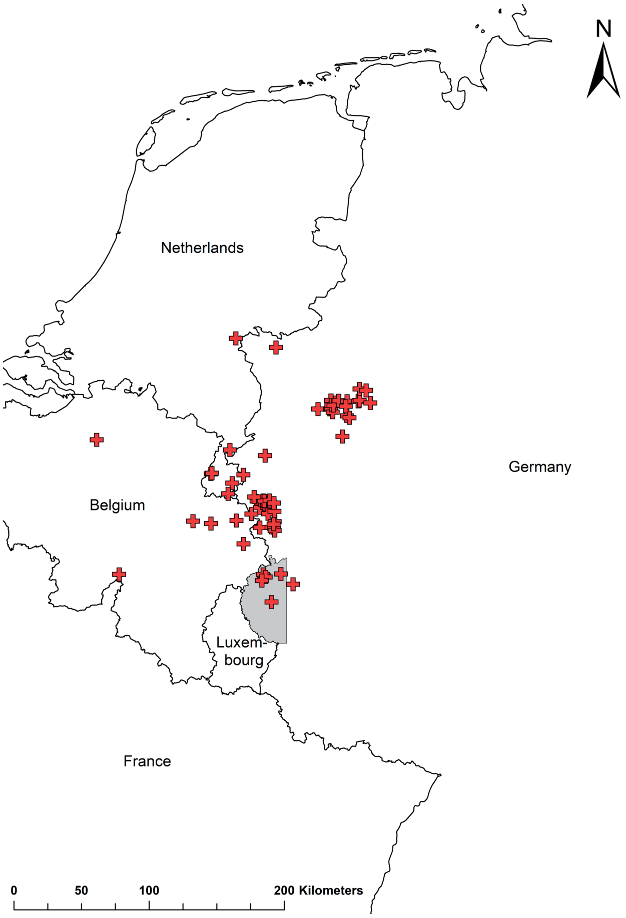

Figure 1. The study area in the Southern Eifel (grey area) is situated in south-western Germany. Red crosses indicate *Bsal*-infected urodelan populations in the Netherlands, Belgium and Germany (cf. Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018, Lötters et al. 2020a in this issue, results of the present study).

The A60 highway divides the study area into a northern and a southern part (Figs 1–3). Highways are well-known barriers for amphibians (Lesbarrères et al. 2006, Emel et al. 2012, van Buskirk et al. 2012) and thus should hamper *Bsal* transmission through the most important transition pathway, direct animal-to-animal contact (Stegen et al. 2017, Schmidt et al. 2017). This does not mean that highways are strict barriers for *Bsal* (especially due to anthropogenic vectors). Rather, we here use the highway as an unprejudiced hypothetical separator between seemingly non-affected areas and areas where *Bsal* has potentially entered amphibian populations (Fig. 1).

Presence-absence mapping of salamander larvae

From May to July 2016, presence-absence mapping of European fire salamander larvae took place in 88 randomly selected forest creeks as wells as creeks where salamanders were recorded in the 1980s/1990s across the study area following a modified removal-sampling of Schmidt et al. (2015) (Fig. 2). Close to the source of each creek, a 75 m section was divided into three 25 m subsections. Each subsection was simultaneously examined for 15 min for presence of larvae by three persons using dip nets. As soon as a larva was found, the search was stopped and the creek was classified as presence locality. It was considered an absence locality when this procedure was replicated in another 75 m section of the creek and still no larva was found. For each subsection, environmental variables of the creek and the surrounding terrestrial habitat (potential habitat of juveniles and adults) were recorded (Table 1).

Modelling habitat suitability

Environmental variables used in the habitat model (Table 1) were: the number of pools with slow-running water within the creek (important microhabitat for European fire salamander larvae: Baumgartner et al. 1999, Werner et al. 2014, Schmidt et al. 2015) and the amount of wooden debris (as shelters: Thiesmeier & Schuhmacher 1990). Further parameters were the substrate (ordered according to increasing grain size, from mainly muddy over sandy to rocky substrate) and water turbidity (potential effect on detection probability of larvae). Capture occasion (date) was included since mortality rate, drift rate and detection probability of salamander larvae may vary over time (Thiesmeier & Schuhmacher 1990, Tanadini et al. 2012, Schmidt et al. 2015).

Female European fire salamanders prefer larval deposition in headwater creeks close to the spring (Thiesmeier 2004). As an indicator for the proximity to the spring, creek width was recorded. Presence of predatory fish, especially salmonids, was recorded since they may influence larval abundance (Sound & Veith 1994, Hecnar & M'Closkey 1997, Ficetola & Bernardi 2004). Creek inclination (which indirectly affects the occurrence of larvae via water flow/drift (Thiesmeier & Schuhmacher 1990, Baumgartner et al. 1999, Werner et al. 2014) and perpendicular slope inclination (which affects the accessibility of a creek for adult females, which prefer gentle slopes for larvae deposition: Manenti et al. 2011) are included as relevant topographical features.

Suitable terrestrial habitat is as important for European fire salamander populations as is suitable aquatic habitat (Thiesmeier 2004). Therefore, various terrestrial habitat parameters were included in the model. European fire salamanders are preferentially found in deciduous forests and we therefore quantified the following land cover types within a radius of 100 m and 500 m around a subsection, respectively creek: deciduous, mixed and coniferous forest, settlements, grassland and arable land (Blaustein & Kiesecker 2002, Thiesmeier 2004). We used CORINE Land Cover Data (https://land.copernicus.eu/pan-european/corine-land-cover) and calculated the proportions of land cover types within buffers using ArcGIS (version 10.5.1). The 100 m radius should cover the core terrestrial habitat of a population, while the 500 m radius should reflect the maximum habitat (Rebelo & Leclair 2003, Schulte et al. 2007). Equally important is a sufficient number of terrestrial hiding places such as rocks and dead wood (Thiesmeier 2004).

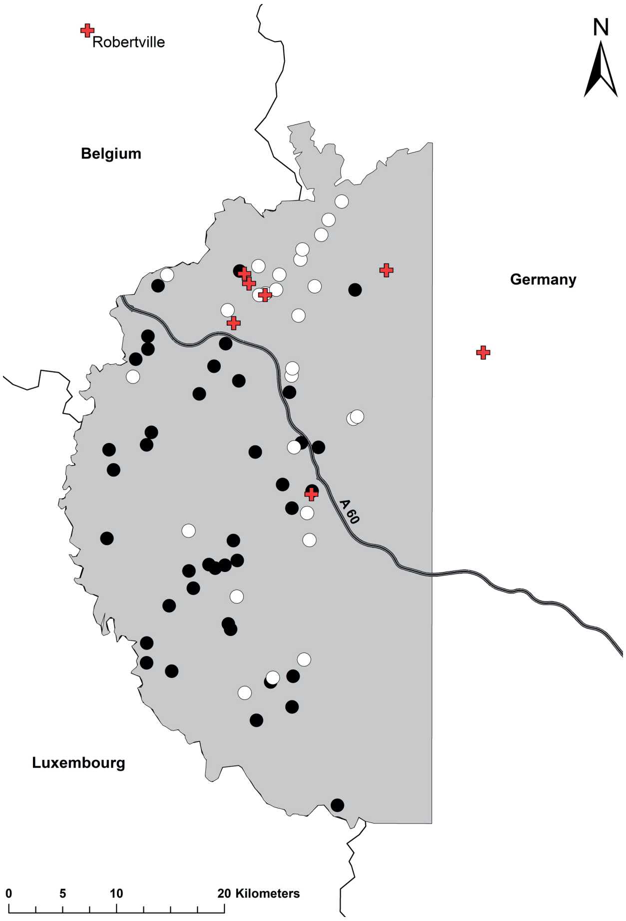

Figure 2. Results of the 2016 presence-absence mapping of fire salamander larvae in the study area (grey area): solid circles = presence, open circles = absence. While 85% of the studied creeks (N = 63) were used for reproduction by European fire salamanders south of the A60 highway, larvae were only present in 28% of the studied creeks (N = 25) north of the A60 highway. Red crosses indicate *Bsal*-infected urodelan populations found in the present study and the infected fire salamander population in Robertville, Belgium (cf. Spitzen-van der Sluijs et al. 2016).

Table 1. Variables used for model building.

Considered variables	Categories
Aquatic habitat	
Water turbidity	Ordinal data (clear – slight turbidity – strong turbidity)
Pools	Count data
Wooden debris in the creek	Ordinal data (few – intermediate – many)
Average creek width	Measured data (cm)
Predatory fish	Binary (presence / absence)
Average creek inclination	Measured data [°]
Average creek prependicular inclination	Measured data [°]
Substrate	Ordinal data (muddy – sandy – rocky)
Terrestrial habitat	
Land use types	Measured data (%)
Hiding places	Ordinal data (few – intermediate – many)

We built a habitat suitability model using only creeks south of the A60 highway (N = 63), since European fire salamanders are abundant there, and neither the analysis of salamander skin swabs nor population monitoring of larvae indicates any *Bsal* outbreak in this area (except for one newt population; see results). Eventually, habitat suitability projections were compiled for the remaining 25 creeks north of the highway using the best fitting logistic regression model. We used the program R (R Core Team 2020) for statistical calculations. Four different candidate generalized linear models (GLMs) (all variables [global], all terrestrial habitat variables, all aquatic habitat variables, and an individual variable selection: Supplementary document 1) with binomial distribution, i.e. logistic regression models, were built. In this method, non-normal distribution is assumed (Dormann & Kühn 2009). Furthermore, all models were automatically simplified to the most important explanatory variables using an information theoretical approach (stepwise AIC) (R-package 'MASS': https://cran.r-project.org/web/packages/MASS/index.html). An area under the (receiver operation) curve (AUC) value was calculated for each model (R-package 'verification': https://cran.r-project.org/web/packages/verification/index.html). Eventually, all eight models were compared to each other by their AICc values (R-package 'MuMIn': https://cran.r-project.org/web/packages/MuMIn/index.html), and the respective best fitting models were selected with a ΔAICc < 2 (Burnham & Anderson 2002).

Presence and absence predictions were tested individually for each model using a Mann-Whitney U-test because the data were not normally distributed (Shapiro-Wilks-test). To validate the models prior to projection, the dataset was divided into ten random groups (R-package 'dismo'). One group was removed from calculations (test dataset), and with the remaining data (training dataset) a new GLM was calculated. For both the test and the training dataset, an AUC value was calculated. The AUC value of the test data set indicates the quality of the model; only models with an AUC value ≥ 0.7 were considered as plausible (Swets 1988). In addition to the validation with AUC, the true skill statistics (TSS) quality measurement was used. It was developed specifically for presence-absence data and, unlike other measurements, it is independent of the prevalence. It is the proportion of validation sites where the species was detected. This avoids calculation errors for an unequal number of presence and absence data (Allouche et al. 2006, 2008). GLM creation and the quality measure calculation were repeated once per group for ten times each. Individual AUC and TSS values were averaged and included in the study as a validation gauge.

Bsal screening

Between 2015 and 2019, we screened European fire salamanders, alpine, palmate and smooth newts as well as syntopic anurans (Supplementary document 2) at 50 sites north and south of the A60 highway for *Bsal* (Fig. 3) using sterile cotton swabs (Medical Wire MW-100). One pair of nitril gloves (Cashins et al. 2008) was used for each specimen to prevent pathogen transmission and false-positive results. For swabbing, we followed the protocols of Hyatt et al. (2007) and van Rooij et al. (2011) and furthermore collected simultaneously two samples (A and B samples) per specimen for quality insurance. Only individuals where *Bsal* was detected in the A and B sample by two independent laboratories (Trier and Braunschweig Universities, respectively) were considered as confirmed *Bsal*-positive. To prevent for *Bsal* transmission between populations, all materials including field workers' boots were disinfected after each site visit using Virkon S (van Rooij et al. 2017).

Laboratory analyses of swabs followed Blooi et al. (2013, 2016), with the modification that only *Bsal* (versus *Bd* and *Bsal* combined) was analyzed in a 'simplex' quantitative realtime PCR (qPCR). DNA was extracted using Prepman (Trier University) or the Quiagen Blood and Tissue Kit (TU Braunschweig). *Bsal*-DNA was amplified and quantified (expressed in GE) using specific primers and fluorescence markers for qPCR as described in Blooi et al. (2013, 2016). In accordance with Thomas et al. (2018), only individuals with a *Bsal* load of ≥ 1 GE were considered as *Bsal*-infected. For each locality, Bayesian 95%-credibility intervals for prevalence were calculated using the software R, the R-package R2WinBUGS (http://cran.r-project.org/web/packages/R2WinBUGS/) and the software WinBUGS (Lunn et al. 2000) following the method described in Lötters et al. (2012).

Results

Eighty-eight potential and previously known reproduction creeks were investigated for the presence of larval European fire salamanders. Larvae were found in 54 out of 63 creeks south of the A60 highway (= 85%), but only in seven out of 25 creeks (= 28%, Table 2) north of the A60 highway, also not in most of the previously known salamander localities (Fig. 2).

Habitat suitability models and projections

A few variables had to be excluded from further analysis due to no or low values (agricultural land and mixed forest within 100 m, agricultural land, settlement and pasture within 500 m radius). The model selection based on the

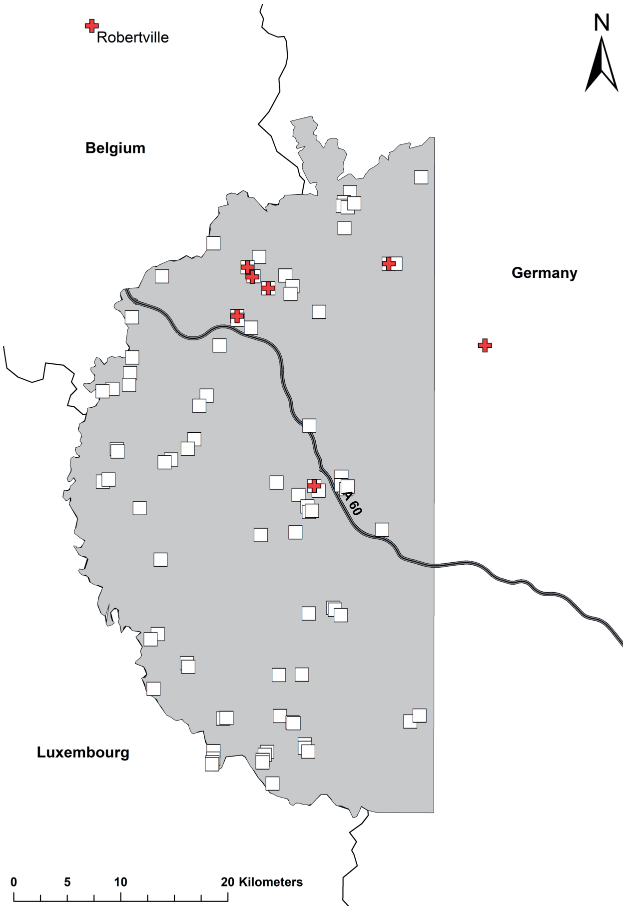

Figure 3. The analysis of 1,526 skin swabs from various amphibian species revealed seven *Bsal*-positive sites confirmed in the A and B samples (red crosses), one confirmed in the A sample only (question mark) and 42 *Bsal*-negative sites (white squares) within and next to the study area (grey area). The northernmost *Bsal*-site in the study area is situated at 26 km from the most proximate *Bsal*-site 'Robertville' (Spitzen-van der Sluijs et al. 2016). Note that more white squares are visible; we merged the coordinates of the swabbed amphibians (white squares) to 42 *Bsal*-negative populations in Supplementary data 2.

Table 2. Mapped creek sections north of the A60 highway with the indication whether larvae were found (1) or not (0), as well as the prognosis of the two best models. Only sites marked with * are considered by a model as unsuitable for European fire salamander larvae, delimited by the respective model-specific threshold.

Creek	Larvae	Model 1	Model 2
Alfbach 1	1	99%	87%
Alfbach 2	0	92%	88%
Dreisbach	0	97%	89%
Ettelbach	0	99%	98%
Grimmelsbach	0	100%	98%
Hennebach	0	91%	96%
Hennebach Seitenarm	0	57% *	89%
Litzenmehlenbach	0	91%	94%
Lünebach	1	93%	98%
Mattelbusch	0	95%	97%
Mausbach	1	61% *	89%
Mehlenbach	0	99%	97%
Mönbach	0	99%	90%
Pittersbach	0	99%	99%
Prüm	0	99%	89%
Scharrenborn	1	100%	99%
Sellerich	0	97%	90%
Steingert	0	79%	88%
Steinrausch	0	82%	86%
Thierbach 1	0	66% *	87%
Thierbach 2	0	99%	99%
Üchenbach	0	81%	92%
Watzbachzufluss	1	100%	100%
Watzbach	1	100%	100%
Willwerath	1	82%	79% *

corrected Akaike information criterion (AICc) showed that two models with a ΔAICc value of 0 and 0.51 could be considered as plausible (Burnham & Anderson 2002). The best fitting model was the automatically simplified global model (Model 1), which took into account the following variables: capture occasion, water turbidity, creek width, creek inclination, substrate, deciduous and mixed forest within 500 m (Supplementary document 3). The two significant predictors were water turbidity and substrate. The turbidity of the water had a significantly negative effect ($Z = -2.25$, $P < 0.05$), and the more rocky the substrate was, the more likely was the presence of larvae ($Z = 2.08$, $P < 0.05$). The AUC value of this model was 0.88, suggesting a 'good' model performance according to Swets (1988).

The second-best fitting model was the automatically simplified model that considered only the aquatic habitat variables (Model 2). It was limited to the variables: capture occasion, water turbidity, creek inclination and substrate (Supplementary document 4). In this model, only the positive influence of rocky substrate on presence of larvae remained ($Z = 2.10$, $P < 0.05$). With 0.83, this model also had a 'good' performance in terms of AUC (Swets 1988). The determined quality measures of the two models, AUC test (Model 1: 0.86 ± 0.25 / Model 2: 0.88 ± 0.13) and TSS (Model 1: 0.85 ± 0.27 / Model 2: 0.82 ± 0.02) of the model validation are in a range of more than 0.80. Model 2 performs better in the evaluation than Model 1 regarding both TSS and AUC.

Based on the thresholds (Model 1: 72%; Model 2: 84%), Model 1 predicts 22 out of the 25 creeks north of the A60 highway as potentially suitable, while Model 2 even predicts 24 creeks as potentially suitable (Table 2). All but one presence creek north of the highway were also predicted as potentially suitable by both models (Table 2).

Regarding the habitat suitability predictions of the investigated creeks north of the A60 highway, divided into the locations where larvae were found during mapping (presence locations, N = 7) and those where no larvae were found (absence locations, N = 18), the box plots of all sections are in the range of 80–100% probability of presence (Fig. 4). Mean values of the presence (Model 1: 91%; Model 2: 93%) and absence locations (Model 1: 90%; Model 2: 92%) are above the threshold values determined. The Mann-Whitney U-test showed no significant differences between the habitat suitability predictions (Model 1: $P = 0.31$; Model 2: $P = 0.48$) of presence and absence sites.

Bsal screening

Swabbing of 1,526 amphibians (1,431 urodelans and 95 anurans) revealed seven confirmed *Bsal*-positive sites. Eleven out of 355 European fire salamanders were infected (3%). Only seven out of 536 alpine newts (1.3%) and two out of 537 palmate newts (0.4%) were infected with *Bsal* (Supplementary document 2). In on site, one alpine newt was

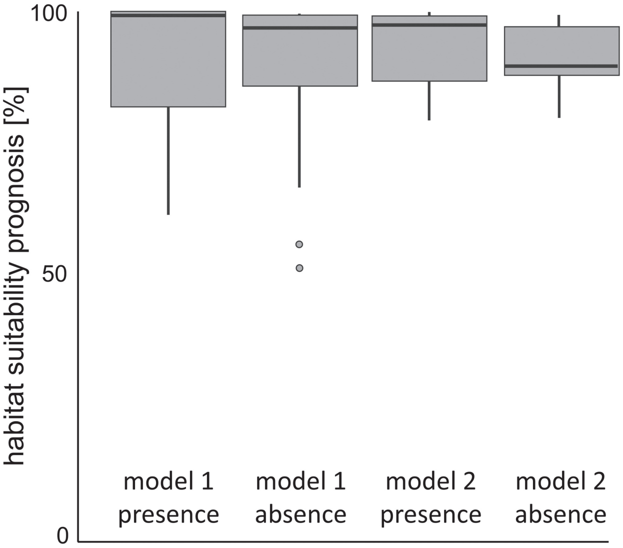

Figure 4. Habitat suitability prognoses for presence and absence creeks north of the A60 highway based on the two best models, respectively.

only positive in the A sample (Supplementary document 2, question mark in Fig. 3). Both swabbed smooth newts and all 43 anurans were *Bsal*-negative (Supplementary document 2). Prevalence was relatively low (7 out of 50 = 14%) and low over all swabbed urodelans (37 out of 1,431 swabs = 2.6%). Bayesian credibility intervals were high for several localities with small sample sizes, while at some localities, sample sizes were reliable (i.e. > 80–90% probability of *Bsal* absence based on Bayesian credibility intervals) to state that *Bsal* was most likely absent (Supplementary document 2).

Six *Bsal*-positive sites were located north of the A60 highway within continuous forest areas where nearly no European fire salamanders or their larvae could be found anymore (cf. Fig. 2). Hence, mainly newts and anurans could be swabbed here, and *Bsal* was detected in seven alpine newts and two palmate newts in the A and B sample (all without any obvious symptoms of chytridiomycosis; Supplementary document 2). In the only swabbed European fire salamander population north of the highway (at the 'Watzbach'), all eleven *Bsal*-positive fire salamanders could be found, while all swabbed fire salamander populations south of the highway tested *Bsal*-negative (Fig. 3, Supplementary document 2). While all specimens swabbed at the 'Watzbach' from 2016 to 2018 were negative for *Bsal*, eleven out of 64 salamanders (17%) were found to be infected in spring and autumn 2019 (Supplementary document 2). We recognized *Bsal*-typical skin lesions in infected individuals (Fig. 5).

One of the *Bsal* sites north of the highway ('Gerolstein') is located east of our study area (Fig. 3) because a suspected case of *Bsal*-caused mortality of several salamanders was reported in spring 2019 via a citizen science project (https://snu.rlp.de/de/projekte/feuersalamander/). We could not find dead (or living) salamanders anymore, but eleven alpine and palmate newts; one alpine newt was positive for *Bsal* (Supplementary document 2).

However, south of the highway, we also found at least one *Bsal*-positive site within a continuous forest area with European fire salamanders (Fig. 3). We did not encounter juvenile or adult fire salamanders at night within this forest, but larvae in creeks. Here, *Bsal* was detected on one alpine newt in the A and B sample (again without obvious signs of disease, but with high GE loads of 853 in the A and 671 in the B sample).

Discussion

Presence-absence mapping in the Southern Eifel Mountains showed an increased absence of European fire salamander larvae north of the A60 highway, while nearly all studied creeks south of the highway yielded records of larvae (Fig. 2). When projecting the best fitting habitat suitability models based on data from creeks south of the A60 highway onto the northern area, nearly all absence creeks there appear to be highly suitable for salamander larvae (Table 2). Both best fitting habitat suitability models are based on reasonable predictors. The capture occasion and water turbidity can be considered as indicators for the detection probability rather than for presence-absence of larvae. Inclusion of these variables in both models could be related to the heavy rainfalls in spring 2016, which most likely decreased detection probability, but also real presence due to an increased flood-induced larval drift (Thiesmeier & Schuhmacher 1990). The significant effect of water turbidity on the absence of salamanders in Model 1 argues for low detection probabilities. Hence, final conclusions on real absence in all absence-creeks cannot be made for 2016, but another presence-absence study in 2019 confirmed the absence of salamander larvae in this area (see Wagner et al. 2020a in this issue). The models also suggest that higher inclination of the studied creek sections is more suitable for salamander larvae in this study area. Inclination also influenced both aquatic and terrestrial habitat parameters in other studies (Baumgartner et al. 1999, Werner et al. 2014), but we hypothesized earlier (in the Material and methods section) that higher inclination automatically enhances drift and should negatively affect presence, which was not statistically supported at least for this study region. Furthermore, in low mountain regions, such as the Eifel, a higher creek inclination is expected close to the spring where female salamanders preferably deposit their larvae (Thiesmeier 2004). Both models suggest that in creeks with a rocky substrate, larvae are more likely to be found. This may be due to an increased number of hiding places and pools suitable for the larvae, but here they can also be detected more easily during fieldwork. Mod-

Figure 5. *Bsal*-infected fire salamander with typical skin lesions, found in autumn 2019 at the Watzbach near Brandscheid (Eifelkreis Bitburg-Prüm). Photograph taken by S. Feldmeier.

el 1 also included creek width and the proportions of deciduous and mixed forest within a 500 m radius (although without significant explanation). Females prefer narrower headwater creeks in proximity to the spring to deposit larvae (Thiesmeier 2004). The positive, but not significant, relationship between proportions of deciduous and mixed forest in the terrestrial habitat is also plausible because juvenile and subadult/adult European fire salamanders are predominantly found there (Thiesmeier 2004, Ficetola et al. 2009, Manenti et al. 2009). In addition to the plausible predictor variables in both models, the quality measures AUC and TSS were used to verify them. Both values are above 0.80 for each model so that they can be considered validated (Swets 1988).

Of course, also other factors such as land-use changes, chemical pollutants or competitive interaction with other species are known drivers of amphibian extinction events (Blaustein & Kiesecker 2002). None of them was included in our habitat models. However, they are not likely to have simultaneously eradicated all salamander populations in one part of the study area while not having a similar effect in the adjacent part. In additions, all study sites are inside sustainably managed forests, which are known to provide comparatively stable conditions for biodiversity over long periods (Paillet et al. 2010). In conclusion, the models are suitable to test creeks for their suitability but also detection probability of larvae of the European fire salamander. Based on the creeks south of the A60 highway, the models predict 22 and 24, respectively, out of 25 creeks north of the highway to be potentially suitable for European fire salamanders. Given the previously documented presence of European fire salamanders in large parts of this area (Bitz et al. 1996) our results lead us to hypothesize that absence in most creeks in the northern study area may be *Bsal*-related.

In fact, the presence of *Bsal* north of the A60 highway was confirmed by our analyses of skin swabs. *Bsal*-infected European fire salamanders, alpine and palmate newts were found at six sites north of the highway (Fig. 3, Supplementary document 2); however, *Bsal* prevalence and infection loads were mainly low. From an epidemiological perspective, such patterns could arise because the peak of the epidemic has passed and/or because the primary host has already been driven to extinction. Infected newts had usually low GE loads (only the infected alpine newt south of the highway had a high infection load). Stegen et al. (2017) found that *Bsal* is lethal for alpine newts only at high infection loads, but they can act as reservoirs and vectors at low infection loads because they are able to clean themselves within about two weeks in the laboratory. North of the highway, only at the 'Watzbach' and 'Watzbachzufluss', juvenile and subadult/adult European fire salamanders could be detected at surveys between 2015 and 2019. This site is only about 1 km from the next known *Bsal*-infected newt population as well as sites of apparent absence of European fire salamanders. An isolated but still *Bsal*-free salamander population also exists next to the type locality of *Bsal* at Bunderbos in the Netherlands (Spitzen-van der Sluijs et al. 2018). This gave hope that *Bsal* does not spread into all populations in an infected area (Spitzen-van der Sluijs et al. 2018). Sadly, the 'Watzbach' can no longer be regarded as a *Bsal*-free refugium as apparently *Bsal* was introduced into the population between the swabbing events 2018 and 2019. Since all salamanders swabbed until 2019 were found to be *Bsal*-negative (Supplementary document 2) and also large larvae populations were found (see Wagner et al. 2020b in this issue), we conclude that this population was infected recently.

It is difficult to detect disease-driven population declines relying on presence data of previously known amphibian populations only. Spitzen-van der Sluijs et al. (2013) needed monitoring data of about one decade to realize that a population was sharply declining in the Netherlands, an observation that ultimately led to the discovery of *Bsal*. This was only possible because the European fire salamander is a rarity in the Netherlands, and the population was permanently monitored by volunteers and scientists. The same accounts for an infected salamander population in Belgium, which was monitored before and after the *Bsal* outbreak (Stegen et al. 2017). However, in overviews on *Bsal* distribution in the Netherlands, Belgium and Germany, for most infected populations no statement on decline could be made due to missing monitoring data (Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018), in several infected sites not even after five years of standardized larvae monitoring (Wagner et al. 2020b in this issue). Hence, long-term monitoring of at least selected salamander and newt populations within the invasive range of *Bsal* should be one major task for further amphibian conservation.

Conclusions

Most creeks of the northern part of our study area provide suitable habitat for larvae of the European fire salamander. However, the significant predictors water turbidity and substrate may also relate to detection probability. Nevertheless, given the similar or even identical results of presence-absence mapping in 2015 (unpub. data) and 2019 (see Wagner et al. 2020a in this issue), the extensive absence of the species in the northern part of our study area does not appear to be habitat-related. Certainly, the absence of larvae of the European fire salamander does not necessarily have to be the result of a *Bsal* outbreak. Post-infection studies of a susceptible host species usually lack information from the pre-infection period, and therefore conclusions about a potential pathogen-driven extinction scenario must remain speculative. Nevertheless, due to the detection of *Bsal*-infected newts and the recent introduction of *Bsal* into the, up to 2018, *Bsal*-free Watzbach population (Figs 3, 5, Supplementary document 2), it is very likely that a previous *Bsal* outbreak rather than unsuitable habitats account for the lack of European fire salamanders in the northern part of our study area.

The potential large-scale extinction of European fire salamanders in our northern study area is astounding.

We are aware of the problems associated with negative records, but our larvae and adult monitoring efforts since 2015 clearly suggest absence of the species. Conversely, in other *Bsal*-infected areas, such as the Netherlands or the Eifel Mountains in North Rhine-Westphalia (Germany), infected populations declined sharply, but they did not get fully extinct (Spitzen-van der Sluijs et al. 2013, 2016, Stegen et al. 2017, Dalbeck et al. 2018, Schulz et al. 2018). Also, in the *Bsal* area south of the highway A60, European fire salamanders are still present in all creeks, which are situated in the continuous forest area where *Bsal* was detected (cf. Fig. 2 and results from larvae monitoring in Wagner et al. 2020a,b in this issue). The same accounts for *Bsal*-infected fire salamander populations in the northern Eifel (see results from larvae monitoring in Wagner et al. 2020b). It therefore remains unclear why European fire salamanders are nearly completely extinct (or at best are only present in such low numbers that we could neither detect terrestrial nor aquatic life-stages in all seemingly negative sites) in the northern part of our study area. Perhaps these populations were already small, but unfortunately, there is no historical data on abundance (see Bitz et al. 1996). Thus, our study illustrates how difficult it can be to identify a presumably disease-driven decline once the decline has happened.

Acknowledgements

We thank the Federal Agency for Nature Conservation ('Bundesamt für Naturschutz'; BfN, F+E project 'Monitoring und Entwicklung von Vorsorgemaßnamen zum Schutz vor der Ausbreitung des Chytridpilzes *Batrachochytrium salamandrivorans* („*Bsal*") im Freiland') and the Nikolaus-Koch-Stiftung for financial support. The 'Struktur and Genehmigungsdirektion Nord' (SGD Nord) kindly provided the permissons for swabbing amphibians in the study area. We thank Karin Fischer for the laboratory analyses at Trier University. We are thankful to the following colleagues (in alphabetical order) for support during field work: Joscha Beninde, Katja Bredimus, Daniel Christiansen, Lara Feiler, Gaetano Fichera, Bernd Fontaine, Florian Keltsch, Mario Harzheim, Luis Fernando Marin da Fonte, Luis Pfeifer, Malwina Schafft, Ulrich Schulte, Jonas Viebahn, Maren Wagner and Katja Wallrich. All field work was carried out in accordance with relevant guidelines and regulations. Swabbing of amphibians and capturing European fire salamander larvae was conducted with permission of the 'Struktur and Genehmigungsdirektion Nord' (SGD Nord), permission number 425-104.232.1502. After removal sampling, all larvae were placed back into their waters.

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Supplementary data

The following data are available online:

Supplementary document 1. Candidate models, which were compared using information-theoretical approaches.

Supplementary document 2. Overview on amphibians, which have been swabbed in the whole study area from spring 2015 to autumn 2019.

Supplementary document 1. Candidate models, which were compared using information-theoretical approaches.

Candidate model	Variables
All variables (global)	Capture occasion, amount of wooden debris in the creek, creek width, presence of predatory (cray) fish, amount of prey items, number of pools, substrate, creek inclination, creek inclination perpendicular, hiding places in the terrestrial habitat, deciduous, mixed and coniferous forest, settlements, grassland and arable land in a buffer (radius) 100 and 500 m
All terrestrial habitat variables	Capture occasion, hiding places, deciduous, mixed and coniferous forest, settlements, grassland and arable land in a buffer (radius) 100 and 500 m
All aquatic habitat variables	Capture occasion, amount of wooden debris, creek width, presence of predatory (cray)fish, amount of prey items, number of pools, substrate, creek inclination, creek inclination perpendicular
Individual selection	Capture occasion, creek width, number of pools according to Schmidt et al. (2015) who found these three parameters as good predictors

Supplementary document 2. Overview on amphibians, which have been swabbed in the whole study area from spring 2015 to autumn 2019. In the table, each for the study area north and south of the highway, sites names are arranged in alphabetical order and date. *Bsal*-positive sites and species are indicated in red. 95% Bayesian credible intervals (CI) refer to yearly prevalence at the site based on all samples of the respective year. If sex and age were noted: m = adult male, f = adult female, j = juvenile/subadult. GE = mean genomic equivalent in A and B samples.

Site	95%-Bayesian CI	Date	Species	N *Bsal*-infected	Ø GE A and B sample	Field workers
North of the highway						
Alfbach	**1/15=6.7% (2–29%)**	16 May 2018	*Ichthyosaura alpestris* (N=6, 4 m, 2 w)	0/6	0	N. Wagner/J. Viebahn/K. Bredimus
			Lissotriton helveticus (N=9, 5 m, 4 w)	1(m)/9	5, 20	
Alfbach	**0/23=0% (0–14%)**	24 May 2019	*Ichthyosaura alpestris* (N=6, 4 m, 2 w)	0/6	0	S. Feldmeier
			Lissotriton helveticus (N=16, 9 m, 7 w)	0/16	0	S. Feldmeier
			Rana temporaira(1 j)	0/1	0	S. Feldmeier
Dreisbach	**2/44=4,6% (2–19%)**	17 May 2018	*Ichthyosaura alpestris* (N=14, 11 m, 3 w)	1(w)/14	19, 54	N. Wagner/J. Viebahn
			Lissotriton helveticus (N=22, 11 m, 11 w)	1(w)/23	2,10	
			Rana temporaria (N=7, 1 w, 6 j)	0/7	0	
Gerolstein	**0/1=0%(–)**	3 July 2018	*Rana temporaria* (N=1, 1 m)	0/1		K. Bredimus/J. Viebahn
Gerolstein	**1/13=8% (2–35%)**	7 June 2019	*Ichthyosaura alpestris* (N=10, 5 m, 5 w)	1(m)/10	1, 43	S. Feldmeier/J. Viebahn
			Lissotriton helveticus (N=1, 1 m)	0/1	0	
			Bufo bufo (N=1, 1 j)	0/1	0	
			Rana temporaria (N=1, 1 j)	0/1	0	
Jünkerath	**0/34=0% (0–11%)**	4, 6, 7 June 2018	*Ichthyosaura alpestris* (N=28)	0/28	0	D. Christiansen
			Lissotriton helveticus (N=6)	0/6	0	
Lindscheid	**0/1=0%(–)**	15 May 2017	*Ichthyosaura alpestris* (N=1, 1 m)	0/1	0	F. Keltsch
Neuendorf	**0/20=0% (0–16%)**	31 May 2017	*Ichthyosaura alpestris* (N=10, 6 m, 4 w)	0/10	0	F. Keltsch
Obermehlen	**0/5=0% (0–44%)**	24 May 2017	*Ichthyosaura alpestris* (N=3, 1 m, 2 w)	0/3	0	F. Keltsch
			Lissotriton helveticus (N=10, 7 m, 3 w)	0/10	0	
Prüm-Quelle	**0/26=0% (0–13%)**	30, 31 May 2017	*Ichthyosaura alpestris* (N=17, 9 m, 8 w)	0/17	0	F. Keltsch
			Lissotriton helveticus (N=2, 1 m, 1 w)	0/2	0	
Prüm-Quelle	**0/22=0% (0–14%)**	3 June 2018	*Ichthyosaura alpestris* (N=7, 4 m, 3 w)	0/7	0	K. Bredimus
			Lissotriton helveticus (N=23, 15 m, 8 w)	0/23	0	
			Rana temporaria (N=1, 1 j)	0/1	0	

Site	95%-Bayesian CI	Date	Species	N *Bsal*-infected	Ø GE A and B sample	Field workers
Schwarzer Mann	**0%(0–70%)**	24 May 2017	*Ichthyosaura alpestris* (N=2, 1 m, 1 w)	0/2	0	F. Keltsch
Schwarzer Mann	**1/15=6.7% (2–29%)**	11 April 2018	*Ichthyosaura alpestris* (N=5, 5 m)	1(m)/5	2, 2	N. Wagner/M. Wagner
			Lissotriton helveticus (N=10, 6 m, 4 w)	0/10	0	
Schwarzer Mann	**0/38=0% (0–9%)**	16 May 2019	*Ichthyosaura alpestris* (N=8, 1 m, 7 w)	0/8	0	N. Wagner/S. Feldmeier
			Lissotriton helveticus (N=29, 9 m, 20 w)	0/29	0	
			Rana temporaria (N=1, 1 j)	0/1	0	
Sellerich	**1/24=4.2% (7–20%)**	9 April 2018	*Ichthyosaura alpestris* (N=16, 13 m, 3 w)	1(m)/16	4, 5	N. Wagner/M. Wagner
			Lissotriton helveticus (N=8, 4 m, 4 w)	0/8	0	
Sellerich	**2/59=3% (1–12%)**	16, 23 May 2019	*Ichthyosaura alpestris* (N=16, 12 m, 12 w)	2(2 m)/24	5–128	N. Wagner/S. Feldmeier
			Lissotriton helveticus (N=34, 19 m, 15 w)	0/34	0	
			Rana temporaria (N=1, 1 j)	0/1	0	
Steinmehlen	**0/1=0%(–)**	24 May 2017	*Ichthyosaura alpestris* (N=1, 1 w)	0/1	0	F. Keltsch
Watzbach/-zufluss	**0/5=0% (0–44%)**	13 April 2016	*Salamandra salamandra* (N=2)	0/2	0	N. Wagner/J. Ewen/C. Kolwelter
			Ichthyosaura alpestris (N=3)	0/3	0	
Watzbach/-zufluss	**0/35=0% (0–10%)**	18 March/ 15 May 2017	*Salamandra salamandra* (N=33)	0/33	0	N. Wagner/F. Keltsch/S. Lötters/S. Feldmeier
			Ichthyosaura alpestris (N=2, 1 m, 1 w)	0/2	0	
Watzbach/-zufluss	**0/32=0% (0–11%)**	10 April 2018	*Salamandra salamandra* (N=27, 2 w, 25 j)	0/27	0	N. Wagner/M. Wagner
			Ichthyosaura alpestris (N=5, 4 m, 1 w)	0/5	0	
Watzbach/-zufluss	**11/65=17% (9–27%)**	2 May/ 7 September 2019	*Salamandra salamandra* (N=63, 33 undetermined, 7 m, 5 w, 18 j)	11/63 (4 undetermined, 4 m, 2 w, 1 j)	2–374,372	S. Feldmeier/J. Viebahn
			Ichthyosaura alpestris (N=1, 1 m)	0/1	0	
			Rana temporaria (N=1, 1 j)	0/1	0	
Winterscheid	**0/13=0% (0–22%)**	17 May 2017	*Ichthyosaura alpestris* (N=11, 8 m, 3 w)	0/11	0	F. Keltsch
			Lissotriton helveticus (N=2, 1 m, 1 w)	0/2	0	
Winterspelt	**0/1=0%(–)**	17 May 2017	*Lissotriton helveticus* (N=1, 1 m)	0/1	0	F. Keltsch
Wolfsschlucht	**0/30=0% (0–11%)**	11 Juli 2018	*Lissotriton helveticus* (N=30, 24 m, 6 w)	0/30	0	K. Bredimus/J. Viebahn

Site	95%-Bayesian CI	Date	Species	N *Bsal*-infected	Ø GE A and B sample	Field workers
South of the highway						
Arzfeld	**0/6=0% (0–43%)**	1 April 2016	*Salamandra salamandra* (N=3)	0/3	0	N. Wagner/U. Schulte
			Ichthyosaura alpestris (N=3)	0/3	0	
Arzfeld	**0/2=0% (0–70%)**	9 May 2017	*Lissotriton helveticus* (N=2 , 2 m)	0/2	0	F. Keltsch
Arzfeld	0/2=0% (0–70%)	5 April 2019	*Bufo bufo* (N=2, 1 m, 1 f)	0/2	0	N. Wagner/S. Feldmeier/J. Viebahn
Bitburg	**0/28=0% (0–12%)**	10 April 2017	*Ichthyosaura alpestris* (N=16, 10 m, 6 w)	0/16	0	F. Keltsch
			Lissotriton helveticus (N=12, 11 m, 1 w)	0/12	0	
Bitburg	**0/26=0% (0–12%)**	21, 23 June 2018	*Ichthyosaura alpestris* (N=15, 3 m, 12 w)	0/15	0	K. Bredimus/J. Viebahn
			Lissotriton helveticus (N=8, 8 w)	0/9	0	
			Rana temporaria (N=3, 1 m, 1 w, 1 j)	0/3	0	
Bitburg	**0/51=0% (0–7%)**	20 March 2019	*Ichthyosaura alpestris* (N=35, 22 m, 13 w)	0/35	0	N. Wagner/J. Viebahn
			Lissotriton helveticus (N=16, 8 m, 8 w)	0/16	0	
Bollendorf	**0/59=0% (0–6%)**	27, 29, 31 March 2017	*Ichthyosaura alpestris* (N=29, 23 m, 6 w)	0/29	0	N. Wagner, J. Beninde, F. Keltsch
			Lissotriton helveticus (N=30, 24 m, 6 w)	0/30	0	
Bollendorf	**0/6=0% (0–43%)**	28 March 2018	*Ichthyosaura alpestris* (N=3, 1 m, 2 w)	0/3	0	N. Wagner
			Lissotriton helveticus (N=3, 2 m, 1 w)	0/3	0	
Bollendorf	**0/51=0% (0–7%)**	19 March 2019	*Ichthyosaura alpestris* (N=30, 21 m, 9 w)	0/30	0	N. Wagner
			Lissotriton helveticus (N=21, 11 m, 10 w)	0/21	0	
Daleiden	**0/5=0% (0–44%)**	9 May 2017	*Ichthyosaura alpestris* (N=1 ,1 w)	0/1	0	F. Keltsch
			Lissotriton helveticus (N=4, 2 m, 2 w)	0/4	0	
Fleißbach	**0/4=0% (0–51%)**	9 March 2017	*Salamandra salamandra* (N=4)		0	N. Wagner/S. Feldmeier
Fleißbach	**0/8=0% (0–31%)**	13 March/ 4 April 2018	*Salamandra salamandra* (N=6, 6 w)	0/6	0	N. Wagner/K. Wallrich
			Rana temporaria (N=2, 2 j)	0/2	0	
Fuhrbach (?)	**0/4=0% (0–51%)**	3 May 2017	*Ichthyosaura alpestris* (N=2, 1 m, 1 w)	0/2 (1(m)/2?)	1, 0	F. Keltsch
			Lissotriton helveticus (N= 2, 1 m, 1 w)	0/2	0	
Fuhrbach	**0/42=0% (0–8%)**	7 May 2019	*Ichthyosaura alpestris* (N=3, 1 m, 2 w)	0/3	0	S. Feldmeier
			Lissotriton helveticus (N= 39, 25 m, 14 w)	0/39	0	

Site	95%-Bayesian CI	Date	Species	N *Bsal*-infected	Ø GE A and B sample	Field workers
Grasmärchen	**0/6=0% (0–43%)**	12 April 2016	*Salamandra salamandra* (N=6)	0/6	0	N. Wagner/J. Beninde/S. Feldmeier
Grasmärchen	**0/3=0% (0–61%)**	21 March 2017	*Salamandra salamandra* (N=3)	0/3	0	N. Wagner/S. Lötters/M. Veith
Großkampenberg	**0/5=0% (0–44%)**	26 April 2015	*Ichthyosaura alpestris* (N=4)	0/4	0	N. Wagner/L. Marin da Fonte/S. Lötters
			Lissotriton helveticus (N=1)	0/1	0	
Großkampenberg	**0%(0–31%)**	11 May 2017	*Ichthyosaura alpestris* (N=4, 2 m, 2 w)	0/4	0	F. Keltsch
			Lissotriton helveticus (N=4, 3 m, 1 w)	0/4	0	
Gutenbach	**0/4=0% (0–51%)**	4 May 2015	*Salamandra salamandra* (N=4)	0/4	0	S. Lötters/L. Marin da Fonte
Gutenbach	**0/16=0% (0–20%)**	25 March 2016	*Salamandra salamandra* (N=16)	0/16	0	N. Wagner/S. Lötters/J. Beninde/G. Fichera
Gutenbach	**0/6=0% (0–43%)**	19 March 2017	*Salamandra salamandra* (N=6)	0/6	0	N. Wagner/K. Wallrich
Gutenbach	**0/1=0%(–)**	2 May 2018	*Salamandra salamandra* (N=1)	0/1	0	N. Wagner/S. Lötters
Habscheid	**0/1=0%(–)**	15 May 2017	*Ichthyosaura alpestris* (N=1, 1 m)	0/1	0	F. Keltsch
Hauchenbach	**0/22=0% (0–15%)**	4 April 2016	*Salamandra salamandra* (N=22)	0/22	0	N. Wagner/J. Beninde/S. Feldmeier
Hauchenbach	**0/2=0% (0–70%)**	15 March 2018	*Bufo bufo* (N=1, 1 w)	0/1	0	N. Wagner/B. Fontaine
		14 May 2018	*Rana temporaria* (N=1, 1 w)	0/1	0	
Hauchenbach	**0/15=0% (0–19%)**	6 March/ 8 April 2019	*Salamandra salamandra* (N=10, 10 w)	0/10	0	N. Wagner/S. Feldmeier/J. Viebahn
			Bufo bufo (N=1, 1 m)	0/1	0	
			Rana temporaria (N=3, 1 m)	0/3	0	
Heisdorf	**0/6=0% (0–43%)**	24 May 2017	*Ichthyosaura alpestris* (N=3, 1 m, 2 w)	0/3	0	F. Keltsch
			Lissotriton helveticus (N=3, 2 m, 1 w)	0/3	0	
Holsthum	**0/1=0%(–)**	27 March 2017	*Lissotriton helveticus* (N=1, 1 m)	0/1	0	F. Keltsch
Hütten	**0/4=0% (0–51%)**	8 May 2017	*Ichthyosaura alpestris* (N=2, 2 m)	0/2	0	F. Keltsch
			Lissotriton helveticus (N=2, 2 w)	0/2	0	
Idenheim	**0/16=0% (0–20%)**	1 April 2017	*Ichthyosaura alpestris* (N=12, 7 m, 5 w)	0/12	0	F. Keltsch
			Lissotriton helveticus (N=4, 2 m, 2 w)	0/4	0	
Idenheim	0/30=0% (0–11%)		*Ichthyosaura alpestris* (N=6, 1 m, 5 w)	0/6	0	N. Wagner
			Lissotriton helveticus (N=24, 19 m, 5 w)	0/24	0	
Ingendorf	**0/1=0%(–)**	18 April 2017	*Ichthyosaura alpestris* (N=1, 1m)	0/1	0	F. Keltsch

Site	95%-Bayesian CI	Date	Species	N *Bsal*-infected	Ø GE A and B sample	Field workers
Irrel	**0/1=0%(–)**	26 April 2015	*Ichthyosaura alpestris* (N=1, 1 w)	0/1	0	N. Wagner/G. Fichera
Irrel	**0/16=0% (0–20%)**	28 March 2016	*Salamandra salamandra* (N=16)	0/16	0	N. Wagner/J. Beninde
Irrel	**0/17=0% (0–19%)**	8, 29 March 2017	*Salamandra salamandra* (N=15)	0/15	0	N. Wagner/F. Keltsch
			Lissotriton helveticus (N=2, 1 m, 1w)	0/2	0	
Irrel	**0/30=0% (0–11%)**	12, 15 March/14 May 2018	*Salamandra salamandra* (N=13, 13 w)	0/13	0	N. Wagner/K. Bredimus/B. Fontaine/K. Wallrich
			Rana temporaria (N=16, 12 m, 3 w, 1 j)	0/16	0	
			Bufo bufo (N=1, 1 w)	0/1	0	
Irrel	**0/42=0% (0–8%)**	5–7 March 2019	*Salamandra salamandra* (N=9, 9 w)	0/9	0	
			Rana temporaria (N=33, 31 m, 2 w)	0/33	0	
Klingendell	**0/4=0% (0–51%)**	30 March 2016	*Salamandra salamandra* (N=4)	0/4	0	N. Wagner/U. Schulte
Körperich	**0/18=0% (0–18%)**	11 April 2017	*Ichthyosaura alpestris* (N=16, 7 m, 9 w)	0/16	0	F. Keltsch
			Lissotriton helveticus (N=2, 1 m, 1 w)	0/2	0	
Läuskopfbach	**0/23=0% (0–14%)**	2, 5 May 2017	*Ichthyosaura alpestris* (N=7, 3 m, 4 w)	0/7	0	F. Keltsch
			Lissotriton helveticus (N=16, 13 m, 3 w)	0/16	0	
Läuskopfbach	**0/22=0% (0–15%)**	20 June 2018	*Ichthyosaura alpestris* (N=4, 2 m, 2 w)	0/4	0	K. Bredimus
			Lissotriton helveticus (N=17, 6 m, 11 w)	0/17	0	
			Bufo bufo (N=1, 1 m)	0/1	0	
Läuskopfbach	0720=0% (0–16%)		*Ichthyosaura alpestris* (N=11, 7 m, 4 w)	0/11	0	S. Feldmeier
			Lissotriton helveticus (N=9, 5 m, 4 w)	0/9	0	
Lichtenborn	**0/22=0% (0–14%)**	1 April 2016	*Salamandra salamandra* (N=1)	0/1	0	N. Wagner/U. Schulte
			Ichthyosaura alpestris (N=10)	0/10	0	
			Lissotriton helveticus (N=11)	0/11	0	
Lichtenborn	**0/72=0% (0–5%)**	3 April 2018	*Salamandra salamandra* (N=31, 6 m, 9 w, 16 j)	0/31	0	N. Wagner/K. Bredimus
			Ichthyosaura alpestris (N=17, 5 m, 12 w)	0/17	0	
			Lissotriton helveticus (N=14, 2 m, 12 w)	0/14	0	
			Rana temporaria (N=2, 1 m, 1 w)	0/2	0	

Site	95%-Bayesian CI	Date	Species	N *Bsal*-infected	Ø GE A and B sample	Field workers
Lichtenborn	**0/72=0% (0–5%)**	3 April 2018	*Bufo bufo* (N=7, 7 m)	0/7	0	
			Alytes obstetricans (N=1, 1 w)	0/1	0	
Lichtenborn	**0/98=0% (0–4%)**	4–5 April 2019	*Salamandra salamandra* (N=83, 14 m, 32 w, 36 j)	0/83	0	N. Wagner/S. Feldmeier/J. Viebahn
			Ichthyosaura alpestris (N=2, 2w)	0/2	0	
			Lissotriton helveticus (N=8, 7 m, 1 w)	0/8	0	
			Rana temporaria (N=2, 1 m, 1 j)	0/2	0	
			Bufo bufo (N=4, 2 m, 2 w)	0/4	0	
Lützkampen	**0/8=0% (0–31%)**	26 April 2015	*Ichthyosaura alpestris* (N=8)	0/8	0	N. Wagner/L. Marin da Fonte/S. Lötters
Lütztkampen	**0/15=0% (0–19%)**	11 May 2017	*Ichthyosaura alpestris* (N=13, 9 m, 4 w)	0/13	0	F. Keltsch
			Lissotriton helveticus (N=2, 2 m)	0/2		
Lützkampen	0/2=0% (0–70%)		*Ichthyosaura alpestris* (N=2)	0/2	0	E. Schommer
Luppertsseifen	**0/21=0% (0–16%)**	5 May 2017	*Ichthyosaura alpestris* (N=15, 10 m, 5 w)	0/15	0	F. Keltsch
			Lissotriton helveticus (N=6, 5 m, 1 w)	0/6	0	
Nimshuscheid	**0/11=0% (0–26%)**	23 May 2017	*Ichthyosaura alpestris* (N=8, 4 m, 4 w)	0/8	0	F. Keltsch
			Lissotriton helveticus (N=3, 2 m, 1 w)	0/3	0	
Nimshuscheid	0/10=0% (0–31%)		*Ichthyosaura alpestris* (N=2, 1 m, 1 w)	0/2	0	S. Feldmeier
			Lissotriton helveticus (N=8, 2 m, 6 w)	0/8	0	
Obersgegen	**0%(0–30%)**	12 April 2017	*Lissotriton helveticus* (N=9, 3 m, 6 w)	0/9	0	F. Keltsch
Obersgegen	**0/1=0%(–)**	21 June 2018	*Lissotriton helveticus* (N=1, 1 w)	0/1	0	K. Bredimus/J. Viebahn
Plütscheid	**1/3=33.3% (8–82%)**	28 April 2017	*Ichthyosaura alpestris* (N=3, 1 m, 2 w)	1(w)/3	853, 671	F. Keltsch
Plütscheid	**0/10=0% (0–31%)**	6 June 2019	*Ichthyosaura alpestris* (N=8, 5 m, 3 w)	0/8	0	S. Feldmeier/J. Viebahn
			Lissotriton helveticus (N=2, 1 m, 1 w)	0/2	0	
Prümzurlay	**0/32=0% (0–11%)**	29 March 2017	*Ichthyosaura alpestris* (N=26, 17 m, 9 w)	0/26	0	F. Keltsch
			Lissotriton helveticus (N=6, 5 m, 1 w)	0/6	0	
Ralingen	**0/3=0% (0–61%)**	28 March 2016	*Salamandra salamandra* (N=3)		0	N. Wagner/J. Beninde
Reiff	**0/16=0% (0–20%)**	9, 10 May 2017	*Ichthyosaura alpestris* (N=14, 3 m, 11 w)	0/14	0	F. Keltsch
			Lissotriton helveticus (N=2, 1 m, 1 w)	0/2	0	

Site	95%-Bayesian CI	Date	Species	N *Bsal*-infected	Ø GE A and B sample	Field workers
Sefferscheid	**0/5=0% (0–44%)**	19 April 2017	*Ichthyosaura alpestris* (N=1, 1 m)	0/1	0	F. Keltsch
			Lissotriton helveticus (N=1, 1 m)	0/1	0	
			Lissotriton vulgaris (N=3, 1 m, 2 w)	0/3	0	
Spielmannsholz	**0/6=0% (0–43%)**	10 May 2017	*Ichthyosaura alpestris* (N=4, 2 m, 2 w)	0/4	0	F. Keltsch
			Lissotriton helveticus (N=2, 1 m, 1 w)	0/2	0	
Steuenbach	**0/7=0% (0–36%)**	13 April 2016	*Salamandra salamandra* (N=7)	0/7	0	N. Wagner/J. Ewen/ C. Kolwelter
Weilerbach	**0/10=0% (0–31%)**	4 May 2015	*Ichthyosaura alpestris* (N=2)	0/2	0	N. Wagner/G. Fichera
			Lissotriton helveticus (N=8)	0/8	0	
Weilerbach	**0/12=0% (0–26%)**	31 March 2017	*Ichthyosaura alpestris* (N=6, 4 m, 2 w)	0/6	0	N. Wagner/J. Beninde/ F. Keltsch
			Lissotriton helveticus (N=6, 4 m, 2 w)	0/6	0	
Weilerbach	**0/4=0% (0–51%)**	26 March 2018	*Lissotriton helveticus* (N=4, 3 m, 1 w)	0/4	0	N. Wagner
Weilerbach	**0/23=0% (0–14%)**	12 March/ 12 April 2019	*Ichthyosaura alpestris* (N=6, 5 m, 1 w)	0/6	0	N. Wagner/ S. Feldmeier/J. Viebahn
			Lissotriton helveticus (N=17, 11 m, 6 w)	0/17	0	

SALAMANDRA **56**(3): 227–229
15 August 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

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Preliminary report on the occurrence of *Batrachochytrium salamandrivorans* in the Steigerwald, Bavaria, Germany

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Manuscript received: 14 July 2020
Accepted: 27 July 2020 by Stefan Lötters

The invasive chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) is one of the most devastating amphibian pathogens that was introduced to Europe, where it is threatening the entire Western Palearctic urodelean diversity, especially the European fire salamander *Salamandra salamandra* (Martel et al. 2013, 2014, 2020, Spitzen-van der Sluijs et al. 2016, Stegen et al. 2017). So far, Germany represents the most affected country with almost 50 *Bsal*-positive sites, mainly concentrated to two regions, Eifel Mountains (Northrhine-Westfalia and Rhineland-Palatine) and Ruhr District (Northrhine-Westfalia) (Dalbeck et al. 2018, Schulz et al. 2018, Wagner et al. 2019a, Lötters et al. 2020 in this issue, Schulz et al. 2020 in this issue).

Here we provide the first evidence for the occurrence of *Bsal* in southern Germany. The pathogen was detected in the nature park Steigerwald, located in the north of the state Bavaria constituting the first record of the pathogen within this state, at a distance of more than 250 km distance from other previously known records (Fig. 1). A dead European fire salamander (*S. salamandra*) was found on 8 May 2020 in the Ebracher Forst, lying in a small stream, a tributary to the Mittelebrach, next to a forest street (coordinates in decimal degrees: 49.864623 N, 10.488179 E). The specimen had characteristic *Bsal* lesions all over the body. It was collected and tested *Bsal*-positive by qPCR analysis (Technische Universität Braunschweig; see Schulz et al. 2020 in this issue for laboratory methods). An independent qPCR analysis carried out by Trier University (S. Lötters pers. comm. June 2020) revealed the same positive result. In addition, a histological examination at Ghent University (see Martel et al. 2013 for methods) confirmed the presence of *Bsal*-induced skin lesions associated with chytrid organisms.

A recent monitoring of the affected fire salamander population in June/July 2020, commissioned by Bayerisches Landesamt für Umwelt, revealed further eight positive animals out of 21 tested. The first analyses (A-samples) identified seven *Bsal*-infected fire salamanders including two dead individuals as well as two individuals with suspicious looking skin aberration. Besides, one Alpine newt *Ichthyosaura alpestris* was also tested *Bsal*-positive. All specimens where found in close proximity of the first case.

The Steigerwald is a low mountain range in Bavaria. The northern part is characterized by a large connected area of deciduous forest, mostly state owned. This area is mainly classified as FFH-Area as well as Special Protection Areas (SPA) of the European Natura 2000 network, which is a popular excursion destination. The Steigerwald represents a hybrid zone where both fire salamander subspecies, *S. salamandra terrestris* and *S. salamandra salamandra*, occur together.

Since 2015, the fire salamander population in the Steigerwald is monitored in the frame of a citizen science project conducted by C. Dittrich (Museum of Natural History Berlin) in cooperation with the Bavarian State Forestry Commission (buergerschaffenwissen.de/projekt/amphibien-taskforce). Although the number of observations fluctuated, there have never been any signs indicating the presence of *Bsal* within that area until the recent detection (Dittrich 2019). Additionally, two studies examined the distribution of *S. salamandra* in the Steigerwald in 2014 and 2017 (Urbaniec 2014, Bandorf 2017), under the direction of M.-O. Rödel, Museum für Naturkunde Berlin. Both studies found the fire salamander distributed across the entire area, larvae being present in almost all of the examined rivers. There was no indication of *Bsal* or any other disease in the seemingly very healthy populations.

The fact that many fire salamander larvae were observed in the stream next to the infected specimen implies that the pathogen was recently introduced to the Steigerwald. The passive transmission and further distribution of spores can occur via contaminated soil, which could stick to hiking shoes, forest machinery, animal paws, or wheels of bikes and cars. Therefore, a profound cleaning and disinfection of shoes, machinery and equipment is necessary, when a contaminated area was visited (van Rooij et al. 2017), in order to minimize further dispersal.

However, the disease dynamic of this pathogen is not well understood, and seems to be even more ambiguous within a huge forest as observed in the Eifel Mountains where the populations are connected and individuals free to migrate giving the possibility that *Bsal* has already been present for a long time (Lötters et al. 2020 in this issue). A further intensive monitoring and screening of the fire salamander population is already planned to gain more information about the current infection status of the population. A larval monitoring will be implemented, not only in the Steigerwald but also as an early warning system in fire salamander habitats all over Bavaria where *Bsal* is still absent.

The apparent spread of *Bsal* towards southern Germany is extremely worrying when taking into account that the new locality narrows the gap towards the Alps, where the alpine salamander, *Salamandra atra,* as well as the even more threatened Lanza's alpine salamander, *Salamandra*

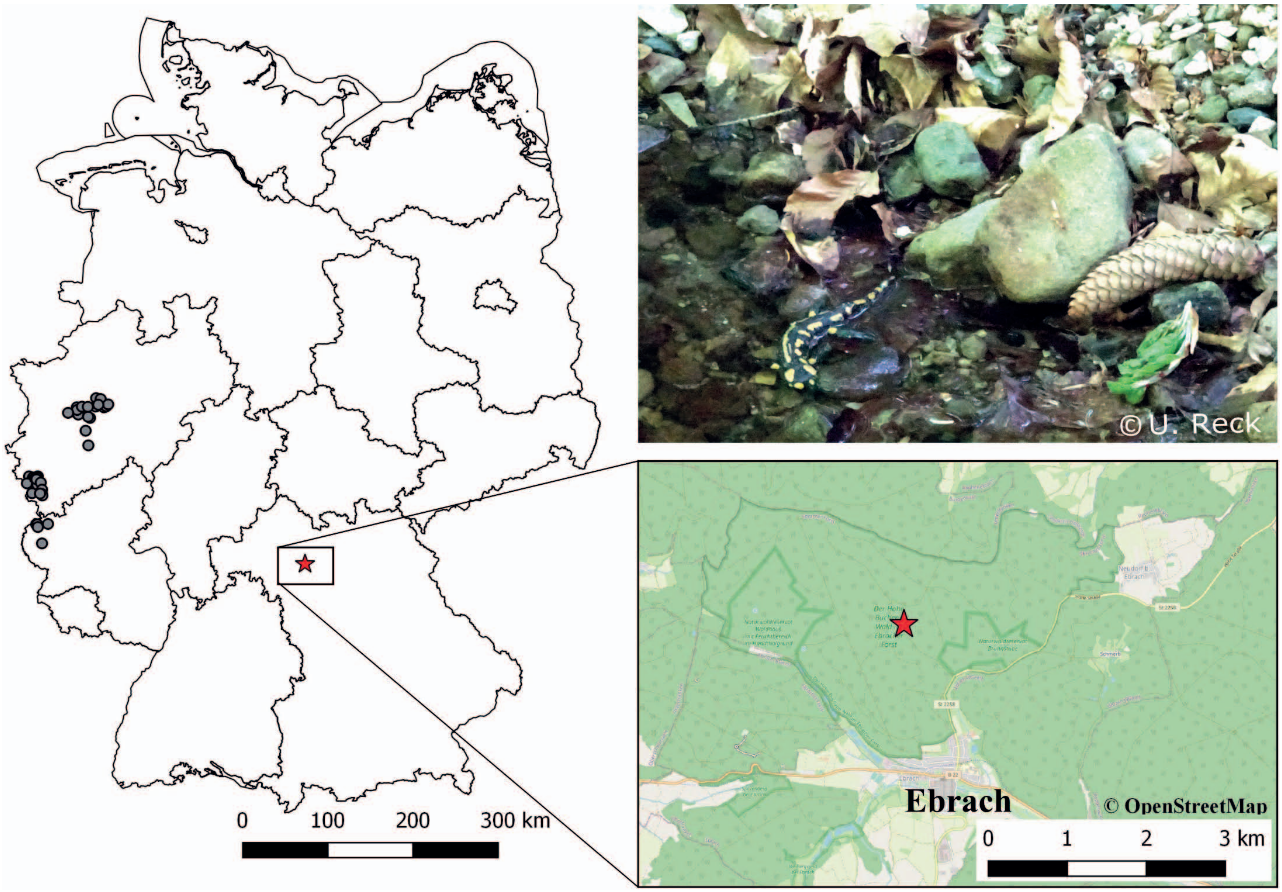

Figure 1. (left) Grey dots indicate *Bsal*-positive sites in Germany (Lötters et al. 2020a in this issue, Schulz et al. 2020 in this issue) (top right) Picture of the dead, *Bsal*-positive tested European fire salamander found in the Steigerwald (Ebracher Forst) taken by U. Reck. Small inset map shows the locality where the dead specimen was found on 8 May 2020. The shape file is based on Bundesamt für Kartographie und Geodäsie, Frankfurt am Main, 2011.

lanzai, occur. These species are likely to be as susceptible to *Bsal* as the European fire salamander. This alarming development supports the need of an urgent German *Bsal* Action Plan mentioned in detail in Lötters et al. (2020 in this issue).

Acknowledgements

We thank Niko Rebhan (who found the first *Bsal*-infected fire salamander in Bavaria and who is member of the „Verein Nationalpark Steigerwald"), Brigitte Weinbrecht (Landratsamt Bamberg), Manfred Scheidler (Höhere Naturschutzbehörde an der Regierung Oberfranken), Ulrich Mergner (Bayerische Staatsforsten AöR Forstbetrieb Ebrach), Andreas von Lindeiner (Landesbund für Vogelschutz in Bayern e.V., LBV), Karin Fischer (Universität Trier), Uwe Friedel (BUND Naturschutz in Bayern) and Mark-Oliver Rödel (Museum für Naturkunde Berlin) for assistance, contribution, examination and organization.

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15 August 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

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Batrachochytrium salamandrivorans kills alpine newts (*Ichthyosaura alpestris*) in southernmost Germany

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Manuscript received: 22 July 2020
Accepted: 24 July 2020 by Stefan Lötters

The European amphibian fauna is threatened by several different pathogens. We currently know of mortalities linked to ranaviruses (Kik et al. 2011), and to two fungal pathogens, the widespread *Batrachochytrium dendrobatidis*, causing amphibian mortalities in several European countries (Fisher et al. 2009), and the invasive chytrid fungus *B. salamandrivorans* (*Bsal*), affecting especially urodeles (Martel et al. 2013, 2014). A modelling study suggests that currently much of the ecological niche of *Bsal* remains unoccupied in Europe (Beukema et al. 2018). A further successful spread of this pathogen is therefore likely. In Europe, Germany has been counting about 50 known *Bsal*-positive sites (Lötters et al. 2020 in this issue) which makes it currently the most severely impacted country.

Several new observations of *Bsal*-related amphibian mortalities in the South of Germany have been made in recent years (Lötters et al. 2020 in this issue), including the first proof of *Bsal* in Bavaria in 2020 (Thein et al. 2020 in this issue). Here we provide evidence of the first *Bsal* occurrence in the German region of Allgovia (northern Alps, Bavaria). Several mortality events of alpine newts (*Ichthyosaura alpestris*) have been observed in garden ponds in a small village north east of the town of Memmingen (Allgovia, Germany, N48.05883°, E10.32297°, Fig. 1) beginning of April 2020. Characteristic *Bsal*-lesions were observed on the skin of these specimens (N = 10, Fig. 2). We tested eight tissue samples of the specimens from one garden pond via qPCR analysis (standard protocol see Blooi et al. 2013), detecting the presence of *Bsal* (mean GE = 36,145.5 ± 12436.8, GE range: 104–94,800). Histological analyses confirmed the presence of sporangia in typical ulcerative skin lesions in five specimens. In the close vicinity of the site of mortality events several additional dead alpine newts were observed, but neither collected nor analyzed.

Alpine newts have been demonstrated to be susceptible to *Bsal* infection (Martel et al. 2014) and identified as potential *Bsal* reservoirs (Stegen et al. 2017). Our report confirms *Bsal*-induced field mortality in this species (Schulz et al. 2018, 2020 in this issue). The impact at the level of populations remains unclear since alpine newts are potentially able to cure themselves when affected by only a low load of *Bsal* zoospores (Stegen et al. 2017).

Transmission pathways of batrachochytrids are poorly understood. *Bsal* was estimated to spread slowly under natural conditions due to the low migration ability of urodeles (Trochet et al. 2014). Long-distance spread may mainly be anthropogenic (Spitzen-van der Sluijs et al. 2018, Martel et al. 2020), especially given that the here reported mortalities were observed in a garden pond. The geographically closest known occurrence of *Bsal* is located at a distance of ca. 170 km of this outbreak in the Bavarian Steigerwald (Thein et al. 2020 in this issue). These new observations of mortalities suggest that *Bsal* is spreading or is being spread by humans across large distances. Aquatic plants bought e.g. in garden centers may be possible vectors, as it is known that amphibians can hide in those plants and then are transported together with pathogens over longer distances and released in suitable habitats (a garden pond, pers. observ. DSS).

Our observation of mortalities in the very south of Germany, close to the German Alps give rise to concern that *Bsal* may now also threaten rare and emblematic species such as the alpine salamander, *Salamandra atra,* and its subspecies, and Lanza's alpine salamander, *S. lanzai.* A German and European threat abatement plan needs to be put in place urgently (Thomas et al. 2019, Lötters et al. 2020 in this issue).

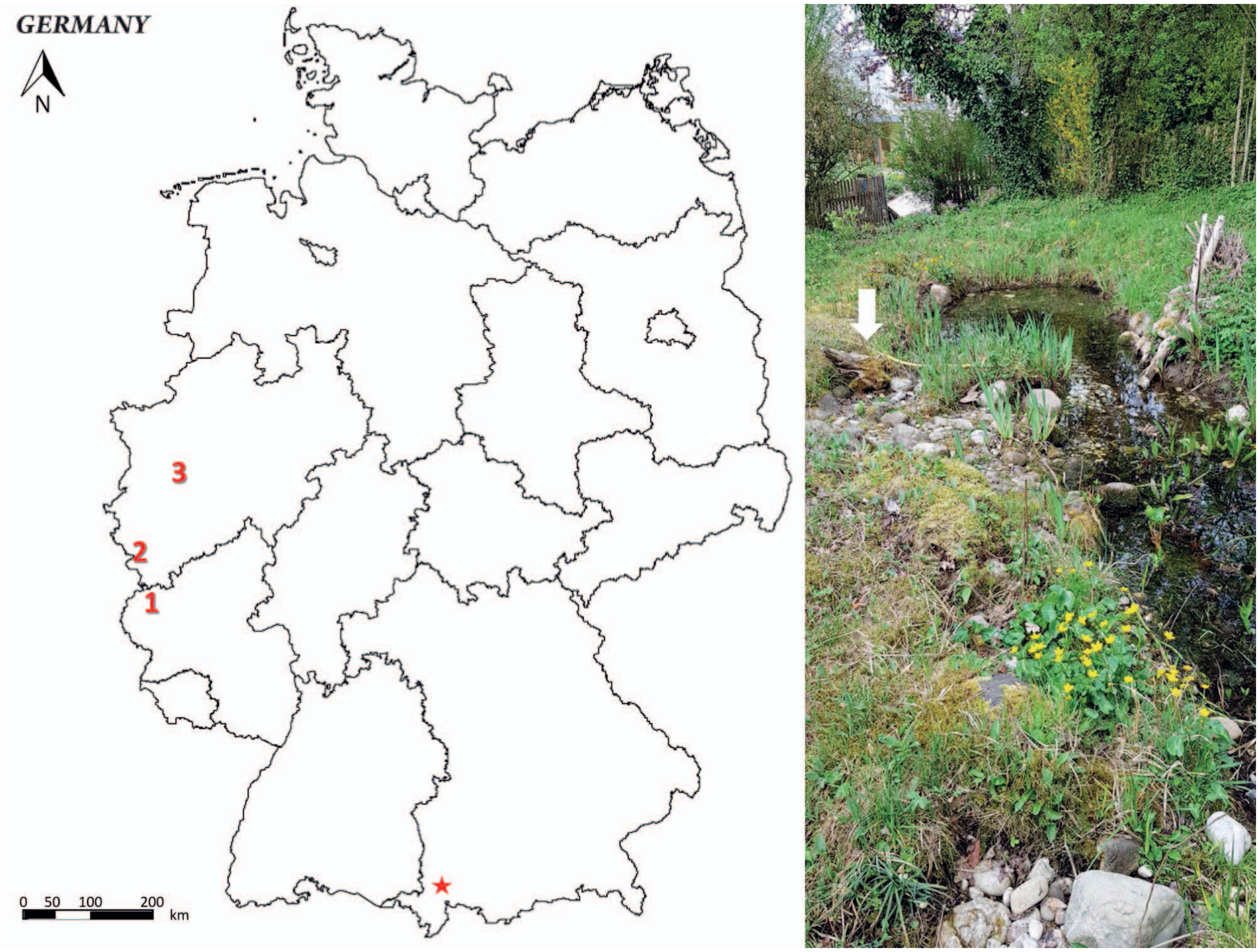

Figure 1. Location of the *Bsal* clusters known for Germany (1 = Southern Eifel cluster, 2 = Northern Eifel cluster, 3 = Ruhr District cluster, for details see Lötters et al. 2020 in this issue). The red star shows the location of the alpine newt mortality site. The arrow in the photo depicting the garden pond shows the location where the dead alpine newts were found.

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Figure 2. Photographs of the observed lesions (white arrows) of alpine newts from a garden pond in Allgovia, southern Bavaria.

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SALAMANDRA **56**(3): 233–238
15 August 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Epidemiological screening of captive salamanders reveals current absence of *Batrachochytrium salamandrivorans* in private collections throughout the federal state of Hesse (Germany)

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Manuscript received: 29 February 2020
Accepted: 5 May 2020 by Stefan Lötters

Abstract. The infamous chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) recently led to the collapse of European fire salamander populations (*Salamandra salamandra*) in The Netherlands. Currently, the pathogen has been rapidly expanding its range and threatens salamander populations throughout Europe, including Germany. Here, *Bsal* is known from wild and captive amphibians and has mostly been reported from the federal state of Northrhine-Westphalia. Due to the geographical proximity of infected areas, its dispersal into neighbouring states is possible. A *Bsal* taskforce was therefore recently formed in the state of Hesse that aims to implement preparative measures for *Bsal* mitigation at different levels. Based on the known *Bsal* susceptibility of salamanders in captivity and their inherent threat potential towards natural populations, an epidemiological screening for *Bsal* prevalence in private amphibian collections throughout the state of Hesse was conducted. We analysed a total of 174 samples from nine private collections of different urodelan species via qPCR and did not detect *Bsal*. We discuss our results and their implications for salamander conservation relative to other surveys of this kind and underscore the importance of tight cooperation between private keepers and conservation scientists in order to protect wild amphibians from the lethal *Bsal* fungus.

Key words. Amphibia, Caudata, *Salamandra salamandra*, *Bsal*, chytrid fungus, emerging infectious diseases, disease monitoring, European fire salamander, herpetoculture.

Introduction

Amphibians are considered the most endangered group of vertebrates (Stuart et al. 2004, Hoffmann et al. 2010). Besides habitat loss and fragmentation, emerging infectious diseases (EIDs) are largely responsible for population declines in amphibians. In particular, the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) (Longcore et al. 1999) has imperilled amphibian communities on a global scale and even caused species extinctions (Fisher et al. 2012). Recently, a newly discovered chytrid fungus, *Batrachochytrium salamandrivorans* (*Bsal*) (Martel et al., 2013), has become a major concern for conservationists in Europe. *Bsal* is notorious for infecting urodelans in a pandemic often referred to as the 'salamander plague'. According to present knowledge, the European fire salamander, *Salamandra salamandra* (Linnaeus, 1758), is the most susceptible species for *Bsal.* Here, this fungus causes an unexceptionally lethal, dose-independent course of disease with characteristic lesions and ulcerations of the skin transmitted via skin contact with infected individuals or contaminated materials (Martel et al. 2013, 2014, Stegen et al. 2017). The inherent threat that *Bsal* poses to the European fire salamander became obvious in The Netherlands: Here, population levels have decreased by 96% due to *Bsal* outbreaks, driving the European fire salamander to the brink of extinction throughout the country (Spitzen-van der Sluijs et al. 2013). However, mass extinction processes have also been taking place in Belgium and Germany (Martel et al. 2014, Stegen et al. 2017, Dalbeck et al.

2018, Schulz et al. 2018, Wagner et al. 2019). Germany is in fact currently considered the 'hot spot' of *Bsal* infections with almost 50 affected sites in the Eifel Mountains as well as the Ruhr District in the federal states Northrhine-Westphalia and Rhineland-Palatinate (Dalbeck et al. 2018, Schulz et al. 2018, 2020 in this issue, Wagner et al. 2019, Lötters et al. 2020 in this issue).

Among the federal states of Germany, Hesse is a state of pivotal importance for salamander conservation: Its borders are located less than 150 km distant from the current *Bsal* epicentre in the Eifel and different models suggest that dispersal of the fungus into Hesse is a likely scenario (Fig. 1) (Feldmeier et al. 2016, Beukema et al. 2018). Hesse probably hosts the highest density of European fire salamander populations with a significant biomass of this species, as it is centrally located in the distribution range of the European fire salamander in Germany (Klewen 1988, Thiesmeier 2004, Deutsche Gesellschaft für Herpetologie und Terrarienkunde 2018). These circumstances have led to the founding of a task force for the protection of European fire salamanders in Hesse in 2018 (Ziemek 2019). This task force aims to develop preventative means in preparation for the arrival of *Bsal* in Hesse and to design strategies to efficiently mitigate its spread within the state.

Recent studies have suggested that *Bsal* was originally introduced to Europe from infected Asian urodelans imported for the pet trade (Martel et al. 2014, Laking et al. 2017, Nguyen et al. 2017, Yuan et al. 2018). This implies that private terrarium collections of amphibians may represent a reservoir for this biodiversity-threatening disease from which novel infections could emerge. Hence, considerable effort was invested into epidemiological tracing of *Bsal* throughout privately kept urodeles across Europe in the recent past (Cunningham et al. 2015, Sabino-Pinto et al. 2015, 2018, Fitzpatrick et al. 2018). These studies have highlighted the infestation of such collections in, e.g., Sweden, Germany, Spain, and the United Kingdom (Cunningham et al. 2015, Sabino-Pinto et al. 2015, 2018, Fitzpatrick et al. 2018), and one of them detected *Bsal*-positive salamanders in one collection in Hesse (Sabino-Pinto et al. 2015, 2018).

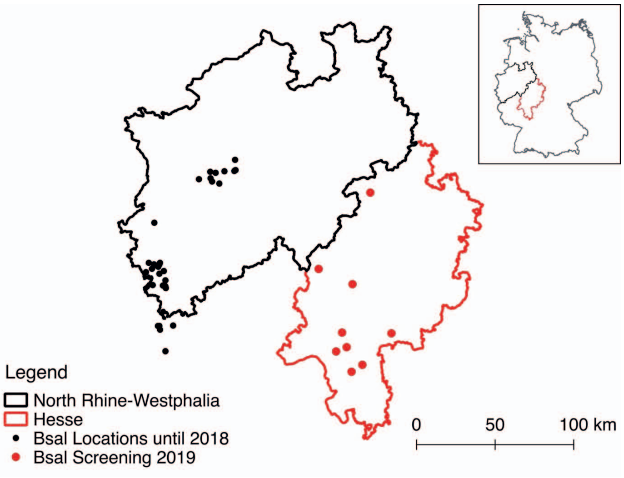

Figure 1. The federal state of Hesse (red) borders Northrhine-Westphalia (black) where Bsal outbreaks in the wild have become known (black spots, following Dalbeck et al. 2018, Schulz et al. 2018). We examined the prevalence of the pathogen in private urodelan collections throughout the state (red spots).

This finding has made us ask to what extent the pathogen may be distributed in private collections within the federal state of Hesse. We opted for a qPCR-based tracing strategy and screened salamander stocks of hobbyists, zoos, universities and professional breeders in order to derive insights about the fungus prevalence in Hesse and to infer hypotheses about the threat potential such collections may pose to European fire salamander populations in the wild.

Material and methods

Throughout 2019, skin swabs of captive urodelans were collected from nine private collections across Hesse (Fig. 1). Animals to be sampled were randomly selected from the keepers' enclosures. First, the habitus of all animals was checked for obvious signs of infections, such as ulcerations and lesions. For the handling of animals, nitrile gloves (one set of gloves per individual) were used in order to avoid potential transmissions of *Bsal*.

Skin swabs were collected as previously described (Hyatt et al. 2007, van Rooij et al. 2011, Blooi et al. 2013): Briefly, the ventral side of each individual was wiped ten times utilizing two sterile cotton swabs (A- and B-samples for data validation), which then were stored separately in sterile Eppendorf tubes. If possible, at least three individuals per enclosure were included in this study, however, if an enclosure housed fewer individuals all available animals were analysed. Negative controls at each locality were generated by opening an unused cotton swab and placing it into a separate tube midway through the sampling process. Used swabs were stored at -20°C until further processing.

For molecular *Bsal* screening, genomic DNA was extracted by using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, DE) following the manufacturer's instructions after a pretreatment with lysozyme. Quantitative polymerase chain reaction (qPCR) was performed as published previously (Blooi et al. 2013, 2016) with the alterations of using a *Bsal*-specific FAM-labelled probe (biomers, Ulm, DE) and the TaqMan^{TM} Fast Universal PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, US) following the manufacturers' instructions as to temperature and cycling conditions. PCR was performed on a RotorGene System (Qiagen, Hilden, DE). Prior to sample analysis, the PCR protocols detection limit was determined by testing a ten-fold dilution series of the positive control with 1000 GE/5 ml. The lower detection limit was demonstrated to be 0.1 GE/5 ml, which conforms with Blooi et al. (2013). The subsequent initial screening of all samples was performed at the Hospital for Birds, Reptiles, Amphibians and Fish of the Justus Liebig University Gießen.

From all obtained samples, 20 B-samples were randomly selected, extracted and analysed a second time in an independent laboratory at the Zoological Institute of the Technische Universität Braunschweig for data validation using the protocol of Blooi et al. (2013). Here, gBlock fragments were used as a standard. The threshold for the ITS copies was set to 100 ITS based on a comparison of Ct values between both standards (1 GE = 33 cycles/100 ITS copies = 35 cycles). Each qPCR plate contained two replicates of samples, two replicates of *Bsal* standard (10-fold dilution scale from 100–10,000,000 ITS copies), and two negative controls. All runs were performed on a CFX96 Real-Time System (Bio-Rad Laboratories Inc., Hercules, CA, US).

Results

For the epidemiological screening of *Bsal* in captive urodelans within Hesse, we sampled animals from nine different localities across the federal state (Fig. 1). Our sampling included 28 taxa, 20 belonging to the family Salamandridae including several subspecies of the European fire salamander as well as the Alpine salamander, *Salamandra atra* Laurenti, 1768, and several newt species (Table 1). The remaining eight taxa included in this study represented the genus *Ambystoma*, family Ambystomatidae (Table 1).

In total, we collected skin swabs from 174 specimens and analysed all of these by means of qPCR. None of the examined animals exhibited any obvious symptoms of the salamander plague, indicating that the pathogen was either recently introduced and had not yet caused any physical symptoms, or was absent from the studied private collections. Our phenotypical examinations were in accordance with our molecular genetic analysis for *Bsal* prevalence in that all analysed skin swabs tested negative (Table 1).

Discussion

As an emerging infectious disease, the salamander plague has been spreading rapidly in Europe and has already caused several local mass extinction events in European fire salamanders (e.g., Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018, Schulz et al. 2018, 2020 in this issue, Wagner et al. 2019, Lötters et al. 2020 in this issue). Previous studies suggested that captive urodelans may represent a reservoir for *Bsal* as indicated by the abundance of *Bsal*-positive specimens that were reported from private collections in Germany, Spain, Sweden and England (Cunningham et al. 2015, Sabino-Pinto et al. 2015, 2018, Fitzpatrick et al. 2018). Apparently, *Bsal* has been introduced to the European amphibian fauna via infected Asian newts imported for the pet trade. It has also been reported that infections of *Bsal* may be asymptomatic and therefore difficult or even impossible to detect (Sabino-Pinto et al. 2018). Recent studies found that captivity as an exter-

Table 1. List of all sampled species and subspecies, number of samples per taxon at each locality (N), and resulting *Bsal* status as identified via qPCR. A-samples were analysed from all specimens, specimens with analysed B-samples are indicated with an asterisk. Coll = Collection.

Coll	Species	N	*Bsal* status
Coll 1	_Salamandra salamandra terrestris_*	9	neg.
	Ichthyosaura alpestris	3	neg.
	Neurergus crocatus	3	neg.
	Neurergus kaiseri	3	neg.
Coll 2	_Salamandra salamandra alfredschmidti_	3	neg.
	Salamandra salamandra bernardezi	3	neg.
	Salamandra salamandra gigliolii	3	neg.
	Salamandra salamandra terrestris	6	neg.
	Tylototriton shanjing	3	neg.
	_Salamandra atra_*	1	neg.
Coll 3	_Salamandra salamandra bernardezi_	4	neg.
	Salamandra salamandra salamandra	10	neg.
Coll 4	_Salamandra salamandra salamandra_	3	neg.
	Ambystoma andersoni	1	neg.
	Ambystoma macrodactylum	7	neg.
	_Ambystoma maculatum_*	5	neg.
	Ambystoma mavortium melanostictus	4	neg.
	Ambystoma mavortium nebulosum	6	neg.
	Ambystoma opacum	5	neg.
Coll 5	_Ambystoma talpoideum_	4	neg.
	_Ambystoma tigrinum_*	10	neg.
	_Salamandra salamandra gallaica_*	5	neg.
	Salamandra salamandra salamandra	2	neg.
	_Salamandra salamandra terrestris_*	7	neg.
	Tylototriton kweichowensis	5	neg.
	_Tylototriton shanjing_*	4	neg.
Coll 6	_Salamandra salamandra_*	8	neg.
	_Ichthyosaura alpestris_*	3	neg.
	Lissotriton vulgaris	3	neg.
	Salamandra atra	3	neg.
	_Salamandra salamandra bernardezi_*	3	neg.
Coll 7	_Salamandra salamandra terrestris_	3	neg.
	Taricha granulosa	4	neg.
	_Triturus cristatus_*	3	neg.
	Triturus marmoratus	3	neg.
Coll 8	_Salamandra salamandra_*	1	neg.
	Ichthyosaura alpestris	3	neg.
	Lissotriton italicus	3	neg.
	Salamandra salamandra bernardezi	3	neg.
Coll 9	_Salamandra salamandra gallaica_	3	neg.
	Salamandra salamandra terrestris	3	neg.
	Triturus carnifex	3	neg.
	Triturus dobrogicus	3	neg.

nal factor negatively influences the microbial communities within the mucosome (i.e., the complex micro-ecosystem of amphibian skin poisons plus their microbial symbionts), which is an important anti-infectious line of defence in amphibians (e.g., Woodhams et al. 2014, Bates et al. 2018, 2019, Lüddecke et al. 2018). As a result of this negative effect, captive animals are probably much more receptive to colonization by pathogens such as *Bsal* (e.g., Bates et al. 2019). On the other hand, animals in private collections are usually kept well fed and in good general condition. In addition, they usually receive good health monitoring so that clinical diseases are detected and can be treated early. In such collections, latent pathogen infections without obvious clinical disease are nevertheless possible (e.g., Sabino-Pinto et al. 2018). Therefore, captive collections might represent a reservoir of pathogens that may cause fatal disease if introduced into naïve free-ranging populations (e.g., Martel et al. 2014, Sabino-Pinto et al. 2015, 2018, Fitzpatrick et al. 2018, Martel et al. 2020).

All these aspects in their totality highlight the importance of close monitoring for *Bsal* in private and public collections as a protective measure, because animals kept thus have an increased likelihood of either harbouring the disease, or are at least at higher risk of being infected if the pathogen is introduced. Since any biological material that is transferred from such collections to the environment (e.g., organic waste, water or soil) constitutes a potential infectious agent, it is of pivotal importance to know the infection status of these. This urgency becomes especially obvious when the potential treatment is taken into account: *Bsal*-infected salamanders can often be treated by keeping the animal at higher temperatures (25°C) for an extended period of time (Blooi et al. 2015). This therapy often results in the eradication of *Bsal*, and many salamanders recover from it when conducted correctly and monitored well. While this therapy may be useful for captive collections with only a limited number of animals being affected, it is not practicable for wild salamander populations due to their sheer numbers and legislative obstacles (Kuzmin et al. 2009, AGAR & FENA 2010). Heat treatment does furthermore not alter the salamander immune system and therefore provides no long-term protection against *Bsal* (Stegen et al. 2017).

In their exploratory study that assessed infection rates in captive salamandrids in Germany and Sweden, Sabino-Pinto et al. (2018) detected several infected animals, with the most severely affected collection being one in Hesse. We therefore expected that several of the screened collections would also contain infected animals. However, none of our qPCR screenings recovered the presence of *Bsal* in any of the analysed localities and consequently, apart from the case documented by Sabino-Pinto et al. (2018), no *Bsal*-positive salamander has been reported in Hesse in 2019.

That said, it is obvious that neither our study across the federal state, nor the study by Sabino-Pinto et al. (2018) are of holistic nature. It should also be kept in mind that our study, like that by Sabino-Pinto et al. (2018), is based on data obtained from collections that voluntarily participated. It may be presumed that collections experiencing clinical problems more regularly are not supportive of such screenings. However – if the pathogen is widely distributed in captive collections in Hesse, it is likely that it would have been detected also in this study – as animals are commonly exchanged between collections. In total, both studies analysed twelve private collections, but this unfortunately represents only a marginal fraction of all such collections, as urodeles and the European fire salamander in particular are widely kept in terraria (e.g., Seidel & Gerhardt 2016). Since even a single infected animal in one of such collections has the potential of causing a massive outbreak of *Bsal* if spores are released into the environment, it remains of critical importance to further monitor such collections and include previously unexamined ones as well (e.g., Martel et al. 2020). Such an initiative, however, can only be successful if scientists studying *Bsal* and private keepers cooperate tightly. Without providing scientists access to the kept animals for subjecting these to analysis, it will be virtually impossible to infer the distribution of *Bsal* in private collections, may it be in one federal state (e.g., Hesse) or across Germany. Furthermore, it is important to conduct epidemiological screenings on a regular basis to the benefit of collections, most importantly when new animals are added, wherever they may come from, or before animals are exchanged (e.g., for breeding attempts). It should also be made mandatory that positive *Bsal*-screening results be reported to a data collection centre so that the distribution and possible spread of *Bsal* can be monitored.

The possible consequences of not sticking to such a dense monitoring for *Bsal* are a serious threat to any native salamander population that happens to exist in proximity to a captive collection and need to be avoided at all costs.

Future Perspectives

Understanding the distribution and prevalence of *Bsal* in captive collections remains an important task for the future. Sampling to this end needs to be continued and even expanded if possible. The *Bsal* task force in Hesse plans to proceed in this critical aspect of disease monitoring and aims to expand its scope by including wild salamander populations. This will be done in tight cooperation with local conservationists as well as forest authorities, utilizing a combined approach of qPCR screening of wild individuals linked to dense monitoring of larval population dynamics in order to detect cryptic population declines.

Acknowledgements

We are grateful to all the private keepers who provided us access to their animals. We also thank Sebastian Steinfartz, Kathleen Preissler, and Miguel Vences for their support and methodological training. Our project was generously funded by the 'Hessischer Biodiversitätsforschungsfonds' of the Hessisches Landesamt für Naturschutz, Umwelt und Geologie (HLNUG).

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15 August 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

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Bsal-driven salamander mortality pre-dates the European index outbreak

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Manuscript received: 28 April 2020
Accepted: 12 Mai 2020 by Miguel Vences

Emerging infectious fungal diseases increasingly become major drivers of global biodiversity loss in the Anthropocene (Daszak et al. 2000, Fisher et al. 2012, Sikes et al. 2018). Amphibians are a textbook example among the deuterostomians. They suffer from fungal skin diseases caused by at least two chytrid fungi (chytridiomycosis), *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*). Both are of Asian origin and have spread to other continents – with human mediation playing a key role – causing massive declines of naïve amphibian hosts (Woodhams et al. 2011, van Rooij et al. 2015, Garner et al. 2016, O'Hanlon et al. 2018, Scheele et al. 2019).

Bsal was only discovered and scientifically described after a drastic population breakdown around the year 2010 in a Dutch population of the European fire salamander, *Salamandra salamandra* (Spitzen-van der Sluijs et al. 2013, Martel et al. 2013). Increased monitoring efforts in recent years have revealed that this pathogen is dramatically spreading. It has since been discovered in three additional European countries in the wild, Belgium, Germany and Spain, confronting us with awkward conservation tasks (Martel et al. 2014, 2020, Spitzen-van der Sluijs et al. 2016, Thomas et al. 2019). *Bsal* exclusively affects caudate amphibians (while at least some anurans might act as reservoirs), with salamandrids, especially the genus *Salamandra*, being highly susceptible (Martel et al. 2014, Stegen et al. 2017). European fire salamander populations, once infected, undergo rapid declines demonstrated through both *in situ* observations and epidemiological modelling, and various mass mortality events have been reported (Spitzen-van der Sluijs et al. 2013, 2016, Stegen et al. 2017, Canessa et al. 2018, Dalbeck et al. 2018).

From what we know, more than half of the ca. 80 *Bsal* records in the pathogen's European invasive range are from Germany (Lötters et al. 2020 in this issue). In this country, *Bsal* was first discovered in 2015 (in *S. salamandra terrestris*) in the wild in the northern Eifel region, about 50 km from the Dutch index outbreak (Spitzen-van der Sluijs et al. 2016). Shortly after its first detection in Germany and reports in the local media, a member of the public reported a European fire salamander mass mortality to us that had previously been observed in 2004 at the Vichtbach, Rott near Roetgen (county of StädteRegion Aachen, federal state of North Rhine-Westphalia; 50.676448, 6.198452) (Dalbeck et al. 2018).

From this mortality event, two specimens, found dead on the forest floor and subsequently stored in 10% formalin, have become available for examination. They have been deposited in the UGent collection (numbers 2015/SL/1 and 2015/SL/2). Macroscopic lesions (Fig. 1) in these specimens are consistent with skin ulcerations that characterize *Bsal* chytridiomycosis under experimental conditions and in natural outbreaks (Martel et al. 2013, Stegen et al. 2017). Histopathology demonstrated the presence of epithelial erosions with intracellular fungal thalli in the epithelial cells bordering the lesions, which is indicative of chytridiomycosis caused by *Bsal* (Fig. 2). In contrast, typical lesions of infections with *Bd* consist of epidermal hyperplasia and hyperkeratosis (e.g. van Rooij et al. 2015). Immunohistochemistry (Thomas et al. 2018) resulted in marked brown staining of structures, consistent with chytrid thallus morphology against a hemaluin counterstained background, confirming the presence of *Batrachochytrium* sp. organisms in the epidermal lesions. Applying the diagnostic qPCR for *Bd* (Boyle et al. 2004) and *Bsal* (Blooi et al. 2013, 2016) on skin tissue after using different protocols for DNA extraction did not result in detectable amplification of chytrid DNA from the samples. The latter is problematic since this would be required for a final diagnosis of *Bsal* infection, and is most probably due to the long-time formalin storage of the samples. However, the combination of highly suggestive clinical signs, compatible microscopic epithelial lesions and positive immunohistochemistry from specimens in a region that is confirmed as a 'hotspot' of *Bsal*-associated declines (Lötters et al. 2020 in this issue) provide multiple lines of evidence to support our classification of this case as *Bsal* infection. No other currently known chytrid fungus or any other pathogen produces similar macroscopic and microscopic pathology (cf. Martel et al. 2013). In addition, histopathology has been proven a reliable method to identify both *Bd* and *Bsal* and to distinguish them (Martel et al. 2013, van Rooij et al. 2015, Weldon et al. 2020).

This finding pre-dates the currently assumed initial *Bsal* introduction into Europe by at least six years and identifies Germany as the first country in which a *Bsal* outbreak has been demonstrated. At that time point, the infection may already have been widespread, since Schulz et al. (2020 in this issue) provide a photograph of a European fire salamander found in 2004 in the Ruhr District of Germany that showed skin lesions consistent with *Bsal* infection (which however cannot be confirmed with full reliability as this specimen was not preserved).

It is noteworthy that *Bsal* has been in the northern Eifel for several years, but remained undetected, as here the disease was diagnosed for the first time in 2015 (Spitzen-van der Sluijs et al. 2016). Given the current *Bsal*-induced population declines of *S. salamandra terrestris* in this region, multiple 'silent' declines of this taxon in the years before are plausible. However, systematic data for this time are lacking as adult and larval monitoring efforts started only in 2014 (and at first at a markedly limited number of sites only; Dalbeck et al. 2018, Wagner et al. 2020 in this issue). On the other hand, ongoing monitoring suggests that numerous populations of the European fire salamander declined due to *Bsal* incursions only within the last five years, i.e. until recently salamanders in a population were relatively common and *Bsal* was not detected, then salamanders disappeared, dying specimens were observed and *Bsal* was detected (Dalbeck et al. 2018, Lötters et al. 2020 in this issue).

Observations suggest that *Bsal* dispersal is difficult to understand. This is further enforced by Spitzen-van der Sluijs et al. (2018) who demonstrated that a Dutch population of the European fire salamander apparently has remained unaffected by *Bsal,* although the pathogen has been present for at least about one decade in a population at a distance of < 1 km. Likewise, puzzling is the fact that – perhaps unexpected – at the Vichtbach (at exactly the area where the dead specimens were found in 2004), a vital European fire salamander population has been monitored since 2017 – without any trace of *Bsal* (Lötters et al. 2020 in this issue).

In conclusion, the long-time undetected presence of *Bsal* and its poorly understood modes of dispersal, combined with new remote outbreaks including Southern Eu-

Figure 1. Ventral view of a 10% formalin fixed specimen of *Salamandra salamandra terrestris,* found dead on the forest floor at Vichtbach, northern Eifel region, Germany, in 2004. The circular black spots represent epidermal erosions, typical of *Bsal* infection.

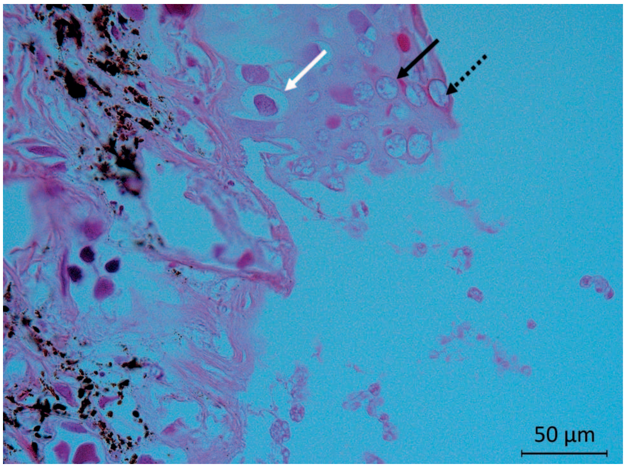

Figure 2. Hematoxylin and eosin staining of a 5 µm thickness skin section (magnification 40×) of a *Salamandra salamandra terrestris,* found dead on the forest floor at Vichtbach, northern Eifel region, Germany, in 2004. Microscopic characteristics are the presence of full thickness necrosis of keratinocytes, which contain (often colonial) thalli that marginate the cell's nucleus. The result is an epidermal erosion. Legend: white arrow – necrotic epithelial cell; black arrow – thallus with spores; stippled arrow – thallus with septae (colonial thallus).

rope, are worrying and prompt installing proper population monitoring and disease surveillance of vulnerable urodele populations in Europe (cf. Martel et al. 2014, 2020, Thomas et al. 2019, Schulz et al. 2020 in this issue).

Acknowledgments

We are grateful to colleagues and collaborators in *Bsal* research and combat from Biologische Station StädteRegion Aachen, Biologische Station im Kreis Düren, Technische Universität Braunschweig, Universität Leipzig, Bundesamt für Naturschutz (BfN) as well as Hans-Joachim Stöhr for making available European fire salamander specimens found dead in 2004. Ché Weldon kindly made valuable comments when reviewing our manuscript.

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SALAMANDRA 56(3): 243–253
15 August 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Long-term monitoring of European fire salamander populations (*Salamandra salamandra*) in the Eifel Mountains (Germany): five years of removal sampling of larvae

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Manuscript received: 2 July 2020
Accepted: 16 July 2020 by Miguel Vences

Abstract. The presence of the parasitic amphibian chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) in the Eifel Mountains, Germany, was confirmed in 2015. Since then, monitoring of selected populations of the European fire salamander (*Salamandra salamandra*) has been established. This species is highly sensitive to the pathogen and infection is commonly lethal for individuals within several days, which gave rise to the term 'salamander plague'. Because of the low detection probability of the terrestrial phase of the salamanders, we assessed the status of populations by monitoring the abundance of aquatic larvae as a proxy. A standardized removal sampling was conducted between 2015 and 2019 in 40 creeks in the Northern Eifel Mountains (21 sites with consistent 5-year data) and in 21 creeks in the Southern Eifel Mountains (17 sites with consistent 5-year data). While in the northern Eifel the pathogen was detected in 2015 and is known to be present since 2004, in the southern Eifel *Bsal*-sites has been confirmed since 2017. In both regions, the number of pools was the best fitting explanatory variable for abundances and detection probabilities of larvae. Indications for larval decline were observed in some creeks with *Bsal* presence while in others capture rates and larval abundances did not significantly change or sometimes even increased when *Bsal* was present. As a result, our data do not indicate an effect of the salamander plague to consistently and immediately causing declines in larval abundance. However, these findings remain tentative, and final conclusions should not be drawn yet. The applied removal sampling method is suggested to represent a time- and cost-effective as well as feasible means to obtain long-term population information on larval abundance in the European fire salamander.

Key words. Amphibia, Caudata, *Bsal,* chytridiomycosis, ecological modelling, larvae monitoring, population trends, salamander plague.

Introduction

Amphibian populations are declining at the global scale because of various factors, often due to synergistic and cumulative effects (Houlahan et al. 2000, Collins & Storfer 2003, Wake & Vredenburg 2008, Stuart et al. 2004, 2010). One important driver, significantly contributing to the decline and extinction of amphibian populations, are emerging infectious diseases (Daszak et al. 2000, Collins & Storfer 2003). Parasitic chytrid skin fungi of the genus *Batrachochytrium* are among the worst pathogens related to amphibian population declines causing chytridiomycosis in susceptible species and populations (Daszak et al. 2003, Fisher et al. 2012, van Rooij et al. 2015). *Batrachochytrium salamandrivorans* (*Bsal*) is the most dangerous known chytrid fungus for many urodelan amphibians; it causes the so called 'salamander plague' (Martel et al. 2013, 2014, Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018, Lötters et al. 2020a in this issue). This pathogen is native to Asia and was recently introduced into Western Europe (Martel et al. 2014, Laking et al. 2017). Here, it is markedly spreading with meanwhile about 80 records known from the wild in Belgium, Germany, the Netherlands and Spain (Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018, Schulz et al. 2018, 2020 in this issue, Lötters et al. 2018, 2020a in this issue, Martel et al. 2020).

So far, the salamander plague particularly affects the European fire salamander, *Salamandra salamandra*, a common and wide-ranging species in Western Europe (Martel et al. 2014, Stegen et al. 2017). *Bsal* is usually lethal to individuals of this species within about two weeks after infection (Martel et al. 2013, 2014, Stegen et al. 2017), resulting in high local mortality rates (Spitzen-van der Sluijs et al. 2013, 2016, Stegen et al. 2017, Dalbeck et al. 2018, Schulz et al. 2018, 2020 in this issue, Lötters et al. 2020a in this issue). However, mortality events could be directly witnessed only in a limited number of cases, suggesting that in most cases they happen 'silently' and are overlooked (Spitzen-van der Sluijs et al. 2016, Stegen et al. 2017, Dalbeck et al. 2018, Schulz et al. 2020 in this issue). This is underpinned by the observation of Schulz et al. (2018), who found dead European fire salamanders hidden in shelters in the German Ruhr District. The occurrence of these silent declines is further supported by the circumstance that the European fire salamander in the 1980's and 1990's was reported from various sites in the Southern Eifel Mountains (Bitz et al. 1996), from which they are widely absent at present (Wagner et al. 2017, 2019a, Sandvoß et al. 2020 in this issue). Nowadays, *Bsal*-infected newts can be found in these areas and in the few sites in this region where European fire salamanders still occur, *Bsal*-infected specimens have been confirmed (Dalbeck et al. 2018, Wagner et al. 2019a, b, Sandvoß et al. 2020 in this issue).

Epidemiological modelling clearly suggests that *Bsal* incursion leads to rapid extinction of the affected salamander populations (Canessa et al. 2018). However, while observations from the wild demonstrate that the salamander plague can severely affect European fire salamander populations, no case of a complete population extinction is known so far. A minimal survival rate of about 1–4% in infected populations has been found (Spitzen-van der Sluijs et al. 2013, 2016, Stegen et al. 2017, Dalbeck et al. 2018, Lötters et al. 2020a in this issue, Schulz et al. 2020 in this issue). Moreover, at least one population, which underwent a mass decline more than 15 years ago (the oldest known case of *Bsal* in Europe) appears to have recovered today, and *Bsal* cannot be detected in it (Lötters et al. 2020b in this issue). These observations show that at the current stage, the long-term effects of *Bsal* on wild European fire salamander populations remain little understood (cf. Lötters et al. 2020a).

Shortly after *Bsal* was first detected in Germany, i.e. in 2015 in the Eifel Mountains (Spitzen-van der Sluijs et al. 2016), a long-term monitoring of selected European fire salamander populations was established to obtain comparative information on abundance trends from *Bsal*-infected and uninfected sites. Furthermore, this monitoring was set up to provide an early-warning-system (EWS) for range expansions of the pathogen (Wagner et al. 2017, Dalbeck et al. 2018). Yet, the abundance of adult European fire salamanders is particularly difficult to estimate (Schmidt et al. 2015). Although individual recognition of post-metamorphic European fire salamanders using photographs of the dorsal patterns is a non-invasive and reliable method (Feldmann 1971, Kopp-Hamberger 1998), it is very challenging due to the cryptic and highly weather-dependent activity of the adults (Thiesmeier 2004). Together with their occasionally large home ranges (Schulte et al. 2007) a reliable and efficient monitoring of large populations by this method is not feasible. Comparable to other amphibians (Skelly & Richardson 2009), we therefore used larvae to establish a monitoring system for the European fire salamander in the Eifel Mountains. Females of this species deposit fully developed larvae in small waterbodies, in the study area usually first order streams, creeks and springs (Thiesmeier 2004). Schmidt et al. (2015) proposed a standardised removal sampling method to monitor the abundance of the larvae as a proxy for the adult population. With regard to population and abundance trends, long-term data on the larval abundance trends are a suitable proxy for the number of reproductively active females. Regarding the larvae monitoring as an EWS for the range expansion of *Bsal*, a strong decline or even disappearance of the larval abundance could point to locations where taking skin swabs of adult European fire salamanders or other syntopic amphibians for molecular *Bsal* detection (Lötters et al. 2020a in this issue) is advisable. In this paper, we present the results from our 2015 to 2019 salamander larvae monitoring and discuss them with the focus on potential population effects of *Bsal*.

Material and methods

In the removal sampling approach sensu Schmidt et al. (2015), the abundance of European fire salamander larvae in distinct portions of a creek are sampled repeatedly (with rotating field workers temporarily removing captured larvae). Abundance in relation to modelled detection probabilities and relevant environmental co-factors can thus be estimated. Since the statistical analysis is based on hierarchical removal sampling models and not on capture-recapture approaches (e.g. Reinhardt et al. 2018), time intensive marking individual recognition of larvae is not necessary (Schmidt et al. 2015). Furthermore, some marking methods can affect the recapture rates (Wagner et al. 2020a in this issue). For a detailed discussion of larval removal sampling in European fire salamanders and its methodology see Wagner et al. (2020a in this issue).

In this study, 75 creek sections next to the spring were selected in reproduction creeks of the European fire salamander in two regions of Western Germany: the Northern and the Southern Eifel Mountains, federal states of North Rhine-Westphalia and Rhineland-Palatinate, respectively (a list of localities is provided in Supplementary document 1). Sampling was carried out between May and June (2015 to 2019). Each stream section was divided into three 25 m subsections, and three field assistants opportunistically captured salamander larvae and temporarily 'removed' them from the creek by containing them in small plastic tubs filled with creek water. After 15 min, field assistants rotated to the next 25 m subsection and again after another 15 min to reduce the observer biases (Schmidt et al. 2015). After 45 min all larvae were released to their respective creek subsections. All equipment and materials including boots were disinfected using 70% ethanol or a 1% solution of Virkon S (van Rooij et al. 2017) on site, to minimize the risk of pathogen transport between sampling sites. The number of pools (as preferred larval microhabitats; Baumgartner et al. 1999, Werner et al. 2014, Schmidt et al. 2015, Wagner et al. 2020b in this issue) within the 75 m section and the average creek width (as proxy for water flow velocity) were recorded as structural environmental co-factors.

For all statistical analyses and modelling, the software R was used (R Developmental Core Team 2012). In our models (described below), we estimated yearly larval abundance and detection probabilities separately for the Northern and the Southern Eifel Mountains and, in addition to this, we included the capture occasion (date) as potential predictor variable, which can affect abundance and detection probabilities due to occasional larval drift (Thiesmeier & Schuhmacher 1990, Reinhardt et al. 2018, Veith et al. 2019), as potential explanatory variable. Presence/absence of *Bsal*, obtained from an ongoing *Bsal* screening at some of the studied creeks (Dalbeck et al. 2018, Wagner et al. 2019a, b, Lötters et al. 2020a in this issue), was either excluded or included as explanatory variable (see below). All potential predictors were normalized prior to analysis.

In a first analysis, we compared 32 candidate models for each year following the hierarchical modelling described in Schmidt et al. (2015). We built different generalized multinomial mixture models (Royle 2004, Dorazio et al. 2005, Royle & Dorazio 2006) using pairwise combinations of the variables described above or a 'constant'-intercept-model for both abundance (Λ) and detection probability (p) (R package 'unmarked'; Fiske & Chandler 2011). We included capture occasion, number of pools and creek width, but excluded *Bsal* presence/absence as explanatory variable in the models because *Bsal* screening was carried out at a limited number of the studied creeks (Supplementary document 1). Survival probability was assumed to be constant as neither emigration/death nor immigration/birth was supposed to have happen during the 45 min of data acquisition. All models were fitted to the data with either a Poisson or a negative binomial abundance model according to Schmidt et al. (2015). The best fitting models were chosen according to ΔAIC < 2 (Burnham & Anderson 2002) using the R package 'AICcmodavg' (Mazerolle 2015). In a second analysis, we only used creeks in which presence/absence monitoring (via quantitative PCR detection from skin swabs taken from metamorphosed salamanders and newts) of *Bsal* was conducted at least once (Supplementary document 1). We included *Bsal* presence/absence as fourth potential predictor variable and compared 50 candidate models for each year.

Results

In our five-year-study, we captured a total of 10,044 European fire salamander larvae (5,497 in the Northern Eifel Mountains, 4,547 in the Southern Eifel Mountains; Supplementary document 1).

The removal sampling was conducted in a total of 40 creek sections in the Northern Eifel Mountains, 21 with consistent data over five years (Supplementary document 1). For 20 of them information on *Bsal* presence/absence was available, with eight being *Bsal*-positive (detection in 2004: Vichtbach; 2015: Belgenbach, Fischbach, Solchbach, Weiße Wehe; 2017: Haftenbach; Sauerbach; 2019: Thönbach). In a few cases, single creeks had to be excluded from our analyses due to extremely high capture rates of larvae that could not be fixed by transformation of data (see Supplementary document 11).

Regarding the plausible (i.e.: ΔAIC < 2) generalized multinomial mixture models using data from all creek sections within the Northern Eifel Mountains, larval abundance was negatively affected by capture occasion (date) in three models, and in another two was affected positively by the number of pools and in one by creek width. Detection probability of larvae was negatively affected by capture occasion in eight models and positively by creek width in five, and positively by number of pools in three cases. In all other models, either a 'constant'-intercept-model was better fitting or no clear effect direction for the variables could be observed. When *Bsal* presence/absence was included as an additional potential predictor, the number of pools positively affected larval abundance in three models. Creek width affected larval abundance three times negatively and one time positively, and capture occasion negatively in another three models. *Bsal* presence had a negative effect on larval abundance in two of the plausible models (in 2017 and 2018). Detection probability of larvae was positively affected by number of pools in nine and negatively by capture occasion in five of the plausible models. Again, in all other models, either a 'constant'-intercept-model was better fitting or no clear effect direction for the variables could be observed. Overall, *Bsal* was the predictor variable in eight out of 21 plausible models (38%); however, mainly with no clear effect direction (Table 1, Supplementary documents 2–13).

Regarding the simple count data, in the eight *Bsal*-positive sites from the Northern Eifel Mountains, the number of captured larvae decreased over the study period (with some peaks in single years) in five of them (Thönbach, Weiße Wehe, Haftenbach, Sauerbach), while it was more or less constant in the Fischbach and the Solchbach. The Vichtbach and the Belgenbach were monitored for a too short period to identify a trend (Fig. 1). The linear trend of the mean captures from *Bsal*-infected sites was negative over the five years ($P < 0.05$, $R^2 = 0.8$). However, this does not account for the mean estimated abundance from both analyses ($P > 0.05$, $R^2 = 0.7$; Supplementary documents 14, 15). Regarding the twelve *Bsal*-negative sites from the Northern Eifel Mountains, no negative trend could be observed for mean captured larvae ($P > 0.05$, $R^2 = 0.5$; Fig. 2) nor for mean estimated abundance ($P > 0.05$, $R^2 = 0.1$; Supplementary documents 16, 17).

In the Southern Eifel Mountains, 21 creek sections were included in our study, 17 with consistent data over five years (Supplementary document 1). For 19 of them information on *Bsal* presence/absence was available, with only four of them *Bsal*-positive (detection in 2017: Fuhrbach, Läuskopfbach; 2019: Watzbach, Watzbachzufluss). In few cases, single creeks had to be excluded from single analyses due to extremely high capture rates of larvae, which again could not be fixed by transformation of data (Supplementary document 1).

Regarding the plausible (i.e.: ΔAIC < 2) generalized multinomial mixture models using data from all creeks within the Southern Eifel Mountains, larval abundance was positively affected by number of pools in seven plausible models and by capture occasion in two models, and negatively by creek width in another two models. Detec-

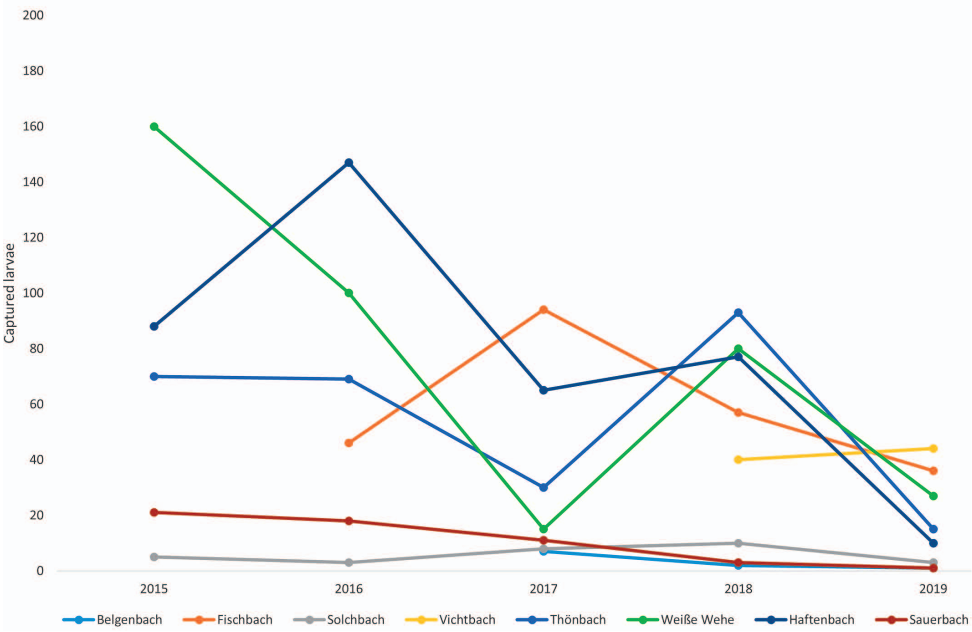

Figure 1. Captured salamander larvae in the *Bsal*-infected sites from the Northern Eifel Mountains. In most creeks, *Bsal* was present from the beginning of the monitoring in 2015, but it was first detected in the Haftenbach and Sauerbach in 2017, and in the Thönbach in 2019.

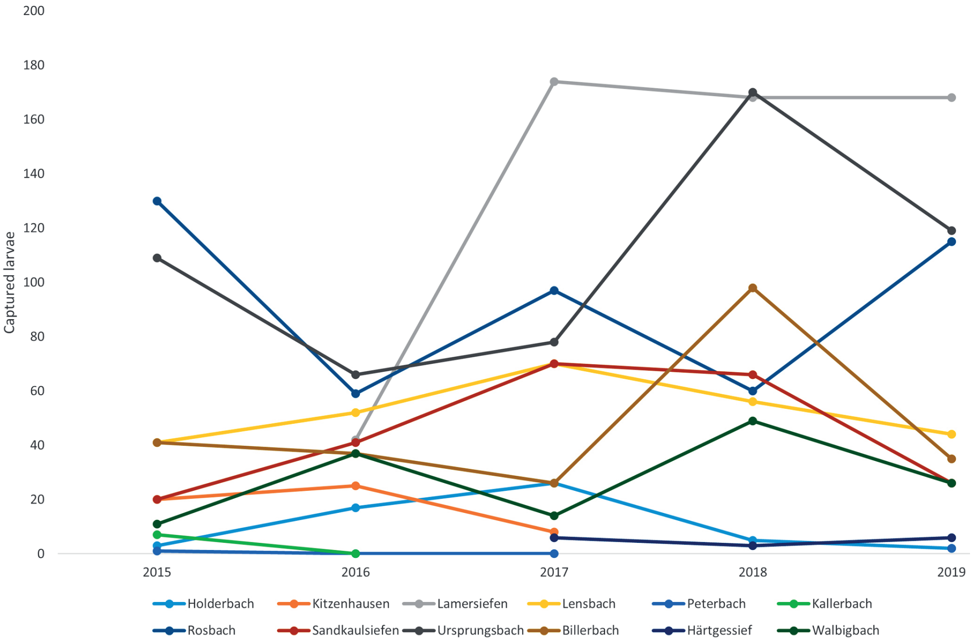

Figure 2. Captured salamander larvae in the 12 sites from the Northern Eifel Mountains, where *Bsal* was not detected.

tion probability was positively affected by creek width in six plausible models, by capture occasion in three models and by number of pools in two models. In all other models, either a 'constant'-intercept-model was better fitting or no clear effect direction for the variables could be observed. When *Bsal* presence/absence was included as potential predictor, the number of pools positively affected larval abundance in four models and capture occasion in one model. *Bsal* presence had a negative effect on larval abundance in two of the plausible models (in 2017 and 2018). Detection probability was positively affected by number of pools in seven models, by capture occasion in two models, by creek width in one model, and one time negatively by capture occasion. *Bsal* presence had a negative effect on detection probability in two plausible models (in 2017). Again, in all other models either a 'constant'-in-

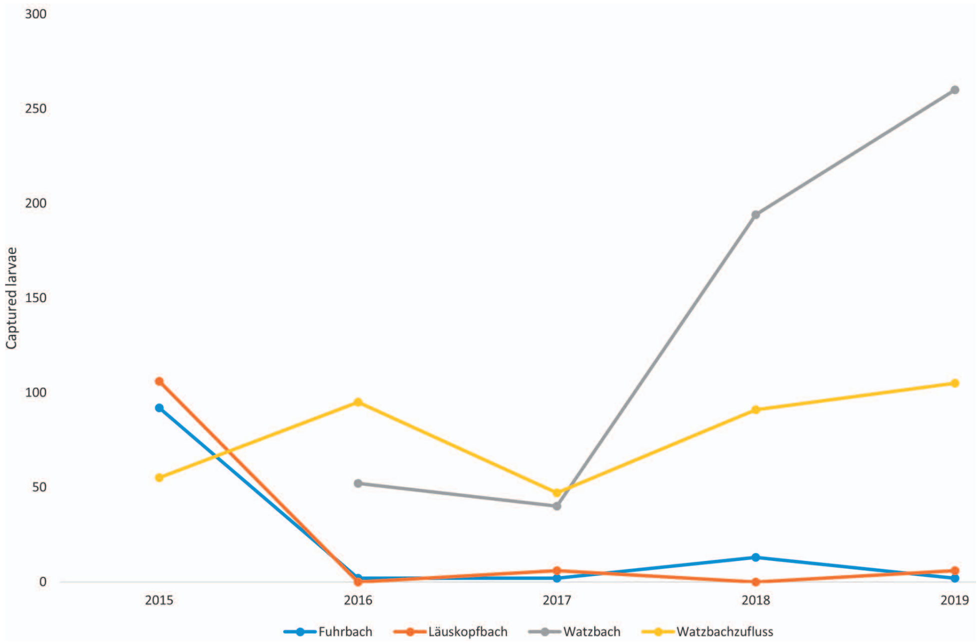

Figure 3. Captured salamander larvae in the *Bsal*-infected sites from the Southern Eifel Mountains. In the Fuhrbach and Läuskopfbach, *Bsal* was first detected in 2017, and in the Watzbach and Watzbachzufluss in 2019.

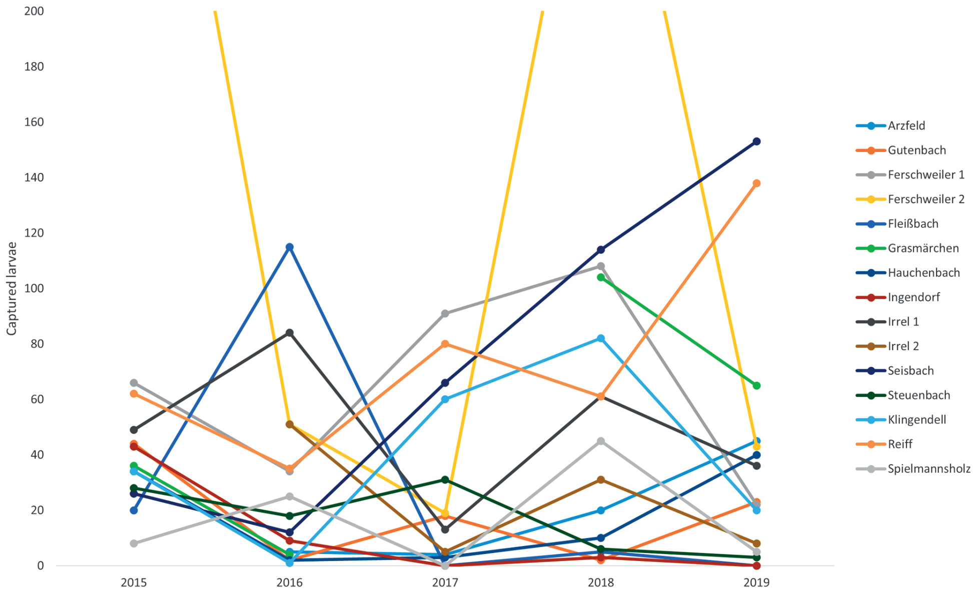

Figure 4. Captured salamander larvae in the 15 sites from the Southern Eifel Mountains, where *Bsal* was not detected.

Table 1. Effects of potential predictors on abundance (Λ) and detection probability (p) of European fire salamander (*S. salamandra*) larvae in the plausible modes of both analyses of removal data from the Northern and Southern Eifel Mountains. In some cases, a 'constant'-intercept-model was best fitting (not shown). Symbols: (Λ) means abundance; (p) means detection probability; arrows indicate positive (↑) or negative (↓) effects; (.) means no observed direction.

	all sites		sites tested for *Bsal*	
	Λ	p	Λ	p
Bsal presence	not included	not included	4 × ↓ 3 × (.)	2 × ↓ 6 × (.)
Number of pools	8 × ↑ 4 × (.)	6 × ↑ 2 × (.)	7 × ↑ 6 × (.)	16 × ↑
Creek width (m)	3 × ↓ 3 × (.)	15 × ↑	1 × ↑ 3 × ↓	1 × ↑
Capture occasion (date)	2 × ↑ 3 × ↓ 2 × (.)	3 × ↑ 4 × ↓ 1 × (.)	1 × ↑ 4 × ↓	2 × ↑ 7 × ↓
N plausible models	33		38	

tercept-model was better fitting or no clear effect direction for the variables could be observed. Overall, *Bsal* was the predictor variable in five out of 17 plausible models (29%), one time with no clear effect direction (Table 1, Supplementary documents 18–30).

Regarding the simple count data from the Southern Eifel Mountains, captured larvae in the two creeks where *Bsal* was detected in the adjacent terrestrial habitat in 2017 already decreased in 2016 and did not recover. Conversely, the capture rates in the two sites where *Bsal* was detected more recently, were stable on a high level or even increased (Fig. 3). Consequently, no linear trend could be observed ($P > 0.05$, $R^2 = 0.1$). The same accounts for the mean estimated larvae from 2015–2019 ($P > 0.05$, $R^2 = 0.2–0.3$; Supplementary documents 31, 32) as well as capture rates and estimates from the 15 sites from the Southern Eifel Mountains, where *Bsal* could not be detected ($P > 0.05$, $R^2 = 0.01–0.02$; Fig. 4, Supplementary documents 33, 34). Strong natural fluctuations in captured and estimated larvae in most likely uninfected populations could be observed (Fig. 4, Supplementary documents 33, 34).

Discussion

Our European fire salamander larvae monitoring in the Northern and Southern Eifel Mountains over so far five years, revealed that larval abundance underlies a remarkable variation. Taking into account the small sample sizes in most sites and difficulties in detecting *Bsal* (Spitzen-van der Sluijs et al. 2016, Lötters et al. 2020a in this issue), final conclusions on the effect of *Bsal* on wild European fire salamander populations cannot be drawn yet. However, despite this lack of a clear pattern overall, a larval decline in the captures and estimates from some creeks with *Bsal* presence is apparent (Figs 1, 3, Supplementary document 1), while in others capture rates and larval abundance did not decrease and sometimes even increased (Figs 1, 3, Supplementary document 1). Hence, the salamander plague apparently has not led to larval abundance declines in all *Bsal*-positive populations per se (at least over the five years studied). Population extinction could not be observed at all. However, this should not give an 'all-clear' sign because of several reasons:

(1) A marked delay of effects in a population after a *Bsal* infection might be expectable, because European fire salamanders only become sexually mature five to six years after metamorphosis (Thiesmeier 2004). Regarding the assumption that 'animal-to-animal' contact is the most important way of *Bsal* transmission (Stegen et al. 2017, Schulz et al. 2018), mainly adults should get infected during their reproduction phase (mating), while juveniles and subadults, with less contact, should have lower infection probabilities.

(2) Immigration of non-infected salamanders into *Bsal* sites from non-affected areas nearby is possible (cf. Schulte et al. 2007), due to the mainly continuous forests with relatively few barriers in the Eifel Mountains (cf. Dalbeck et al. 2018, Wagner et al. 2019a, b). This could lead to misinterpretation of the trends of locally infected populations.

(3) Although *Bsal* had only a negative effect on larval abundance in four of all plausible models and a negative effect on detection probabilities only in two models, *Bsal* was included as explanatory variable in over one third of all plausible models (Table 1). Hence, an overall-effect of *Bsal* infection on larval abundance and detection probability (and thereby indirectly on abundance) could be observed in over one third of all plausible models.

In most cases of our study, the number of pools within a creek positively affected larval abundance and detection probability (Table 1). An increasing number of pools, which can serve as a proxy for the heterogeneity of a studied creek, is known to positively affect the aquatic habitat of European fire salamander larvae (Baumgartner et al. 1999, Werner et al. 2014, Schmidt et al. 2015, Wagner et al. 2020b in this issue). Detection probability furthermore could be positively affected by the increasing number of pools due to easier capturing of larvae (larvae often passively and actively accumulate in these microhabitats such as pools; see Reinhardt et al. 2018). Creek width had no general effect direction on larval abundance when regarding all plausible models, but in most cases a positive effect on detection probability (Table 1). This could be related to larger creek sections, which could be easier monitored or that in wider creek sections – as proxy for water flow – again more larvae are accumulating in pools. Also capture occasion had no general effect direction on larval abundance when looking at all plausible models, but mainly a negative effect on detection probability (Table 1). This should be related to the catastrophic drift events due to heavy rainfalls, which normally increase with later dates of the capture occasions (Thiesmeier & Schuhmacher

1990, Reinhardt et al. 2018, Veith et al. 2019) or simply on-going metamorphosis.

In some, most likely uninfected populations, strong natural fluctuations of the larval abundance could be observed (Figs 2, 4, Supplementary documents 16, 17, 33, 34), and in some cases larval numbers strongly decreased over years (e.g. Fleißbach, Ingendorf; Supplementary document 1) for no obvious reason. This could be related to several factors (e.g. predation pressure due to fish or introduced crayfish), in several years most likely to larval drift just before the capture occasions (Thiesmeier & Schuhmacher 1990, Reinhardt et al. 2018, Veith et al. 2019). Short-term fluctuations in amphibian populations are well-known including what is considered 'natural' fluctuations (e.g. Meyer et al. 1998); however, such information is largely lacking for *S. salamandra* (e.g. Thiesmeier 2004). In some years (e.g. 2016), overall negative effects of – most likely – heavy rainfalls and high rates of larval drift during the capture period could be observed in the entire Eifel region (see also Reinhardt et al. 2018) and, conversely, also general 'good salamander years' (e.g. 2018) that should also be related to favourable local weather conditions (cf. Figs 1–4, Supplementary documents 14–17, 31–34).

One has to take into account that larval abundance is a proxy for adult population size. This especially accounts for species with a life history strategy characterized by low adult mortality, late sexually maturity and relatively low potential reproduction rates, such as the European fire salamander (Schmidt et al. 2005). This strategy is typical for species with an unreliable recruitment rate; in case of salamander larvae, especially catastrophic drift events are important, which can eradicate the larval cohort of a complete reproduction phase (e.g. Thiesmeier & Schuhmacher 1990, Reinhardt et al. 2018, Veith et al. 2019).

Hence, strong fluctuations in larval abundance between single years should be natural, especially when heavy rainfalls cause larval drift. Conversely, steady decreases of larval numbers over years can show decreases in adult population size (Schmidt et al. 2005). This is another example showing that distinguishing between well-known natural (Meyer et al. 1998) and non-natural (disease-driven) population fluctuations is only possible on the basis of long-term observation data. Even after five years of larvae monitoring in the *Bsal*-affected Eifel region, we still can only speculate on general effects of the salamander plague on salamander populations, and various relevant factors are still unknown. Hence, monitoring has to be conducted over longer time periods. Although capture-mark-recapture (CMR) studies of terrestrial life stages have been conducted (Feldmann 1971, Kopp-Hamberger 1998, Stegen et al. 2017, Spitzen-van der Sluijs et al. 2018), this is unrealistic at a larger scale and for large populations so that larval abundance has to be taken as raw proxy for the adult population size (see above). Even for Habitats Directive species of European interest, only in a few selected Special Areas of Conservation monitoring is conducted only every 4–6 years in the member states by applying simple count methods (https://ec.europa.eu/environment/nature/knowledge/rep_habitats/index_en.htm). Thus, a yearly removal sampling of larvae is highly ambitious. There are other methods to monitor larval populations of salamander species in creeks (summarized and discussed in Skelly & Richardson 2009), for instance, by capturing larvae using traps (leaf litter bags; cf. Pauley & Little 1998). However, results from such traps cannot easily relate to overall abundance (Waldron et al. 2003). Furthermore, Wilson & Dorcas (2003) compared dip-netting (i.e. capture per unit effort) to funnel trapping in small streams in larval salamanders in North America and found that funnel traps captured more aquatic adults and thereby species (which is irrelevant regarding our salamander monitoring as *S. salamandra* is the only stream breeding salamander species in Central/Western Europe), while dip-netting resulted in higher amounts of salamander larvae compared to funnel trapping. However, Nowakoswki & Maerz (2009) described a size-bias; that is, leaf-litter traps captured higher proportion of larger salamander larvae from streams, whereas dip-netting yielded a higher proportion of smaller larvae. In the European fire salamander (cf. Thiesmeier & Schuhmacher 1990, Reinhardt et al. 2018, Veith et al. 2019), catastrophic drift after heavy rainfalls severely effects larval abundance in the studied creek sections over time. Thus, installing drift and upstream migration traps on a studied transect over longer time (analogous to macrozoobenthos sampling; cf. Goedmakers 1980) might lead to higher larval capture rates, at least in the European fire salamander (Veith et al. 2019, Wagner et al. 2020a in this issue).. Abundance is highly underestimated using the here applied removal sampling method compared to CMR studies (see Wagner et al. 2020a). However, CMR methods and longer study times are highly time and cost intensive and unrealistic to apply in real-world nature conservation just like CMR studies using terrestrial life stages. The same accounts for removal sampling at night. Our experiences from the first five years show that it is already hard to coordinate (and pay) field workers to conduct removal sampling by daytime in a larger study area in the defined time period.

To sum up, standardized monitoring by capturing larvae in defined creek sections and time intervals seems to be the only feasible way to get information on European fire salamander populations over many consecutive years. Its potential for detecting population break-downs, and thus its potential as EWS, is obvious, since population size monitoring does not primarily depend on absolute estimates and their reliability; rather reliably population trends need to be identified (however, note limitations of the use of larval numbers as proxy for the adult population size discussed above). Using the removal method proposed by Schmidt et al. (2015) requires three fieldworkers. In a 'minimalistic' scenario, it should also be feasible for one (or two) person(s) to conduct the removal sampling. However, simple count methods may also suffice (see Wagner et al. 2020a), especially when it comes to spatially large-scale monitoring at the *Bsal* expansion front.

Acknowledgements

We are grateful to all colleagues and collaborators who conducted larvae monitoring from Biologische Station StädteRegion Aachen, Biologische Station im Kreis Düren and Trier University. Special thanks to Benedikt R. Schmidt for his valuable help with the data analyses. Funding was made available via the Regierungsbezirk Köln, Nikolaus-Koch-Stiftung, Zoo Landau, Deutsche Gesellschaft für Herpetologie und Terrarienkunde (DGHT) and Bundesamt für Naturschutz (BfN).

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Supplementary data

The following data are available online:

Supplementary document 1. Captures and estimated abundance data for creeks in the Northern and Southern Eifel Mountains 2015–2019, ordered after districts, then alphabetically after site names.

Supplementary document 2. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 35 creeks (Northern Eifel Mountains, 2015).

Supplementary document 3. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 15 creeks with *Bsal* screening (Northern Eifel Mountains, 2015).

Supplementary document 4. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 29 creeks (Northern Eifel Mountains, 2016).

Supplementary document 5. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 17 creeks with *Bsal* screening (Northern Eifel Mountains, 2016).

Supplementary document 6. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 30 creeks (Northern Eifel Mountains, 2017).

Supplementary document 7. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 18 creeks with *Bsal* screening (Northern Eifel Mountains, 2017).

Supplementary document 8. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 26 creeks (Northern Eifel Mountains, 2018).

Supplementary document 9. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 17 creeks with *Bsal* screening (Northern Eifel Mountains, 2018).

Supplementary document 10. Model selection overview of the only plausible hierarchical model with ΔAIC < 2 considering removal data from 28 creeks (Northern Eifel Mountains, 2019).

Supplementary document 11. Effect of date on detection probability and number of pools on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 28 creeks (Northern Eifel Mountains, 2019).

Supplementary document 12. Model selection overview of the only plausible hierarchical model with ΔAIC < 2 considering removal data from 21 creeks with *Bsal* screening (Northern Eifel Mountains, 2019).

Supplementary document 13. Effect of date on detection probability and number of pools on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 21 creeks with *Bsal* screening (Northern Eifel Mountains, 2019).

Supplementary document 14. Estimated larvae according to the first analysis of removal data from *Bsal*-infected sites from the Northern Eifel Mountains.

Supplementary document 15. Estimated larvae according to the second analysis of removal data from *Bsal*-infected sites from the Northern Eifel Mountains.

Supplementary document 16. Estimated larvae according to the first analysis of removal data from sites from the Northern Eifel Mountains where *Bsal* was not detected.

Supplementary document 17. Estimated larvae according to the second analysis of removal data from sites from the Northern Eifel Mountains where *Bsal* was not detected.

Supplementary document 18. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 17 creeks (Southern Eifel Mountains, 2015) (first *Bsal* detection in 2017).

Supplementary document 19. Effect of creek width on detection probability and number of pools on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 17 creeks (Southern Eifel Mountains, 2016) (first *Bsal* detection in 2017).

Supplementary document 20. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 21 creeks (Southern Eifel Mountains, 2016) (first *Bsal* detection in 2017).

Supplementary document 21. Effect of number of pools on detection probability and creek width on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 21 creeks (Southern Eifel Mountains, 2016) (first *Bsal* detection in 2017).

Supplementary document 22. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 20 creeks (Southern Eifel Mountains, 2017).

Supplementary document 23. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 18 creeks with *Bsal* screening (Southern Eifel Mountains, 2017).

Supplementary document 24. Effect of number of pools on detection probability and on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 20 creeks (Southern Eifel Mountains, 2018).

Supplementary document 25. Effect of number of pools on detection probability and on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 20 creeks (Southern Eifel Mountains, 2018).

Supplementary document 26. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 17 creeks with *Bsal screening* (Southern Eifel Mountains, 2018).

Supplementary document 27. Effect of *Bsal* on detection probability and number of pools on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 17 creeks with *Bsal* screening (Southern Eifel Mountains, 2018).

Supplementary document 28. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 20 creeks (Southern Eifel Mountains, 2019).

Supplementary document 29. Effect of date on detection probability and number of pools on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 20 creeks (Southern Eifel Mountains, 2019).

Supplementary document 30. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 18 creeks with *Bsal* screening (Southern Eifel Mountains, 2019).

Supplementary document 31. Estimated larvae according to the first analysis of removal data from *Bsal*-infected sites from the Southern Eifel Mountains.

Supplementary document 32. Estimated larvae according to the second analysis of removal data from *Bsal*-infected sites from the Southern Eifel Mountains.

Supplementary document 33. Estimated larvae according to the first analysis of removal data from sites from the Southern Eifel Mountains where *Bsal* was not detected.

Supplementary document 34. Estimated larvae according to the second analysis of removal data from sites from the Southern Eifel Mountains where *Bsal* was not detected.

Supplementary document 1. Captures and estimated abundance data for creeks in the Northern and Southern Eifel Mountains 2015–2019, ordered after districts, then alphabetically after site names. Legend: * results of the *Bsal*-screening according to Lötters et al. (2020a); ** estimated abundance according to the best fitting model with ΔAIC = 0; red indicates finding of *Bsal*-positive salamanders or newts in sample sites in the surrounding of the creeks; capture occasion dates are given as 'day.month.year' or 'year' only.

Site	County	Capture occasion	*Bsal*-record* (prevalence with Bayesian 95%-credible intervals)	Captured larvae	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width)	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width, *Bsal*-presence)	N of pools	Creek width (cm)	Remarks
Northern Eifel Mts.									
North Rhine-Westphalia, Beverbach 1	StädteRegion Aachen	19.06.2015	no *Bsal*-screening	0	3.9 (0; 19)	no *Bsal*-screening	54	80	site was exluded after 2015
North Rhine-Westphalia, Beverbach 2	StädteRegion Aachen	22.06.2015	no *Bsal*-screening	0	4.6 (0; 22)	no *Bsal*-screening	33	90	site was exluded after 2015
North Rhine-Westphalia, Belgenbach	StädteRegion Aachen	2015	96% (79–99%)	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	
		25.05.2017	0% (0–26%)	7	16.8 (9; 28)	17.8 (10; 29)	12	146	only larvae could be swabbed
		10.05.2018	no *Bsal*-screening	2	3.2 (2; 6)	5.3 (2; 12)	16	100	
		22.05.2019	0% (0–70%)	1	2.6 (1; 7)	2.0 (1; 5)	14	180	only anurans could be swabbed
North Rhine-Westphalia, Dreilägerbach	StädteRegion Aachen	11.06.2015	no *Bsal*-screening	1	6.1 (1; 18)	no *Bsal*-screening	54	90	
		25.05.2016	no *Bsal*-screening	0	0.4 (0; 2)	no *Bsal*-screening	23	177	
		14.05.2017	no *Bsal*-screening	0	0.6 (0; 3)	no *Bsal*-screening	23	150	
North Rhine-Westphalia, Entenpfuhl	StädteRegion Aachen	22.06.2015	no *Bsal*-screening	0	4.6 (0; 22)	no *Bsal*-screening	53	70	site was exluded after 2015
North Rhine-Westphalia, Ermesbach	StädteRegion Aachen	24.06.2015	no *Bsal*-screening	0	5.4 (0; 26)	no *Bsal*-screening	18	70	site was exluded after 2015
North Rhine-Westphalia, Eselsbach	StädteRegion Aachen	17.06.2015	no *Bsal*-screening	10	65.8 (35; 107)	no *Bsal*-screening	15	60	
		02.06.2016	no *Bsal*-screening	11	18.1 (13; 26)	no *Bsal*-screening	17	120	
		12.05.2017	no *Bsal*-screening	9	16.1 (10; 24)	no *Bsal*-screening	24	40	
		24.05.2018	no *Bsal*-screening	0	0.8 (0; 5)	no *Bsal*-screening	40	50	
		22.05.2019	no *Bsal*-screening	1	2.7 (1; 7)	no *Bsal*-screening	32	50	
North Rhine-Westphalia, Fischbach	StädteRegion Aachen	2014	0% (0–13%)	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	
		2015	2% (1–9%)	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	
		09.06.2016	13% (1–30%)	46	70.6 (60; 84)	70.5 (60; 83)	20	70	
		11.05.2017	0%(0–14%)	94	110.3 (102; 119)	109.2 (102; 118)	50	90	
		19.05.2018	0% (0–8%)	57	110.1 (92; 131)	119.2 (99; 143)	27	45	
		14.05.2019	0% (0–9%)	36	167.8 (167; 168)	167.8 (166; 168)	41	130	

Site	County	Capture occasion	*Bsal*-record* (prevalence with Bayesian 95%-credible intervals)	Captured larvae	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width)	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width, *Bsal*-presence)	N of pools	Creek width (cm)	Remarks
North Rhine-Westphalia, Holderbach	StädteRegion Aachen	24.06.2015	no *Bsal*-screening	3	34.2 (9; 76)	31.8 (10; 68)	56	120	
		27.05.2016	no *Bsal*-screening	17	18.3 (17; 21)	17.6 (17; 20)	56	113	
		25.05.2017	0% (0–61%)	26	70.1 (51; 93)	74.0 (54; 99)	6	200	
		09.05.2018	0% (0–70%)	5	7.3 (5; 12)	6.9 (5; 10)	9	110	
		24.05.2019	0% (0–61%)	2	4.8 (2; 10)	3.7 (2; 8)	18	120	
North Rhine-Westphalia, Inde, Zufluss	StädteRegion Aachen	19.06.2015	no *Bsal*-screening	3	25.5 (7; 56)	no *Bsal*-screening	4	50	
		26.05.2016	no *Bsal*-screening	16	33.7 (23; 47)	no *Bsal*-screening	4	70	
		10.05.2017	no *Bsal*-screening	18	49.0 (33; 69)	no *Bsal*-screening	6	53	
North Rhine-Westphalia, Kitzenhausen	StädteRegion Aachen	05.06.2015	no *Bsal*-screening	20	56.6 (39; 78)	53.3 (37; 73)	69	110	
		22.05.2016	no *Bsal*-screening	25	42.8 (33; 55)	44.2 (34; 57)	14	130	
		18.05.2017	0% (0–31%)	8	12.9 (9; 19)	13.2 (9; 20)	29	240	
North Rhine-Westphalia, Lamersiefen	StädteRegion Aachen	2014	0% (0–19%)	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	
		2015	0% (0–11%)	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	
		08.06.2016	0% (0–31%)	42	62.5 (53; 74)	61.9 (52; 73)	22	90	
		24.05.2017	0% (0–13%)	174	179.8 (178; 180)	179.7 (178; 180)	52	70	
		05.05.2018	0% (0–36%)	168	191.0 (187; 192)	191.0 (187; 192)	15	95	
		10.05.2019	0% (0–20%)	168	168 (168; 168)	168.0 (168; 168)	27	98	
North Rhine-Westphalia, Lensbach	StädteRegion Aachen	05.06.2015	no *Bsal*-screening	41	114.9 (89; 145)	107.3 (84; 135)	60	100	
		25.05.2016	no *Bsal*-screening	52	83.9 (71; 99)	85.0 (72; 100)	17	180	
		28.05.2017	0% (0–61%)	70	90.9 (81; 102)	90.3 (81; 101)	41	225	
		28.05.2018	0%	56	153.0 (124; 184)	72.8 (64; 83)	16	53	
		13.06.2019	0% (0–13%)	44	56.4 (49; 65)	55.0 (49; 63)	35	70	
North Rhine-Westphalia, Lejbeschsloch	StädteRegion Aachen	17.06.2015	no *Bsal*-screening	4	28.3 (10; 57)	no *Bsal*-screening	80	53	
		27.05.2016	no *Bsal*-screening	5	5.1 (5; 6)	no *Bsal*-screening	80	60	
		25.05.2017	no *Bsal*-screening	3	4.1 (3; 7)	no *Bsal*-screening	42	235	
		09.05.2018	no *Bsal*-screening	0	0.2 (0; 2)	no *Bsal*-screening	9	70	
		24.05.2019	no *Bsal*-screening	3	6.6 (3; 13)	no *Bsal*-screening	16	120	

Site	County	Capture occasion	*Bsal*-record* (prevalence with Bayesian 95%-credible intervals)	Captured larvae	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width)	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width, *Bsal*-presence)	N of pools	Creek width (cm)	Remarks
North Rhine-Westphalia, Peterbach	StädteRegion Aachen	2014	0% (0–20%)	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	
		20.05.2015	no *Bsal*-screening	1	1.5 (1; 4)	1.6 (1; 4)	16	70	
		02.06.2016	no *Bsal*-screening	0	0.6 (0; 3)	0.8 (0; 4)	11	60	
		11.05.2017	no *Bsal*-screening	0	0.7 (0; 4)	0.9 (0; 5)	20	60	
		2018	0% (0–51%)	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	
		23.05.2019	0% (0–6%)	2	4.7 (2; 10)	3.8 (2; 8)	40	70	
North Rhine-Westphalia, Saarscher Bach	StädteRegion Aachen	17.06.2015	no *Bsal*-screening	1	9.6 (1; 28)	no *Bsal*-screening	24	110	
North Rhine-Westphalia, Schleebach	StädteRegion Aachen	15.04.2015	no *Bsal*-screening	0	0 (0; 0)	no *Bsal*-screening	41	70	
North Rhine-Westphalia, Solchbachquelle	StädteRegion Aachen	2014	0% (0–13%)	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	
		24.05.2015	5% (1–25%)	5	7.8 (5; 13)	8.0 (5; 13)	93	270	
		31.05.2016	0% (0–31%)	3	5.8 (3; 11)	6.2 (3; 12)	12	240	
		21.05.2017	4% (2–15%)	8	11.8 (8; 17)	11.9 (8; 17)	34	125	
		18.05.2018	2% (1–11%)	10	19.1 (12; 28)	21.9 (14; 33)	17	167	
		23.05.2019	0% (0–9%)	3	6.2 (3; 12)	5.1 (3; 9)	15	160	
North Rhine-Westphalia, Tiefenbach	StädteRegion Aachen	17.06.2015	no *Bsal*-screening	0	3.3 (0; 16)	no *Bsal*-screening	9	105	
North Rhine-Westphalia, Vichtbach	StädteRegion Aachen	2004	100% (26–99%)	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	
		2017	0% (0–11%)	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	
		21.05.2018	0% (0–11%)	40	83.2 (66; 103)	84.0 (67; 104)	62	83	
		01.05.2019	0% (0–10%)	44	46.2 (44; 50)	47.5 (44; 52)	40	150	
North Rhine-Westphalia, Weihrauchbend	StädteRegion Aachen	24.05.2015	no *Bsal*-screening	7	10.8 (7; 16)	no *Bsal*-screening	42	118	
		31.05.2016	no *Bsal*-screening	31	64.5 (50; 82)	no *Bsal*-screening	4	145	
		21.05.2017	no *Bsal*-screening	26	59.9 (44; 79)	no *Bsal*-screening	12	110	
		18.05.2018	no *Bsal*-screening	57	106.3 (89; 126)	no *Bsal*-screening	17	167	
		18.05.2019	no *Bsal*-screening	35	51.8 (43; 62)	no *Bsal*-screening	16	127	
North Rhine-Westphalia, Giesenbach	Düren	20.05.2015	no *Bsal*-screening	18	23.7 (19; 30)	no *Bsal*-screening	19	90	
		25.05.2016	no *Bsal*-screening	28	43.1 (35; 53)	no *Bsal*-screening	20	50	
		10.05.2017	no *Bsal*-screening	26	49.5 (38; 64)	no *Bsal*-screening	20	50	
		08.05.2018	no *Bsal*-screening	30	41.8 (35; 51)	no *Bsal*-screening	27	30	
		22.05.2019	no *Bsal*-screening	9	16.8 (11; 25)	no *Bsal*-screening	30	50	

Site	County	Capture occasion	*Bsal*-record* (prevalence with Bayesian 95%-credible intervals)	Captured larvae	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width)	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width, *Bsal*-presence)	N of pools	Creek width (cm)	Remarks
North Rhine-Westphalia, Kallerbach	Düren	22.05.2015	0% (0–15%)	7	9.9 (7; 15)	10.1 (7; 15)	10	45	
		10.05.2016	0% (0–30%)	0	0.6 (0; 4)	0.8 (0; 4)	10	45	
		2017	0% (0–51%)	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	
North Rhine-Westphalia, Morlesief	Düren	20.05.2015	no *Bsal*-screening	30	39.3 (33; 47)	no *Bsal*-screening	17	50	
		02.06.2016	no *Bsal*-screening	15	24.1 (18; 32)	no *Bsal*-screening	18	120	
		22.05.2017	no *Bsal*-screening	29	55.1 (43; 70)	no *Bsal*-screening	20	40	
		24.05.2018	no *Bsal*-screening	0	0.8 (0; 5)	no *Bsal*-screening	27	50	
		23.05.2019	no *Bsal*-screening	31	58.7 (46; 74)	no *Bsal*-screening	38	70	
North Rhine-Westphalia, Mückensiefen	Düren	20.05.2015	no *Bsal*-screening	11	14.5 (11; 19)	no *Bsal*-screening	61	70	
		21.05.2016	no *Bsal*-screening	2	2.3 (2; 4)	no *Bsal*-screening	51	90	
		14.05.2017	no *Bsal*-screening	7	10.6 (7; 16)	no *Bsal*-screening	33	120	
		11.05.2018	no *Bsal*-screening	21	31.7 (25; 40)	no *Bsal*-screening	36	100	
		18.05.2019	no *Bsal*-screening	11	16.8 (12; 23)	no *Bsal*-screening	21	76	
North Rhine-Westphalia, Rinnebach	Düren	17.06.2015	no *Bsal*-screening	9	59.6 (30; 99)	no *Bsal*-screening	3	120	
		25.05.2016	no *Bsal*-screening	2	4.7 (2; 10)	no *Bsal*-screening	6	130	
		11.05.2017	no *Bsal*-screening	0	0.7 (0; 4)	no *Bsal*-screening	20	60	
		24.05.2018	no *Bsal*-screening	0	0.8 (0; 5)	no *Bsal*-screening	27	80	
		16.05.2019	no *Bsal*-screening	11	15.9 (12; 22)	no *Bsal*-screening	39	95	
North Rhine-Westphalia, Rosbach	Düren	19.05.2015	0% (0–7%)	130	161.7 (152; 168)	161.6 (152; 168)	24	75	
		09.05.2016	0%	59	78.4 (69; 89)	75.7 (67; 85)	30	40	
		04.05.2017	0% (0–15%)	97	167.0 (149; 180)	168.3 (151; 180)	23	50	
		08.05.2018	0% (0–31%)	60	83.4 (73; 95)	78.0 (69; 88)	41	45	
		16.05.2019	0% (0–11%)	115	158.9 (146; 168)	151.9 (139; 165)	53	70	
North Rhine-Westphalia, Sandkaulsiefen	Düren	26.05.2015	no *Bsal*-screening	20	32.9 (25; 43)	32.4 (25; 42)	24	97	
		29.05.2016	no *Bsal*-screening	41	65.1 (54; 78)	65.7 (54; 79)	18	120	
		13.05.2017	no *Bsal*-screening	70	138.1 (117; 162)	141.4 (119; 166)	18	90	
		01.05.2018	0% (0–51%)	66	79.6 (72; 88)	85.8 (77; 96)	11	77	
		07.05.2019	0% (0–43%)	26	29.3 (26; 33)	29.7 (26; 34)	23	167	

Site	County	Capture occasion	*Bsal*-record* (prevalence with Bayesian 95%-credible intervals)	Captured larvae	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width)	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width, *Bsal*-presence)	N of pools	Creek width (cm)	Remarks
North Rhine-Westphalia, Schiefersiefen	Düren	26.05.2015	no *Bsal*-screening	54	88.8 (75; 104)	no *Bsal*-screening	32	108	
		20.05.2016	no *Bsal*-screening	101	108.5 (104; 114)	no *Bsal*-screening	56	2	
		12.05.2017	no *Bsal*-screening	66	169.6 (149; 180)	no *Bsal*-screening	3	110	
		19.05.2018	no *Bsal*-screening	225	excluded from analysis	no *Bsal*-screening	22	229	
		17.05.2019	no *Bsal*-screening	56	81.2 (70; 94)	no *Bsal*-screening	30	127	
North Rhine-Westphalia, Schüllbach	Düren	20.05.2015	no *Bsal*-screening	30	39.3 (33; 47)	no *Bsal*-screening	30	30	
		25.05.2016	no *Bsal*-screening	62	128.2 (107; 152)	no *Bsal*-screening	4	60	
		11.05.2017	no *Bsal*-screening	75	138.3 (118; 161)	no *Bsal*-screening	21	50	
		08.05.2018	no *Bsal*-screening	122	169.2 (154; 185)	no *Bsal*-screening	27	50	
		22.05.2019	no *Bsal*-screening	64	113.8 (97; 133)	no *Bsal*-screening	32	60	
North Rhine-Westphalia, Thönbach	Düren	22.05.2015	no *Bsal*-screening	70	97.7 (86; 111)	96.9 (86; 110)	75	165	
		28.05.2016	no *Bsal*-screening	69	136.4 (116; 158)	145.5 (124; 162)	6	220	
		13.05.2017	no *Bsal*-screening	30	78.6 (59; 102)	82.6 (61; 108)	7	180	
		07.05.2018	0% (0–8%)	93	126.1 (114; 140)	120.7 (110; 133)	10	107	
		12.05.2019	4% (2–8%)	15	18.9 (15; 24)	18.6 (15; 23)	29	220	
North Rhine-Westphalia, Ursprungsbach	Düren	22.05.2015	no *Bsal*-screening	109	151.5 (138; 166)	150.3 (137; 165)	56	15	
		27.05.2016	no *Bsal*-screening	66	136.0 (114; 158)	146.3 (124; 162)	4	213	
		13.05.2017	no *Bsal*-screening	78	159.1 (137; 178)	162.1 (140; 179)	16	50	
		08.05.2018	0% (0–16%)	170	191.4 (189; 192)	191.1 (188; 192)	26	80	
		12.05.2019	0% (0–31%)	119	148.6 (137; 161)	146.3 (136; 158)	51	130	
North Rhine-Westphalia, Weiße Wehe	Düren	17.05.2015	18% (5–50%)	160	167.7 (166; 168)	167.7 (166; 168)	49	70	
		28.05.2016	30% (11–61%)	100	152.3 (138; 163)	152.5 (138; 163)	19	90	
		20.05.2017	0% (0–8%)	15	21.8 (17; 29)	21.9 (17; 29)	34	125	
		11.05.2018	0% (0–11%)	80	120.0 (106; 136)	165.8 (142; 189)	31	77	
		18.05.2019	0% (0–6%)	27	40.7 (33; 50)	37.1 (31; 45)	25	112	
North Rhine-Westphalia, Billerbach	NP Eifel	22.05.2015	no *Bsal*-screening	41	57.3 (49; 67)	56.9 (49; 67)	8	80	
		24.05.2016	no *Bsal*-screening	37	55.2 (46; 66)	54.6 (46; 65)	22	110	
		10.05.2017	no *Bsal*-screening	26	53.0 (40; 69)	54.6 (41; 71)	17	140	
		07.05.2018	0% (0–31%)	98	132.9 (120; 147)	127.2 (116; 140)	46	95	
		13.05.2019	0% (0–20%)	35	45.0 (39; 53)	43.8 (38; 51)	35	120	

Site	County	Capture occasion	*Bsal*-record* (prevalence with Bayesian 95%-credible intervals)	Captured larvae	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width)	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width, *Bsal*-presence)	N of pools	Creek width (cm)	Remarks
North Rhine-Westphalia, Härtgessief	NP Eifel	2014	0% (0–19%)	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	since 2015, no more adult salamanders could be found
		25.05.2017	no *Bsal*-screening	6	12.5 (7; 21)	13.1 (7; 22)	18	130	
		10.05.2018	no *Bsal*-screening	3	4.6 (3; 8)	4.2 (3; 7)	24	197	
		23.05.2019	0%	6	11.4 (7; 19)	9.4 (6; 15)	11	190	
North Rhine-Westphalia, Haftenbach	NP Eifel	01.06.2015	0–8%	88	163.3 (152; 168)	162.3 (149; 168)	19	90	
		12.05.2016	0–14%	147	162.3 (160; 163)	162.2 (159; 163)	32	90	
		17.05.2017	10% (3–25%)	65	145.4 (121; 173)	167.3 (147; 180)	22	200	
		23.05.2018	0–11%	77	164.9 (143; 180)	135.8 (114; 161)	13	70	
		29.05.2019	0% (0–26%)	10	25.9 (16; 40)	18.7 (12; 28)	20	170	
North Rhine-Westphalia, Lorbach	NP Eifel	01.06.2015	no *Bsal*-screening	37	81.9 (64; 103)	no *Bsal*-screening	10	230	
		12.05.2016	no *Bsal*-screening	32	55.6 (44; 69)	no *Bsal*-screening	13	230	
		17.05.2017	no *Bsal*-screening	26	68.3 (50; 91)	no *Bsal*-screening	7	70	
		23.05.2018	no *Bsal*-screening	10	23.0 (14; 35)	no *Bsal*-screening	11	90	
		12.06.2019	no *Bsal*-screening	5	6.4 (5; 9)	no *Bsal*-screening	11	70	
North Rhine-Westphalia, Riffelsbach	NP Eifel	24.06.2015	no *Bsal*-screening	0	5.4 (0; 26)	no *Bsal*-screening	16	170	
North Rhine-Westphalia, Sauerbach	NP Eifel	01.06.2015	0% (0–14%)	21	46.8 (34; 63)	44.9 (33; 60)	11	80	
		12.05.2016	0% (0–15%)	18	29.9 (22; 39)	30.5 (23; 40)	16	90	
		17.05.2017	22% (7–55%)	11	27.3 (17; 41)	28.7 (18; 43)	10	60	
		28.05.2018	100%	3	9.2 (3; 19)	7.4 (3; 15)	4	80	
		29.05.2019	no *Bsal*-screening	1	3.9 (1; 10)	2.6 (1; 7)	12	90	
North Rhine-Westphalia, Walbigbach	NP Eifel	24.06.2015	no *Bsal*-screening	11	107.3 (59; 160)	95.1 (52; 149)	4	40	
		24.05.2016	no *Bsal*-screening	37	56.0 (47; 67)	55.7 (46; 67)	21	35	
		10.05.2017	no *Bsal*-screening	14	34.5 (23; 50)	36.2 (24; 52)	10	30	
		07.05.2018	0% (0–26%)	49	66.6 (58; 77)	63.8 (56; 73)	28	35	
		13.05.2019	0% (0–26%)	26	33.2 (28; 40)	32.3 (28; 38)	21	40	
Total sites Northern Eifel Mts.: 40 (21 with 5 year data)			Total captured larvae:	5498					

Site	County	Capture occasion	*Bsal*-record* (prevalence with Bayesian 95%-credible intervals)	Captured larvae	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width)	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width, *Bsal*-presence)	N of pools	Creek width (cm)	Remarks
Southern Eifel Mts.									
Rhineland-Palatinate, Arzfeld	Bitburg-Prüm	18.06.2016	0% (0–43%)	5	7.5 (5; 12)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	50	50	
		12.06.2017	0% (0–70%)	4	6.5 (4; 11)	6.5 (4; 11)	25	60	
		07.06.2018	no *Bsal*-screening	20	31.8 (24; 41)	28.9 (23; 37)	27	50	
		30.05.2019	0% (0–70%)	45	61.5 (53; 72)	62.6 (54; 73)	62	50	
Rhineland-Palatinate, Ernzen Gutenbach	Bitburg-Prüm	28.05.2015	0% (0–51%)	44	46.5 (44; 50)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	8	350	
		26.05.2016	0% (0–20%)	2	2.9 (2; 6)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	50	300	
		24.05.2017	0% (0–43%)	18	28.1 (21; 37)	28.0 (21; 37)	23	250	
		14.05.2018	0%	2	4.1 (2; 9)	3.3 (2; 7)	11	77	
		16.05.2019	no *Bsal*-screening	23	40.5 (31; 52)	33.6 (27; 42)	52	180	
Rhineland-Palatinate, Ferschweiler 1	Bitburg-Prüm	03.06.2015	no *Bsal*-screening	66	128.3 (108; 151)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	11	80	
		28.05.2016	no *Bsal*-screening	34	54.6 (44; 67)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	29	70	
		21.05.2017	0% (0–11%)	91	129.2 (121; 133)	129.2 (120; 133)	60	30	
		22.05.2018	no *Bsal*-screening	108	143.2 (138; 145)	141.8 (134; 145)	41	45	
		18.05.2019	no *Bsal*-screening	22	36.5 (28; 47)	34.5 (27; 44)	38	100	
Rhineland-Palatinate, Ferschweiler 2	Bitburg-Prüm	03.06.2015	no *Bsal*-screening	362	excluded from analysis	first *Bsal*-detection in the Southern Eifel Mts. in 2017	11	130	
		28.05.2016	no *Bsal*-screening	51	88.7 (74; 106)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	14	100	
		21.05.2017	0% (0–11%)	19	29.6 (23; 38)	29.6 (23; 38)	25	40	
		22.05.2018	no *Bsal*-screening	308	excluded from analysis	excluded from analysis	27	50	
		18.05.2019	no *Bsal*-screening	43	70.7 (59; 85)	69.6 (58; 83)	31	70	
Rhineland-Palatinate, Fleißbach	Bitburg-Prüm	04.06.2015	no *Bsal*-screening	20	47.2 (34; 64)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	8	50	
		28.05.2016	no *Bsal*-screening	115	145.0 (139; 147)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	52	50	
		29.05.2017	0% (0–51%)	0	0.3 (0; 2)	0.3 (0; 2)	14	10	
		16.05.2018	0% (0–44%)	5	9.2 (5; 15)	7.6 (5; 12)	15	95	
		16.05.2019	no *Bsal*-screening	0	0.6 (0; 3)	0.3 (0; 2)	58	15	
Rhineland-Palatinate, Fuhrbach	Bitburg-Prüm	02.07.2015	no *Bsal*-screening	92	156.0 (142; 163)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	36	100	two swabbing site in the same forest (newts)
		04.06.2016	no *Bsal*-screening	2	4.0 (2; 9)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	11	150	

Site	County	Capture occasion	*Bsal*-record* (prevalence with Bayesian 95%-credible intervals)	Captured larvae	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width)	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width, *Bsal*-presence)	N of pools	Creek width (cm)	Remarks
Rhineland-Palatinate, Fuhrbach	Bitburg-Prüm	05.06.2017	0% (0–51%), 33% (8–82%)	2	3.4 (2; 7)	3.4 (2; 7)	30	150	
		28.05.2018	no *Bsal*-screening	13	21.4 (15; 30)	93.1 (55; 136)	24	197	
		11.06.2019	0% (0–16%), 0% (0–31%)	2	1.3 (2; 4)	3.1 (2; 6)	57	40	
Rhineland-Palatinate, Gentingen, Grasmärchen	Bitburg-Prüm	04.06.2015	no *Bsal*-screening	36	78.0 (61; 97)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	11	60	
		19.06.2016	0–43%	4	6.8 (4; 12)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	30	50	
		2017	0–61%	no monitoring	no monitoring	no monitoring	no monitoring	no monitoring	
		17.05.2018	no *Bsal*-screening	104	138.1 (128; 145)	140.6 (131; 145)	10	107	
		20.05.2019	no *Bsal*-screening	65	105.5 (91; 122)	77.8 (71; 86)	104	40	
Rhineland-Palatinate, Gemünder Bach	Bitburg-Prüm	10.06.2015	no *Bsal*-screening	43	89.4 (72; 109)	no *Bsal*-screening	20	70	
		06.06.2016	no *Bsal*-screening	1	2.2 (1; 6)	no *Bsal*-screening	20	50	
		31.05.2017	no *Bsal*-screening	2	3.4 (2; 7)	no *Bsal*-screening	50	50	
		22.05.2018	no *Bsal*-screening	33	51.8 (42; 63)	no *Bsal*-screening	46	95	
		20.05.2019	no *Bsal*-screening	22	35.9 (28; 46)	no *Bsal*-screening	65	40	
Rhineland-Palatinate, Hauchenbach	Bitburg-Prüm	01.06.2015	no *Bsal*-screening	34	49.5 (41; 59)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	11	170	
		28.05.2016	0% (0–15%)	2	4.0 (2; 9)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	11	150	
		08.06.2017	0% (0–70%)	3	4.9 (3; 9)	4.9 (3; 9)	10	75	
		09.06.2018	0% (0–44%)	10	18.9 (12; 28)	14.6 (11; 20)	27	80	
		25.05.2019	0% (0–31%)	40	58.1 (49; 69)	66.6 (55; 81)	26	150	
Rhineland-Palatinate, Heimbach	Bitburg-Prüm	10.06.2015	no *Bsal*-screening	28	41.2 (34; 50)	no *Bsal*-screening	16	170	
		11.06.2016	no *Bsal*-screening	4	6.9 (4; 12)	no *Bsal*-screening	20	200	
		07.06.2017	no *Bsal*-screening	11	17.3 (12; 24)	no *Bsal*-screening	40	100	
		28.05.2018	no *Bsal*-screening	8	13.2 (9; 20)	no *Bsal*-screening	27	30	
		27.05.2019	no *Bsal*-screening	11	16.0 (12; 22)	no *Bsal*-screening	63	50	
Rhineland-Palatinate, Ingendorf	Bitburg-Prüm	01.07.2015	no *Bsal*-screening	43	93.2 (75; 115)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	20	60	
		18.06.2016	no *Bsal*-screening	9	15.7 (10; 23)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	19	80	
		11.06.2017	0%	0	0.3 (0; 2)	0.3 (0; 2)	3	40	
		11.06.2018	no *Bsal*-screening	3	5.3 (3; 10)	4.8 (3; 9)	40	50	
		25.05.2019	no *Bsal*-screening	0	0.3 (0; 2)	0.3 (0; 2)	42	50	

Site	County	Capture occasion	*Bsal*-record* (prevalence with Bayesian 95%-credible intervals)	Captured larvae	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width)	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width, *Bsal*-presence)	N of pools	Creek width (cm)	Remarks
Rhineland-Palatinate, Irrel 1	Bitburg-Prüm	28.05.2015	0%	49	92.0 (76; 110)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	4	90	
		26.05.2016	0% (0–20%)	84	133.5 (118; 146)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	28	40	
		21.05.2017	0% (0–19%)	13	20.3 (15; 28)	20.2 (15; 28)	8	30	
		22.05.2018	0% (0–22%)	61	100.3 (86; 117)	87.1 (76; 100)	28	35	
		15.05.2019	0% (0–30%)	36	63.0 (51; 78)	55.9 (46; 68)	39	40	
Rhineland-Palatinate, Irrel 2	Bitburg-Prüm	26.05.2016	0% (0–20%)	51	91.0 (76; 109)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	11	60	
		21.05.2017	0% (0–19%)	5	8.1 (5; 13)	8.1 (5; 13)	40	55	
		22.05.2018	0% (0–22%)	31	52.4 (42; 65)	44.4 (37; 54)	26	80	
		15.05.2019	0% (0–30%)	8	14.5 (9; 22)	12.1 (8; 18)	48	70	
Rhineland-Palatinate, Läuskopfbach	Bitburg-Prüm	07.07.2015	no *Bsal*-screening	106	155.1 (142; 163)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	18	150	two swabbing site in the same forest (newts)
		11.06.2016	no *Bsal*-screening	0	0.5 (0; 3)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	16	50	
		07.06.2017	0% (0–14%), 33% (8–82%)	6	9.5 (6; 15)	9.5 (6; 15)	6	100	
		04.06.2018	0% (0–16%)	0	0.5 (0; 3)	7.1 (0; 26)	62	83	
		21.05.2019	0% (0–16%), 0% (0–31%)	6	9.7 (6; 15)	9.8 (6; 15)	36	80	
Rhineland-Palatinate, Lützkampen, Seisbach	Bitburg-Prüm	19.06.2015	0% (0–31%)	26	49.7 (38; 64)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	31	100	
		20.06.2016	no *Bsal*-screening	12	16.7 (13; 22)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	60	50	
		26.06.2017	0% (0–19%)	66	102.5 (89; 118)	102.2 (89; 118)	40	50	
		30.05.2018	no *Bsal*-screening	114	142.3 (136; 145)	143.2 (138; 145)	17	167	
		03.06.2019	0% (0–70%)	153	164.6 (163; 165)	164.5 (162; 165)	88	100	
Rhineland-Palatinate, Mauel, Steuenbach	Bitburg-Prüm	07.07.2015	no *Bsal*-screening	28	52.7 (41; 67)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	17	100	
		11.06.2016	0% (0–36%)	18	31.4 (23; 42)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	14	130	
		05.06.2017	no *Bsal*-screening	31	48.6 (39; 60)	48.5 (39; 60)	80	45	
		28.05.2018	no *Bsal*-screening	6	10.3 (6; 16)	9.0 (6; 14)	31	77	
		10.06.2019	no *Bsal*-screening	3	3.7 (3; 6)	4.8 (3; 9)	44	70	

Site	County	Capture occasion	*Bsal*-record* (prevalence with Bayesian 95%-credible intervals)	Captured larvae	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width)	Estimated larvae ± 95%-credible interval** (considered variables: date, pools, creek width, *Bsal*-presence)	N of pools	Creek width (cm)	Remarks
Rhineland-Palatinate, Olmscheid, Hof Klingendell	Bitburg-Prüm	16.06.2015	no *Bsal*-screening	34	53.0 (44; 64)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	21	150	
		11.06.2016	0% (0–51%)	1	2.2 (1; 6)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	20	50	
		07.06.2017	no *Bsal*-screening	60	93.2 (80; 108)	93.0 (80; 108)	40	80	
		28.05.2018	no *Bsal*-screening	82	116.7 (104; 131)	117.6 (105; 132)	16	100	
		27.05.2019	no *Bsal*-screening	20	28.8 (23; 37)	29.7 (23; 38)	49	40	
Rhineland-Palatinate, Reiff	Bitburg-Prüm	16.06.2015	no *Bsal*-screening	62	138.5 (116; 160)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	33	50	
		18.06.2016	no *Bsal*-screening	35	47.1 (40; 56)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	65	50	
		19.06.2017	0% (0–20%)	80	121.6 (108; 133)	121.4 (108; 132)	18	40	
		30.05.2018	no *Bsal*-screening	61	87.3 (76; 100)	87.6 (76; 100)	36	100	
		30.05.2019	no *Bsal*-screening	138	163.7 (159; 165)	162.9 (157; 165)	85	45	
Rhineland-Palatinate, Spielmannsholz	Bitburg-Prüm	19.06.2015	no *Bsal*-screening	8	20.1 (12; 31)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	17	70	
		18.06.2016	no *Bsal*-screening	25	43.2 (34; 55)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	16	100	
		06.06.2017	0% (0–43%)	0	0.3 (0; 2)	0.3 (0; 2)	5	30	
		31.05.2018	no *Bsal*-screening	45	79.0 (65; 95)	64.1 (55; 75)	9	122	
		19.06.2019	no *Bsal*-screening	5	5.6 (5; 8)	9.0 (5; 15)	21	40	
Rhineland-Palatinate, Watzbach	Bitburg-Prüm	23.06.2016	0% (0–44%)	52	86.4 (73; 102)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	23	40	
		06.06.2017	0% (0–10%)	40	62.1 (52; 74)	62.0 (52; 74)	30	40	
		31.05.2018	0% (0–32%)	194	excluded from analysis	excluded from analysis	27	45	
		22.06.2019	23% (12–41%)	260	excluded from analysis	excluded from analysis	52	50	
Rhineland-Palatinate, Watzbachzufluss	Bitburg-Prüm	25.06.2015	no *Bsal*-screening	55	101.4 (85; 120)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	34	100	
		23.06.2016	0% (0–44%)	95	143.0 (134; 147)	first *Bsal*-detection in the Southern Eifel Mts. in 2017	20	80	
		06.06.2017	0% (0–10%)	47	73.6 (62; 87)	73.4 (62; 87)	100	25	
		31.05.2018	0% (0–32%)	91	124.4 (112; 138)	130.0 (117; 143)	22	229	
		22.06.2019	23% (12–41%)	105	113.6 (108; 120)	160.0 (149; 165)	26	30	
Total sites Southern Eifel Mts.: 21 (17 with 5 year data)			Total captured larvae:	4542					

Supplementary document 2. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 35 creeks (Northern Eifel Mountains, 2015). Symbols: (Λ) means abundance; (p) means detection probability; (nb) negative-binomial distribution; arrows indicate positive (↑) or negative (↓) effects; (.) means no observed direction.

Model	AIC	ΔAIC	AIC weight
Λ=constant (.), p=date (↓), nb	-3601.16	0.00	0.37
Λ=date (↓), p=date (↓), nb	-3601.08	0.08	0.35
Λ=pools (.), p=date (↓), nb	-3599.30	1.90	0.14

Supplementary document 3. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 15 creeks with *Bsal screening* (Northern Eifel Mountains, 2015). Symbols as in Supplementary document 2. Red indicates effects of *Bsal.*

Model	AIC	ΔAIC	AIC weight
Λ=constant (.), p=date (↓), nb	-3176.79	0.00	0.34
Λ=creek width (↓), p=date (↓), nb	-3176.16	0.63	0.24
Λ=date (↓), p=date (↓), nb	-3174.98	1.81	0.14
Λ=*Bsal* (.), p=date (↓), nb	-3174.82	1.97	0.13
Λ=pools (↑), p=date (↓), nb	-3174.80	1.99	0.12

Supplementary document 4. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 29 creeks (Northern Eifel Mountains, 2016). Symbols as in Supplementary document 2.

Model	AIC	ΔAIC	AIC weight
Λ=constant (.), p=creek width (↑)	-4008.11	0.00	0.30
Λ=constant (.), p=creek width (↑), nb	-4008.11	0.00	0.30
Λ=creek width (.), p=creek width (↑), nb	-4006.67	1.44	0.15
Λ=pools (.), p=creek width (↑), nb	-4006.52	1.60	0.14
Λ=date (.), p=creek width (↑), nb	-4006.24	1.87	0.12

Supplementary document 5. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 17 creeks with *Bsal screening* (Northern Eifel Mountains, 2016). Symbols as in Supplementary document 2. Red indicates effects of *Bsal.*

Model	AIC	ΔAIC	AIC weight
Λ=constant (.), p=pools (↑), nb	-4008.11	0.00	0.30
Λ=creek width (↑), p=pools (↑), nb	-4008.11	0.00	0.30
Λ=pools (.), p=pools (↑), nb	-4006.67	1.44	0.15
Λ=*Bsal* (.), p=pools (↑), nb	-4006.24	1.87	0.12

Supplementary document 6. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 30 creeks (Northern Eifel Mountains, 2017). Symbols as in Supplementary document 2.

Model	AIC	ΔAIC	AIC weight
Λ=constant (.), p=pools (↑), nb	-4240.66	0.00	0.37
Λ=creek width (↓), p=pools (↑), nb	-4008.11	0.00	0.30
Λ=date (↓), p=pools (↑), nb	-4006.24	1.87	0.12

Supplementary document 7. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 18 creeks with *Bsal screening* (Northern Eifel Mountains, 2017). Symbols as in Supplementary document 2. Red indicates effects of *Bsal*.

Model	AIC	ΔAIC	AIC weight
Λ=constant (.), p=pools (↑), nb	-3425.06	0.00	0.32
Λ= creek width (↓), pools (↑), nb	-3424.43	0.63	0.23
Λ=date (↓), p=pools (↑), nb	-3423.66	1.40	0.16
Λ=*Bsal* (↓), p=pools (↑), nb	-3423.55	1.51	0.15
Λ=pools (↑), p= pools (↑), nb	-3423.43	1.63	0.14

Supplementary document 8. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 26 creeks (Northern Eifel Mountains, 2018). Symbols as in Supplementary document 2.

Model	AIC	ΔAIC	AIC weight
Λ=constant (.), p=date (↓), nb	-5615.84	0.00	0.44
Λ=date (↓), p=date (↓), nb	-5614.54	1.30	0.23
Λ=pools (↑), p=date (↓), nb	-5613.98	1.86	0.17
Λ=creek width (.), p=date (↓), nb	-5613.86	1.98	0.16

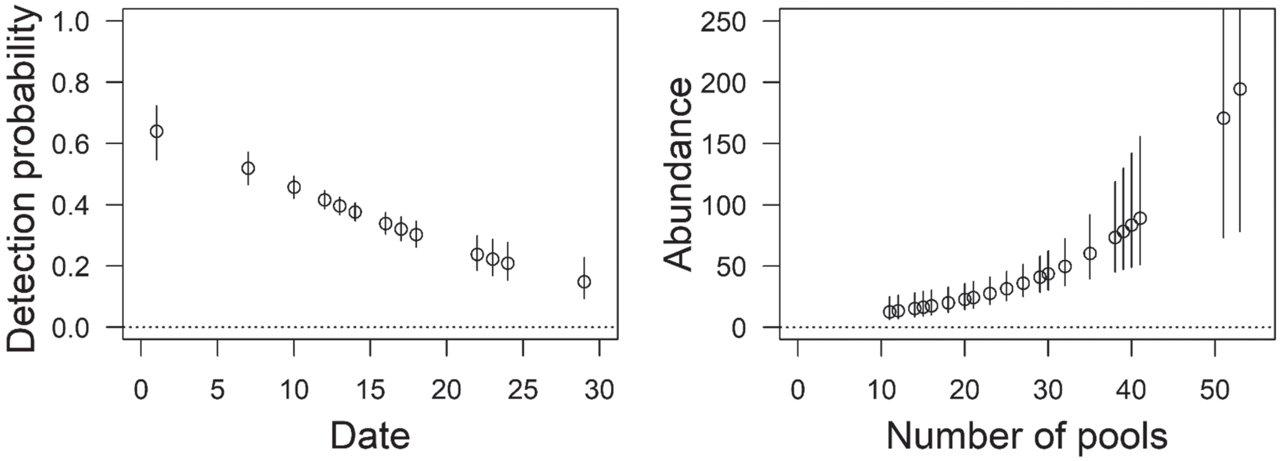
Supplementary document 9. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 17 creeks with *Bsal* screening (Northern Eifel Mountains, 2018). Symbols as in Supplementary document 2. Red indicates effects of *Bsal*.

Model	AIC	ΔAIC	AIC weight
Λ=constant (.), p=*Bsal* (.), nb	-4517.86	0.00	0.19
Λ=pools (.), p=*Bsal* (.), nb	-4517.77	0.09	0.18
Λ=date (↓), p=*Bsal* (.), nb	-4517.77	0.09	0.18
Λ=creek width (↓), p=*Bsal* (.), nb	-4517.58	0.28	0.17
Λ=*Bsal* (↓), p=*Bsal* (.), nb	-4516.44	1.42	0.10
Λ=constant (.), p=date (↓), nb	-4516.26	1.60	0.09

Supplementary document 10. Model selection overview of the only plausible hierarchical model with ΔAIC < 2 considering removal data from 28 creeks (Northern Eifel Mountains, 2019). Symbols as in Supplementary document 2.

Model	AIC	ΔAIC	AIC weight
Λ=pools (↑), p=date (↓), nb	-4151.21	0.00	0.97

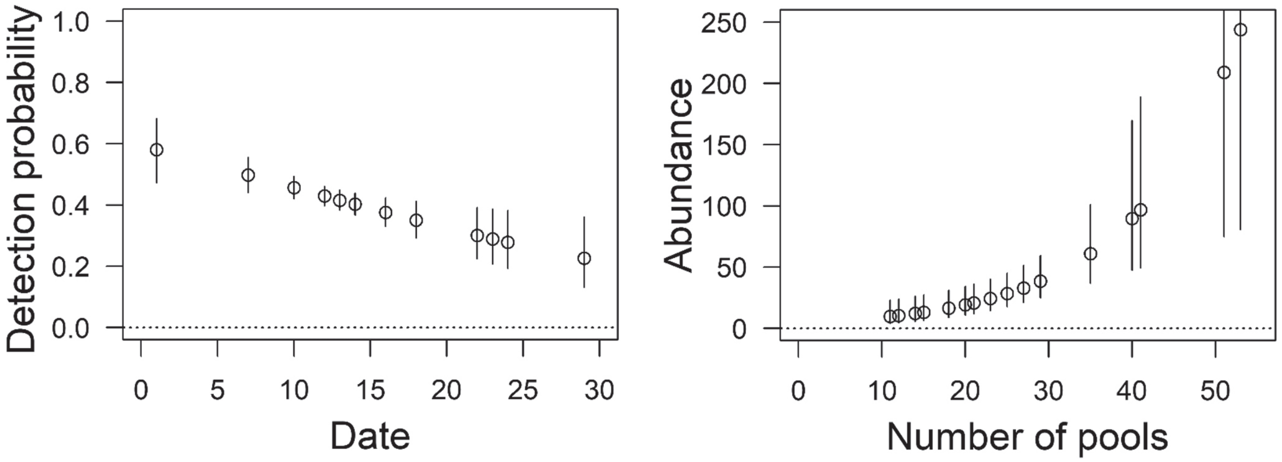
Supplementary document 11. Effect of date on detection probability and number of pools on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 28 creeks (Northern Eifel Mountains, 2019).



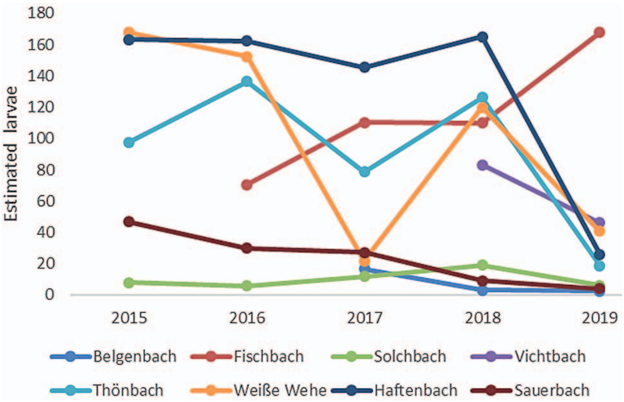
Supplementary document 12. Model selection overview of the only plausible hierarchical model with ΔAIC < 2 considering removal data from 21 creeks with *Bsal screening* (Northern Eifel Mountains, 2019). Symbols as in Supplementary document 2.

Model	AIC	ΔAIC	AIC weight
Λ=pools (↑), p=date (↓), nb	-3544.74	0.00	0.60

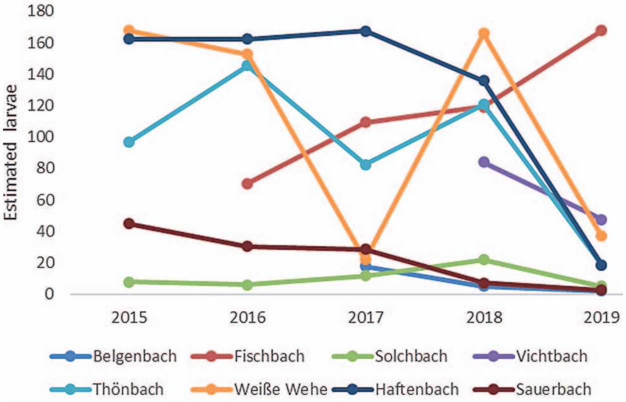
Supplementary document 13. Effect of date on detection probability and number of pools on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 21 creeks with *Bsal screening* (Northern Eifel Mountains, 2019).



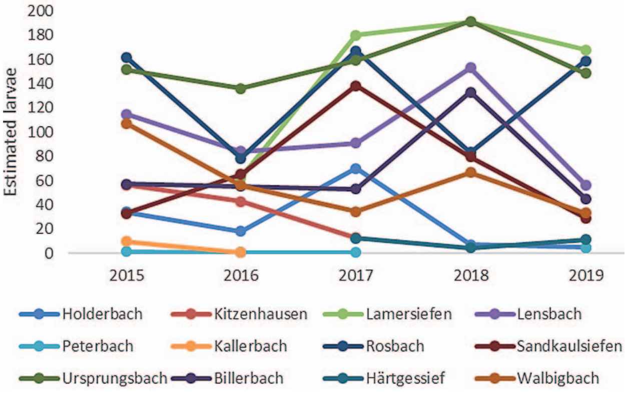
Supplementary document 14. Estimated larvae according to the first analysis of removal data from *Bsal*-infected sites from the Northern Eifel Mountains. In most creeks, *Bsal* was present from the beginning of the monitoring, but was first detected in 2017 in the Haftenbach and Sauerbach and in 2019 in the Thönbach. Confidence intervals can be found in Supplementary document 1.



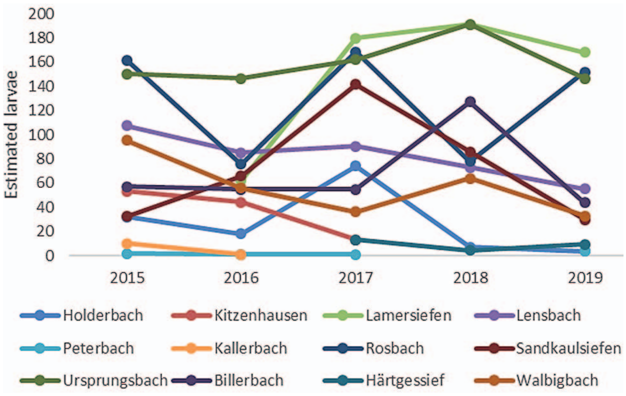
Supplementary document 15. Estimated larvae according to the second analysis of removal data from *Bsal*-infected sites from the Northern Eifel Mountains. In most creeks, *Bsal* was present from the beginning of the monitoring, but was first detected in 2017 in the Haftenbach and Sauerbach and in 2019 in the Thönbach. Confidence intervals can be found in in Supplementary document 1.



Supplementary document 16. Estimated larvae according to the first analysis of removal data from sites from the Northern Eifel Mountains where *Bsal* was not detected. Confidence intervals can be found in in Supplementary document 1.



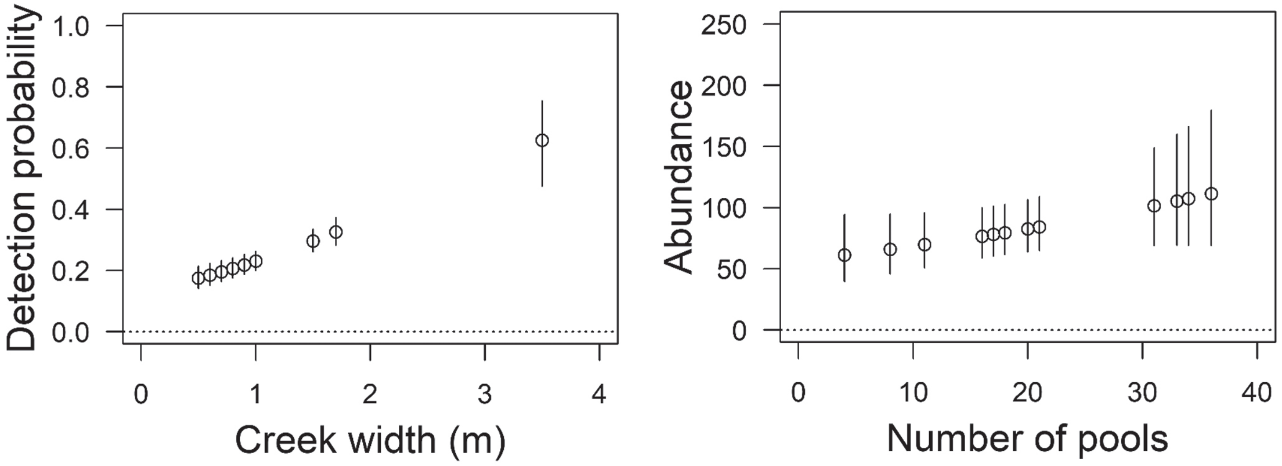
Supplementary document 17. Estimated larvae according to the second analysis of removal data from sites from the Northern Eifel Mountains where *Bsal* was not detected. Confidence intervals can be found in in Supplementary document 1.



Supplementary document 18. Model selection overview of plausible hierarchical models with ΔAIC < 2 considering removal data from 17 creeks (Southern Eifel Mountains, 2015) (first *Bsal* detection in 2017). Symbols as in Supplementary document 2.

Model	AIC	ΔAIC	AIC weight
Λ=pools (↑), p=creek width (↑), nb	-2827.00	0.00	0.25
Λ=constant (.), p=creek width (↑)	-2826.70	0.29	0.21
Λ=constant (.), p=creek width (↑), nb	-2826.70	0.29	0.21
Λ= creek width (↓), p=creek width (↑), nb	-2826.16	0.84	0.16
Λ=date (↑), p=creek width (↑), nb	-2825.76	1.23	0.13

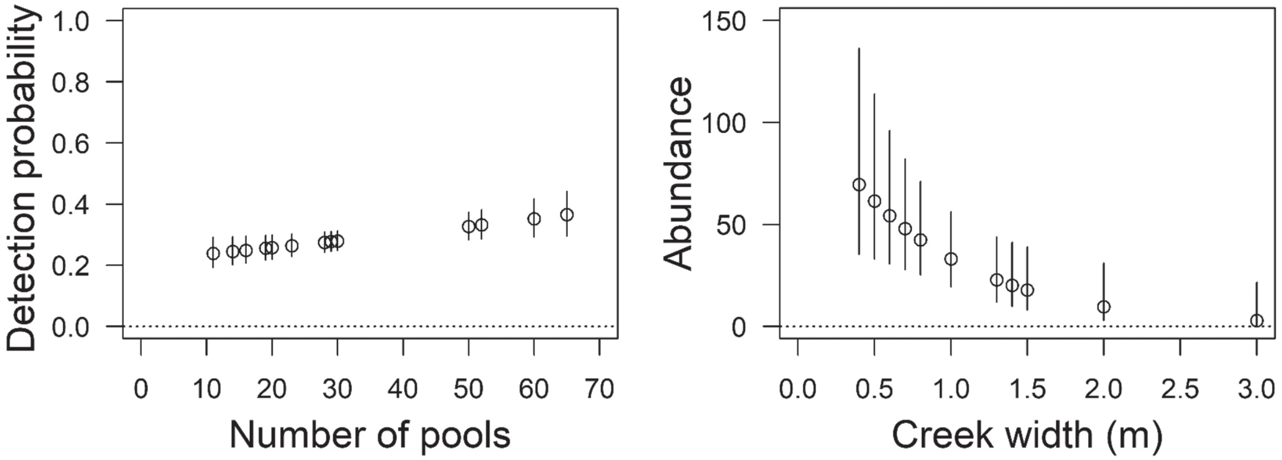
Supplementary document 19. Effect of creek width on detection probability and number of pools on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 17 creeks (Southern Eifel Mountains, 2016) (first *Bsal* detection in 2017).



Supplementary document 20. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 21 creeks (Southern Eifel Mountains, 2016) (first *Bsal* detection in 2017). Symbols as in Supplementary document 2.

Model	AIC	ΔAIC	AIC weight
Λ=creek width (↓), p=pools (↑), nb	-2827.00	0.00	0.25

Supplementary document 21. Effect of number of pools on detection probability and creek width on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 21 creeks (Southern Eifel Mountains, 2016) (first *Bsal* detection in 2017).



Supplementary document 22. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 20 creeks (Southern Eifel Mountains, 2017). Symbols as in Supplementary document 2.

Model	AIC	ΔAIC	AIC weight
Λ=pools (↑), p=constant (.), nb	-1655.19	0.00	0.18
Λ=constant (.), p=constant (.), nb	-1654.17	1.02	0.11
Λ=pools (↑), p=creek width (↑), nb	-1654.04	1.15	0.10
Λ=pools (↑), p=date (.), nb	-1653.53	1.66	0.10
Λ=pools (↑), p=pools (.), nb	-1653.33	1.86	0.08

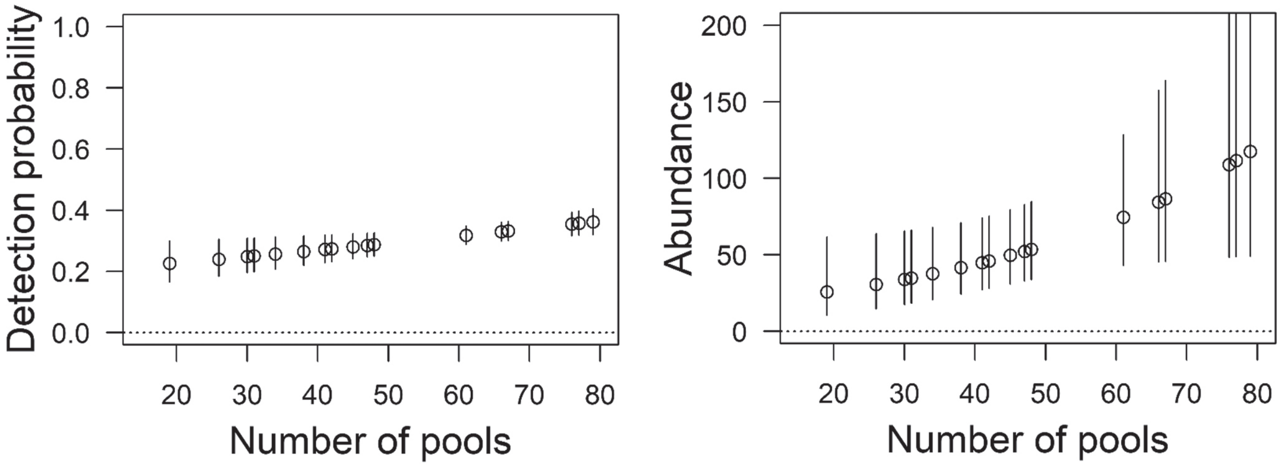
Supplementary document 23. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 18 creeks with *Bsal screening* (Southern Eifel Mountains, 2017). Symbols as in Supplementary document 2. Red indicates effects of *Bsal.*

Model	AIC	ΔAIC	AIC weight
Λ=pools (↑), p=constant (.), nb	-1661.95	0.00	0.12
Λ=constant (.), p=creek width (↑), nb	-1660.98	0.97	0.08
Λ=*Bsal* (↓), p=constant (.), nb	-1660.85	1.10	0.07
Λ=pools (↑), p=*Bsal* (↓), nb	-1660.79	1.16	0.07
Λ=constant (.), p=constant (.), nb	-1660.56	1.39	0.06
Λ=pools (↑), p= date (↓), nb	-1660.31	1.64	0.05
Λ=constant (.), p=*Bsal* (↓), nb	-1660.21	1.74	0.05
Λ=pools (↑), p=pools (↑), nb	-1660.09	1.86	0.05

Supplementary document 24. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 20 creeks (Southern Eifel Mountains, 2018). Symbols as in Supplementary document 2.

Model	AIC	ΔAIC	AIC weight
Λ=pools (↑), p=pools (.), nb	-1661.95	0.00	0.12
Λ=constant (.), p=pools (↑), nb	-1660.09	1.86	0.05

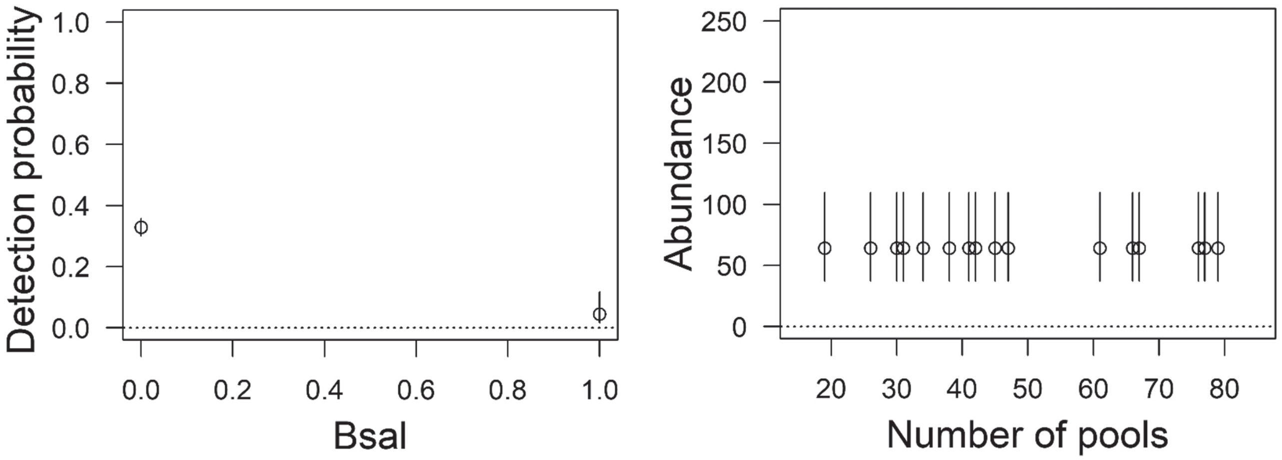
Supplementary document 25. Effect of number of pools on detection probability and on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 20 creeks (Southern Eifel Mountains, 2018).



Supplementary document 26. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 17 creeks with *Bsal screening* (Southern Eifel Mountains, 2018). Symbols as in Supplementary document 2. Red indicates effects of *Bsal*.

Model	AIC	ΔAIC	AIC weight
Λ=pools (.), p=*Bsal* (.), nb	-3141.82	0.00	0.26
Λ=*Bsal* (↓), p=pools (↑), nb	-3140.69	1.13	0.15
Λ=pools (.), p=pools (↑), nb	-3140.65	1.16	0.15

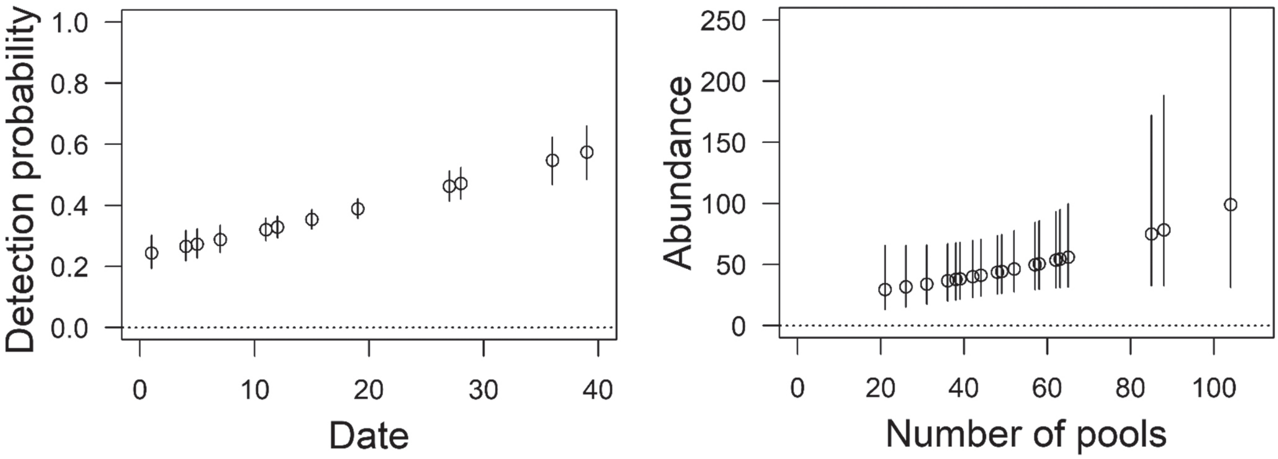
Supplementary document 27. Effect of *Bsal* on detection probability and number of pools on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 17 creeks with *Bsal screening* (Southern Eifel Mountains, 2018).



Supplementary document 28. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 20 creeks (Southern Eifel Mountains, 2019). Symbols as in Supplementary document 2.

Model	AIC	ΔAIC	AIC weight
Λ=pools (↑), p=date (↑), nb	-2973.00	0.00	0.24
Λ=constant (.), p=date (↑), nb	-2972.87	0.13	0.22
Λ=constant (.), p=pools (↑), nb	-2971.94	1.06	0.14
Λ=date (↑), p=date(↑), nb	-2971.03	1.97	0.09

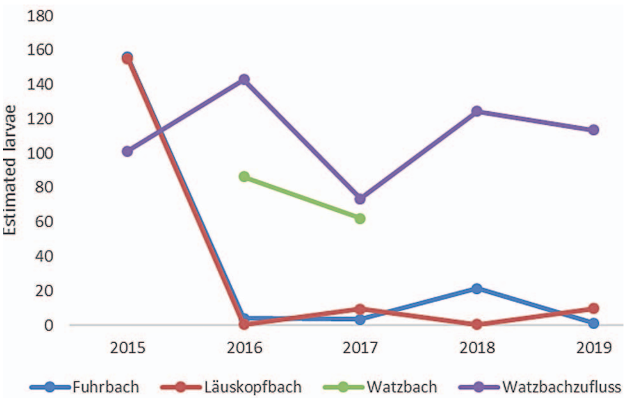
Supplementary document 29. Effect of date on detection probability and number of pools on larval abundance according to the best fitting model with ΔAIC = 0 considering removal data from 20 creeks (Southern Eifel Mountains, 2019).



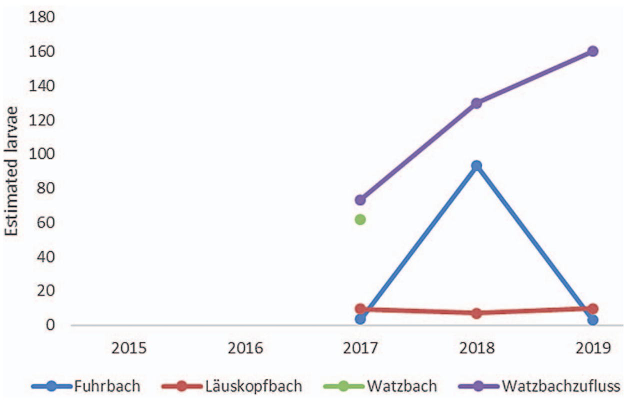
Supplementary document 30. Model selection overview of plausible hierarchical model with ΔAIC < 2 considering removal data from 18 creeks with *Bsal* screening (Southern Eifel Mountains, 2019). Symbols as in Supplementary document 2. Red indicates effects of *Bsal.*

Model	AIC	ΔAIC	AIC weight
Λ=constant (.), p=pools (↑), nb	-2934.18	0.00	0.18
Λ=pools (.), p=date(↑), nb	-2933.97	0.21	0.16
Λ=constant (.), p=date(↑), nb	-2933.85	0.33	0.15
Λ=pools (.), p=pools(↑), nb	-2933.32	0.86	0.12
Λ=date (↑), p=pools (↑), nb	-2932.87	1.31	0.09
Λ=*Bsal* (.), p=pools (↑), nb	-2932.20	1.99	0.07

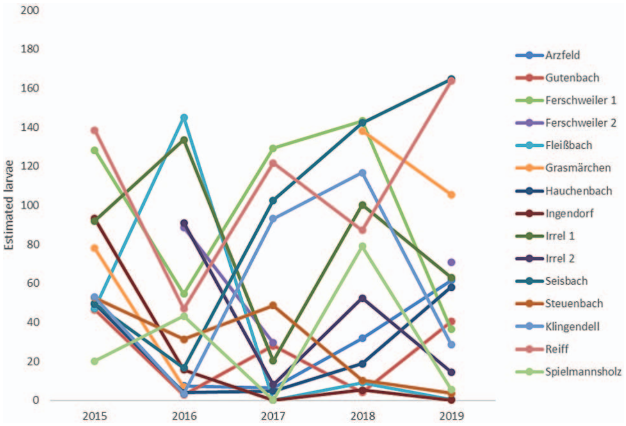
Supplementary document 31. Estimated larvae according to the first analysis of removal data from *Bsal*-infected sites from the Southern Eifel Mountains. In the Fuhrbach and Läuskopfbach, *Bsal* was first detected in 2017 and in 2019 in the Watzbach and Watzbachzufluss. Confidence intervals can be found in in Supplementary document 1.



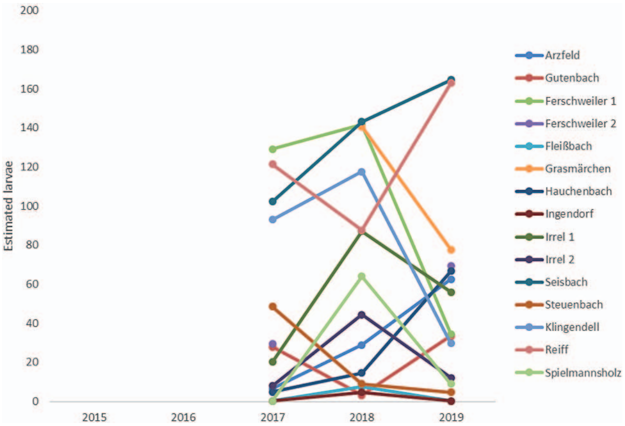
Supplementary document 32. Estimated larvae according to the second analysis of removal data from *Bsal*-infected sites from the Southern Eifel Mountains. In the Fuhrbach and Läuskopfbach, *Bsal* was first detected in 2017 and in 2019 in the Watzbach and Watzbachzufluss. Confidence intervals can be found in in Supplementary document 1.



Supplementary document 33. Estimated larvae according to the first analysis of removal data from sites from the Southern Eifel Mountains where *Bsal* was not detected. Confidence intervals can be found in in Supplementary document 1.



Supplementary document 34. Estimated larvae according to the second analysis of removal data from sites from the Southern Eifel Mountains where *Bsal* was not detected. Confidence intervals can be found in in Supplementary document 1.



SALAMANDRA **56**(3): 254–264
15 August 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Do habitat preferences of European fire salamander (*Salamandra salamandra*) larvae differ among landscapes? A case study from Western Germany

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Manuscript received: 29 March 2020
Accepted: 5 June 2020 by Miguel Vences

Abstract. The European fire salamander (*Salamandra salamandra*) is a widespread species that occurs in a variety of habitats throughout its range. We studied if different environmental parameters influence presence as well as abundance of larvae within different local landscape units across a more than 5,000 km² large Central European study area. This knowledge is crucial to differentiate between habitat specific absence/low abundance and externally triggered extinctions or population declines, e.g. through newly emerging infectious diseases. Within our study area, the salamander plague, caused by the invasive fungus *Batrachochytrium salamandrivorans*, has recently been invoked as major factor for population declines in *S. salamandra*. We estimated larval habitat preferences of 135 European fire salamander populations (presence-absence only), and estimated larval abundances in 85 out of them. In the entire study area, regardless of landscape units, presence of European fire salamander larvae was positively affected by low elevation, a high number of pools (preferred larval microhabitats) and a high amount of consumable macrozoobenthos in the reproduction creeks as well as a high proportion of forest cover in the surrounding terrestrial habitats. Apart from some minor differences among landscape units when they were analysed separately, we observed in many cases a positive effect of a high number of pools (11 out of 56 overall models = 20%) and, furthermore, a negative effect of a late mapping date (mainly due to larval drift caused by heavy rainfalls in early summer and metamorphosis) on larval abundances (12 out of 56 overall models = 21%). Consequently, at least in this Central European study area, which includes mountainous regions up to 700 m a.s.l. ('West- and Osteifel', 'Hunsrück'), a mainly agriculturally used lime soil plateau ('Gutland') and a river valley ('Moseltal'), these habitat preferences can be used to differentiate between habitat-caused and disease-caused absence of European fire salamanders and also population declines using the larval population.

Key words. Amphibia, Caudata, chytridiomycosis, habitat suitability, *Batrachochytrium salamandrivorans* salamander plague, larval ecology, stream habitat.

Introduction

Amphibians are dramatically declining at the global scale (Stuart et al. 2004, 2010, Wake & Vredenburg 2008). Habitat change, environmental pollution, alien species, over-exploitation, global change and emerging infectious diseases are main factors for amphibian population declines and extinctions (e.g. Daszak et al. 2000, Collins & Storfer 2003). This also applies to the European fire salamander, *Salamandra salamandra*, a common species in Western Europe (Thiesmeier 2004). Beside conversion of deciduous into coniferous forests (Thiesmeier 2004) or pollution of larval habitats (Mandrillon & Saglio 2007), this species is now locally threatened by amphibian chytridiomycosis, an emerging infectious disease: While in the Pyrenees, population declines were described due to the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bosch & Martínez-Solano 2006), a second chytrid fungus, *B. salamandrivorans* (*Bsal*), is responsible for local population declines in Central Europe, especially Germany (Martel et al. 2013, 2014, Spitzen-van der Sluijs et al. 2016, Stegen et al. 2017, Dalbeck et al. 2018, Lötters et al. 2020 in this issue, Schulz et al. 2020 in this issue). Unfortunately, according to these authors, disease-driven declines of European fire salamander populations may occur unnoticed.

With the goal to better understand potential past declines in Germany, Sandvoß et al. (2020 in this issue) analysed the characteristics of larval habitats in a *Bsal*-free area. Using an ecological modelling approach, they then compared their results with data from a mountain chain where, despite several former records, European fire salamanders are only sparsely found today. They focussed on the larval populations since European fire salamander larvae can be easily detected using standard methods within their mostly lotic freshwater habitats, while the strong cryptic behaviour of the terrestrial life-stages hampers a large-scale mapping of the species within a given area (cf. Thiesmeier 2004, Skelly & Richardson 2009). For this purpose, Sandvoß et al. (2020 in this issue) applied a standardised removal sampling approach proposed by Schmidt et al. (2015), which allows a rapid quantitative assessment of the larval population. Sandvoß et al. (2020 in this issue) then used a presence-absence habitat suitability model built upon data from the disease-free region to show that most creeks in the area where species is sparse today in fact constitute highly suitable larval habitats. They concluded that *Bsal* might have driven these populations to extinction, as in addition to their modelling results, the pathogen was observed in alpine (*Ichthyosaura alpestris*) and palmate newts (*Lissotriton helveticus*) and – most recently – in one of the last remaining European fire salamander populations in this area (Sandvoß et al. 2020 in this issue).

Habitat suitability models are frequently used in conservation planning to quantify a species' habitat requirements, to understand species-habitat relationships and to predict species occurrences (e.g. Ahmadi-Nedushan et al. 2006). They come in different methodological flavours, with mere presence-absence data being the simplest way to feed such models, often just to verify the use of a habitat by a species rather than the success of that use. Major input variables are local biotic and abiotic habitat characteristics, which affect the presence (e.g. Sandvoß et al. 2020 in this issue) and abundance and thus the range of a focal species (Schmidt et al. 2015, Wagner et al. 2020 in this issue). Habitat suitability models based on local data may capture both general ecological and potential local adaptations. Hence, it may be questioned that they will assign meaningful suitability values to habitats outside this area, so in consequence they may lead to insufficient or even false management decisions.

To differentiate between naturally caused absence or low abundance of European fire salamander larvae (e.g. due to unsuitable aquatic or terrestrial habitats) and extinctions/population declines due to emerging infectious diseases caused by invasive pathogens, especially *Bsal*, we here present the results of a presence-absence mapping and abundance estimations of European fire salamander larvae based on standardized removal sampling in a more than 5,000 km² large study area in Western Germany. To test the performance of local models, we divided our study area into natural landscape units (Fig. 1) to compare local habitat preferences of the study species. We conducted presence-absence mapping in the German federal state of Rhineland-Palatinate in different regions including mountainous areas ('Eifel', 'Hunsrück'), a mainly agriculturally used plateau ('Gutland') and the river valley of the Moselle between 2016 and 2019. Furthermore, we included the presence-absence data from Sandvoß et al. (2020 in this issue) from the 'Eifel' and the 'Gutland' from 2016 in the analysis to get an even larger data set. The goal of the study was to identify the main biotic and abiotic variables, which explain presence-absence as well as abundances and detection probabilities of European fire salamander larvae. Since *S. salamandra* has colonized Central Europe only after the last ice age (Veith 1992) and thus constitutes a genetically rather uniform population here (Steinfartz et al. 2000), we expected that local habitat suitability models developed from different areas would identify similar biotic and abiotic predictors of the species' occurrence and abundance.

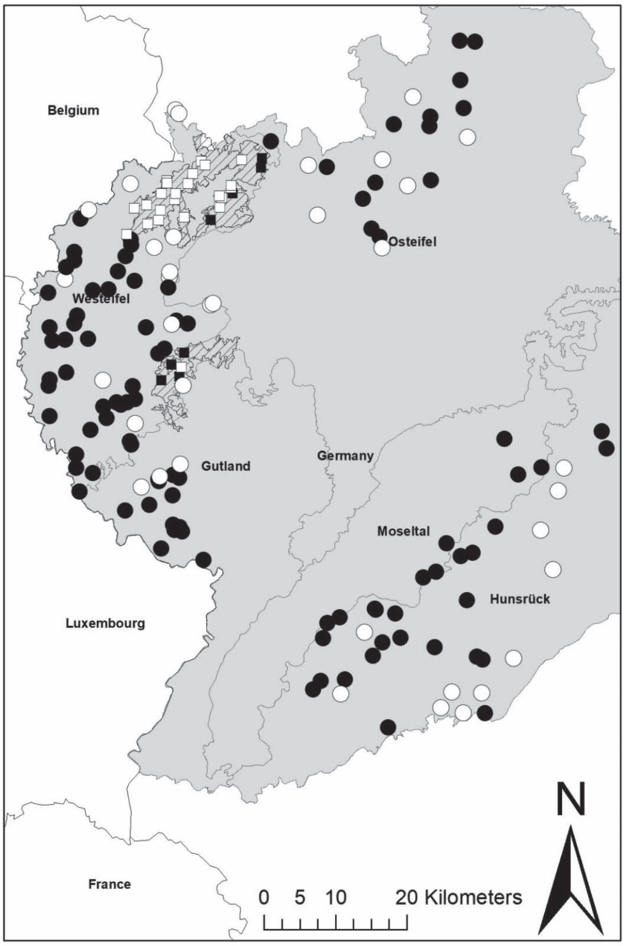

Figure 1. Study area in western Germany, divided into five landscape units. Hatched polygons indicate the continuous forest areas in which *Bsal* was detected and no creeks were considered for neither habitat suitability nor abundance modelling. Solid symbols indicate the localities of European fire salamander reproduction creeks, and open symbols creeks, where larvae of the species were not found. Circles indicate creeks, which were used in the analysis; squares those from the *Bsal*-affected forest areas that were excluded from the analysis.

Materials and methods

Study area

We conducted our study in the north-western part of the German federal state of Rhineland-Palatinate. The study area encompasses more than 5,000 km² and, according to Meynen & Schmithüsen (1962), can be divided into five landscape units: 'Hunsrück', 'Moseltal' (Moselle valley), 'Gutland', 'Westeifel', 'Osteifel' (Fig. 1). The landscape units 'Osteifel' and 'Westeifel' are the northern highlands of the study area and are part of the Rhenish Slate Mountains, with elevations up to ca. 700 m a.s.l. and with high proportions of planted coniferous forests (mainly *Picea abies* or *Pseudotsuga menziesii*). However, especially at the western slopes with red sand stone, at the border to Luxembourg, deciduous and mixed forests as well as large *S. salamandra* populations are found (Wagner et al. 2017). The 'Gutland' is a plateau with mainly lime soils; therefore, agricultural land use is predominant here, but with several remaining deciduous or mixed forest, especially at the western part (partly red sand stone) into the direction of Luxembourg. The 'Moseltal' and the 'Hunsrück' are also parts of the Rhenish Slate Mountains. The 'Moseltal' is characterised by high proportions of vineyards on its steep slopes, but also residual forest patches and creeks that flow into the Moselle. In the 'Hunsrück', the southern highland of the study area with elevations up to above 600 m a.s.l,, natural forests have been replaced by exotic *P. abies* and partly *P. menziesii*.

European fire salamander occurrences

In suitable habitats within the entire study area (especially deciduous forests with small first and second order creeks: Thiesmeier 2004), large and stable populations of *S. salamandra* have continuously been reported in all five landscape units (Bitz et al. 1996, Wagner et al. 2017). However, the amphibian pathogen *Bsal* was recently detected in two forest areas of the region, which are almost entirely situated in the 'Westeifel' (Fig. 1). Twenty-four creeks with absence and eight creeks with presence of salamander larvae from these two sites were therefore excluded from habitat suitability analysis and abundance modelling (Fig. 1) because absence or low abundances could be already disease-caused here.

Data acquisition

Presence-absence of larvae and larvae population size were used as response variables. Since the terrestrial life-stages show pronounced cryptic behaviour, they can only be detected during specific weather conditions at night (temperatures above 5°C, rainfall) (Thiesmeier 2004), and their terrestrial habitat can by quite large (Schulte et al. 2007). This is similar to many other amphibian species where larval sampling is a more effective way to monitor presence and abundances (Skelly & Richardson 2009). Fieldwork was carried out from May to July 2016–2018. We consistently choose 75 m long sections next to the springs of 135 creeks, the preferred region where adult females of this larviparous species give birth to their offspring (mainly between March and May; Thiesmeier 2004). Hence, with beginning of our mapping, most larvae should have been deposited. We used the protocol described by Schmidt et al. (2015) to conduct a removal sampling in 85 of the creeks. Each 75 m section was subdivided into three 25 m subsections and in each subsection one person was capturing larvae using a dip net for 15 min. All larvae were removed from the creek and kept in plastic aquaria until the end of the sampling. Then, all persons rotated two times, so that finally nine capture events were conducted within 45 min. Subsequently, all larvae were released back to their subsections. For presence-absence monitoring in the remaining 50 creeks, data acquisition stopped when the first larva was discovered (confirmed presence); otherwise, after three unsuccessful capture attempts (i.e. a full removal sampling attempt without captures), a second 75 m section was chosen, and after another three unsuccessful capture events larvae were considered as truly absent. Several environmental variables were recorded in order to characterise each creek (larval habitat) and its surrounding terrestrial (adult) habitat (Table 1). After field work and when changing between localities, we carefully disinfected all boots and materials for at least 5 min using 0.5% Virkon S® solution to prevent transfer of potentially present zoospores (van Rooij et al. 2017).

Statistical analysis

The software R 2.15.1 was used for all analyses (R Development Core Team 2012). In a first step, logistic regression models (function glm, Generalized Linear Models, GLMs, with logit link) were calculated for presence-absence data. We used the landscape unit as categorical variable and included it into all models (Fig. 1). Further potential predictor variables for presence and abundance of larvae (Table 1) include the record date because it is known that there are natural fluctuations in amphibian population sizes not only between years (e.g. Meyer et al. 1998) but also within a year due to, for instance, predation, but here mainly due to larval drift (especially catastrophic drift after heavy rainfalls: Thiesmeier & Schumacher 1990, Reinhardt et al. 2018, Veith et al. 2019). Elevation was used as a potential predictor because in the neighbouring federal state of North Rhine-Westphalia an elevational limit of 600 m a.s.l. for the European fire salamander is described (Thiesmeier & Dalbeck 2011). With regard to the larval habitat, the number of pools within the creek, creek width, the water flow and the presence of predatory fish (especially salmonids; presence-absence coded) are important factors known to influence the abundance of larvae (Baumgartner et al. 1999, Werner et al. 2014, Schmidt et al. 2015). The tilt (°) parallel to the studied creek section influ-

Table 1. Variables recorded at each studied creek to model presence-absence probabilities as well as larval abundances and detection probabilities.

Variable	Potential effect on detection probability	Reason	Potential effect on larval presence/ abundance	Reason
Date	(Yes)	Indirectly over larval abundances	Yes	Larval drift, predation
Elevation	(Yes)	Indirectly over larval abundances	Yes	Due to known elevational limits of the species
Aquatic habitat				
Number of pools	Yes	Accumulation of larvae in pools	Yes	Preferred larval microhabitat
Creek width	Yes	Visibility of larvae, influence on water flow, larval drift	Yes	Influence on water flow, larval drift
Presence of predatory fish	(Yes)	Indirectly over larval abundances	Yes	Predation of larvae
Tilt parallel to creek section	(Yes)	Indirectly over larval abundances	Yes	Influence on water flow, larval drift
Tilt perpendicular to creek section	(Yes)	Indirectly over larval abundances	Yes	Accessibility of creek section for adult females
Quantity of prey items (ordinal, 1–3)	(Yes)	Indirectly over larval abundances	Yes	Obligate for survival (also decrease of cannibalism)
Substrate type (ordinal, 1–3)	(Yes)	Indirectly over larval abundances	Yes	'Stony creeks' with more hiding places
Terrestrial habitat				
Hiding places in 100 m buffer (ordinal, 1–3)	(Yes)	Indirectly over larval abundances	Yes	Core habitat preferences of adults
Land use (%) in 100 m buffer	(Yes)	Indirectly over larval abundances	Yes	Core habitat range of adults

ences the water flow and thereby larval drift (Thiesmeier & Schumacher 1990, Reinhardt et al. 2018, Veith et al. 2019). The tilt (°) perpendicular to the creek section is supposed to influence the accessibility for adult females, which are migrating to the reproduction waters for larvae deposition (Manenti et al. 2009). The quantity of prey items for larvae, i.e. consumable macrozoobenthos (especially gammarids: Ruff & Maier 2000) was estimated according to the by-catches during larval monitoring and in relation to all studied creeks (ordinal data: 'low', 'medium', 'high'). The substrate type of the creek section (ordered according to increasing grain size, from mainly muddy over sandy to stony substrate) was included as a potential factor that influences larval abundance and detection probability. Regarding the terrestrial habitat, the quantity of potential suitable hiding places (dead wood, stones, etc.) for adult European fire salamanders was estimated in a 100 m buffer and in relation to all studied creeks (ordinal data: 'low', 'medium', 'high'). Finally, the proportions of land use in a 100 m buffer was calculated using ArcMap 10 and the CORINE land cover data (see Supplementary documents 1–8, merged to 'deciduous forest', 'mixed forest', 'coniferous forest', 'agricultural used land', 'grasslands', 'settlements'). The 100 m buffer around the reproduction creeks should cover the core area of the adult (and subadult/juvenile) population, although larger migratory distances are known (e.g. Thiesmeier 2004, Schulte et al. 2007).

We build the following models (Tabs. 2, 4, 6): (1) a 'Global model' containing all potential predictors; (2) the 'Simplified global model' using the stepAIC function from the R-package 'MASS' (i.e. model simplification by stepwise removing variables without explanatory power using model selection according to their AIC values, Burnham & Anderson 2002); (3) a model only containing the variables of the larval habitats and the landscape units ('Aquatic habitat model'); (4) a model only containing the variables of the terrestrial habitats and the landscape units ('Terrestrial habitat model'); (5) a simple model only containing the elevation as potential predictor and the landscape units ('Elevation model'). Because female European fire salamanders mainly deposit their larvae from March to May (Thiesmeier 2004) and catastrophic drift after heavy rainfall can substantially decrease larval abundance (and thereby detectability) in upper stream sections (Thiesmeier & Schumacher 1990, Reinhardt et al. 2018, Veith et al. 2019), we finally (6) built a simple 'Time model', only containing the date of field work occasions as potential predictor and the landscape units. All models were compared according to their AICc values (R package 'MuMIn'). Models with ΔAICc values < 2 compared to the best model were considered as equally plausible (Burnham & Anderson 2002). Pairwise comparison of predictors of larval presence in presence and absence creeks from the different landscapes were conducted using t-tests or Wilcoxon-tests depending on normality of distribution and homogeneity of variances (tested with Shapiro-Wilk and F-tests).

Besides presence-absence mapping, we also collected abundance data in 85 out of the 135 studied creeks by removal sampling according to Schmidt et al. (2015). Hence, in a second analysis, we compared different hierarchical models for removal sampling data (Royle 2004, Dorazio et al. 2005, Royle & Dorazio 2006). The categorical variable 'landscape unit' could not be integrated in these models; instead we first analysed the complete dataset from the whole study area (i.e. all 85 creeks were used for hierarchical modelling), and in a second step the dataset was analysed for each landscape unit separately (Fig. 1). All considered potential predictor variables (Table 1) were normalized prior to analysis. We built different generalized multinomial mixture models using pairwise combinations of the variables described above or a 'constant'-intercept-model for both abundance and detection probability (R package 'unmarked'). Survival probability was always kept constant as neither emigration/death nor immigration/birth was supposed to have taken place during the 45 min of data acquisition. All models were fitted to the data with either a Poisson or a negative binomial abundance model (see Schmidt et al. [2015] for details). The best fitting models were chosen according to the AIC values (ΔAIC values < 2: Burnham & Anderson 2002) using the R package 'AICcmodavg'.

Results

We recorded presence-absence of larval European fire salamanders in 50 creeks including the data from Sandvoß et al. (2020 in this issue) to get a larger data set and conducted removal sampling in 85 additional creeks. Consequently, we used presence-absence data from 135 creeks and quantitative data from 85 creeks (Supplementary documents 1–2).

Logistic regression models

The best-fitting model was the 'Simplified global model' (Table 2). Only elevation of the creek above sea level had a highly significant negative effect on the presence of European fire salamander larvae, while the number of pools, the abundance of potential prey and the proportion of any forest type in a 100 m buffer around the creeks had a significant positive effect (Table 3).

The factorial variable 'landscape unit' did not explain variance and was, together with several other factors, excluded from the final best-fitting GLM. Comparing these predictors in presence and absence creeks from the different landscape units revealed that presence creeks were always lower in elevation, although highly significant only in the landscape unit 'Westeifel' (Fig. 2A; W = 121.5, P < 0.001). The number of pools was always higher in presence creeks; this difference was highly significant in the 'Westeifel' (W = 494.5, P < 0.01) and the 'Hunsrück' (t = 4.3, df = 30.4, P < 0.001) and significant (t = 2.6, df = 17.9, P < 0.05) in the 'Osteifel' (Fig. 2B). Likewise, the quantity of consumable macrozoobenthos was always higher in presence creeks; this difference was significant in the 'Osteifel' (W = 75, P < 0.05) and the 'Gutland' (W = 44, P < 0.05) (Fig. 2C). Moreover, the proportion of forest cover was always higher in 100 m surroundings of the presence creeks; this difference was significant (W = 165, P < 0.05) in the 'Hunsrück' (Fig. 2D).

Table 2. Model selection overview of logistic regression models considering presence-absence data from 135 creeks in the study area.

Model	Degrees of freedom	Log Likelihood	AICc	Delta AICc	AICc weight
Simplified global model	9	-37.69	95.00	0.00	1.00
Global model	20	-32.68	113.60	18.61	0.00
Aquatic habitat model	11	-48.19	120.80	25.8	0.00
Elevation model	6	-68.54	149.80	54.79	0.00
Terrestrial habitat model	12	-62.52	151.60	56.66	0.00
Time model	6	-75.90	164.50	69.5	0.00

Table 3. Best-fitting logistic regression ('simplified global') model considering presence-absence data from 135 creeks in the study area.

Coefficient	Estimate ± SE	Z value	P value
y-intercept	-15.4 ± 8.6	-1.8	0.07
Elevation a.s.l. (m)	-0.0 ±0.0	-3.4	< 0.001
Number of pools	0.1 ± 0.0	3.7	< 0.001
Substrate	0.5 ± 0.4	1.6	0.11
Prey	1.4 ± 0.5	2.6	< 0.01
Deciduous forest	0.2 ± 0.1	2.2	< 0.05
Mixed forest	0.2 ± 0.1	2.2	< 0.05
Coniferous forest	0.2 ± 0.1	2.0	< 0.05
Grassland	0.2 ± 0.1	1.8	0.06

Hierarchical models

From the 85 creeks with quantitative data, two ('Ferschweiler2' and 'Watzbach'; Supplementary document 2) had to be excluded from analysis as outliers because of their high number of captured larvae (308 and 194, respectively; cf. overall average of only 34 ± 59 larvae, see Supplementary document 2). All attempts to transform the removal data to include all creeks in hierarchical modelling failed. Furthermore, the environmental variable 'substrate' could not be used here due to missing data from the 'Moseltal' and the 'Hunsrück'. Hence, 512 candidate models were compared, and there was only one best-fitting, negative binomial model in which high larval abundances were best predicted by a high number of pools and detection probability was positively influenced by later mapping date (Fig. 3, Table 4).

Considering only the removal data of the 20 creeks from the 'Osteifel' (Fig. 1), there was neither agricultural land

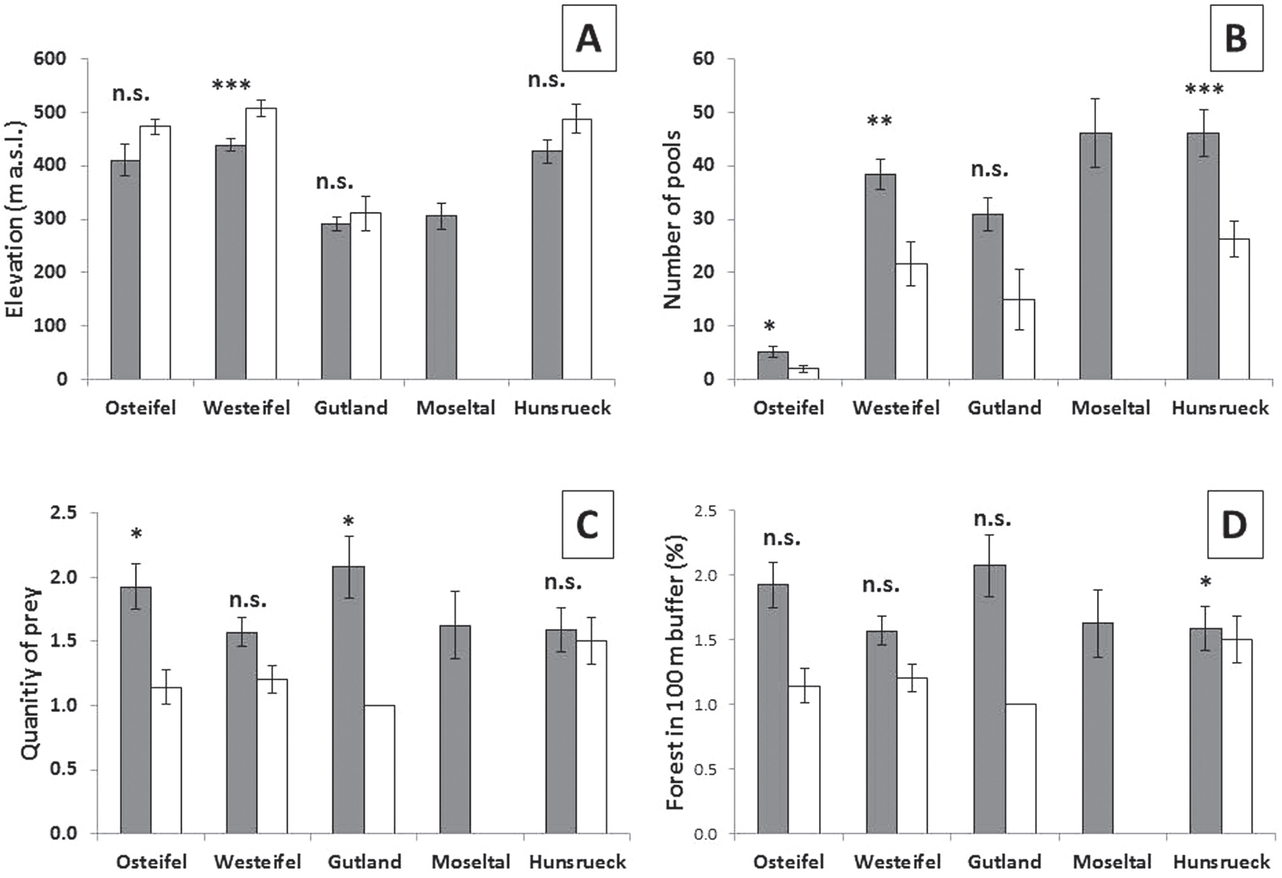

Figure 2. Pairwise comparison of predictor variables (A = elevation, B = number of pools C = quantity if potential prey, D = proportion of forest cover within 100 m), which were significant in the best logistic regression model, between presence (grey bars) and absence (open bars) creeks and per landscape unit; all values are means ± SE; significance levels are given: $P < 0.001$ (***), $P < 0.01$ (**), $P < 0.5$ (*) and $P > 0.5$ (n.s.); absence creeks were missing in the Moseltal.

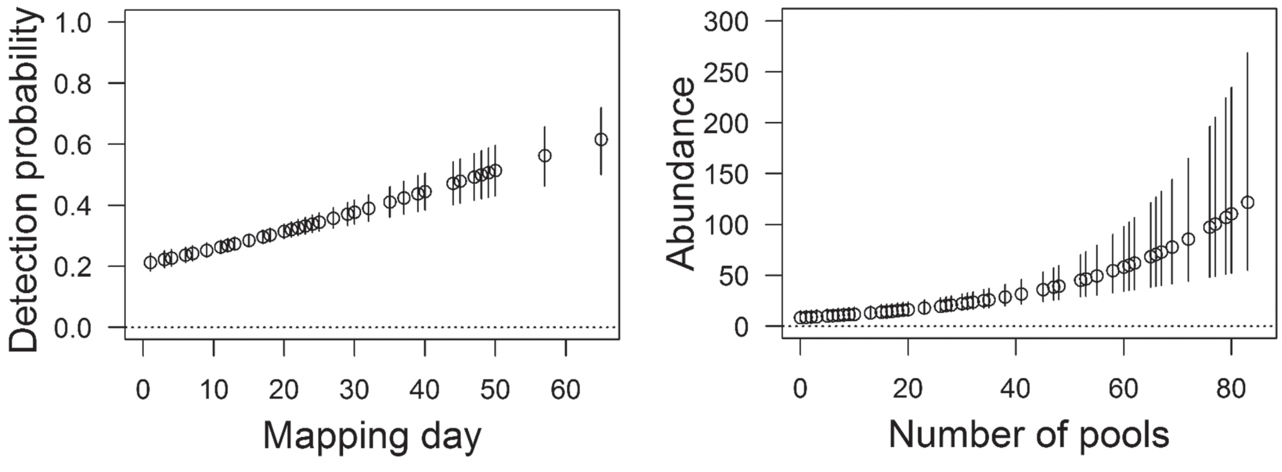

Figure 3. In the best fitting hierarchical model, detection probabilities of European fire salamander larvae increased with later capture occasions and the larval abundances increased with the number of pools in the studied creek sections. All values are predicted means ± 95% confidence intervals.

Table 4. Predictors of larval abundances and detection probabilities according to the best-fitting hierarchical model for the entire study area and for landscape units, respectively. All data were fitted to a negative binomial model. Arrows indicate positive (↑) or negative (↓) influence of factors on larval abundance.↑↓

Landscape Unit	ΔAIC	Abundance (Lambda)	Detection probability (p)
All data (N = 83)	0	Number of pools ↑	Mapping day ↑
Osteifel (N = 20)	0	Number of pools ↑	Coniferous forest ↓
	0.23	Number of pools ↑	Tilt parallel ↑
	0.28	Prey ↑	Coniferous forest ↓
	0.46	Substrate ↑	Coniferous forest ↓
	0.51	Prey ↑	Tilt parallel ↑
	0.64	Substrate ↑	Tilt parallel ↑
	0.74	Tilt perpendicular ↑	Tilt parallel ↑
	0.76	Number of pools ↑	Hiding places ↓
	0.86	Tilt perpendicular ↑	Coniferous forest ↓
	0.98	Prey ↑	Hiding places ↓
	1.22	Substrate ↑	Hiding places ↓
	1.39	Number of pools ↑	Deciduous forest ↑
	1.42	Tilt perpendicular ↑	Hiding places ↓
	1.64	Prey ↑	Deciduous forest ↑
	1.76	Substrate ↑	Deciduous forest ↑
Westeifel (N = 16)	0	Number of pools ↑	Width of creek ↑
	0.55	Number of pools ↑	Tilt perpendicular ↓
	0.56	Number of pools ↑	Hiding places ↓
	0.89	Number of pools ↑	Number of pools ↑
	1.56	Number of pools ↑	constant (.)
Gutland (N = 7)	0	Predatory fish ↓	Substrate ↑
	0	Coniferous forest ↓	Substrate ↑
	0.35	Deciduous forest ↑	Substrate ↑
	0.55	Number of pools ↑	Substrate ↑
	0.58	Width of creek ↓	Substrate ↑
	0.6	constant (.)	Substrate ↑
	0.69	Hiding places ↓	Substrate ↑
Gutland (continued)	0.71	Agriculture ↓	Substrate ↑
	1.05	Elevation a.s.l. ↓	Substrate ↑
	1.2	Agriculture ↓	Tilt parallel ↑
	1.52	Predatory fish ↓	Tilt parallel ↑
	1.52	Coniferous forest ↓	Tilt parallel ↑
	1.53	Mapping day ↓	Substrate ↑
	1.98	Grassland ↑	Substrate ↑
Moseltal (N = 8)	0	Mapping day ↓	constant (.)
	0.25	Tilt perpendicular ↓	Width of creek
	0.25	Width of creek ↑	Tilt perpendicular ↓
	0.48	Mapping day ↓	Hiding places ↓
	0.78	Prey ↑	constant (.)
	1.15	Prey ↑	Hiding places ↓
	1.16	Mapping day ↓	Width of creek
	1.24	Prey ↑	Tilt perpendicular ↓
	1.27	Agriculture ↓	Tilt perpendicular ↓
	1.34	Mapping day ↓	Tilt perpendicular ↓
	1.79	Mapping day ↓	Width of creek
	1.83	Mapping day ↓	Number of pools ↑
	1.85	Tilt perpendicular ↓	Agriculture
	1.86	Mapping day ↓	Coniferous forest
	1.88	Mapping day ↓	Deciduous forest
	1.96	Mapping day ↓	Agriculture
	1.99	Mapping day ↓	Prey
	1.99	Mapping day ↓	Mapping day
Hunsrück (N = 32)	0	Number of pools ↑	Width of creek ↓
	1.44	Agriculture ↓	Width of creek ↓
	1.48	Mapping day ↓	Width of creek ↓
	1.89	Settlement ↓	Width of creek ↓

nor settlements around the studied creeks and no predatory fish were found, so these three variables were excluded from further analysis. Consequently, 392 candidate models were compared, from which 15 negative binomial models with ΔAICc values < 2 were considered as plausible (Burnham & Anderson 2002). The best-fitting model estimated again more larvae in creek sections with more pools and, furthermore, an (indirect) negative effect of the terrestrial habitat (here, proportion of coniferous forest in 100 m buffer) on the detection probability (Table 4). Each of the four remaining 14 plausible models estimated a positive effect of number of pools, a high amount of prey or stony substrate on larval abundances (Table 4). Similarly, in four models, detection probability was negatively influenced indirectly by the proportion of coniferous forest in the vicinity of 100 m (Table 4).

When only the 17 creeks from the landscape unit 'Westeifel' (Fig. 1) were considered, again, neither predatory fish nor agriculture nor settlement around the creeks could be observed, so these variables were again excluded from further analysis. In addition, the 'Watzbach' once more had to be excluded as outlier. 392 candidate models were compared, and in all four plausible negative binomial models the number of pools positively affected larval abundance, while no or varying factors influenced detection probabilities (Table 4).

Also from the dataset 'Gutland' (eight creeks only), 'Ferschweiler2' had to be excluded as outlier (see above). Here, no settlement was in the 100 m surroundings of creeks, so this variable was excluded. 512 candidate models were fitted, and 14 negative binomial models were considered plausible. Varying factors influenced larval abundances, in two models each, presence of predatory fish, agriculture and proportions of coniferous forest around creeks negatively affected abundance (Table 4). In eleven models, stony substrate enhanced detection probability (Table 4).

Data from eight creeks only were available in the 'Moseltal' (Fig. 1). No predatory fish, mixed forest, grasslands and settlements could be observed, and for half of the creeks no data on substrate was available. Hence, only eleven factors were considered in modelling. Among the 18 plausible models, larval abundances mostly decreased with increasing date (ten times), and detection probability was most often negatively affected by the perpendicular tilt (Table 4).

From the landscape unit 'Hunsrück', 32 creeks could be considered for abundance modelling (Fig. 1), but the variable 'substrate' had to be excluded due to missing data, so that 512 candidate models were compared. Among the four plausible models, different variables affected abundances, but creek width always negatively influenced detection probabilities (Table 4).

Discussion

Presence-absence of European fire salamander populations

With regard to habitat suitability, no influence of the natural landscape units could be observed, suggesting that only the predictors identified by our best model should influence the occurrences of European fire salamander larvae in the entire study region. Elevation a.s.l. had a negative effect on European fire salamander presence (Table 3). This is in accordance with Thiesmeier & Dalbeck (2011), who found an elevational distribution limit of 600 m a.s.l. of the species in the federal state of North Rhine-Westphalia, which adjoins our study area to the north. In the 'Westeifel', presence creeks were significantly lower in elevation compared to absence creeks (Fig. 2A). Thiesmeier & Dalbeck (2011) described the elevational limit of 600 m a.s.l. in North Rhine-Westphalia on the basis of more recent distribution data; however, in the 1990s, single European fire salamanders in this region were also found at higher elevations. Moreover, Feldmann & Klewen (1981) reported European fire salamander populations in Westphalia from even above 800 m a.s.l.. In Rhineland-Palatinate, several European fire salamander records from the 1980/90s were above 600 m a.s.l. (Bitz et al. 1996). Nevertheless, a preference of the European fire salamander for lower elevations in mountainous areas is consistent in both federal states. Therefore, we assume that the European fire salamander populations at high elevations of our study area (Bitz et al. 1996, Wagner et al. 2017) live under sub-optimal conditions. Moreover, such areas are almost completely covered with conifers (Wagner et al. 2017).

An increasing number of pools, which can serve as a proxy for the heterogeneity of a study creek, is known to positively affect the aquatic habitat of European fire salamander larvae (Baumgartner et al. 1999, Werner et al. 2014, Schmidt et al. 2015, Wagner et al. 2020 in this issue) (Table 4). This could be observed in all our landscape units (Fig. 2B), although not always significantly. The higher the estimated amount of prey, in our creeks mainly *Gammarus fossarum*, the more likely European fire salamander larvae were present (Fig. 2, Table 3), which also makes sense biologically. Finally, high proportions of forest around the creeks led to high presence probability, but without an observed negative effect of coniferous forests (Table 3), although land-use change from deciduous into coniferous forests in the terrestrial habitat of European fire salamanders is listed as a major threat for the species (e.g. Thiesmeier 2004). When comparing predictor variables of presence and absence creeks from different landscape units separately, obvious differences are found (Fig. 2). To sum up, a typical reproduction creek for *S. salamandra* in this case study across our study region is at low elevation, contains a high number of pools and consumable macrozoobenthos, and is situated inside a forest. This statistically confirms the well-known preferences of the species (e.g. Thiesmeier 2004, Thiesmeier & Dalbeck 2011).

Larval abundance and detection probability

With regard to larval abundance, we found both similarities and differences in predictors among landscape units (Fig. 3, Table 4). Like for presence probability, there was a positive influence of increasing number of pools on larval abundance (Table 4) when creeks from the entire study area were included in hierarchical modelling (Fig. 3, Table 4) as well as for the landscape units 'Osteifel', 'Westeifel' and 'Hunsrück' (Table 4). Similarly, the later the capture occasion the lower the estimated larval abundance was estimated in our respective likely models (Table 4). Later in the field season, numerous larvae are likely to have been drifted down-stream, out of the studied creek sections (cf. Thiesmeier & Schumacher 1990, Reinhardt et al. 2018, Veith et al. 2019). In early summer 2016 and 2018, heavy rainfalls increased water flow in many study creeks. In addition, larval abundance will decrease with time due to the onset of metamorphosis. The positive effect of higher quantities of consumable macrozoobenthos on the abundance of European fire salamander larvae (Table 4) seems plausible; it should reduce cannibalism of earlier larvae by older conspecifics (see Degani et al. 1980 for *S. infraimmaculata*). Increasing agricultural land-use near creeks negatively affects larval abundance of European fire salamanders in the landscape units 'Gutland', 'Moseltal' and 'Hunsrück' (Table 4). Apart from the absence of suitable forests as terrestrial habitats (historical agricultural expansion), the application of agrochemicals may affect larval European fire salamanders. They may affect terrestrial and aquatic life-stages (reviewed by Mann et al. 2009). In a laboratory study, environmentally relevant herbicide concentrations led to lethargy and decreased predatory behaviour in larval European fire salamanders (Mandrillon & Saglio 2007). In the field, herbicide contamination of creeks may result in decreased body size of European fire salamander larvae, which vice versa can increase drift rates of smaller larvae into creek section with predatory fish (cf. Veith et al. 2019). Hence, long-term agrochemical contamination of creeks could affect populations of European fire salamanders via the larval stages in direct and indirect ways. Finally, in the 'Osteifel' four out of 15 plausible models suggest a positive effect of stony substrate on larval abundance (Table 4). This may have an immediate positive effect on the aquatic habitat (i.e. the slate stones in creeks from this landscape unit forms suitable hiding places for larvae, which could also lower predatory pressure) or an indirect effect (capturing larvae under slate stones is easier and results in a higher detection probability). Further predictors are plausible (e.g. decreasing abundances with increasing elevation [see above] or presence of predatory fish: Table 4), while others may be non-causal artefacts (e.g. increasing abundance with increasing perpendicular tilt: Table 4).

When creeks from the entire study area are considered, detection probabilities increase with later field season (Fig. 3, Table 4). At first sight, this seems counterintuitive to the often observed negative effect of later capture occasions on larval abundances (Table 4). However, this may be explained by the larger body size of later larval stages or lower water levels in later season, which both increase detection, or the simple fact that at least some field workers improved their detection efforts. The negative effect of coniferous forest and the positive effect of deciduous forest surrounding the creeks should primarily affect the adult population, so their effect on larval abundance is indirect. The adult European fire salamander populations are usually smaller in coniferous and larger in deciduous forests (Thiesmeier 2004), and consequently less or more larvae will be deposited in the respective creeks. Likewise, increasing perpendicular tilt lowers accessibility of creek sections for females (Manenti et al. 2009), and likewise the positive effect of the (parallel) tilt of the creek section could be indirect. A faster water flow throughout the year could force larvae to accumulate in pools and under stones/dead wood, where they are easier to detect. In the 'Gutland', a positive effect of a coarse, stony substrate on the detection probability is observed (Table 4; cf. discussion above on effects on abundance). In the 'Hunsrück', increasing width of the creek section decreased detection probability, which could be explained with widths up to 2–3.5 m in this landscape unit (Supplementary documents 1–2). In such wide streambeds many larvae should be overlooked.

Conclusions

In our study, local habitat suitability models developed from different areas mostly identified the same biotic and abiotic parameters as major predictors of the abundance of larval European fire salamanders. Presence of European fire salamanders in north-western Rhineland-Palatinate, Germany, is positively affected mainly by low elevation, high numbers of pools, abundant consumable macrozoobenthos within creeks and high proportions of forest cover around creeks. Larval abundance is usually positively affected by higher number of pools (11 out of 56 overall models = 20%) and negatively by later mapping dates (12 out of 56 overall models = 21%). Detection probability is explained by various factors in the best fitting models, but using all data sets, again later mapping day had a negative effect. Consequently, such preferences of this wide-ranging urodelan species, which are easy to determine, can be used to differentiate between naturally caused absence or low abundance of European fire salamander larvae (e.g. due to unsuitable aquatic or terrestrial habitats, heavy rainfalls causing catastrophic drift) and extinctions/population declines due to emerging infectious diseases caused by invasive pathogens.

Acknowledgements

We would like to thank the SGD Nord (Koblenz) for the permissions to conduct the removal sampling with larval European fire salamanders. For funding we are grateful to the DGHT Wilhelm-Peters-Fonds, the Zoo Landau, the Nikolaus-Koch-Stiftung and especially the Federal Agency for Nature Conservation (Bundesamt für Naturschutz, BfN) within the frame of the R+D project "Monitoring und Entwicklung von Vorsorgemaßnahmen zum Schutz vor der Ausbreitung des Chytridpilzes *Batrachochytrium salamandrivorans* ("*Bsal*") im Freiland"). The following student collaborators kindly contributed to field work: in alphabetical order, Anna-Lea Beckenbauer, Daniel Christiansen, Melissa Dzafic, Sarah Ehresmann, Charlotte Faul, Lara Feiler, Lisa Geimer, Mareike Hansen, Mario Harzheim, Marie-Josiane Haupert, Nicolas Janecke, Phillip König, Alanah Kripp, Hannah Kruft, Sammy-Jo Lorenz, Lisa Mahla, Florian Merten, Marlene Mürz, Leonie Nikolai, Luis Pfeifer, Niklas Richter, Simone Ruf, Marie Sandvoß, Malwina Schafft, Lisa Schmitz, Elena Schommer, Hannah Schürmann, Katrin Schwerdtfeger, Annika Segschneider and Giorgia Stincone.

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Supplementary data

The following data are available online:

Supplementary document 1. Raw data from presence-absence mapping of larval European fire salamanders.

Supplementary document 2. Raw data from removal sampling of larval European fire salamanders.

Supplementary document 3. Proportions of different land use in a 100 m buffer around all studied creeks, divided into presence and absence sites.

Supplementary document 4. Proportions of different land use in a 100 m buffer around the studied creeks from the 'Westeifel', divided into presence and absence sites.

Supplementary document 5. Proportions of different land use in a 100 m buffer around the studied creeks from the 'Osteifel', divided into presence and absence sites.

Supplementary document 6. Proportions of different land use in a 100 m buffer around the studied creeks from the 'Gutland', divided into presence and absence sites.

Supplementary document 7. Proportions of different land use in a 100 m buffer around the studied creeks from the 'Moseltal'.

Supplementary document 8. Proportions of different land use in a 100 m buffer around the studied creeks from the 'Hunsrück', divided into presence and absence sites.

Supplementary document 1. Raw data from presence-absence mapping of larval fire salamanders. For variable definitions see Table 1; NA = not applicable.

LandscapeUnit	Creek	Presence	Date	Day	Elevation(m)	Pools	Width(m)	Fish	Tilt(°)	Tilt_perpendicular(°)	Substrate	Prey	Hiding	Decid100	Mixed100(%)	Conif100(%)	Agri100(%)	Grass100(%)	Settl100(%)	Decid500(%)	Mixed500(%)	Conif500(%)	Agri500(%)	Grass500(%)	Settl500(%)
Gutland	Diebach	1	15 June 2016	21	270	42	0.4	0	15	5	3	1	3	30.1	0	50.4	0	19.5	0	36.1	0	39.5	0	24.4	0
Gutland	Eschbach_2	1	25 June 2016	31	344	21	0.8	0	8	14	2	2	3	0	0	100	0	0	0	0	0	63.8	27.5	8.7	0
Gutland	Ferschweiler_1	1	18 May 2018	8	321	45	0.5	0	15	6	3	2	3	75.6	24.4	0	0	0	0	44.8	11.9	0	1.6	40.2	1.6
Gutland	Ferschweiler_2	1	18 May 2018	8	324	21	1.5	0	5	11	3	1	2	100	0	0	0	0	0	87.7	0	0	3.2	9.1	0
Gutland	Finkenbach	0	27 May 2016	2	399	16	0.7	0	10	4	1	1	3	0	0	0	0	100	0	0	8.2	0	17.1	74.7	0
Gutland	Fleissbach	1	16 May 2018	6	357	31	0.5	1	30	7	3	1	3	0	0	100	0	0	0	3.9	0	96.1	0	0	0
Gutland	Gutenbach	1	14 May 2018	4	288	30	1.9	0	8	19	1	3	3	98.8	0	0	1.2	0	0	46.8	0	6.0	47.2	0	0
Gutland	Hauchenbach	1	9 June 2018	30	256	19	1.5	0	2	7	3	3	3	12.9	85.9	0	0	1.2	0	31.6	44.8	0	5.0	18.6	0
Gutland	Ingendorf	1	11 June 2018	32	352	47	0.5	0	3	1	1	3	3	100	0	0	0	0	0	75.0	0	0	25.0	0	0
Gutland	Irrel_1	1	18 May 2018	8	233	38	0.5	0	5	15	2	3	2	100	0	0	0	0	0	70.3	0	0	23.1	6.6	0
Gutland	Irrel_2	1	18 May 2018	8	217	34	1.0	0	2	6	1	2	1	69	0	0	0	31	0	64.5	0	0.3	11.5	21.5	2.2
Gutland	Mattelsbach	1	5 June 2016	11	277	12	0.5	0	5	3	1	3	2	100	0	0	0	0	0	53.8	22.9	0	15.3	8.0	0
Gutland	Pfaffenderbach	1	15 June 2016	21	249	38	2.0	0	10	1	1	1	3	54.7	0	0	0	45.3	0	31.4	0	0	20.5	47.5	0.6
Gutland	Schanzbach	1	1 June 2016	7	300	24	0.8	0	4	2	3	2	2	100	0	0	0	0	0	70.1	0	2.7	27.2	0	0
Gutland	Schleidbach	0	5 June 2016	11	309	16	0.5	0	6	3	2	1	1	100	0	0	0	0	0	65.4	3.6	14.8	15.7	0.5	0
Gutland	Silberbach	0	5 June 2016	11	291	28	2.0	0	8	2	1	1	3	80.2	0	0	19.4	0.4	0	51.4	0	0	27.9	20.6	0
Gutland	Stockheimerbach	0	1 June 2016	7	245	0	0.5	0	1	7	1	1	2	51.8	0	0	48.2	0	0	54.1	0	0	24.9	21.0	0
Hunsrueck	Alkenbach	1	23 May 2017	9	327	62	0.8	0	5	15	3	3	2	100	0	0	0	0	0	63.7	7.4	0	0	28.8	0
Hunsrueck	Altbach	0	12 June 2017	29	484	17	3.5	1	1	19	3	1	2	0	0	100	0	0	0	4.9	14.0	33.6	2.1	28.3	17.1
Hunsrueck	AltlayerNebenbach	1	11 June 2016	17	358	80	0.4	0	8	3	1	1	2	100	0	0	0	0	0	75.3	0	0	24.7	0	0
Hunsrueck	Antenbachsgraben	1	12 June 2016	18	302	28	0.5	0	5	2	3	1	2	0	26.0	0	0	74.0	0	26.4	13.4	0	0	60.2	0
Hunsrueck	Bernsbach	1	29 May 2017	15	309	15	1.0	0	5	5	NA	3	2	100	0	0	0	0	0	100	0	0	0	0	0
Hunsrueck	Dammfloesschen	0	12 June 2017	29	541	38	0.5	0	7	6	3	1	2	0	0	95.5	0	0	4.5	0	0	69.1	0	2.1	28.8
Hunsrueck	DrohnerNebenbach	0	11 July 2016	47	563	0	0.3	0	6	1	1	1	2	0	0	100	0	0	0	0	24.8	75.2	0	0	0
Hunsrueck	Eisbach	1	10 June 2017	27	585	35	1.0	0	5	7	3	1	2	100	0	0	0	0	0	78.9	0	21.1	0	0	0
Hunsrueck	Eschbach	0	18 June 2016	24	403	38	1.5	0	3	3	3	1	2	0	53.2	0	46.8	0	0	0	18.2	0	77.9	0	3.9
Hunsrueck	Forstelbach	0	20 June 2017	37	559	32	1.3	0	2	12	3	1	2	37.5	0	62.5	0	0	0	72.1	6.2	20.8	0	1	0
Hunsrueck	Gehresbach	1	27 June 2017	44	311	20	1.0	0	5	9	NA	1	2	100	0	0	0	0	0	100	0	0	0	0	0
Hunsrueck	Hottenbach	1	7 June 2017	24	577	66	1.0	0	3	2	NA	1	3	0	82.4	17.6	0	0	0	0	38.9	31.1	0	0	0
Hunsrueck	Kalmbach	1	6 June 2017	23	439	30	1.0	0	8	1	NA	2	3	39.2	0	60.8	0	0	0	71.1	0	12.5	0	16.4	0

LandscapeUnit	Creek	Presence	Date	Day	Elevation(m)	Pools	Width(m)	Fish	Tilt(°)	Tilt_perpendicular(°)	Substrate	Prey	Hiding	Decid100	Mixed100(%)	Conif100(%)	Agri100(%)	Grass100(%)	Settl100(%)	Decid500(%)	Mixed500(%)	Conif500(%)	Agri500(%)	Grass500(%)	Settl500(%)
Hunsrueck	Kieselbornbach	1	23 June 2016	29	449	69	1.5	1	6	2	3	1	2	74.2	0	25.8	0	0	0	32.2	0	67.8	0	0	0
Hunsrueck	Kiweringsbach	1	23 May 2017	9	441	83	1.0	0	2	3	3	1	1	96.8	0	3.2	0	0	0	52.8	0	45.6	0	1.5	0
Hunsrueck	Koenigsbach	0	10 June 2017	27	577	30	1.5	1	2	1	1	1	3	38.8	0	61.2	0	0	0	59.1	1.0	39.9	0	0	0
Hunsrueck	Korlingen	1	15 May 2017	1	254	77	0.8	0	7.5	9	1	3	2	99.1	0	0	0	0	0.9	44.2	0	0	15.9	14.9	25.0
Hunsrueck	Krebsbach	1	31 May 2017	17	548	60	1.5	0	3	7	2	1	3	0.3	0	99.7	0	0	0	39.6	0	60.4	0	0	0
Hunsrueck	Lautenbach	1	9 July 2016	45	543	62	0.5	0	13	7	3	1	1	93.9	0	6.1	0	0	0	81.5	0	18.5	0	0	0
Hunsrueck	Liedchenbach	1	13 July 2017	60	360	52	1.0	0	1	9	1	2	2	100	0	0	0	0	0	59.4	0	0	5.0	35.7	0
Hunsrueck	Liefbach	1	29 May 2017	15	516	61	0.5	0	1	2	NA	1	2	100	0	0	0	0	0	20.5	0	79.5	0	0	0
Hunsrueck	LorscheiderNebenbach	1	31 May 2016	6	468	20	0.5	0	7	2	3	1	2	53.2	0	38.5	8.3	0	0	17.7	2.1	41.2	38.9	0	0
Hunsrueck	Markenbach	1	29 June 2016	35	388	45	0.4	0	18	6	1	2	2	0	0	100	0	0	0	0	22.1	33.7	0	44.1	0.0
Hunsrueck	Misselbach	1	26 May 2016	1	509	27	1.0	1	2	3	1	1	2	0	49.9	50.1	0	0	0	0	18.9	81.1	0	0	0
Hunsrueck	Moertschelbach	1	21 May 2017	7	315	53	1.5	0	10	9	NA	3	3	38.5	0	0	0	61.5	0	25.8	0	0	36.6	37.6	0
Hunsrueck	Morschbach	1	11 June 2016	17	407	41	1.0	0	2	6	3	2	2	100	0	0	0	0	0	42.9	0.4	0	56.7	0	0
Hunsrueck	Rauruwer	0	5 June 2016	11	480	30	0.5	0	1	1	3	1	1	0	0	0	0	0	0	20.1	0	79.9	0	0	0
Hunsrueck	Riedfloss	0	31 May 2017	17	588	30	1.5	1	2	2	1	1	1	0	0	41.1	0	58.9	0	22.9	0	45.9	0	31.2	0
Hunsrueck	Sattelbach	1	24 June 2016	30	566	62	2.0	1	8	3	1	1	3	42.1	0	57.9	0	0	0	53.9	0	46.1	0	0	0
Hunsrueck	Schastebach	1	29 May 2017	15	425	55	1.0	0	4	1	3	2	2	100	0	0	0	0	0	40.9	4.5	50.3	0	4.3	0
Hunsrueck	Steierbach	0	18 July 2017	65	315	23	2.0	0	5	11	NA	1	2	88.4	0	11.6	0	0	0	45.9	0	53.4	0.7	0	0
Hunsrueck	Struntbach	0	11 July 2016	47	443	35	0.4	0	1	2	1	3	2	0	0	50.8	0	49.2	0	15.0	0	39.3	17.0	28.7	0
Hunsrueck	Thommerbach	0	26 May 2016	1	412	16	1.5	0	4	6	3	1	2	98.4	0	0	0	1.6	0	30.1	0	0	7.6	51.8	10.6
Moseltal	Morschbach	1	11 June 2016	17	407	41	1.0	0	2	6	3	2	2	100	0	0	0	0	0	42.9	0.4	0	56.7	0	0
Moseltal	AhringsbachNebenbach	1	18 June 2016	24	345	65	0.5	0	10	9	3	1	2	88.4	0	0	11.6	0	0	55.0	0	0	45.0	0	0
Moseltal	CompenerBaechelchen	1	6 June 2017	23	230	45	1.0	0	3	7	NA	1	3	100	0	0	0	0	0	97.2	0	0	0	0	2.8
Moseltal	Lierenbach	1	16 June 2016	22	275	58	1.0	0	5	10	3	2	3	8.5	0	91.5	0	0	0	55.6	0	44.4	0	0	0
Moseltal	Schafhausbach	1	8 June 2017	25	395	18	0.2	0	3	3	NA	1	2	5.4	0	79.1	15.5	0	0	33.1	0	18.3	48.6	0	0
Moseltal	Speinbach	1	5 June 2017	22	280	28	0.5	0	5	9	NA	2	3	68.7	0	31.3	0	0	0	37.5	0.4	56.0	6.1	0	0
Moseltal	Viebach	1	8 June 2017	25	278	41	0.7	0	3	11	NA	1	3	86.4	0	0	13.6	0	0	38.3	0	28.6	33.1	0	0
Moseltal	Waldrach	1	17 May 2017	3	233	72	0.8	0	15	2	3	3	3	87.4	0	12.6	0	0	0	45.4	0	34.0	0	0	20.6
Osteifel	Aelsbach	1	19 June 2018	40	471	2	1.0	0	3	6	3	1	1	66.2	0	0	0	33.8	0	32.7	0	22.2	0	45.0	0
Osteifel	Ahbach	1	29 June 2018	49	501	13	0.9	0	4	18	3	2	3	65.9	0	34.1	0	0	0	70.4	0	28.9	0	0.6	0
Osteifel	Alfenbach	1	20 June 2018	41	344	7	0.8	0	4	20	3	2	2	41.1	0	58.9	0	0	0	40.4	0	35.5	0	24.1	0
Osteifel	Auschsbach	1	14 June 2016	20	237	3	0.5	0	5	21	3	2	1	100	0	0	0	0	0	80.5	0	4.0	0	0	15.4

LandscapeUnit	Creek	Presence	Date	Day	Elevation(m)	Pools	Width(m)	Fish	Tilt(°)	Tilt_perpendicular(°)	Substrate	Prey	Hiding	Decid100	Mixed100(%)	Conif100(%)	Agri100(%)	Grass100(%)	Settl100(%)	Decid500(%)	Mixed500(%)	Conif500(%)	Agri500(%)	Grass500(%)	Settl500(%)
Osteifel	Baubach	0	6 June 2018	27	499	1	0.5	0	3	1	1	1	1	0	0	100	0	0	0	38.5	0	58.7	0	2.8	0
Osteifel	BodenbacherBach	0	19 June 2018	40	540	0	0.5	0	3	3	3	1	3	0	86.0	14.0	0	0	0	0	33.4	59.2	0.3	7.2	0
Osteifel	Drasselbach	1	20 June 2018	41	323	2	0.2	0	1	8	3	1	1	0	0	100	0	0	0	40.5	0	42.9	0	0	10.4
Osteifel	Exbach	0	24 June 2018	44	443	1	0.5	0	3	3	3	2	1	2.4	46.0	0	0	51.6	0	38.0	35.3	0	0	26.7	0
Osteifel	Gilgenbach	1	27 June 2018	47	380	6	0.5	0	4	22	3	2	2	0	21.9	78.1	0	0	0	0	39.6	60.4	0	0	0
Osteifel	Girrelsbach	1	27 June 2018	47	377	9	0.5	0	3	16	3	2	3	35.9	0	64.1	0	0	0	62.7	0	37.3	0	0	0
Osteifel	Gruenbach	1	29 June 2018	49	468	7	0.5	0	5	2	3	2	1	25.7	0	74.3	0	0	0	39.3	0	40.0	0	20.7	0
Osteifel	Hasbach_1	1	6 June 2018	27	575	0	0.6	0	4	9	3	2	1	100	0	0	0	0	0	5.0	0	81.5	0	13.6	0
Osteifel	Hasbach_2	1	6 June 2018	27	516	6	0.5	0	6	10	1	3	1	100	0	0	0	0	0	100	0	0	0	0	0
Osteifel	Kaulbach	0	30 June 2018	50	429	2	1.5	0	4	1	3	1	2	0	0	0	0	100	0	0	0	2.6	23.2	72.5	1.6
Osteifel	Krekelbach	1	20 June 2018	41	409	8	0.3	0	7	17	3	2	1	100	0	0	0	0	0	65.8	0	0	0	34.2	0
Osteifel	Laufenbach	0	24 June 2018	44	440	1	0.3	0	24	22	1	1	1	99.4	0	0	0	0.6	0	64.9	0	10.1	6.1	19.0	0
Osteifel	Mirbach	1	14 June 2016	20	225	2	0.4	0	3	1	3	1	1	25.2	0	74.8	0	0	0	55.7	0	36.8	0	0.1	7.4
Osteifel	Nohnerbach	0	29 June 2018	49	500	6	0.5	0	5	11	1	1	1	20.5	0	79.5	0	0	0	29.8	0	57.5	0	12.7	0
Osteifel	Rudersbach	1	30 June 2018	50	509	3	0.7	0	2	7	1	3	1	38.6	0	61.4	0	0	0	25.1	4.9	58.6	0	11.5	0
Osteifel	Wiesbach	0	30 June 2018	50	462	3	0.5	0	1	2	1	1	2	100	0	0	0	0	0	6.2	24.6	69.2	0	0	0
Westeifel	Arzfeld	1	6 June 2018	27	493	47	0.6	0	8	6	3	2	1	95.0	0	0	0	5.0	0	70.6	9.4	0	9.4	20.1	0
Westeifel	Berkotherbach	1	25 June 2016	31	439	49	1.2	0	15	18	1	2	2	0	100	0	0	0	0	24.0	31.5	14.0	10.5	20.0	0
Westeifel	Bisselbach	1	7 July 2018	57	511	5	1.7	0	3	7	1	3	1	22.2	77.8	0	0	0	0	31.3	0	56.1	0	12.6	0
Westeifel	Bosborn	1	8 July 2016	44	502	43	0.6	0	15	5	1	1	3	100	0	0	0	0	0	38.3	0	33.2	20.3	8.2	0
Westeifel	Braubach	1	28 June 2016	34	405	15	0.8	0	8	30	1	1	3	0	50.4	49.6	0	0	0	0	37.2	26.2	4.4	0	15.0
Westeifel	Bueschbach	1	17 June 2018	38	462	22	0.5	0	6	8	2	2	2	86.0	0	0	0	10.3	0	31.0	0	0	0	68.6	0.5
Westeifel	Eiergraben	1	18 June 2016	24	446	28	0.3	0	20	17	1	1	3	98.2	0	0	0	1.8	0	45.7	0	16.2	0	38.0	0
Westeifel	Eigelscheid	1	12 June 2017	29	469	46	0.3	0	4	1	1	1	2	0	0	100	0	0	0	0	0	98.3	0	1.7	0
Westeifel	Eschweiler	1	9 July 2016	45	451	23	2.0	0	5	25	1	1	3	93.6	0	0	6.4	93.6	0	46.3	13.4	0	36.0	4.3	0
Westeifel	Ettelbach	0	10 July 2016	46	490	21	0.6	1	3	13.5	1	1	1	0	0	73.0	0	27.0	0	0.3	6.4	52.0	13.2	28.1	0
Westeifel	Ettelbach_2	0	23 May 2018	13	517	7	0.5	0	2	11	1	1	2	0.0	0	100.0	0	0	0	24.9	0	60.4	3.6	11.0	0
Westeifel	Fussbach	1	14 June 2016	20	360	16	1.0	0	7	19	1	1	3	86.5	0	0	0	13.5	0	48.8	0	0	8.5	42.8	0
Westeifel	Gaybach	1	19 June 2016	25	333	19	0.6	0	3	12	1	1	3	100	0	0	0	0	0	92.9	0	7.1	0	0	0
Westeifel	Gecklenbach_Seitenarm	1	19 June 2016	25	467	23	1.0	0	6	14	1	1	2	15.9	0	0	0	84.1	0	14.0	0.0	0	45.9	40.0	0
Westeifel	Gemuenderbach	1	18 May 2018	8	416	48	0.8	0	15	15	2	1	3	0	98.3	1.7	0	0	0	13.0	42.0	13.1	31.9	0	0
Westeifel	Grasmaerchen	1	17 May 2018	7	225	77	0.5	0	15	6	3	2	2	29.0	0	0	0	71.0	0	31.3	0	0	12.8	55.9	0

LandscapeUnit	Creek	Presence	Date	Day	Elevation(m)	Pools	Width(m)	Fish	Tilt(°)	Tilt_perpendicular(°)	Substrate	Prey	Hiding	Decid100	Mixed100(%)	Conif100(%)	Agri100(%)	Grass100(%)	Settl100(%)	Decid500(%)	Mixed500(%)	Conif500(%)	Agri500(%)	Grass500(%)	Settl500(%)
Westeifel	Grieselbergerbach	1	25 June 2016	31	478	59	0.4	0	7	10	1	1	2	99.3	0	0.7	0	0	0	39.0	0	17.7	0	43.3	0
Westeifel	Grimbach	0	28 June 2016	34	447	8	0.7	0	3	31	1	1	1	93.2	0	0	0	6.8	0	26.5	0	0	7.9	65.6	0
Westeifel	Heilhausen	1	2 July 2016	38	454	40	0.3	0	15	1	1	1	2	72.8	0	0	0	27.2	0	32.6	0	0	7.9	59.5	0
Westeifel	Heimbach	1	28 May 2018	18	350	45	1.2	0	10	19	1	2	3	100	0	0	0	0	0	84.0	0	1.2	14.8	0	0
Westeifel	Heimersdell	1	18 June 2016	24	390	31	1.0	0	5	21	1	2	2	97.7	0	2.3	0	0	0	49.4	8.3	40.3	0	2.1	0
Westeifel	Heisdorferbach	1	24 June 2016	30	526	36	0.3	0	10	6	2	3	3	100	0	0	0	0	0	82.0	0	15.5	1.3	1.2	0
Westeifel	Hennebach	0	22 June 2016	28	502	33	0.9	0	7	1	1	2	2	0	0	100	0	0	0	0	0	96.6	0	3.4	0
Westeifel	Hennebach_Seitenarm	0	2 July 2016	38	506	65	0.3	0	10	7	1	1	3	0	0	100	0	0	0	0	0	93.2	1.0	5.8	0
Westeifel	Herreler_Graben	1	14 June 2016	20	364	26	0.4	0	10	7	1	1	3	98.3	0	0	1.7	0	0	70.7	0	0	27.0	2.3	0
Westeifel	Hofswaldbach	1	8 July 2016	44	457	35	0.5	0	5	12	1	2	2	0	0	100	0	0	0	4.2	0	52.3	0	43.5	0
Westeifel	Irsen	0	9 July 2016	45	548	32	0.8	0	2	6	1	1	2	0	0	100	0	0	0	8.6	1.1	61.0	10.4	18.9	0
Westeifel	Klingendell	1	14 May 2018	4	385	67	0.8	0	20	26	1	2	2	100	0	0	0	0	0	72.8	0	0	0.2	26.9	0
Westeifel	Koerperich	1	19 June 2016	25	312	21	0.7	0	2	11	1	2	3	0	0	0	1.6	98.4	0.0	0	0	0	55.9	36.0	8.0
Westeifel	Kotzelsbach	1	28 June 2016	34	411	32	0.7	0	15	17	1	1	3	0	0	100	0	0	0	1.3	4.2	38.7	15.3	40.6	0
Westeifel	Lammbach	1	25 June 2016	31	456	10	0.5	0	3	6	1	1	3	100	0	0	0	0	0	44.1	0	0	0	55.9	0
Westeifel	Luenebach	1	22 June 2016	28	541	31	0.2	0	10	8	2	3	2	0	0	100	0	0	0	0	0	82.9	17.1	0	0
Westeifel	Mattelbusch	0	16 July 2016	49	498	31	0.4	0	35	13	1	2	2	0	0	46.9	0	53.1	0	0	0	45.5	0	54.5	0
Westeifel	Mausbach	1	2 July 2016	38	459	39	0.4	0	10	7	1	1	3	0	0	96.1	0	3.9	0	0	0	34.2	0	65.8	0
Westeifel	Michelbach	0	18 June 2016	24	323	14	2.0	0	4	3	1	1	1	0	0	0	0	100	0	3.5	0	0	14.2	68.3	14.0
Westeifel	Moehnbach	0	28 May 2018	18	509	10	0.3	0	4	1	1	1	2	0	0	95.0	0	5.0	0	0	0	58.6	0	41.4	0
Westeifel	Pittenbach	1	15 July 2016	48	422	44	0.8	0	6	15	1	1	2	100	0	0	0	0	0	43.7	0	0	22.0	34.3	0
Westeifel	Reiff	1	30 May 2018	20	452	66	0.5	0	15	19	1	2	3	99.5	0	0	0.5	0	0	69.9	0	0	0	30.1	0
Westeifel	Reinzelbach_1	0	11 May 2018	1	588	7	0.3	0	2	16	1	1	3	3.6	0	96.4	0	0	0	29.4	0	70.6	0	0	0
Westeifel	Reinzelbach_2	0	11 May 2018	1	592	15	0.5	0	6	2	1	1	2	0	0	100	0	0	0	0.7	0	99.3	0	0	0
Westeifel	Reipeldingen	1	29 June 2016	35	450	39	1.3	0	4	18	1	2	3	0	0	100	0	0	0	9.4	0	54.0	27.1	9.5	0
Westeifel	Roscheid	1	12 June 2017	29	420	22	0.4	1	3	10	1	1	1	84.7	0	0	0	15.3	0	48.4	0	20.4	11.9	19.3	0
Westeifel	Scharrenborn	1	12 June 2017	29	485	40	0.2	0	7	7	2	1	1	0	0	100	0	0	0	0	20.2	76.8	0	3.0	0
Westeifel	Schirmbach	1	8 July 2016	44	509	54	0.6	0	7	1	2	1	3	0	0	94.3	0	5.7	0	0	0	60.7	0	39.3	0
Westeifel	Seisbach	1	30 May 2018	20	462	79	1.3	0	10	1	1	2	2	42.6	0	57.3	0	0.1	0	30.2	0	38.7	10.2	20.9	0
Westeifel	Sengerich	1	9 July 2016	45	531	42	0.3	0	15	4	2	1	2	89.1	0	0	10.9	0	0	29.4	0	0	68.0	2.7	0
Westeifel	Spielmannsholz	1	31 May 2018	21	500	26	1.0	0	5	22	3	3	3	100	0	0	0	0	0	52.7	0	24.5	9.6	13.3	0
Westeifel	Steuenbach	1	28 May 2018	18	386	41	1.0	0	15	20	3	3	2	100	0	0	0	0	0	75.1	0	0	0	24.9	0

LandscapeUnit	Creek	Presence	Date	Day	Elevation(m)	Pools	Width(m)	Fish	Tilt(°)	Tilt_perpendicular(°)	Substrate	Prey	Hiding	Decid100	Mixed100(%)	Conif100(%)	Agri100(%)	Grass100(%)	Settl100(%)	Decid500(%)	Mixed500(%)	Conif500(%)	Agri500(%)	Grass500(%)	Settl500(%)
Westeifel	Taubenbach	0	23 May 2018	13	530	3	0.5	0	4	16	1	1	2	86.5	0	0	0	13.5	0	24.5	0.2	0	0	75.4	0
Westeifel	Teggelbach	1	19 June 2016	25	248	23	0.4	0	3	17	1	1	1	18.1	0.0	81.9	0	0	0	24.0	8.5	41.2	22.1	4.2	0
Westeifel	Thierbach_1	0	26 June 2016	32	501	23	0.8	0	7	18	1	1	3	0	0	100	0	0	0	15.7	0	46.0	0	38.4	0
Westeifel	Thierbach_2	0	26 June 2016	32	521	26	1.1	0	10	12	2	1	2	0	0	100	0	0	0	0.8	0	36.0	6.3	57.0	0
Westeifel	Wallertbach	1	9 July 2016	45	498	36	0.5	0	4	9	1	2	2	0	0	100	0	0	0	0	0	98.9	0	1.1	0
Westeifel	Watzbach	1	31 May 2018	21	523	55	1	0	5	20	3	1	2	100	0	0	0	0	0	45.4	0	5.0	0	49.6	0
Westeifel	Watzbachzufluss	1	31 May 2018	21	499	76	0.3	0	10	10	1	1	2	0	0	72.7	0	27.3	0	20.8	0	43.5	0	35.8	0
Westeifel	Weiherbach	0	24 June 2016	30	534	30	0.3	0	4	9	2	2	3	100	0	0	0	0	0	52.7	0	2.1	0	45.2	0
Westeifel	Winterspelterbach	1	8 July 2016	44	491	37	1.0	0	10	5	1	2	1	0	0	100	0	0	0	0	0.6	65.6	0	33.8	0

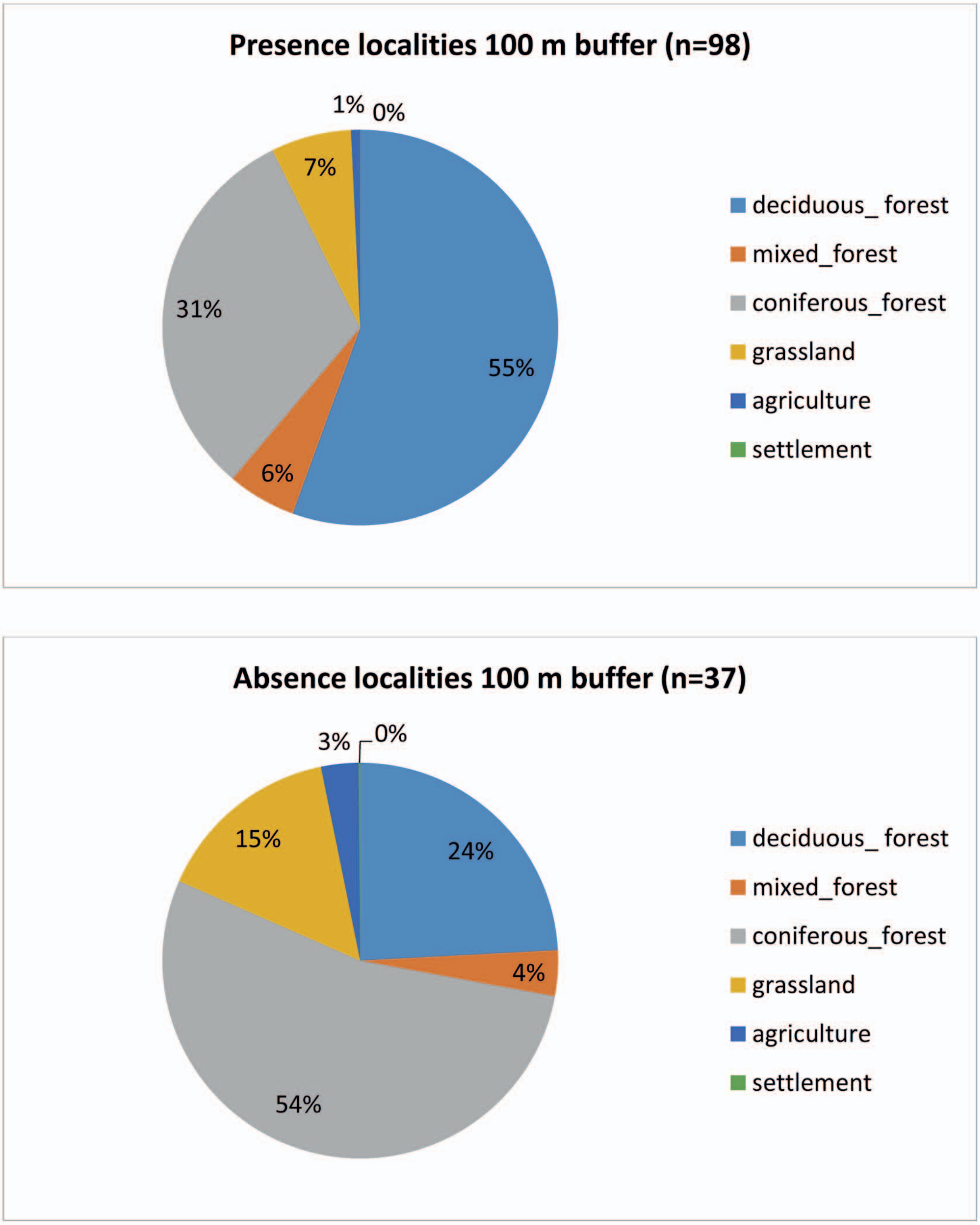
Supplementary document 2. Raw data from removal sampling of larval fire salamanders. Larvae1 = captured larvae from the first occasion of removal sampling, Larvae2 = captured larvae from the second occasion of removal sampling, Larvae3 = captured larvae from the third occasion of removal sampling.

LandscapeUnit	Creek	Larvae1	Larvae2	Larvae3	Total_captured	Date	Day	Elevation (m)	Pools	Width (m)	Fish	Tilt (°)	Tilt_perpendicular (°)	Substrate	Prey	Hiding	Decid100	Mixed100 (%)	Conif100 (%)	Agri100 (%)	Grass100 (%)	Settl100 (%)	Decid500 (%)	Mixed500 (%)	Conif500 (%)	Agri500 (%)	Grass500 (%)	Settl500 (%)
Gutland	Gutenbach	2	0	0	2	14 May 2018	4	288	30	1.9	0	8	19	1	3	3	98.8	0	0	1.2	0	0	46.8	0	6.0	47.2	0	0
Gutland	Fleissbach	3	2	0	5	16 May 2018	6	357	31	0.5	1	30	7	3	1	3	0	0	100	0	0	0	3.9	0	96.1	0	0	0
Gutland	Ferschweiler_1	40	40	28	108	22 May 2018	12	321	45	0.5	0	15	6	3	2	3	75.6	24.4	0	0	0	0	44.8	11.9	0	1.6	40.2	1.6
Gutland	Ferschweiler_2	133	153	22	308	22 May 2018	12	324	21	1.5	0	5	11	3	1	2	100	0	0	0	0	0	87.7	0	0	3.2	9.1	0
Gutland	Irrel_1	7	23	31	61	22 May 2018	12	233	38	0.5	0	5	15	2	3	2	100	0	0	0	0	0	70.3	0	0	23.1	6.6	0
Gutland	Irrel_2	7	10	14	31	22 May 2018	12	217	34	1.0	0	2	6	1	2	1	69	0	0	0	31	0	64.5	0	0.3	11.5	21.5	2.2
Gutland	Hauchenbach	5	5	0	10	9 June 2018	30	256	19	1.5	0	2	7	3	3	3	12.9	85.9	0	0	1.2	0	31.6	44.8	0	5.0	18.6	0
Gutland	Ingendorf	1	0	2	3	11 June 2018	32	352	47	0.5	0	3	1	1	3	3	100	0	0	0	0	0	75.0	0	0	25.0	0	0
Hunsrueck	Misselbach	2	1	0	3	26 May 2016	1	509	27	1.0	1	2	3	1	1	2	0	49.9	50.1	0	0	0	0	18.9	81.1	0	0	0
Hunsrueck	Thommerbach	0	0	0	0	26 May 2016	1	412	16	1.5	0	4	6	3	1	2	98.4	0	0	0	1.6	0	30.1	0	0	7.6	51.8	10.6
Hunsrueck	LorscheiderNebenbach	0	0	2	2	31 May 2016	6	468	20	0.5	0	7	2	3	1	2	53.2	0	38.5	8.3	0	0	17.7	2.1	41.2	38.9	0	0
Hunsrueck	Rauruwer	0	0	0	0	5 June 2016	11	480	30	0.5	0	1	1	3	1	1	0	0	100	0	0	0	20.1	0	79.9	0	0	0
Hunsrueck	AltlayerNebenbach	6	5	4	15	11 June 2016	17	358	80	0.4	0	8	3	1	1	2	100	0	0	0	0	0	75.3	0	0	24.7	0	0
Hunsrueck	Antenbachsgraben	0	2	1	3	12 June 2016	18	302	28	0.5	0	5	2	3	1	2	0	26.0	0	0	74.0	0	26.4	13.4	0	0	60.2	0
Hunsrueck	Eschbach	0	0	0	0	18 June 2016	24	403	38	1.5	0	3	3	3	1	2	0	53.2	0	46.8	0	0	0	18.2	0	77.9	0	3.9
Hunsrueck	Kieselbornbach	5	10	9	24	23 June 2016	29	449	69	1.5	1	6	2	3	1	2	74.2	0	25.8	0	0	0	32.2	0	67.8	0	0	0
Hunsrueck	Sattelbach	0	0	1	1	24 June 2016	30	566	62	2.0	1	8	3	1	1	3	42.1	0	57.9	0	0	0	53.9	0	46.1	0	0	0
Hunsrueck	Markenbach	1	0	1	2	29 June 2016	35	388	45	0.4	0	18	6	1	2	2	0	0	100	0	0	0	0	22.1	33.7	0	44.1	0.0
Hunsrueck	Lautenbach	19	18	14	51	9 July 2016	45	543	62	0.5	0	13	7	3	1	1	93.9	0	6.1	0	0	0	81.5	0	18.5	0	0	0
Hunsrueck	DrohnerNebenbach	0	0	0	0	11 July 2016	47	563	0	0.3	0	6	1	1	1	2	0	0	100	0	0	0	0	24.8	75.2	0	0	0
Hunsrueck	Struntbach	0	0	0	0	11 July 2016	47	443	35	0.4	0	1	2	1	3	2	0	0	50.8	0	49.2	0	15.0	0	39.3	17.0	28.7	0
Hunsrueck	Altbach	0	0	0	0	12 July 2016	48	484	17	3.5	1	1	19	3	1	2	0	0	100	0	0	0	4.9	14.0	33.6	2.1	28.3	17.1
Hunsrueck	Dammfloesschen	0	0	0	0	12 July 2016	48	541	38	0.5	0	7	6	3	1	2	0	0	95.5	0	0	4.5	0	0	69.1	0	2.1	28.8
Hunsrueck	Liedchenbach	0	2	1	3	13 July 2016	49	360	52	1.0	0	1	9	1	2	2	100	0	0	0	0	0	59.4	0	0	5.0	35.7	0
Hunsrueck	Korlingen	4	4	0	8	15 May 2017	1	254	77	0.8	0	7.5	9	1	3	2	99.1	0	0	0	0	0.9	44.2	0	0	15.9	14.9	25.0
Hunsrueck	Moertschelbach	3	9	9	21	21 May 2017	7	315	53	1.5	0	10	9	NA	3	3	38.5	0	0	0	61.5	0	25.8	0	0	36.6	37.6	0
Hunsrueck	Kiweringsbach	21	25	15	61	23 May 2017	9	441	83	1.0	0	2	3	3	1	1	96.8	0	3.2	0	0	0	52.8	0	45.6	0	1.5	0
Hunsrueck	Alkenbach	34	26	14	74	23 May 2017	9	327	62	0.8	0	5	15	3	3	2	100	0	0	0	0	0	63.7	7.4	0	0	28.8	0
Hunsrueck	Liefbach	33	24	25	82	29 May 2017	15	516	61	0.5	0	1	2	NA	1	2	100	0	0	0	0	0	20.5	0	79.5	0	0	0

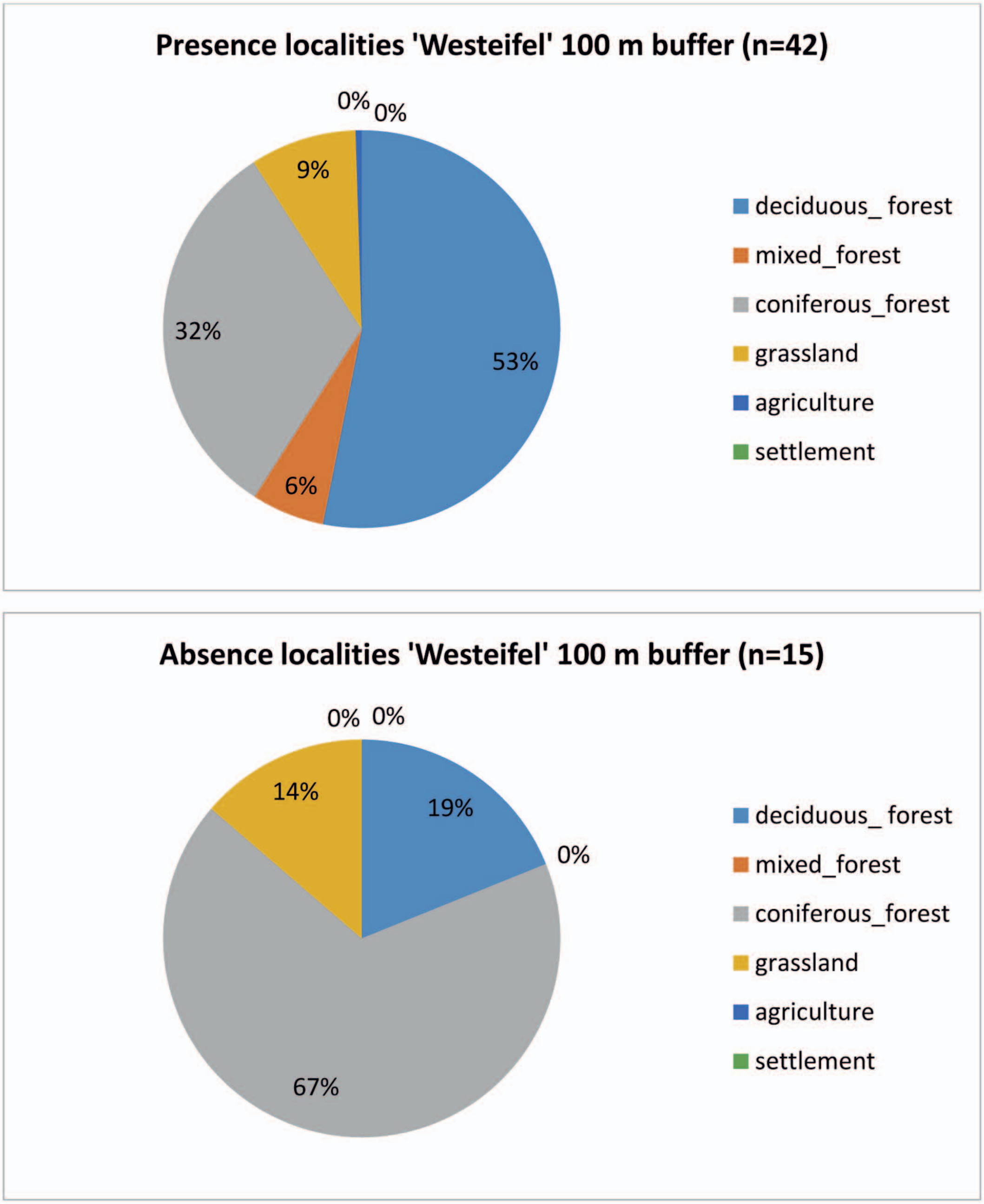
LandscapeUnit	Creek	Larvae1	Larvae2	Larvae3	Total_captured	Date	Day	Elevation (m)	Pools	Width (m)	Fish	Tilt (°)	Tilt_perpendicular (°)	Substrate	Prey	Hiding	Decid100	Mixed100 (%)	Conif100 (%)	Agri100 (%)	Grass100 (%)	Settl100 (%)	Decid500 (%)	Mixed500 (%)	Conif500 (%)	Agri500 (%)	Grass500 (%)	Settl500 (%)
Hunsrueck	Schastebach	19	25	12	56	29 May 2017	15	425	55	1.0	0	4	1	3	2	2	100	0	0	0	0	0	40.9	4.5	50.3	0	4.3	0
Hunsrueck	Bernsbach	15	16	19	50	29 May 2017	15	309	15	1.0	0	5	5	NA	3	2	100	0	0	0	0	0	100	0	0	0	0	0
Hunsrueck	Krebsbach	7	24	10	41	31 May 2017	17	548	60	1.5	0	3	7	2	1	3	0.3	0	99.7	0	0	0	39.6	0	60.4	0	0	0
Hunsrueck	Riedfloss	0	0	0	0	31 May 2017	17	588	30	1.5	1	2	2	1	1	1	0	0	41.1	0	58.9	0	22.9	0	45.9	0	31.2	0
Hunsrueck	Kalmbach	12	15	8	35	6 June 2017	23	439	30	1.0	0	8	1	NA	2	3	39.2	0	60.8	0	0	0	71.1	0	12.5	0	16.4	0
Hunsrueck	Hottenbach	17	24	22	63	7 June 2017	24	577	66	1.0	0	3	2	NA	1	3	0	82.4	17.6	0	0	0	0	38.9	31.1	0	0	0
Hunsrueck	Eisbach	14	10	10	34	10 June 2017	27	585	35	1.0	0	5	7	3	1	2	100	0	0	0	0	0	78.9	0	21.1	0	0	0
Hunsrueck	Koenigsbach	0	0	0	0	10 June 2017	27	577	30	1.5	1	2	1	1	1	3	38.8	0	61.2	0	0	0	59.1	1.0	39.9	0	0	0
Hunsrueck	Forstelbach	0	0	0	0	20 June 2017	37	559	32	1.3	0	2	12	3	1	2	37.5	0	62.5	0	0	0	72.1	6.2	20.8	0	1	0
Hunsrueck	Gehresbach	3	0	0	3	27 June 2017	44	311	20	1.0	0	5	9	NA	1	2	100	0	0	0	0	0	100	0	0	0	0	0
Hunsrueck	Steierbach	0	0	0	0	18 July 2017	65	315	23	2.0	0	5	11	NA	1	2	88.4	0	11.6	0	0	0	45.9	0	53.4	0.7	0	0
Moseltal	Morschbach	13	7	5	25	11 June 2016	17	407	41	1.0	0	2	6	3	2	2	100	0	0	0	0	0	42.9	0.4	0	56.7	0	0
Moseltal	Lierenbach	10	7	8	25	16 June 2016	22	275	58	1.0	0	5	10	3	2	3	8.5	0	91.5	0	0	0	55.6	0	44.4	0	0	0
Moseltal	AhringsbachNebenbach	1	0	0	1	18 June 2016	24	345	65	0.5	0	10	9	3	1	2	88.4	0	0	11.6	0	0	55.0	0	0	45.0	0	0
Moseltal	Waldrach	36	13	28	77	17 May 2017	3	233	72	0.8	0	15	2	3	3	3	87.4	0	12.6	0	0	0	45.4	0	34.0	0	0	20.6
Moseltal	Speinbach	0	0	1	1	5 June 2017	22	280	28	0.5	0	5	9	NA	2	3	68.7	0	31.3	0	0	0	37.5	0.4	56.0	6.1	0	0
Moseltal	CompenerBaechelchen	8	4	5	17	6 June 2017	23	230	45	1.0	0	3	7	NA	1	3	100	0	0	0	0	0	97.2	0	0	0	0	2.8
Moseltal	Schafhausbach	6	3	1	10	8 June 2017	25	395	18	0.2	0	3	3	NA	1	2	5.4	0	79.1	15.5	0	0	33.1	0	18.3	48.6	0	0
Moseltal	Viehbach	1	1	2	4	8 June 2017	25	278	41	0.7	0	3	11	NA	1	3	86.4	0	0	13.6	0	0	38.3	0	28.6	33.1	0	0
Osteifel	Hasbach_1	2	2	0	4	6 June 2018	27	575	0	0.6	0	4	9	3	2	1	100	0	0	0	0	0	5.0	0	81.5	0	13.6	0
Osteifel	Hasbach_2	1	0	0	1	6 June 2018	27	516	6	0.5	0	6	10	1	3	1	100	0	0	0	0	0	100	0	0	0	0	0
Osteifel	Baubach	0	0	0	0	6 June 2018	27	499	1	0.5	0	3	1	1	1	1	0	0	100	0	0	0	38.5	0	58.7	0	2.8	0
Osteifel	Auschsbach	17	10	1	28	14 June 2018	35	237	3	0.5	0	5	21	3	2	1	100	0	0	0	0	0	80.5	0	4.0	0	0	15.4
Osteifel	Mirbach	1	0	0	1	14 June 2018	35	225	2	0.4	0	3	1	3	1	1	25.2	0	74.8	0	0	0	55.7	0	36.8	0	0.1	7.4
Osteifel	Aelsbach	3	0	0	3	19 June 2018	39	471	2	1.0	0	3	6	3	1	1	66.2	0	0	0	33.8	0	32.7	0	22.2	0	45.0	0
Osteifel	BodenbacherBach	0	0	0	0	19 June 2018	39	540	0	0.5	0	3	3	3	1	3	0	86.0	14.0	0	0	0	0	33.4	59.2	0.3	7.2	0
Osteifel	Alfenbach	21	8	2	31	20 June 2018	40	344	7	0.8	0	4	20	3	2	2	41.1	0	58.9	0	0	0	40.4	0	35.5	0	24.1	0
Osteifel	Drasselbach	2	0	0	2	20 June 2018	40	323	2	0.2	0	1	8	3	1	1	0	0	100	0	0	0	40.5	0	42.9	0	0	10.4
Osteifel	Krekelbach	63	11	3	77	20 June 2018	40	409	8	0.3	0	7	17	3	2	1	100	0	0	0	0	0	65.8	0	0	0	34.2	0
Osteifel	Exbach	0	0	0	0	24 June 2018	44	443	1	0.5	0	3	3	3	2	1	2.4	46.0	0	0	51.6	0	38.0	35.3	0	0	26.7	0
Osteifel	Laufenbach	0	0	0	0	24 June 2018	44	440	1	0.3	0	24	22	1	1	1	99.4	0	0	0	0.6	0	64.9	0	10.1	6.1	19.0	0
Osteifel	Gilgenbach	17	8	1	26	27 June 2018	47	380	6	0.5	0	4	22	3	2	2	0	21.9	78.1	0	0	0	0	39.6	60.4	0	0	0

LandscapeUnit	Creek	Larvae1	Larvae2	Larvae3	Total_captured	Date	Day	Elevation (m)	Pools	Width (m)	Fish	Tilt (°)	Tilt_perpendicular (°)	Substrate	Prey	Hiding	Decid100	Mixed100 (%)	Conif100 (%)	Agri100 (%)	Grass100 (%)	Settl100 (%)	Decid500 (%)	Mixed500 (%)	Conif500 (%)	Agri500 (%)	Grass500 (%)	Settl500 (%)
Osteifel	Girrelsbach	29	14	9	52	27 June 2018	47	377	9	0.5	0	3	16	3	2	3	35.9	0	64.1	0	0	0	62.7	0	37.3	0	0	0
Osteifel	Ahbach	6	1	0	7	29 June 2018	49	501	13	0.9	0	4	18	3	2	3	65.9	0	34.1	0	0	0	70.4	0	28.9	0	0.6	0
Osteifel	Gruenbach	8	4	3	15	29 June 2018	49	468	7	0.5	0	5	2	3	2	1	25.7	0	74.3	0	0	0	39.3	0	40.0	0	20.7	0
Osteifel	Nohnerbach	0	0	0	0	29 June 2018	49	500	6	0.5	0	5	11	1	1	1	20.5	0	79.5	0	0	0	29.8	0	57.5	0	12.7	0
Osteifel	Kaulbach	0	0	0	0	30 June 2018	50	429	2	1.5	0	4	1	3	1	2	0	0	0	0	100	0	0	0	2.6	23.2	72.5	1.6
Osteifel	Rudersbach	3	0	0	3	30 June 2018	50	509	3	0.7	0	2	7	1	3	1	38.6	0	61.4	0	0	0	25.1	4.9	58.6	0	11.5	0
Osteifel	Wiesbach	0	0	0	0	30 June 2018	50	462	3	0.5	0	1	2	1	1	2	100	0	0	0	0	0	6.2	24.6	69.2	0	0	0
Westeifel	Reinzelbach_1	0	0	0	0	11 May 2018	1	588	7	0.3	0	2	16	1	1	3	3.6	0	96.4	0	0	0	29.4	0	70.6	0	0	0
Westeifel	Reinzelbach_2	0	0	0	0	11 May 2018	1	592	15	0.5	0	6	2	1	1	2	0	0	100	0	0	0	0.7	0	99.3	0	0	0
Westeifel	Klingendell	42	23	17	82	14 May 2018	4	385	67	0.8	0	20	26	1	2	2	100	0	0	0	0	0	72.8	0	0	0.2	26.9	0
Westeifel	Grasmaerchen	41	36	27	104	17 May 2018	7	225	77	0.5	0	15	6	3	2	2	29.0	0	0	0	71.0	0	31.3	0	0	12.8	55.9	0
Westeifel	Gemuenderbach	14	10	9	33	22 May 2018	12	416	48	0.8	0	15	15	2	1	3	0	98.3	1.7	0	0	0	13.0	42.0	13.1	31.9	0	0
Westeifel	Ettelbach_2	0	0	0	0	23 May 2018	13	517	7	0.5	0	2	11	1	1	2	0.0	0	100.0	0	0	0	24.9	0	60.4	3.6	11.0	0
Westeifel	Taubenbach	0	0	0	0	23 May 2018	13	530	3	0.5	0	4	16	1	1	2	86.5	0	0	0	13.5	0	24.5	0.2	0	0	75.4	0
Westeifel	Steuenbach	0	3	3	6	28 May 2018	18	386	41	1.0	0	15	20	3	3	2	100	0	0	0	0	0	75.1	0	0	0	24.9	0
Westeifel	Heimbach	4	2	2	8	28 May 2018	18	350	45	1.2	0	10	19	1	2	3	100	0	0	0	0	0	84.0	0	1.2	14.8	0	0
Westeifel	Moehnbach	0	0	0	0	28 May 2018	18	509	10	0.3	0	4	1	1	1	2	0	0	95.0	0	5.0	0	0	0	58.6	0	41.4	0
Westeifel	Reiff	26	21	14	61	30 May 2018	20	452	66	0.5	0	15	19	1	2	3	99.5	0	0	0.5	0	0	69.9	0	0	0	30.1	0
Westeifel	Seisbach	45	36	33	114	30 May 2018	20	462	79	1.3	0	10	1	1	2	2	42.6	0	57.3	0	0.1	0	30.2	0	38.7	10.2	20.9	0
Westeifel	Spielmannsholz	18	13	14	45	31 May 2018	21	500	26	1.0	0	5	22	3	3	3	100	0	0	0	0	0	52.7	0	24.5	9.6	13.3	0
Westeifel	Watzbachzufluss	29	40	22	91	31 May 2018	21	499	76	0.3	0	10	10	1	1	2	0	0	72.7	0	27.3	0	20.8	0	43.5	0	35.8	0
Westeifel	Watzbach	69	52	73	194	31 May 2018	21	523	55	1	0	5	20	3	1	2	100	0	0	0	0	0	45.4	0	5.0	0	49.6	0
Westeifel	Arzfeld	10	5	5	20	6 June 2018	27	493	47	0.6	0	8	6	3	2	1	95.0	0	0	0	5.0	0	70.6	9.4	0	9.4	20.1	0
Westeifel	Bisselbach	8	4	1	13	7 July 2018	57	511	5	1.7	0	3	7	1	3	1	22.2	77.8	0	0	0	0	31.3	0	56.1	0	12.6	0

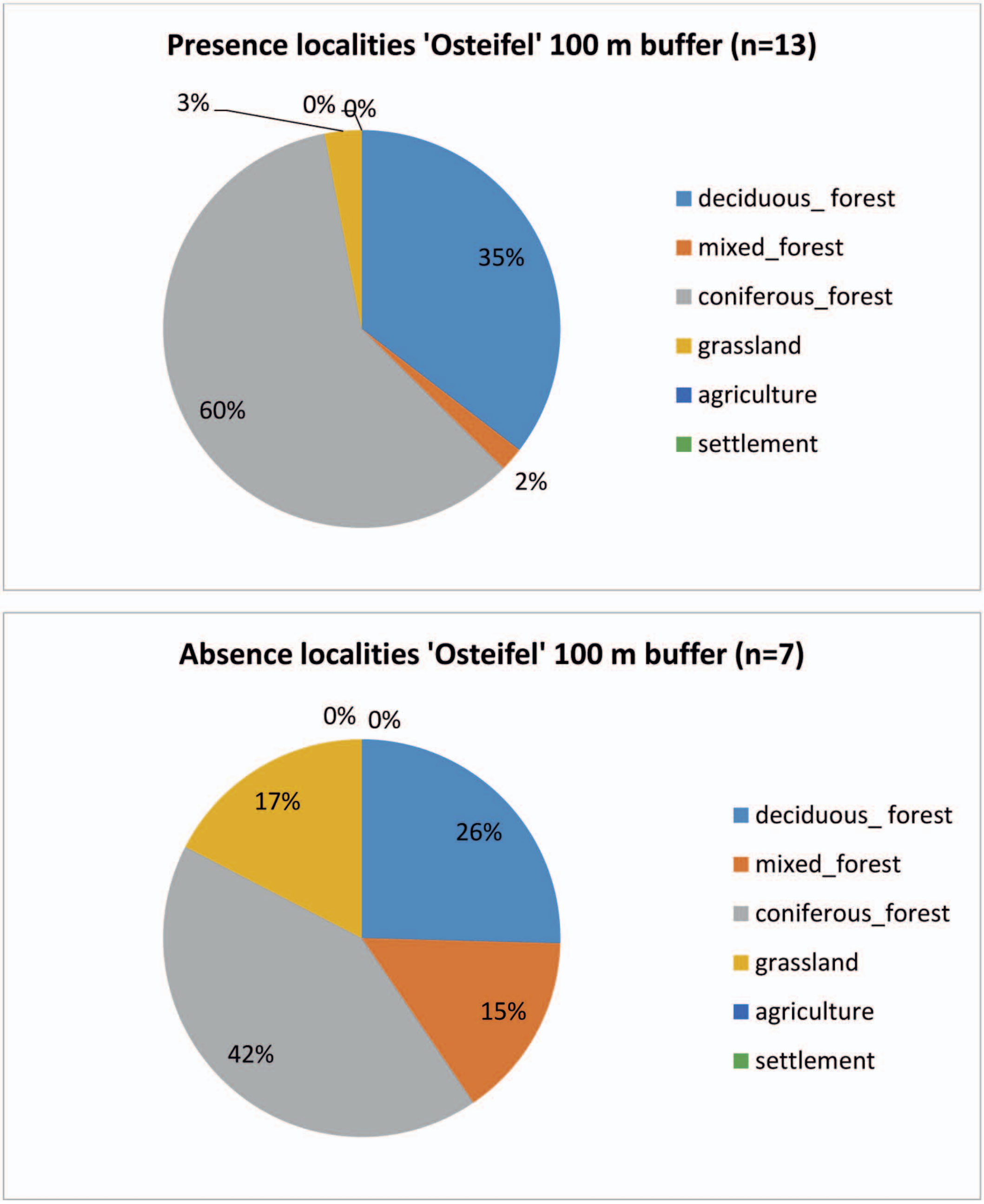
Supplementary document 3. Proportions of different land use in a 100 m buffer around all studied creeks, divided into presence and absence sites.



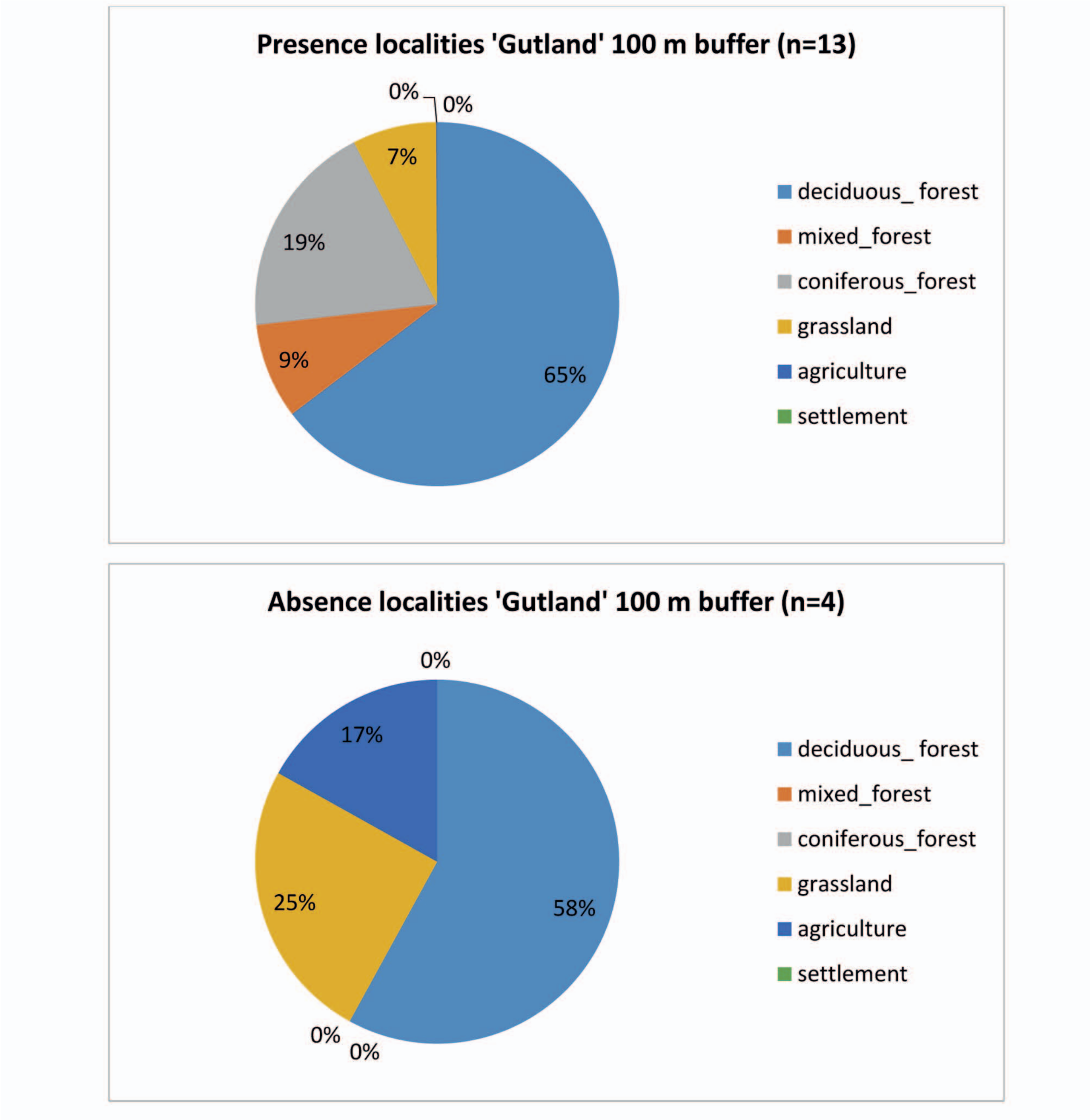
Supplementary document 4. Proportions of different land use in a 100 m buffer around all studied creeks from the 'Westeifel', divided into presence and absence sites.



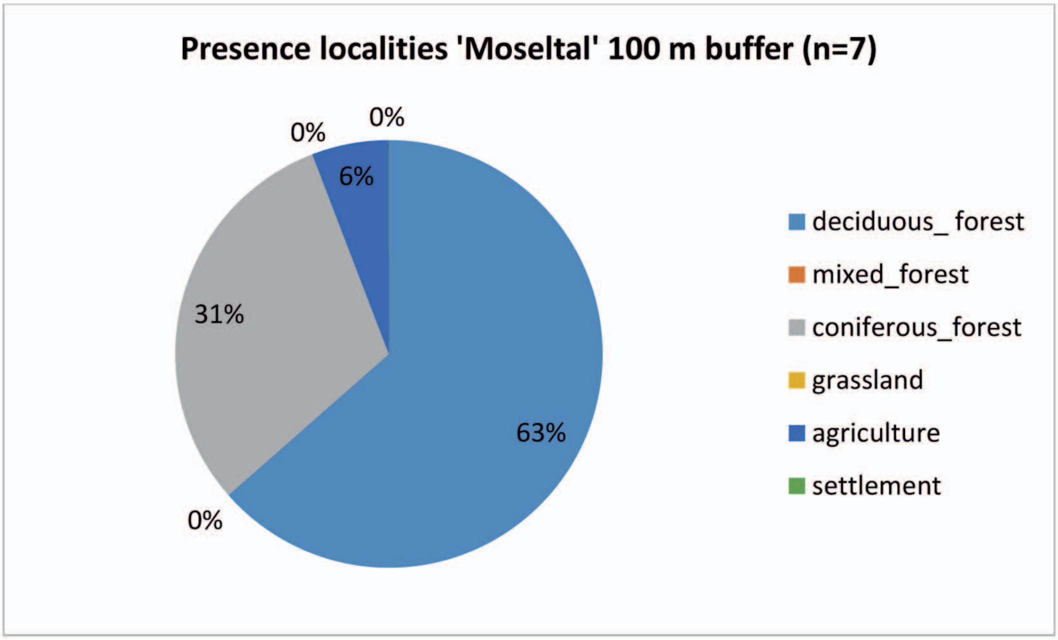
Supplementary document 5. Proportions of different land use in a 100 m buffer around all studied creeks from the 'Osteifel', divided into presence and absence sites.



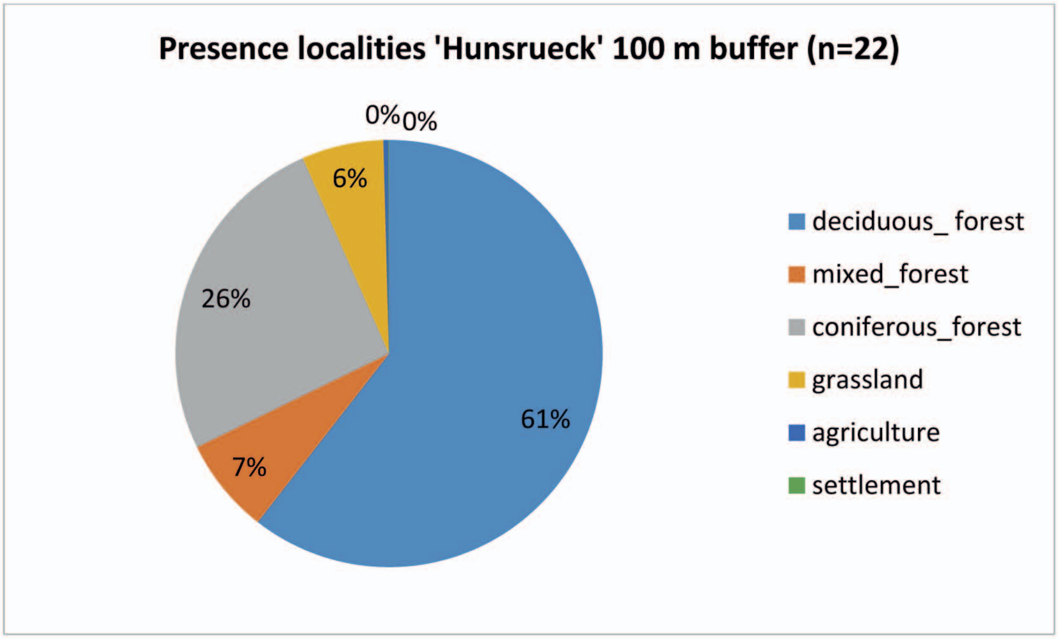
Supplementary document 6. Proportions of different land use in a 100 m buffer around all studied creeks from the 'Gutland', divided into presence and absence sites.

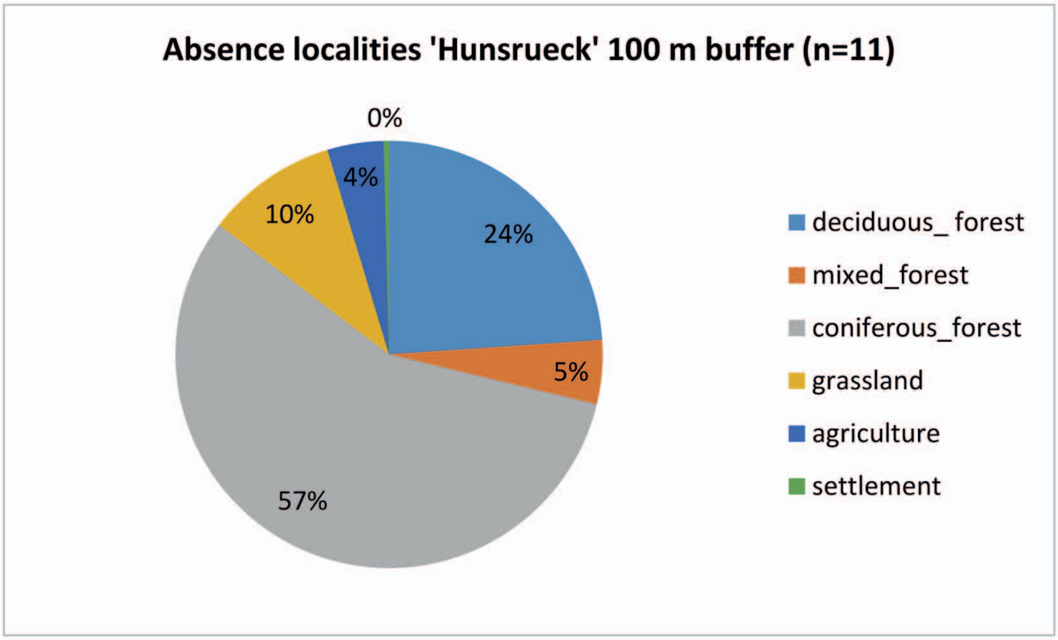


Supplementary document 7. Proportions of different land use in a 100 m buffer around all studied creeks from the 'Moseltal'.



Supplementary document 8. Proportions of different land use in a 100 m buffer around all studied creeks from the 'Hunsrück', divided into presence and absence sites.





SALAMANDRA **56**(3): 265–274
15 August 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Comparison of different methods to estimate abundances of larval fire salamanders (*Salamandra salamandra*) in first-order creeks

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Manuscript received: 27 May 2019
Accepted: 26 August 2019 by Edgar Lehr

Abstract. The European urodelan diversity is threatened by the recent range expansion of the chytrid fungus *Batrachochytrium salamandrivorans*. The fire salamander can be classified as especially sensitive as infection of individuals with this parasitic fungus usually leads to chytridiomycosis and rapid mortality. Hence, to observe effects at the population level, it is crucial to monitor population sizes and trends. The abundance of adult fire salamanders is far more difficult to estimate compared to larval stages. Therefore, population monitoring of fire salamanders focusses on the aquatic larvae. We examined abundances of larval fire salamanders in two different first-order creeks. Four different methods were compared: two simple count methods, and two abundance estimates using removal sampling and capture-mark-recapture (CMR), respectively. The results of the count methods strongly correlated with the abundance estimates. Furthermore, the results of the removal sampling correlated with the estimates using CMR data. The estimates of the CMR study should get closer to real larval abundances in the creeks compared to the estimates of the removal sampling approach. Count methods but also removal sampling analysis underestimate real larval abundances. Due to (i) the strong correlations of the results, (ii) less time and cost effort of removal sampling compared to CMR, we suggest the removal sampling method, keeping in mind that real larval population is larger.

Key words. Amphibia, Caudata, Capture-mark-recapture (CMR), program MARK, program POPAN, removal sampling, *Batrachochytrium salamandrivorans.*

Introduction

Europe's urodelan diversity is severely threatened by the invasive and recently expanding chytrid fungus *Batrachochytrium salamandrivorans (Bsal)* (Martel et al. 2013, 2014, Spitzen-van der Sluijs et al. 2016, Stegen et al. 2017). Especially, the European fire salamander (*Salamandra salamandra* (Linnaeus, 1758)) can be classified as extremely sensitive towards this pathogene because usually infection leads to chytridiomycosis and individuals die within about two weeks (Martel et al. 2014, Stegen et al. 2017). In European fire salamander populations in The Netherlands, Belgium and Germany (Spitzen-van der Sluijs et al. 2013, 2016, Dalbeck et al. 2018, Stegen et al., 2017, Lötters et al. 2020 in this issue) mass mortalities followed infection. In such cases, the rapid population collapse of adults should be followed by a strong downturn in numbers of larvae in the following years. The same was observed by Bosch & Martínez-Solano (2006), who found significantly decreasing larvae counts after a mass mortality event in a Spanish fire salamander population caused by the chytrid fungus *Batrachochytrium dendrobatidis (Bd)*. Until today – after 18 years – this Spanish salamander population did not recover from this population decline (while other more resistant species in this amphibian community profited by the decline of the populations of susceptible species) (Bosch et al. 2018). Similar strong effects of *Bsal* on salamander populations are described in Belgium by Stegen et al. (2017) after mass mortality events.

However, in the recent exotic range of *Bsal* (Belgium, Germany, the Netherlands and Spain), the observed prevalence of infected salamanders in a population is usually very low (mostly far < 10%: Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018). Furthermore, decreases of several *Bsal*-infected salamander populations are described without observed mass mortalities (Dalbeck et al. 2018, Lötters et al. 2020 in this issue). Hence, mortality events may be overseen in many cases, and individuals may die 'silent', for instance inside their hiding places. Therefore, long-term population monitoring is needed to get further information on population sizes and trends.

The abundances of adult European fire salamanders are difficult to estimate (Schmidt et al. 2015), which, for instance, has also been described for adult plethodontid salamanders in the United States (Bailey et al. 2004). Although, individual recognition of adult fire salamanders using photographs of the dorsal patterns is a well-known non-invasive and reliable method (Feldmann 1971, Kopp-Hamberger 1998), the main arguments against using adults for population monitoring include their weather-dependent activity (Thiesmeier 2004) and larger home ranges (Schulte et al. 2007) compared to larvae. Hence, higher detection probabilities and recapture rates can be reached with the same effort using larval stages. Schmidt et al. (2015) proposed a removal sampling method to monitor abundances of fire salamander larvae. The analysis of such removal sampling data estimates abundances in relation to modelled detection probabilities and relevant environmental co-factors. Individual recognition of larvae is not necessary because the statistical analysis is based on hierarchical removal sampling models (Schmidt et al. 2015, Wagner et al. 2020 in this issue).

In the German parts of the Eifel where *Bsal* is recently expanding (Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018, Wagner et al. 2019, Lötters et al. 2020 in this issue), a monitoring network in reproduction creeks of the fire salamander was established in 2015 to estimate larval abundances and long-term population trends. Based on removal sampling of larvae in > 40 reproduction creeks this monitoring should act as early-warning-system (Dalbeck et al. 2018, Wagner et al. 2017, 2020 in this issue) by identifying population breakdowns in potentially *Bsal*-infected populations.

To estimate abundance in a single locality, the hierarchical removal sampling models (Royle 2004, Dorazio et al. 2005, Royle & Dorazio 2006) as suggested by Schmidt et al. (2015) require data from spatially distinct populations. Furthermore, the performance of removal sampling estimators regarding one closed population is usually poor (Borchers et al. 2002, and see Schmidt et al. 2015), and the 'population' of fire salamander larvae within a defined section of a reproduction creek can only be regarded as 'closed' during a short sampling period. If a single larval population is sampled and monitored over a longer time period, it has to be regarded as 'open population' because emigration and immigration (here, mainly due to larval drift: Thiesmeier & Schuhmacher 1990, Reinhardt et al. 2018, Veith et al. 2019) as well as death and birth (i.e. newly deposited larvae) may occur.

We here compare the results of (I) modelling larval abundances for 'closed' populations using removal sampling (Schmidt et al. 2015) with (II) capture-mark-recapture (CMR) results for 'open' populations (modified Jolly-Seber approach using 'POPAN': Wagner et al. 2011, Schwarz & Arnason 2017) gained from the same creek sections. With the latter we especially accounted for drift events (Thiesmeier & Schuhmacher 1990, Reinhardt et al. 2018, Veith et al. 2019). We finally compared these different population estimates with two simple time-count methods.

Materials and methods

Study region

We studied the larval populations in two first-order creeks in Rhineland-Palatinate (Germany), with two independent sections each (ca. 500 m apart), to obtain representative results from four spatially distinct populations/subpopulations. Both creeks are situated in the 'Hunsrück' mountain area: the 'Beresbach' (hereafter named BB, with sections BB-A and BB-B) to the East of the city of Trier (N 49°45'52", E 6°50'06"), and the 'Weilerbach' (lower section)/'Ebersbach' (upper section) (hereafter named WB, with sections EB-A and EB-B) to the South of Koblenz (N 50°11'00", E 7°37'45"). Each section of 75 m was subdivided into three 25 m subsections (see Schmidt et al. 2015 and Fig. 1). The study sections have similar but also differing habitat parameters (Table 1). All sections are surrounded by mixed deciduous forest with varying tree species composition and with many hiding places (dead wood, stones), making the terrestrial habitat of the adult populations comparable. They differ slightly in inclination and consequently water velocity (which might result in more larval drift), with upper section BB-B being steepest (7–8°) and lower section EB-A being the flattest (ca. 2°). Creek sections differ in, i.e. the number of available pools which are known to positively affect presence of larvae (e.g., Werner et al. 2014). Estimated quantity of gammarids (Ruff & Maier 2000) and other consumable benthos and creek width (Schmidt et al. 2015) (Table 1) are similar among sections. Consequently, the four sections cover a broad spectrum of habitats of fire salamander larvae. The recorded parameters (Table 1) were furthermore used for modelling larval abundances using removal data (see below).

Field work

In the BB, the subspecies *Salamandra salamandra terrestris* Lacépède, 1788 occurs, while the EB population lies at the western edge of a subspecies intergradation zone of *S. s. salamandra* × *terrestris* (Veith 1992). Mating of adults on land mainly occurs around July (ranges from March to September) and deposition of larvae in April and May (Thiesmeier 2004). Consequently, field work took place in June and July 2017 when most larvae were present in the creeks.

At the beginning and the end of each 75 m study transect, slightly modified drift and upstream migration traps of Goedmakers (1980) were installed (Fig. 1; see also Veith et al. 2019). Each pair of traps completely blocked the creek (with the aid of additional lateral barriers) and all water had to pass the traps. The single opening (30 cm wide) of a drift trap faced up to capture larvae moving downstream, while the two openings of an upstream migration trap (together 20 cm wide) faced down to capture animals moving upstream.

We used fluorescent Visible Implant Alpha (VI Alpha) Tags of Northwest Marine Technology Inc. (size 1.2 ×

Table 1. Habitat characteristics of the studied transects (75 m) within the two creeks. No predatory fish was present in these four sections.

Locality	Terrestrial habitat		Aquatic habitat			
	Forest (100 m radius)	Hiding places (100 m radius)	Average inclination (°)	Estimated quantity of larval food	Creek width (m)	Number of pools
BB-B *Upper part*	dominated by *Fagus sylvatica* and *Acer pseudoplatanus*	Many	about 7–8° (365–355 m a.s.l.)	Many	0.7–0.8	Ca. 50
BB-A *Lower part*	dominated by *Fagus sylvatica* and *Acer pseudoplatanus*	Many	about 5° downstream (318–312 m a.s.l.)	Many	0.7–0.8	Ca. 50
EB-B *Upper part*	dominated by *Quercus* spec. and *Carpinus betulus*	Intermediate	about 2° (250–248 m a.s.l.)	Many	0.6–0.7	Ca. 15
EB-A *Lower part*	dominated by *Alnus glutinosa* and *Fagus sylvatica*	Intermediate	about 5° (230–224 m a.s.l.)	Many	0.6–0.7	Ca. 20

2.7 mm) to conduct a CMR study between 10/06/2017 and 19/07/2017 in the BB and between 15/06/2017 and 10/07/2017 in the EB. First, drift and upstream migration traps were checked for captured larvae every 2–3 days. Furthermore, larvae were captured in each 25 m subsection for 15 min by opportunistic search and with the aid of a dip net. This corresponds to the first cycle of the hierarchical removal sampling models of Schmidt et al. (2015). Each larva was weighted to the nearest 0.001 g using a Kern EMB 200-3 field balance. Alpha-Tags were laterally injected using the

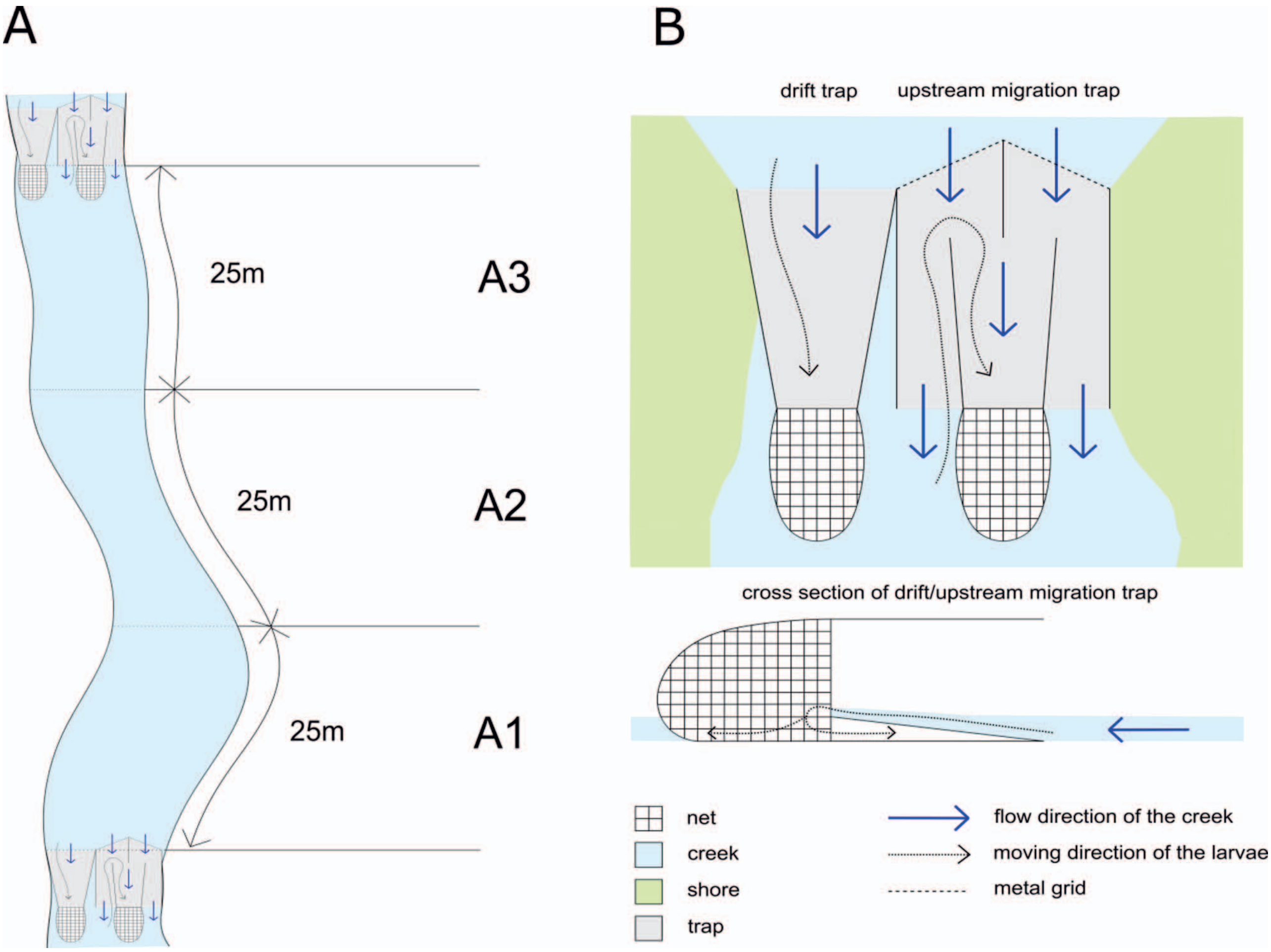

Figure 1. Schematic drawing of a study transect (A) and of a drift and upstream migration trap (B).

specific injector of Northwest Marine Technology Inc. The identification code consisted of a letter followed by a two-digit number. Each larva was photographed from above on graph paper using a digital cam and with black light from the side in a glass tub fixed in a special construction. By doing so, the Alpha-Tag number of each individual could be easily read. In cases where the tag was hardly readable due to bad tag position or increasing pigmentation we used the tail pattern for individual photographic identification later on in the lab, a method considered suitable for only small numbers of larvae (up to 20 individuals according to Eitam & Blaustein 2002). At the end of the study day, all larvae were released next to the capture place or, if captured in a trap, one meter upstream or downstream of the respective trap. Hence, natural immigration and emigration by drift was allowed ('open population').

In addition, we conducted removal sampling (for technical details see Schmidt et al. 2015) in all sections; at 28/06/2017 and 04/07/2017 in the BB and at 30/06/2017 and 04/07/2017 in the EB. Three field workers each conducted simultaneous capture of larvae in each section (Schmidt et al. 2015). Afterwards, drift and upstream migration traps were checked to ensure that during each search period the population was closed. When moving between different sections, all equipment including gumboots was disinfected using Virkon S® according to Van Rooij et al. (2017).

As two different time-count methods we used the sum of all larvae captured by one field worker in all three subsections of a transect within 45 min (i.e. captures/day during CMR) and the sum of all larvae captured by three field workers in all three subsections of a transect within 45 min (i.e. captures/day during removal sampling).

Data analysis

Photographs of larvae on graph paper were used for measuring snout-to-vent-length (SVL) and head width using the software MB-Ruler (Version 5.3) (©MB-Software solutions). The individual body index according to Hemmer & Kadel (1972) was calculated using the formula
$\log(b) = \log(\text{mass}) - 3 \times \log(\text{SVL})$.

To assess a potential invasiveness of Alpha-Tags, individual body indices were merged into two groups (newly captured and recaptured larvae), and the means were compared after testing for normal distribution (Shapiro-Wilks tests) and variance homogeneity (Levene tests) (Rudolf & Kuhlisch 2008).

CMR data were analyses using the program MARK (White & Burnham 1999, Cooch & White 2017). Death of individuals during the study time was possible and emigration by larval drift allowed (see above), also metamorphosing individuals at the end of the study period. Birth by newly deposited larvae is possible by single females during the whole study period (Thiesmeier 2004), furthermore immigration to the study sections by drift was also allowed. Hence, we choose the POPAN-approach for 'open populations' integrated in the program MARK (White & Burnham 1999, Wagner et al. 2011, Cooch & White 2017, Schwarz & Arnason 2017). The POPAN-approach ('super-population approach') is a modified Jolly Seber model that not only estimates the population sizes at each occasion but also for the 'super-population' over the entire study period (Wagner et al. 2011). We built 4–6 different models for each data set (i.e. section) by manipulating the parameters 'survival probability' (φ), 'detection probability' (p), and the probability of an individual to enter the 'super-population (b_i) from 'time-varying' to 'constant'. Furthermore, we used the sinus or logit function for φ and p. For b_i, we always used the Mlogit link function and for N the log-link function (as recommended by Schwarz & Arnason 2017). Best fitting models were chosen by their small sample Akaike Information Criterion values (AICc: Burnham & Anderson 2002) and used for parameter estimation. Because both studied creeks were affected by dryness and subsequent flood between 21/06/2017 and 25/06/2017, we furthermore split the data sets at 25/06/2017 to estimate abundances also for a time period with relatively constant conditions.

The removal sampling data from our four sections were merged with removal sampling data from 75 m sections of 50 further creeks of the same area ('Hunsrück' mountains). This was necessary because the hierarchical removal sampling models used by us (Royle 200, Dorazio et al. 2005, Royle & Dorazio 2006) require data from spatially distinct populations in order to estimate abundance in a single locality. Between end of May and middle of June 2017, these data including eight habitat parameters were obtained using the same standard methods as in the four sections of the present study (unpublished data). Co-variables of the larval habitat were the number of pools per section (preferred larval micro habitats: Thiesmeier 2004, Schmidt et al. 2015), presence/absence of predatory fish (Thiesmeier 2004), estimated quantity of gammarids and other consumable benthos (Ruff & Maier 2000), sampling date, creek width (m) and average inclination (°) as a proxy for larval drift during the study period (Thiesmeier & Schuhmacher 1990, Reinhardt et al. 2018, Veith et al. 2019); within 100 m surrounding each sections we characterised the terrestrial habitat through the quantity of hiding places (dead wood, stones) and forest types (deciduous, mixed or coniferous forest) (Thiesmeier 2004) (Table 1). Predictor variables were normalized prior to statistical analysis. We built different generalized multinomial mixture models using pairwise combinations of the eight environmental variables or a 'constant'-intercept-model for both abundance and detection probability. The software R (R Development Core Team 2012) and the R-package 'unmarked' (Fiske & Chandler 2011) were used for calculations. All models were fitted to the data with either a Poisson or a negative binomial abundance model (see Schmidt et al. [2015] for further details). Hence, 126 candidate models were fitted to the data and ranked by their AIC values (Burnham & Anderson 2002) using the R-package 'AICcmodavg' (Mazerolle 2015). To obtain site-specific estimates, we used the Bayesian approach implemented in the package ‘unmarked’ (function ‘ranef’: Fiske & Chandler 2011).

To compare the different methods, the estimates derived from CMR data were correlated with the results from the first time-count method and the estimates from using removal sampling data, and the latter estimates were furthermore correlated with the results of both time-count methods using IBM SPSS Statistics (Version 24.0). After testing for normal distribution (using Shapiro-Wilks tests) and variance homogeneity (using Levene tests), either a Pearson or a Spearman correlation coefficient was calculated. P-values were Bonferroni-corrected for alpha adjustment (Rudolf & Kuhlisch 2008).

Results

CMR study

In BB-A, the sum of captured larvae increased from beginning of June to beginning of July followed by a steady decrease of captures until the end of July. At the first four occasions in BB-A, only one larva could be recaptured, but from end of June the recapture rates increased from 18%–57%.

In BB-B, a steady decrease of captured larvae from beginning to the end of the study could be observed. Similar to the upper section, recapture rates were low at the beginning of the study and increased from end of June to end of July from 20%–67%.

In EB-A and EB-B, the captures increased at the first three occasions (with relatively low recapture rates), but drastically fell after a dry period followed by heavy rain falls at the end of June. However, the recapture rate of the few larvae, which were captured after these events, increased to 100% by the end of July.

Model selection tables and estimates for survival (φ), capture (p) and entry to the super-population (pent) probabilities can be found in the Supplementary Table 1. In BB-A, the estimates increased to nearly 2,000 larvae at the third occasion (but with large 95% CI) and stabilized by end of June (ca. 500–1,000 larvae, with narrow 95% CI) (Fig. 2). The super-population of BB-A is estimated to be 4,829 larval salamanders (3,844–6,262; 95% CI) regarding the whole sampling period. Due to the dry period and following heavy rainfalls in the locality of the EB, also a shortened time period (beginning at 26/06/2017) was analysed for all four transects (see Supplementary Fig. 1). When only these eight capture occasions were considered, the super-population estimate of BB-A is 2,003 (1,847; 2,193 95% CI).

Similar to BB-A, the estimates at the first occasions are high (ca. 1,400–2,100 larvae with larvae 95% CI), but with a low estimate (ca. 150 larvae) at the third occasion (Fig. 2). Also here, the estimates stabilized by end of June (300–600 larvae, narrow 95% CI) and decrease to about 100 individuals by the end of the study. The super-population estimate of BB-B is 3,912 larvae (3,121–4,973; 95% CI) for the whole sampling period and 693 (584; 857; 95% CI) for the shortened period.

Table 2. Count data of the removal sampling (i.e. sum of larvae captured in the 75 m transect by three persons in 45 min) and estimates obtained from hierarchical modelling in the studied creeks.

Date	Creek	Count data (number of larvae)	Estimated larvae ± 95% CI
28/06/2017	BB-A	33	67 (52; 85)
28/06/2017	BB-B	8	17 (10; 26)
30/06/2017	EB-A	11	19 (13; 27)
30/06/2017	EB-B	5	9 (5; 16)
04/07/2017	BB-A	49	100 (81; 121)
04/07/2017	BB-B	19	39 (28; 53)
04/07/2017	EB-A	1	2 (1; 6)
04/07/2017	EB-B	6	11 (7; 18)

In EB-A, high estimates (up to 600 larvae, large 95% CI) are reached until end of June, followed by always less than 100 individuals estimated until the end of the study (Fig. 2). The super-population estimate of EB-A is 1,083 larvae (886–1,389; 95% CI) for the whole sampling period and 299 larvae (268; 355 95% CI) for the shortened period.

In EB-B, the estimates increase until end of June to more than 700 larvae (large 95% CI) followed by a steady decrease to about 150–200 individuals (Fig. 2). The super-population estimate of EB-B is 833 larvae (687–1,069; 95% CI) for the whole sampling period and 212 larvae (191–246; 95% CI) for the shortened period.

Removal sampling

In BB-A and BB-B, the sum of all captured larvae increased between the first and second sampling occasion, conversely, the captures decreased in EB-A and EB-B (Table 2). There was only one best fitting (negative binomial abundance) model with ΔAIC value ≤ 2. Detection probability was negatively affected by creek width (m) and abundance was positively affected by the number of pools (Fig. 3). Comparable to the count data, the estimates for the two WB sections increased and for the two EB sections decreased between sampling occasions (Table 2).

Comparison of the different methods

The POPAN-estimates (CMR) positively correlated (Spearman correlation coefficient: 0.73; Bonferroni-corrected p-value < 0.001) with the results of the first time-count method (the sum of captures/day during CMR). Likewise, the estimates gained from the removal sampling data strongly correlate (Spearman correlation coefficient: 1.0; Bonferroni-corrected p-value < 0.001) with the results of the second time-count method (the sum of captures/day during removal sampling). All other correlations did not reach the level of significance (see Supplementary Fig. 2).

Discussion

The main goal of the present study was to compare the results of two different modelling approaches used for population size estimation. Their estimates differed by a factor of about 20. The large super-population estimates are in accordance to Thiesmeier & Sommerhäuser (1995), which in an extreme case found up to 383 larvae/m², with average densities of 18.1 and 19.0 larvae/m² in flowing parts and pools, respectively. The best fitting hierarchical model using removal sampling data suggests decreasing detection probability with increasing creek width (Fig. 3). Detection probability decreased from about 40% in creeks with < 1 m width to < 10% in creeks with 3 m width (Fig. 3). Hence, also this 'closed population approach' suggests that many larvae are not found during capture occasions and thus indicates large larvae populations. This is in accordance with many studies on imperfect detection probabilities in wild animal populations (e.g., Kéry 2002, Schmidt et al. 2002, Kéry & Schmidt 2008, Kéry et al. 2009, Wagner et al. 2011), and the super-population estimates of partly > 2,000 larvae per study section seem plausible. Thiesmeier & Schuhmacher (1990) found that 83% of drifted larvae were newly deposited or in early developmental stages. Because our study took place in June and July, a large part of the drifted larvae remained unconsidered. Consequently, between March and May the larval populations could have been even larger.

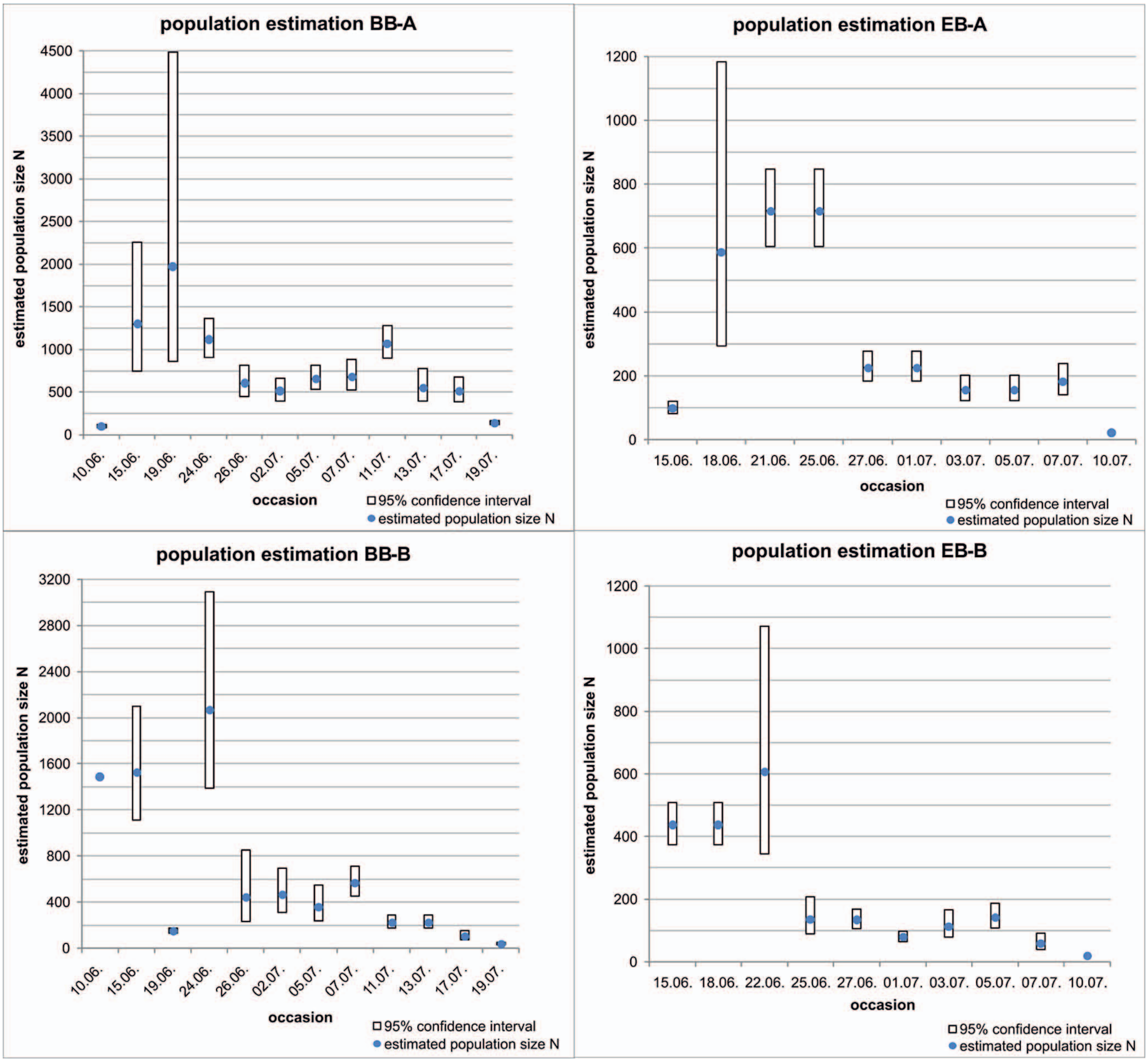

Figure 2. Population size estimates for each capture occasions and for each transect A and B in the BB and EB using CMR data of marked fire salamander larvae.

With regard to the assumption of a 'closed' larvae population using removal sampling data, it has to be taken into account that sampling took place within only 45 minutes and that lack of larvae captured in the respective drift and upstream migration traps proved a closed population status. Hence, the survival probability was always considered constant in the hierarchical models whereas the calculation using CMR data considered the larval population as 'open' because both birth and drift was possible during this study time over four weeks.

Regardless imperfect detection probabilities (they differ by a factor of about 40 to the POPAN-estimates), our strong correlation between CMR estimates and simple time-count methods suggest that the minimal time-consuming time-count method, as already applied by e.g., Sound & Veith (1994), can provide first insights into population size variation over time. They may even suffice the necessities of a long-term population monitoring programs that are conducted to detect drastic break-downs of larval populations, e.g., due to the currently range-expanding *Bsal* fungus (Spitzen-van der Sluijs et al. 2016, Wagner et al. 2017, 2019, Lötters et al. 2020 in this issue). Nevertheless, we suggest to continue to apply the removal sampling with hierarchical modelling in cases where monitoring of fire salamander larvae has already started based on this approach. However, one should keep in mind that the modelled abundances using removal data apparently drastically underestimate true larval population sizes (as estimated based on the open population approach based on CMR data). This effect was shown by us to be even

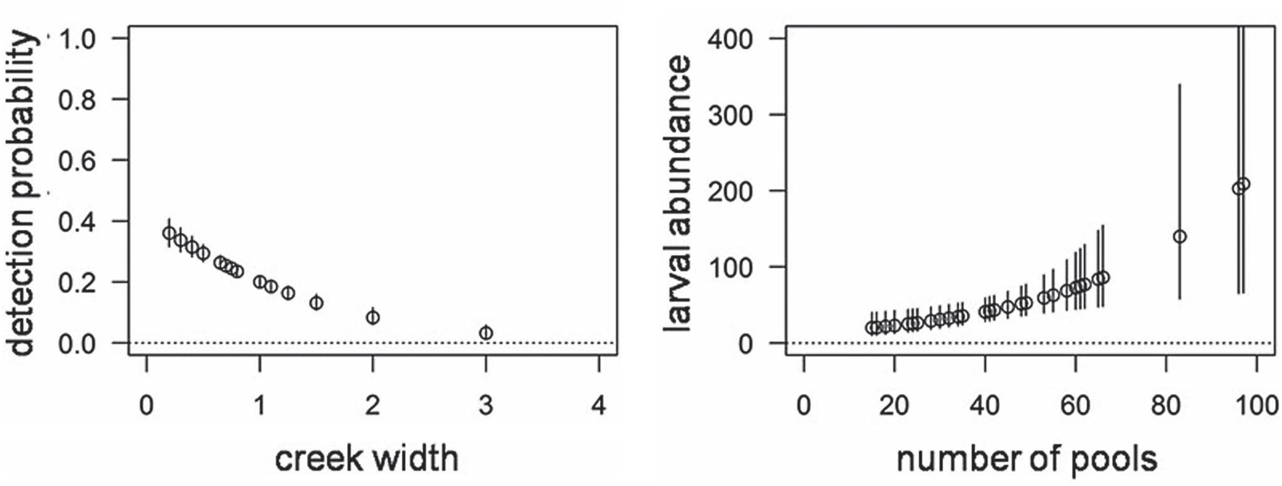

Figure 3. Decreasing detection probability with increasing creek width (m) and increasing larval abundance with increasing number of pools according to the best fitting hierarchical model using removal sampling data.

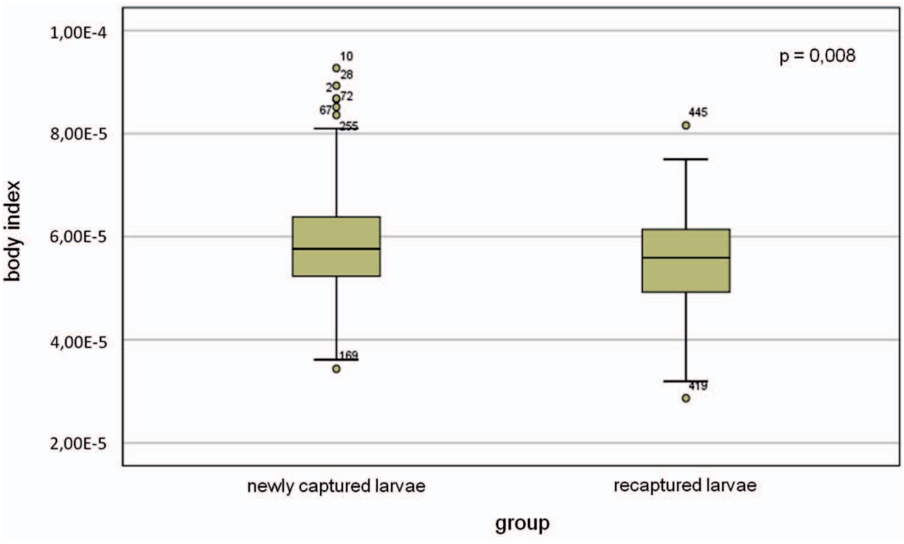

Figure 4. Comparison of means of the body index of newly captured and recaptured larvae suggest a significant impact of the Alpha-tags.

stronger when applying the simple sum of captures per time interval method (applied during the CMR). However, these time-count values correlated even better with the CMR estimates for open populations. Therefore, when financial and personal resources are small compared to the size of a given study area, one could argue that newly starting larvae monitoring programs may even apply the more simple time count methods. However, neither time count methods nor the removal sampling will suffice to gain realistic population size estimates. Up to now, we only studied four transects, and robust transformation factors for simply multiplying count data to gain realistic population size data are not yet available. Furthermore, detection probabilities may vary site-specifically (e.g., Kéry 2002, Schmidt et al. 2002, Kéry & Schmidt 2008, Kéry et al. 2009, Wagner et al., 2011). Therefore, only the CMR method will provide the option to estimate more realistic population sizes of fire salamander larvae. Our recaptured marked larvae had significantly lower body indices compared to newly captured ones (Fig. 4), and we cannot even exclude that marked larvae suffer from an increased mortality; this would inevitably lead to an overestimation of population size. In consequence, the CMR method based on individual marking with Alpha-Tags may be too invasive for being applied (see also Lunghi & Veith [2017] for problems with cave salamanders, genus *Hydromantes*), and hence may bias CMR population sizes estimates based on this individual marking technique. Marking techniques based on digit amputation (e.g., Reinhardt et al. 2018) are invasive per se (see also the comment by May [2004]) and should not be used as long as a negative impact on larval survival cannot be excluded. Non-invasive individual photographic identification of larvae may be an alternative to be applied in CMR studies. However, as long as this approach cannot deal with capture quantities larger than 20 (see Eitam & Blaustein 2002) and the applicability of computer-based identification programs as Wild-ID is not tested, photo-identification also seems to be no option.

Acknowledgements

We thank the 'Struktur- und Genehmigungsdirektion Nord' (Koblenz) for all necessary permission to conduct the present study. Furthermore, we are grateful to all students who had conducted the removal sampling (in alphabetical order): Michélle Fuchs, Wiebke Harms, Julia Hermes, Marina Kiweler, Martina Mittelstädt, Mona-Lisa Steiger, Marina Zentarra.

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Supplementary data

The following data are available online:

Supplementary Table S1. Model selection table and estimates for survival, capture and entry to the super-population) probabilities.

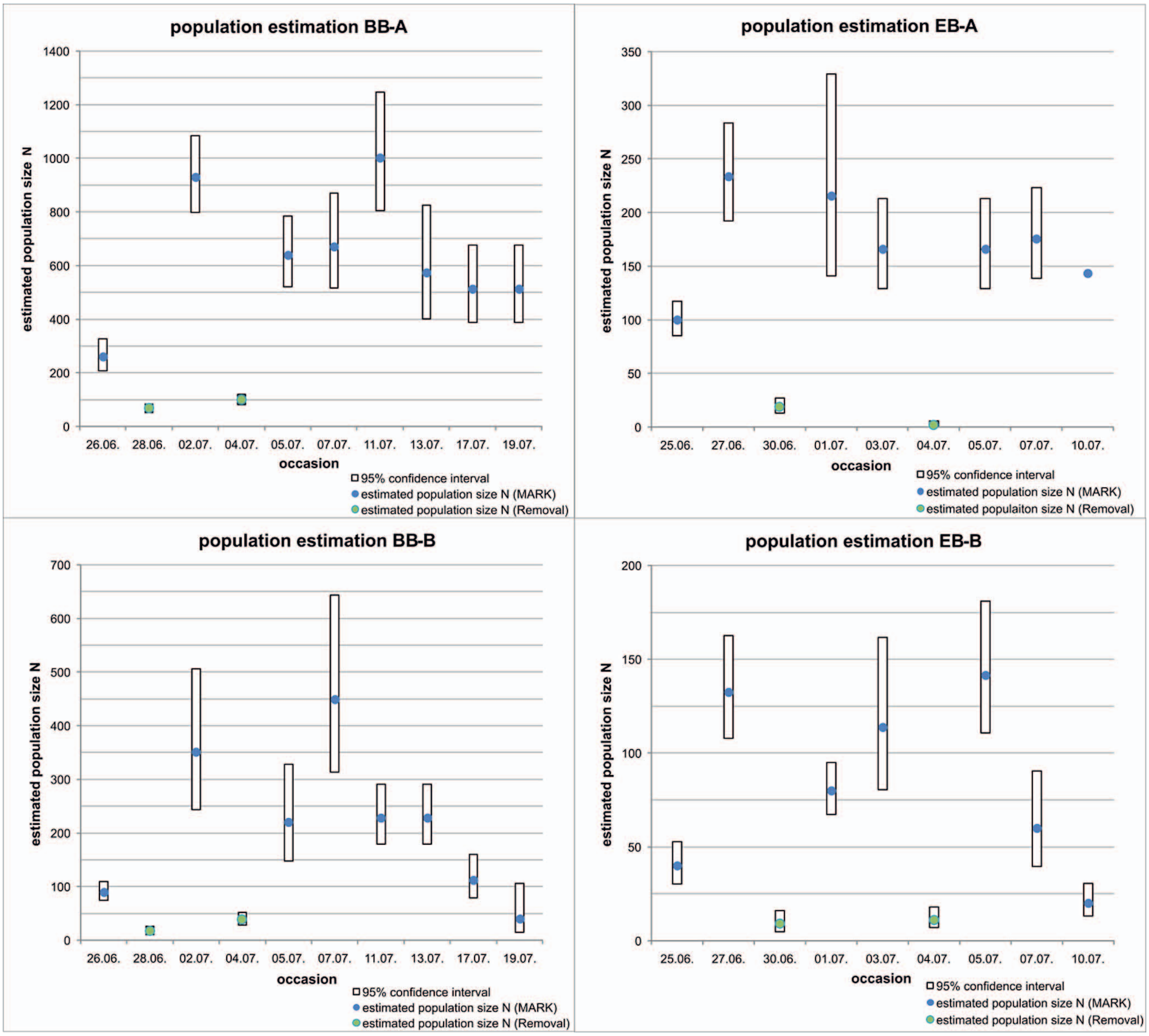
Supplementary Figure S1. Population size estimates for a shortened capture period and for each transect.

Supplementary Figure S2. Correlations between results gained from the two model approaches and the two count methods.

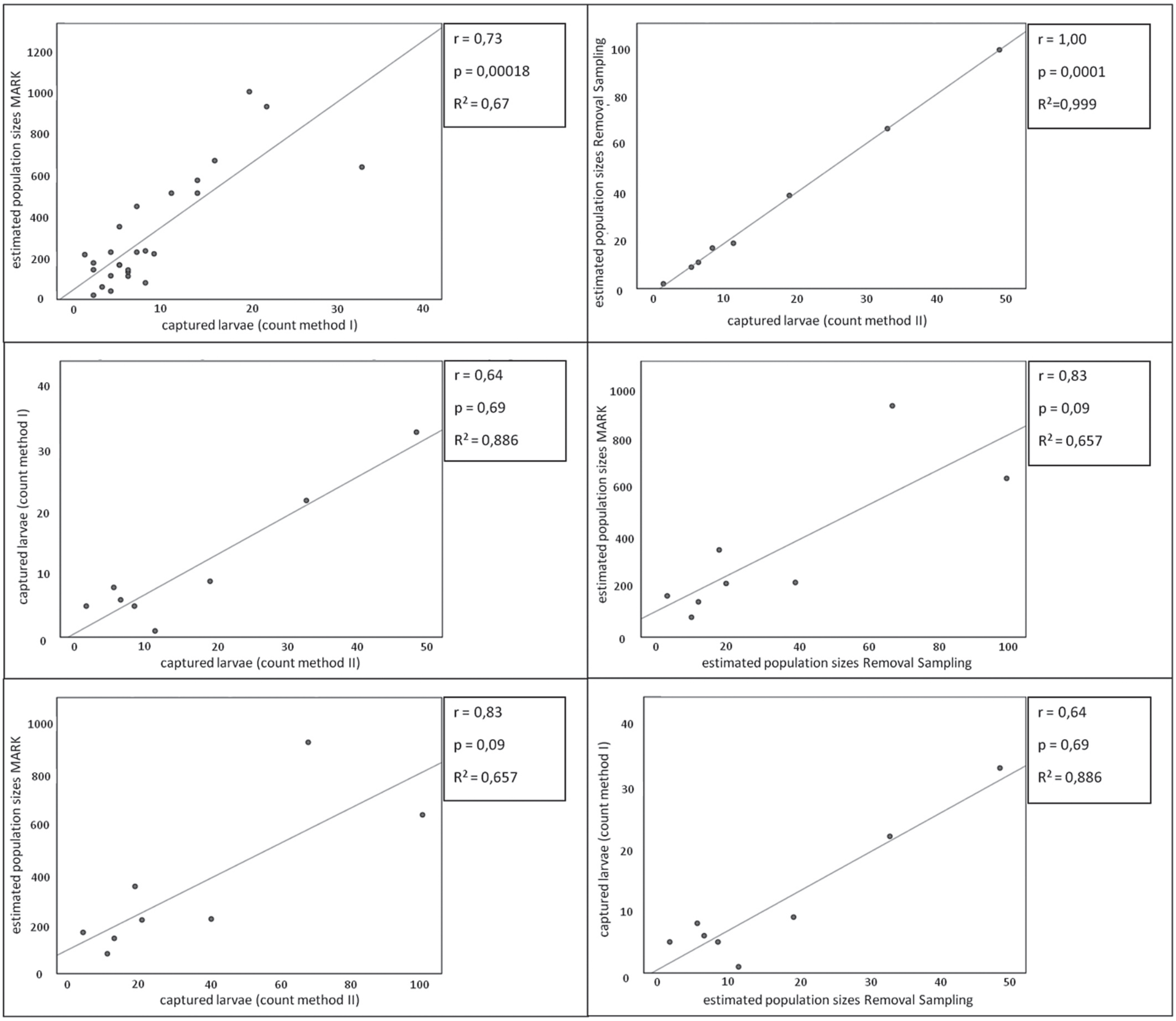
Supplementary document 1. Model selection table and estimates for survival (phi), capture (p) and entry to the super-population (pent) probabilities.

Creek	Model	AICc	Delta AICc	AICc Weight	Model Likelihood	No. Par.	Deviance
EB-A	Logit_Phi(t)_p(t)_pent(t)_N(id)	1590	0	0.70	1.00	16	-1223
	Logit_Phi(t)_p(t)_pent(t)_N(log)	1592	2	0.21	0.32	20	-1230
	Sin_Phi(t)_p(t)_pent(t)_N(id)	1595	4	0.08	0.11	21	-1230
	Sin_Phi(t)_p(t)_pent(t)_N(log)	1600	10	0.01	0.01	21	-1224
	Sin_Phi(t)_p(.)_pent(t)_N(id)	1624	34	0.00	0.00	13	-1183
	Logit_Phi(t)_p(.)_pent(t)_N(id)	1717	127	0.00	0.00	6	-1075
EB-B	Logit_Phi(t)_p(t)_pent(t)_N(id)	1388	0	0.46	1.00	21	-924
	Logit_Phi(t)_p(t)_pent(t)_N(log)	1388	0	0.46	1.00	21	-924
	Sin_Phi(t)_p(t)_pent(t)_N(id)	1392	4	0.06	0.13	23	-924
	Sin_Phi(t)_p(t)_pent(t)_N(log)	1394	6	0.02	0.04	24	-924
	Logit_Phi(t)_p(.)_pent(t)_N(id)	1477	89	0.00	0.00	17	-827
	Sin_Phi(t)_p(.)_pent(t)_N(id)	1487	99	0.00	0.00	13	-809
BB-A	Logit_Phi(t)_p(t)_pent(t)_N(id)	3427	0	0.83	1.00	27	-7133
	Logit_Phi(t)_p(t)_pent(t)_N(log)	3430	3	0.15	0.19	29	-7134
	Sin_Phi(t)_p(t)_pent(t)_N(id)	3436	9	0.01	0.01	32	-7134
	Sin_Phi(t)_p(t)_pent(t)_N(log)	3436	9	0.01	0.01	32	-7134
	Logit_Phi(t)_p(.)_pent(t)_N(id)	3480	53	0.00	0.00	21	-7068
	Logit_Phi(t)_p(.)_pent(t)_N(log)	3482	55	0.00	0.00	22	-7068
BB-B	Logit_Phi(t)_p(t)_pent(t)_N(log)	1508	0	0.99	1.00	23	-3019
	Sin_Phi(t)_p(t)_pent(t)_N(log)	1518	11	0.01	0.01	28	-3019
	Sin_Phi(t)_p(t)_pent(t)_N(id)	1520	13	0.00	0.00	27	-3013
	Logit_Phi(t)_p(t)_pent(t)_N(id)	1540	33	0.00	0.00	21	-2981
	Logit_Phi(t)_p(.)_pent(t)_N(log)	1607	100	0.00	0.00	18	-2907
	Sin_Phi(t)_p(.)_pent(t)_N(log)	1612	105	0.00	0.00	20	-2907

Supplementary document 2. Population size estimates for a shortened capture period (only after heavy rainfalls) and for each transect A and B in the BB and EB using CMR data of marked fire salamander larvae.



Supplementary document 3. Correlations between results gained from the two model approaches and the two count methods.



SALAMANDRA **56**(x): 275–284
15 August 2020 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Evidence of *Batrachochytrium dendrobatidis* and other amphibian parasites in the Green toad (*Bufotes viridis*), syntopic amphibians and environment in the Cologne Bay, Germany

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Manuscript received: 24 October 2019
Accepted: 26 January 2020 by Stefan Lötters

Abstract. Chytridiomycosis, a disease induced by the chytrid fungi *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*), has strongly contributed to the ongoing worldwide amphibian conservation crisis. While *Bd* infection has caused amphibian declines for decades on several continents, *Bsal* is a novel threat to Central European salamanders and newts, being responsible for the collapse of Fire Salamander populations in the Netherlands, Belgium, and Germany. However, numerous other parasites causing harm to amphibians exist, yet have received much less attention than the chytrid fungi. The goal of the present study was to contribute to the understanding of declines of the Green Toad, *Bufotes viridis,* at its northwestern distribution border, in the area of Cologne, Germany. We combined the data from four years of *Bd* monitoring with a metabarcoding approach to detect other, mainly unicellular parasites, from amphibian feces and environmental samples, and also report results from *Bsal* testing in 2019. Skin swabs of approximately 280 amphibians were tested for *Bd* and 66 for *Bsal*, and 150 cloacal swabs and environmental samples from five sites were tested for other pathogens and parasites. We found *Bd* in all sampled locations with high prevalences and partly high individual infection loads but without clinical signs of chytridiomycosis. None of the samples tested for *Bsal* was positive for this pathogen. We further detected eight additional potential amphibian pathogens from fecal samples: three metamonads (*Tritrichomonas augusta, Trichomitus batrachorum* and *Hexamita inflata*), three ciliates (*Balantidium duodeni, Nyctotherus cordiformis* and *N. hubeiensis*), one stramenopile (*Blastocystis* sp.) and one metazoan (the nematode *Rhabdias ranae*). In the environmental samples, we detected OTUs of nine organisms potentially harmful for amphibians: *Blastocystis* sp., *Hexamita inflata*, *Tritrichomonas augusta*, *Trichomitus batrachorum*, two oomycetes (*Leptolegnia* sp., *Saprolegnia* sp.), two ichthyosporeans (*Amphibiocystidium ranae*, *Anurofeca* sp.) and the myxozoan *Myxobolus* sp.

Key words. Amphibia, *Bd, Bsal,* chytrid infection, pathogens, North Rhine-Westphalia.

Introduction

Chytridiomycosis has been characterized as the panzootic associated to the greatest recorded loss of biodiversity arising from a disease (Scheele et al. 2019), substantially contributing to ongoing declines of amphibian diversity worldwide. This skin-affecting disease is caused by the chytridiomycete fungi *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*). It affects mainly the epidermis (stratum corneum and stratum granulosum), leading to a strong disturbance of its biological functions (van Rooij et al. 2015). While *Bd* has a broader host-range and can affect representatives of all major amphibian clades, *Bsal* appears to be restricted to urodelan hosts, i.e., salamanders and newts (Stegen et al. 2017), although it recently was shown to likewise be present on anurans, viz. the small-webbed fire-bellied toads (*Bombina microdeladigitora*) (Nguyen et al. 2017). Yet, *Bsal*, in many species seems to be even more aggressively causing mortality of infected animals. According to recent studies (Laking et al. 2017, O'Hanlon et al. 2018) the origin of these pathogens is in Asia. Upon its discovery in the late 1990's *Bd* was mostly studied in the tropics but later also found in temperate zones; in 1999 it was first detected in Germany (MUTSCHMANN et al. 2000), predominantly in captive animals. Since then, several studies have traced its occurrence in the wild in Europe (GARNER et al. 2005, LÖTTERS et al. 2018) where most amphibian species appear to be able to coexist with *Bd*, but others are affected by chytridiomycosis (GARNER et al. 2005).

Since 2016, *Bd* monitoring has been carried out in the Cologne bay (Western Germany) in the framework of a conservation management project for *Bufotes viridis.* This project, initiated by the NABU (Naturschutzbund Deutschland e.V.), Technische Universität Braunschweig and the Cologne Zoo and subsequently supported also by the Stadtentwässerungsbetriebe Köln (StEB) AöR, targeted the severe population declines of *B. viridis* in this area, including conservation genetics approaches (VENCES et al. 2019) and ex-situ rearing (ZIEGLER et al. 2019). Additionally, in 2018, cloacal swabs of around 150 anuran individuals plus a series of environmental samples were tested for the occurrence of amphibian parasites that may be contributing to the declines of *B. viridis* in Cologne. Intestinal protist parasites or commensals like metamonads or ciliates are quite common in amphibians (BAKER 2008) yet their harmfulness is strongly dependent on their grade of infestation and the health state of their host. This especially applies when the animals are burdened with multiple infections. We applied a metabarcoding approach to obtain reliable information on parasites from environmental DNA (BASS et al. 2015, RYTKÖNEN et al. 2018), especially for protists that may be difficult to cultivate in culture media. Since Cologne is close to current *Bsal* outbreaks (DALBECK et al. 2018, LÖTTERS et al. 2020, SCHULZ et al. 2020, this issue), we also screened for this second pathogen in 2019.

Thus, we here present *Bd* monitoring data from 2016–2019 and *Bsal* monitoring data from 2019. We also summarize records of amphibian pathogens and parasites detected via metabarcoding in the same populations, and discuss the relevance of these findings for conservation management of amphibians, especially *B. viridis*, in Cologne.

Materials and methods

Sample collection

Skin swabs were collected in 2016–2019 from wild amphibians at nine sampling sites in and around the municipal area of Cologne (Fig. 1, Table 1). Sites were selected depending on amphibian species diversity and the occurrence of our main target, *Bufotes viridis*. A total of 288 samples were taken from five different anuran and urodelan species (*B. viridis*, *Bufo bufo*, *Epidalea calamita*, *Pelophylax* kl. *esculentus* and *Lissotriton vulgaris*). Until further examination, the swabs were kept at -20°C.

Cloacal swabs were taken from anurans from six of the selected sampling sites (not all sites were revisited every year) in 2018. All animals were probed with a sterile small swab (MW113, MWE Medical Wire, Corsham, UK) inserted in the cloaca. The inserted swab was gently rotated around three times, extracted from the cloaca, and immediately transferred to a clean, pre-labeled 1.5 ml microtube (Roth, Karlsruhe, Germany). The tubes were frozen at -20°C until further processing.

Two-liter water samples were taken in 2018 from five sites (where *B. viridis* occured), transferred to the laboratory, pre-filtered with a 100 µm gauze to remove larger debris and, with the help of a hand-pump, up to 600 ml water were pulled over a cellulose nitrate filter with a pore size of 1.2 µm (Sartorius, Göttingen, Germany). Samples were taken in duplicates or triplicates. Filters were preserved individually each in 5 ml DESS (YODER et al. 2006) at room temperature.

Chytrid detection via quantitative PCR (qPCR)

DNA from skin swabs was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following BLETZ et al. (2015). We used the manufacturer's pretreatment protocol for gram negative bacteria, expanding the initial incubation to 1 h and carrying out the second incubation at 70°C (BELDEN et al. 2015), then followed by the protocol for animal tissues. Samples from 2016–2018 were analyzed for *Bd* with qPCR assays using the *Bd*-specific primers according to BOYLE et al. (2004), following a standard mixture using KlearKall Master Mix (LGC genomics, Middlesex, UK) each time investing 5 µl DNA and 20 µl Mix. In 2016 and 2017 we implemented an initial step at 95°C/10 min, followed by 49 cycles of 95°C/15 s and 62°C/1 min on a CFX96 Real-Time System (Bio-Rad Laboratories Inc., Hercules, USA). In 2018 we implemented an initial denaturation step of 95°C for 15 min followed by 43 cycles of 15 s/95°C and 60 s/60°C on a StepOne™ Real-Time System (Applied Biosystems, Foster City, USA). In 2016–2018, we used the *Bd* isolate JEL310 (obtained from the laboratory of D. WOODHAMS at the University of Massachusetts, originally from that of J. LONGCORE at the University of Maine) as a standard, in a dilution of 1–10,000 zoospore genome equivalents. For 2016, the threshold was set at 1 zoospore for positive detection. Due to technical difficulties with implementing the standards, no exact loads could be determined for 2017 and 2018. For samples from these two years, we implemented two qualitative approaches considering a sample as a putative positive when the shape of the amplification curve was logarithmic with a certain cycle threshold (Ct-value). Following a very conservative approach according to the Techne (Cole-Palmer, Staffordshire, UK) qPCR test manual for *Bd* detection and our own experience, a sample would then be a putative positive if it had amplified at cycle 28 ± 3. After a more inclusive approach, following a protocol of the Institute for Research in Immunology and Cancer (IRIC) of the University of Montreal, curves that amplify between cycle 15 and 35 would be considered a positive detection for *Bd*.

Since Cologne is spatially close to current *Bsal* outbreaks (DALBECK et al. 2018), in 2019 we analyzed the samples for *Bd* and *Bsal*. Different than in 2016–2018 we used

Table 1. Sampling sites in the municipal area of Cologne where amphibians were studied for pathogen infections. *Bb* (*Bufo bufo*), *Bv* (*Bufotes viridis*), *Ec* (*Epidalea calamita*) *Lv* (*Lissotriton vulgaris*), *Pe* (*Pelophylax* kl. *esculentus*).

Sampling site	Longitude, Latitude	N samples studied for *Bd*	Species studied
Baadenberger Senke (BS)	50.9878, 6.8518	13	*Bb*
Decksteiner Weiher (DW)	50.9070, 6.8992	2	*Bb*
Ginsterpfad (GP)	50.9850, 6.9301	118	*Bb, Bv, Ec, Lv, Pe*
Kiesgrube Esser (KE)	50.8368, 6.9300	6	*Bv*
NBI (NB)	50.8514, 6.9451	37	*Bv*
Porz Wahn (PW)	50.8618, 7.0773	12	*Bv*
R2.12 (R2)	50.8454, 6 9397	13	*Bv, Ec, Lv*
Vogelacker (VA)	50.8449, 6.9535	2	*Bv*
Westhovener Aue (WA)	50.9037, 7.0085	81	*Bb, Bv, Pe*

gBlocks® gene fragments instead of zoospores as standard to account for ITS copy number variations between strains, as described by Rebollar et al. (2017). We implemented a duplex real time PCR, following the protocol of Blooi et al. (2013) to detect *Bd* and *Bsal* simultaneously, again with the use of KlearKall Master Mix (LGC genomics, Middlesex, UK) but with the alteration of 15 min/95°C for the first step and investing 10 µl Mix and 5 µl DNA. Final concentration of the ordered stock solution was 1 ng/µl. We used a logarithmic standard dilution from 10^7 to 10^2 ITS copies/5µl. Given that Rebollar et al. (2017) estimated around 73 copies zsp.$^{-1}$ for strain JEL310, we set the threshold at 100 ITS copies for the 2019 samples (next logarithmic scale to 73) for positive detection.

Metabarcoding of cloacal swab samples

DNA from cloacal swabs was isolated with the Quick-gDNA prep kit (Zymo Research, Irwing, USA) following the swab isolation protocol suggested by the manufacturer. We amplified a fragment of the hypervariable V9 region

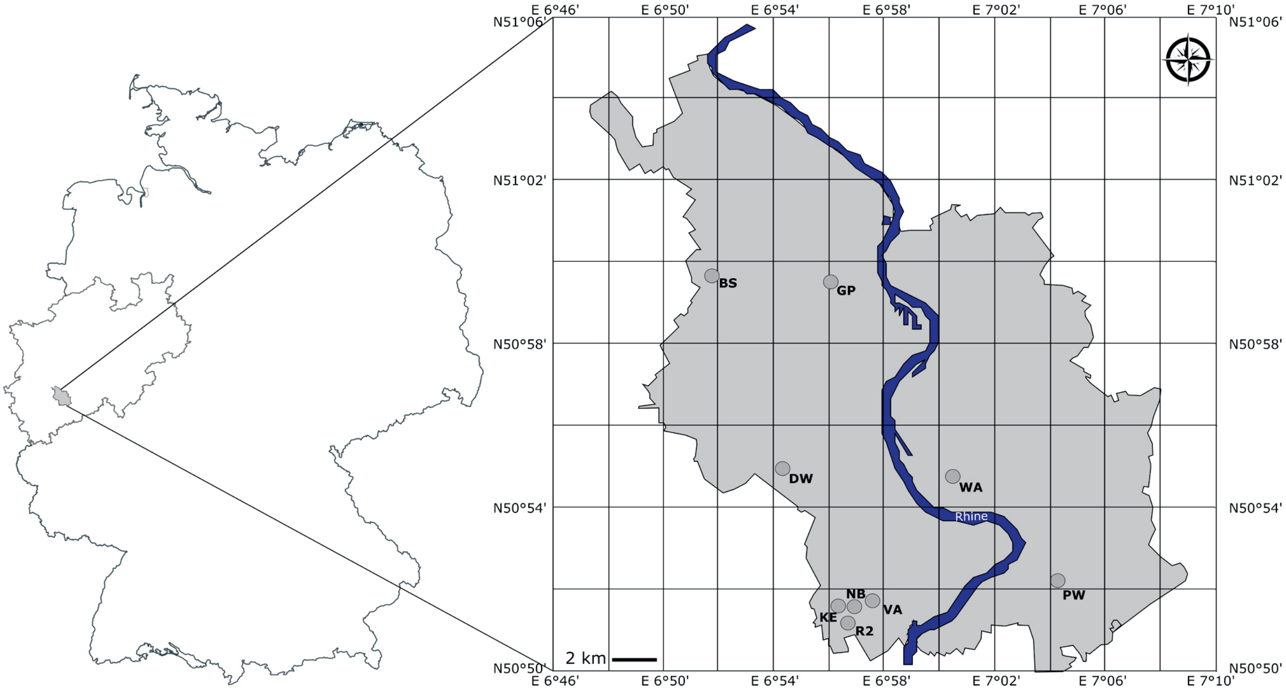

Figure 1. Maps of sampling locations within the area of Cologne. Germany map ("Lage von Köln in Nordrhein-Westfalen, Deutschland") was created by TUBS and is under the Creative Attribution-Share Alike 3.0 Unported license (https://creativecommons.org/licenses/by-sa/3.0/deed.en). It can be found on https://commons.wikimedia.org/wiki/File:Locator_map_K_in_Germany.svg_. The map was re-colored and some elements were removed. BS (Baadenberger Senke), GP (Ginsterpfad), DW (Decksteiner Weiher), KE (Kiesgrube Esser), NB (Neues Biotop Immendorf-NBI), R2 (R2.12), VA (Vogelacker), PW (Porz Wahn), WA (Westhovener Aue).

of the 18S small subunit rDNA (Amaral-Zettler et al. 2009) in duplicate, with uniquely indexed primers that also contained Illumina adapters, as described in Vences et al. (2016). To decrease the amplification of the host species we added a vertebrate blocking primer (Vestheim & Jarman 2008). Samples were pooled in approximately equal concentrations based on band strength on agarose electrophoresis, cleaned up with the PCR clean-up gel extraction kit (Macherey-Nagel, Düren, Germany), quantified using a Qubit fluorometer (Invitrogen, Carlsbad, USA), and sequenced on an Illumina MiSeq instrument (250 bp, paired-end).

Metabarcoding of environmental samples

DNA isolation from water filters took place following a DNAzol (Alfa Aesar, Haverhill, USA) protocol. Filters were rinsed thoroughly with their own supernatants (DESS). The liquids (~5 ml) were transferred to fresh 50 ml tubes (Roth, Karlsruhe, Germany) and centrifuged at 4,000 x g for 20 min at 4°C (Megafuge 2.0R, Heraeus Instruments, Hanau, Germany), followed by the manufacturer's protocol. Pellets were dried in a Speedvac (Concentrator 5301, Eppendorf, Hamburg, Germany) at 45°C and re-suspended with 30 µl elution-buffer from the Quick-gDNA prep kit (Zymo Research, Irving, USA). To remove all remaining salts, all samples were centrifuged at 10,000 x g for 10 min, afterwards discarding the supernatant. The remaining pellet was washed twice with each 1 ml ddH_2O and centrifuged at 10,000 x g for 10 min, each time discarding the supernatant after centrifugation. The remaining pellets were re-suspended with 30 µl elution-buffer. Amplification (in triplicate) of the V9 region of 18S took place with the same primers as used for cloacal swabs, but not containing Illumina adapters. The three replicates of each (filter) replicate were combined and purified with the PCR Purification Kit (Jena Bioscience, Jena, Germany), starting with step 1b for High Yield Sample preparation, and subsequently following the manufacturer's protocol. Samples were quality-checked with Nanodrop (Spectrophotometer ND-1000; Peqlab, Erlangen, Germany) and sent to the sequencing facility of the Cologne Center of Genomics (CCG) for library preparation and a subsequent sequencing on an Illumina HiSeq instrument, sharing a lane with 37 samples in total for a 2 × 150 bp paired-end run.

The raw reads of all metabarcoded samples (environmental and cloacal swabs) were submitted to the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/) and are accessible under BioProject number PRJNA611823.

Statistical data processing

Sequences from cloacal swab metabarcoding were analyzed with MACQIIME version 1.9.1 (Caporaso et al. 2010) using only the forward reads. After demultiplexing, sequences were denoised and quality filtered using Deblur (Amir et al. 2017) with a subsequent clipping to 150 bp. For taxonomic assignment, sequences were aligned to the external SILVA database (Quast et al. 2013, Yilmaz et al. 2014) and clustered into operational taxonomic units (OTUs) at 90% sequence identity. The dataset was rarefied to a minimum read count of 1,000 reads per sample. Because of incompleteness of the SILVA database, around 50% of the OTUs could not be assigned. They were once again blasted to a second database as described for the environmental samples below, which allowed all sequences to be assigned. Sequences identified as OTUs potentially relevant for amphibian health were then once again blasted against the NCBI/GenBank database to verify their identity.

For further statistical analysis we assigned OTUs to categories based on the number of reads per OTU and sample. An OTU with > 500 reads in a sample was considered to be unambiguously detected in this sample, and possibly detected with 1–499 reads. Assuming that technical replicates underlie only small differences due to technical variances associated with Illumina (Marioni et al. 2008), the reads of both (technical) replicates were summed for each OTU and sample. The categories < 500 and > 500 reads per sample and OTU were analyzed with R (Version 3.3.2, R Core Team 2015) regarding their frequency distribution in the different hosts and sampling sites. To test for significant differences between the hosts and sites in terms of frequency distribution of parasite categories we used Exact Fisher tests (all used tests are part of the native R stats package). In cases where the contingency table was too large for the algorithm of the Fisher Test, a Pearson Chi^2 test (also native R stats package) was computed, although it needs to be taken into account that this test is designed for larger sample sizes (and could lead to ambiguous results). In this case, the Exact Fisher test was used as a post hoc test for pairwise analysis between sampling sites and hosts, respectively with a Holm correction as p-value adjustment.

The data received from HiSeq Ilumina sequencing of environmental samples were filtered for high-quality V9 reads and clustered into operational taxonomic units (OTUs). Forward and reverse reads were quality encoded and merged using VSEARCH v2.6.2 (Rognes et al. 2016). Only sequences including the primer sequences were retained, sequences including ambiguous nucleotides (Ns) were discarded. The filtered reads were converted into fasta files, dereplicated with VSEARCH and then clustered with Swarm v2.2.2 (Mahé et al. 2015). Chimeras were identified with VSEARCH and only non-chimeric OTUs were used for further analysis. For taxonomic assignment, OTUs were globally pairwise aligned with VSEARCH using a reference database build from the Tara Ocean V9 database (de Vargas et al. 2015), the Protist Ribosomal Reference database PR^2 (v4.10.0, Guillou et al. 2013), the SILVA All Species Living Tree Project (Quast et al. 2013, Yilmaz et al. 2014), as well as 84 sequences of marine protist strains of the Heterotrophic Flagellate Collection Cologne (HFCC). Amplicons were assigned to their best hit/hits using the Stampa pipeline (Mahé 2016).

Other statistical analyses (e.g. ANOVAs) for the years 2016 and 2019 (where exact loads could be determined)

Table 2. Prevalence of *Bd* and *Bsal* in 2016 and 2019 (*Bsal* only tested in 2019) in percent per year, ordered according to year, site, and prevalence. NA = not applicable (not tested).

Year of sampling	Sampling site	N samples	Samples *Bd* positive (%)	Samples *Bsal* positive (%)
2016	Ginsterpfad	41	63	NA
2016	Decksteiner Weiher	2	50	NA
2016	Kiesgrube Esser	6	17	NA
2016	NBI	37	70	NA
2016	Porz Wahn	12	58	NA
2016	R2.12	13	31	NA
2016	Vogelacker	2	100	NA
2016	Westhovener Aue	20	45	NA
2019	Ginsterpfad	22	41	0
2019	Westhovener Aue	44	14	0

were conducted with R (Version 3.3.2) regarding a possible influence of season, species or sampling site on the strength of infection of individuals.

Results

Chytrid infection prevalence and loads

Bd infection was confirmed at all sites sampled, with a persistent presence in the sampled time period between 2016 and 2019 (Fig. 2, Table 2, Supplementary document 1). In 2016 we found almost 60% of the 133 screened animals being positive for *Bd*. In 2016 the three species of toads, *Bufotes viridis* (~57%), *Epidalea calamita* (66%) and *Bufo bufo* (75%, but only 4 specimens tested overall) had the highest prevalence for *Bd* infection (Fig. 3, Table 3). For the years 2017 and 2018 no exact loads could be determined. However, our results indicate that *Bd* was nevertheless very probably present in the system. Using a very conservative approach (only qPCR curves between 28 ± 3 cycles), 5 putative positives were observed in 2017 and 2018 (2 *B. viridis* and 1 *B. bufo* from Ginsterpfad, 2 *B. bufo* from Baadenberger Senke). In a more inclusive approach (qPCR curves between 15 and 35), 18 additional *Bd* positives (Supplementary document 2) were observed (1 *Lissotriton vulgaris* and 9 *B. viridis* from Ginsterpfad, 2 *B. viridis* from Westhovener Aue and 6 *B. bufo* from Baadenberger Senke).

In 2019 the highest frequency of *Bd* positives was found in *B. viridis*, with 22% of 55 specimens being infected. Though the highest *Bd* load in 2016 was detected in May, the season seemed to have no significant influence on the infection load of the individuals ($p > 0.05$).

In 2016 the highest load, with 30,202.46 zoospore equivalents/ swab (zeq/swab) was found on an individual (*B. viridis*) at the sampling site "Ginsterpfad". In this case, species (*B. viridis*) and site were those with overall highest loads in 2016 ($p < 0.05$). Despite methodological differences in the qPCR protocol, similar patterns were observed in 2019. Again, the season seemed to play no statistically significant role in the strength of infection ($p > 0.05$) but the highest load was again detected in May. In 2019 the sampling site with the significantly highest loads was again "Ginsterpfad" ($p < 0.05$), and *B. viridis* was the species with (not significantly) highest loads.

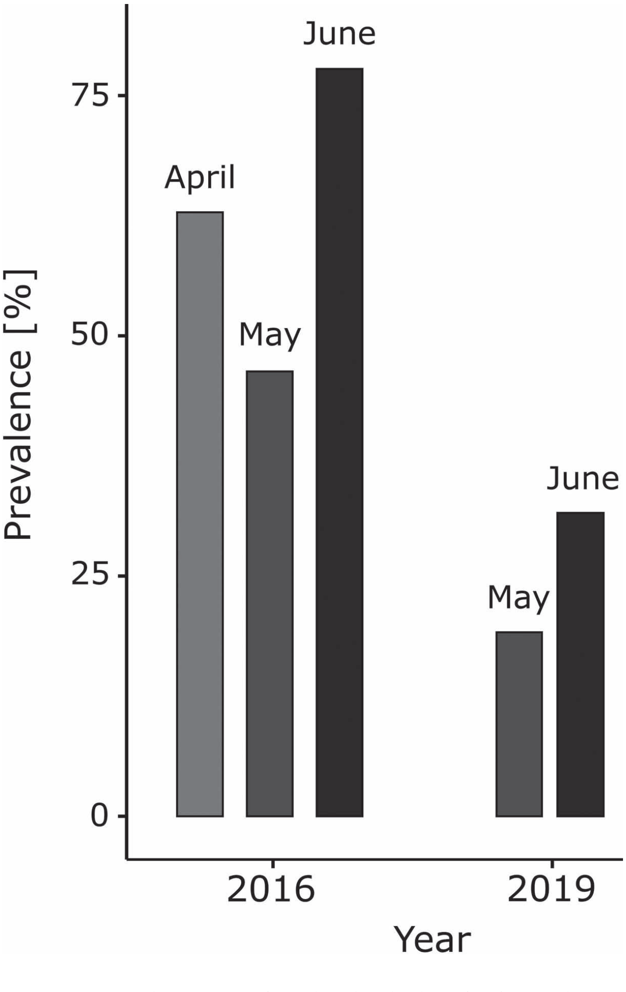

Figure 2. Prevalence [% infected individuals] of *Bd* in Cologne, by month, for the two years 2016 and 2019.

Table 3. Prevalence of *Bd* and *Bsal* in 2016 and 2019 (*Bsal* only for 2019) in percent per year, and sampled species. NA = not applicable (not tested).

Year of sampling	Sampled species	N samples	Samples *Bd* positive (%)	Samples *Bsal* positive (%)
2016	*Bufo bufo* (*Bb*)	4	75.0	NA
2016	*Bufotes viridis* (*Bv*)	91	57.1	NA
2016	*Epidalea calamita* (*Ec*)	24	66.7	NA
2016	*Lissotriton vulgaris* (*Lv*)	9	33.3	NA
2016	*Pelophylax* kl. *esculentus* (*Pe*)	5	40.0	NA
2019	*Bufotes viridis* (*Bv*)	55	21.8	0
2019	*Epidalea calamita* (*Ec*)	6	33.3	0
2019	*Lissotriton vulgaris* (*Lv*)	4	0.0	0
2019	*Pelophylax* kl. *esculentus* (*Pe*)	1	100.0	0

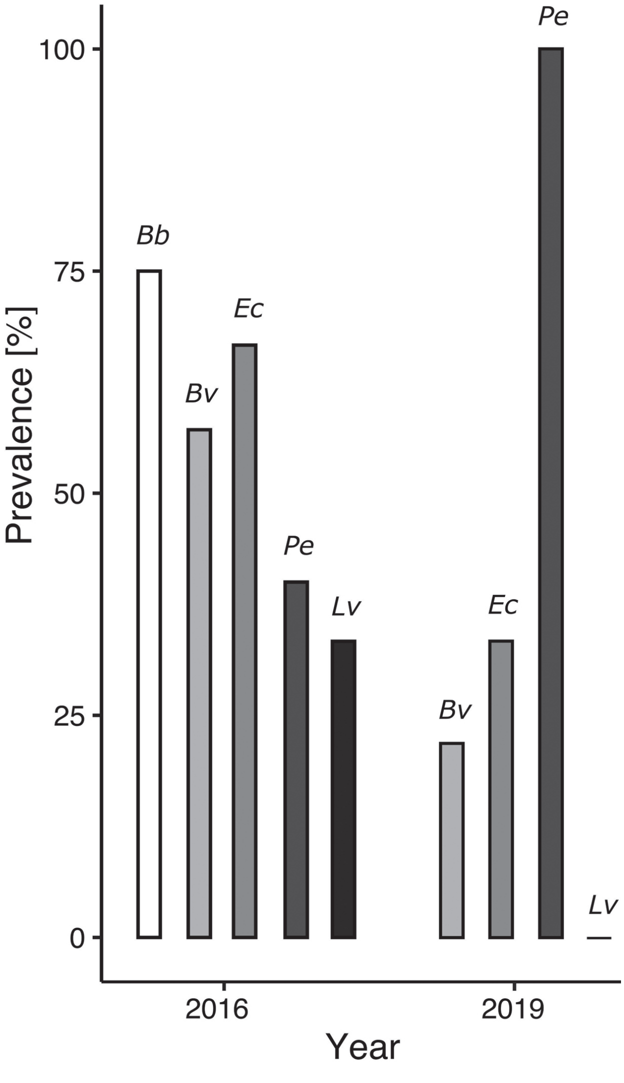

Figure 3. Prevalence [% infected individuals] of *Bd* in Cologne, per species and year, for 2016 and 2019. Abbreviations: *Bb* (*Bufo bufo*), *Bv* (*Bufotes viridis*), *Ec* (*Epidalea calamita*), *Pe* (*Pelophylax kl. esculentus*), *Lv* (*Lissotriton vulgaris*).

Loads ranged from 1.44–30,202.46 zeq/swab in 2016, and from 1,649–2,073,797 ITS copies/swab in 2019. Regardless of the partially high loads of *Bd*, none of the animals showed the typical symptoms of chytridiomycosis (i.e., lethargy, emaciation or skin lesions). From outward appearance, all individuals were found to have lesion-free skin and seemed to be in a healthy, vital condition.

The sample record of 2019 consisted of 66 samples including four *L. vulgaris* and no evidence for *Bsal* was found in these samples.

Pathogen and parasite metabarcoding

Nine different OTUs of potentially harmful organisms were found in the 157 samples originating from cloacal swabs (Table 4, Supplementary document 3): three metamonads (*Tritrichomonas augusta, Trichomitus batrachorum* and *Hexamita inflata*), three ciliates (*Balantidium duodeni, Nyctotherus cordiformis* and *N. hubeiensis*), one stramenopile (*Blastocystis* sp.) and one metazoan (a nematode: *Rhabdias ranae*). Most of these organisms are known as gut commensals with a potential to harm their host, depending on the strength of infestation. *Bd* was only found in one cloacal swab sample with a low read number.

In the category > 500 reads (considered as unambiguously identified) *E. calamita* showed the highest relative parasite load (Exact Fisher test, $p < 0.01$). In the category < 500 reads per sample and OTU, the Chi^2 test revealed a significant difference between host species ($\chi^2 = 73.503$, df = 32 and $p = 4.145e^{-5}$), but the post-hoc Exact Fisher test showed no significant differences in pairwise comparisons. For both categories, the Chi^2 test ($\chi^2 = 102.69$, df = 40, $p < 0.01$, < 500 reads) as well as the Exact Fisher test ($p < 0.01$, > 500 reads) supported significant differences between the sampling sites, with “Ginsterpfad” being the site with the highest frequency of parasitic loads.

In the environmental samples, we detected OTUs of nine organisms potentially harmful for amphibians (Table 5, Supplementary document 4): *T. augusta* and

Table 4. Parasitic organisms from fecal samples of *Bufotes viridis*, *Pelophylax* kl. *esculentus*, *Bufo bufo* and *Epidalea calamita* taken from six sampling locations around Cologne in 2018 and identified using MiSeq amplicon-sequencing of the V9 region of the small subunit rDNA. *Bb* (*B. bufo*), *Bv* (*B. viridis*), *Ec* (*E. calamita*), *Pe* (*P.* kl. *esculentus*); resp. BS (Baadenberger Senke), AV (Am Vogelacker), NB (NBI, Neues Biotop Immendorf), PW (Porz Wahn), GP (Ginsterpfad); OTU (operational taxonomic unit).

OTU	BLAST Identity (%)	OTUs identified (>500 reads)	OTUs (percent)	OTUs included (<500 reads)	OTUs (percent)	Total no reads	Localities	Species
Tritrichomonas augusta	100	43/157	27.30%	109/157	69.40%	60082	BS, AV, NB, PW, GP, WA	*Bb, Ec, Bv, Pe*
Rhabdias ranae	98	9/157	5.70%	74/157	47.13%	10957	BS, AV, NB, PW, GP, WA	*Bb, Ec, Bv, Pe*
Balantidium duodeni	99	2/157	1.30%	102/157	64.96%	6048	BS, AV, NB, PW, GP, WA	*Bb, Ec, Bv, Pe*
Blastocystis sp.	98	5/157	3.20%	20/157	12.74%	5652	BS, AV, NB, PW, GP, WA	*Bb, Ec, Bv, Pe*
Trichomitus batrachorum	94	1/157	0.60%	41/157	26.11%	1795	AV, NB, PW, GP, WA	Bb, Ec, Bv,
Nyctotherus cordiformis	99	0/157	0%	1/157	0.60%	279	NB	*Pe*
Nyctotherus hubeiensis	98	0/157	0%	1/157	0.60%	73	NB	*Pe*
Hexamita inflata	95	0/157	0%	1/157	0.60%	47	AV	*Bb*
Batrachochytrium dendrobatidis	99	1/157	0.60%	1/157	0.60%	2	GP	*Bv*

Table 5. OTUs of parasitic or potentially harmful organisms found in the water samples from 2018 with the help of HiSeq-sequencing of the V9 region of the small subunit rDNA. Occurrence per sampling sites with BLAST identity in percent, total number of reads and localities. Abbreviations stand for: PW (Porz Wahn), GP (Ginsterpfad), NB (NBI), WA (Westhovener Aue), OTU (operational taxonomic unit).

OTU	BLAST Identity (%)	Total no reads	Localities
Trichomitus batrachorum	98	1434	PW, GP
Tritrichomonas augusta	100	397	PW, GP
Leptolegnia caudata	100	292	NB
Anurofeca sp.	98	57	PW
Hexamita inflata	76.9	51	WA
Blastocystis sp.	65.7	18	NB
Saprolegnia sp.	99.2	11	PW, GP, NB, WA
Myxobolus sp.	97	5	PW, GP, NB, WA
Amphibiocystidium ranae	93.9	1	NB

T. batrachorum, the latter one with the highest number of reads, *H. inflata* and *B.* sp. (both with very low BLAST identities), and two oomycetes, *Saprolegnia* sp. and *Leptolegnia* sp. as well as two ichthyosporeans, *Anurofeca* sp. and *Amphibiocystidium ranae*. Furthermore, the myxozoan *Myxobolus* sp. was present in all water samples with a low number of reads (BLAST identity 97%). Overall, the water sample data included only one relevant OTU (*T. batrachorum*) above the chosen threshold of 500 reads, while many OTUs had substantially fewer reads and partially low BLAST identities.

Discussion

Our data show that *Bd* is common in amphibian populations in the Cologne area, and was detected across the whole sampling period from March to June (at least in 2016 and 2019), without clear seasonal trends. Seasonal fluctuations of the occurrence of *Bd* can be explained based on its temperature optimum between 17–25°C (van Rooij et al. 2015) which supports its prevalence being higher in spring. Individual *Bd* loads in our study are difficult to compare across sampling years due to methodological differences, and technical qPCR problems in 2017 and 2018. Reliable data are available for 2016 and 2019 but were obtained with different qPCR standards. Applying the estimate of 73 ITS copies/zoospore for strain JEL310 (Rebollar et al. 2017), the highest loads for 2019 (2,073,797 ITS copies/73 ~28,408 zeq/swab) are comparable to those of 2016 (~30,202 zeq/swab). These values are similar to those detected in other amphibians (e.g., Briggs et al. 2010), but their interpretation is limited by our lack of knowledge on the *Bd* strain involved. This also impedes precise comparison of loads which can be over- or underestimated without strain-specific custom standards for qPCR. The use of gBlocks® gene fragments can be seen as a methological improvement as it allows a correlation between ITS copies and zoospores. Vredenburg et al. (2009) set the threshold for a population collapse at an average ~10^4–10^5 zoospores; in 2016 we measured an average *Bd* load of 579.44 (± 3,311.64) zeq/swab, a value that is by far lower than the threshold.

The city of Cologne is geographically situated in between the two documented *Bsal* hotspots in Germany, i.e., the Eifel Mountains and the Ruhr district (Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018), both at a distance of 60–70 km from Cologne. Since *Bsal* is apparently spreading in western Germany (Dalbeck et al. 2018), screening for this pathogen in Cologne is highly relevant. In the limited number of samples studied in 2019 we could not detect this pathogen, but it can be expected to reach the area in the next years, for example via the many hikers that frequently visit the Eifel Mountains from Cologne.

In addition to *Bd*, we found amphibian populations in Cologne to be regularly infected with a variety of mostly unicellular organisms that may be harmful to their hosts. Our data suggest that protists such as *Tritrichomonas* very frequently inhabit the intestines of amphibians in the study area, and it is known that these, if present in high densities and depending on the physical condition of the host, can be harmful for amphibians (Baker 2008). Despite the high prevalence of *Tritrichomonas*, we observed no signs of illness or malnutrition in the infected amphibians. The same applies for the infection with the nematode *Rhabdias* that is known to be quite common in the lungs of anurans and can cause pneumonitis as well as tumors when its larvae migrate and form cysts (Baker 2008). Still, many Green toads, as in our case, stay asymptomatic and pathogenic changes only occur when the individual is heavily burdened. In the water samples, other organisms were present that could have an effect on amphibian health. The so called 'watermolds', like *Saprolegnia* or *Leptolegnia*, can have strong impact on amphibian eggs, causing significant egg mortality (Blaustein et al. 1994) and increasing mortality of larvae (Romansic et al. 2009); but again, we have no evidence for negative effects on amphibians in Cologne. In case of the environmental samples, one must also take in account that due to the methodical choices (pre-filtering), there is a possibility that some organisms of interest may have been removed prior to analysis.

Unfortunately, we carried out the parasite metabarcoding on samples collected in 2018, one of the years in which our *Bd* screening was handicapped by technical difficulties; a correlative analysis of parasite infection and *Bd* infection loads was therefore not possible.

So far, no disease-driven mass mortality in amphibians has been observed in our study area. Yet, populations of *B. viridis* have dwindled and disappeared at various sites in Cologne. This decline is especially well documented for the population "Ginsterpfad", which currently is one of the northernmost populations of the species in western Germany. Despite intensive habitat management, only few individuals currently occur at this site, while a large population was present some decades ago. Our data suggest that, in this population, prevalence of *Bd* and of additional potential amphibian parasites is particularly high. Furthermore, we found that the highest loads in both 2016 and 2019 indeed corresponded to *B. viridis*. This agrees with a recent study from Sweden, where *E. calamita* and *B. viridis* (formerly considered as *B. variabilis*; see Dufresnes et al. 2019) had substantially higher *Bd* prevalences than other tested anurans (Kärvemo et al. 2018), possibly related to their highly keratinized skin. While during our surveys, all sampled adult toads appeared to be in good health, future attention should be directed to juveniles, in particular directly after metamorphosis. Studies on North American toads suggest that these early terrestrial stages may be particularly vulnerable to chytridiomycosis (Kueneman et al. 2015), possibly due to a major restructuring of the immune system and skin during metamorphosis (Rollins-Smith et al. 2011). While the high prevalence of *Bd* and other pathogens in the "Ginsterpfad" and other Cologne populations probably has little effect on adult toads, we speculate that it may drive "silent" declines (Pasmans et al. 2018) via increased juvenile mortality, highlighting the need to test this hypothesis in future studies.

Acknowledgements

We are grateful to several friends and colleagues (Malou Raschewski, Oliver Töplitzer, Luke Schluckebier, Laura Rader) for assistance during field work, and to Gabriele Keunecke, Meike Kondermann and Rosita Bieg for help in the laboratory. Frank Pasmans and An Martel (Ghent University) contributed helpful advice. Permits were granted by the Untere Naturschutzbehörde Cologne. Thanks to Theo Pagel (Cologne Zoo) for supporting the project which was funded by intramural funds of Technische Universität Braunschweig and Cologne Zoo. Furthermore, VS was supported by a project of the Bundesamt für Naturschutz and JSP by the Deutsche Bundesstiftung Umwelt.

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Supplementary data

The following data are available online:

Supplementary document 1. Overview of *Bd* sampling in 2016 and 2019.

Supplementary document 2. Overview of *Bd* sampling in 2017 and 2018.

Supplementary document 3. Overview of amphibians sampled for the gut parasite metabarcoding study in 2018.

Supplementary document 4. Sampling sites of environmental samples in 2018.

Supplementary document 1. Overview of *Bd* sampling in 2016 and 2019 sorted by sample ID, species, *Bd* detection (pos (positive) or neg (negative), load (either zeq/swab (zoospore equivalent/swab) or ITS copies (last column), date of sampling, sampling site and sex (m [male], f [female]); nA (not determined).

Sample ID	Species	Bd (pos./neg.)	BdLoad	Date	SamplingSite	Sex	Type
WK 001	*Lissotriton vulgaris*	neg	0	06.04.16	Ginsterpfad	m	Zeq/swab
WK 002	*Lissotriton vulgaris*	pos	3.31	06.04.16	Ginsterpfad	m	Zeq/swab
WK 003	*Bufo bufo*	pos	158.3	06.04.16	Decksteiner Weiher	m	Zeq/swab
WK 004	*Bufo bufo*	neg	0	06.04.16	Decksteiner Weiher	m	Zeq/swab
WK 005	*Bufotes viridis*	pos	13.25	09.04.16	NBI	m	Zeq/swab
WK 006	*Bufotes viridis*	pos	4.39	09.04.16	NBI	m	Zeq/swab
WK 007	*Bufotes viridis*	neg	0	09.04.16	NBI	m	Zeq/swab
WK 008	*Bufotes viridis*	neg	0	09.04.16	NBI	m	Zeq/swab
WK 009	*Bufotes viridis*	pos	1.74	09.04.16	NBI	m	Zeq/swab
WK 010	*Bufotes viridis*	pos	6.17	09.04.16	NBI	m	Zeq/swab
WK 011	*Lissotriton vulgaris*	pos	6.26	06.04.16	Ginsterpfad	f	Zeq/swab
WK 012	*Bufotes viridis*	pos	65.37	09.04.16	NBI	m	Zeq/swab
WK 013	*Bufotes viridis*	pos	3613.68	09.04.16	NBI	m	Zeq/swab
WK 014	*Bufotes viridis*	pos	408.55	09.04.16	NBI	m	Zeq/swab
WK 015	*Bufotes viridis*	pos	67.06	09.04.16	NBI	m	Zeq/swab
WK 016	*Bufotes viridis*	pos	16.1	09.04.16	NBI	m	Zeq/swab
WK 017	*Bufotes viridis*	neg	0	09.04.16	NBI	f	Zeq/swab
WK 019	*Bufotes viridis*	pos	38.15	09.04.16	NBI	m	Zeq/swab
WK 020	*Bufotes viridis*	neg	0	09.04.16	NBI	m	Zeq/swab
WK 021	*Bufotes viridis*	pos	26.83	09.04.16	NBI	m	Zeq/swab
WK 022	*Epidalea calamita*	pos	2.67	09.04.16	Ginsterpfad	m	Zeq/swab
WK 023	*Epidalea calamita*	pos	6.58	09.04.16	Ginsterpfad	m	Zeq/swab
WK 024	*Epidalea calamita*	pos	16.7	09.04.16	Ginsterpfad	m	Zeq/swab
WK 025	*Bufo bufo*	pos	2.53	09.04.16	Ginsterpfad	m	Zeq/swab
WK 026	*Lissotriton vulgaris*	neg	0	09.04.16	Ginsterpfad	f	Zeq/swab
WK 027	*Lissotriton vulgaris*	neg	0	09.04.16	Ginsterpfad	f	Zeq/swab
WK 028	*Lissotriton vulgaris*	pos	6.36	09.04.16	Ginsterpfad	m	Zeq/swab
WK 029	*Bufotes viridis*	pos	1.52	12.04.16	Westhovener Aue	m	Zeq/swab
WK 030	*Bufotes viridis*	pos	1.44	12.04.16	Westhovener Aue	m	Zeq/swab
WK 031	*Bufotes viridis*	neg	0	12.04.16	Westhovener Aue	m	Zeq/swab
WK 032	*Bufotes viridis*	neg	0	12.04.16	Westhovener Aue	m	Zeq/swab
WK 033	*Bufotes viridis*	pos	2.21	12.04.16	Westhovener Aue	m	Zeq/swab
WK 034	*Bufotes viridis*	neg	0	12.04.16	Westhovener Aue	m	Zeq/swab
WK 035	*Bufotes viridis*	pos	39.53	12.04.16	Porz Wahn	m	Zeq/swab
WK 036	*Bufotes viridis*	pos	2.87	12.04.16	Porz Wahn	m	Zeq/swab
WK 037	*Bufotes viridis*	neg	0	12.04.16	Porz Wahn	m	Zeq/swab
WK 038	*Bufotes viridis*	neg	0	12.04.16	Porz Wahn	m	Zeq/swab
WK 040	*Bufotes viridis*	pos	3.85	12.04.16	Porz Wahn	m	Zeq/swab

Sample ID	Species	Bd (pos./neg.)	BdLoad	Date	SamplingSite	Sex	Type
WK 041	*Bufotes viridis*	pos	1.45	12.04.16	Porz Wahn	m	Zeq/swab
WK 042	*Bufotes viridis*	pos	1.93	12.04.16	Porz Wahn	m	Zeq/swab
WK 043	*Bufotes viridis*	pos	6.32	12.04.16	Porz Wahn	m	Zeq/swab
WK 044	*Epidalea calamita*	pos	2.31	14.04.16	Ginsterpfad	m	Zeq/swab
WK 045	*Epidalea calamita*	neg	0	14.04.16	Ginsterpfad	m	Zeq/swab
WK 046	*Bufotes viridis*	neg	0	21.04.16	NBI	m	Zeq/swab
WK 047	*Bufotes viridis*	pos	2.45	21.04.16	NBI	m	Zeq/swab
WK 048	*Bufotes viridis*	pos	22.57	21.04.16	NBI	m	Zeq/swab
WK 049	*Bufotes viridis*	pos	575.53	21.04.16	NBI	m	Zeq/swab
WK 050	*Bufotes viridis*	pos	2.48	21.04.16	NBI	m	Zeq/swab
WK 051	*Bufotes viridis*	pos	41.02	21.04.16	NBI	m	Zeq/swab
WK 052	*Bufotes viridis*	neg	0	21.04.16	NBI	m	Zeq/swab
WK 053	*Bufotes viridis*	pos	5.53	21.04.16	NBI	f	Zeq/swab
WK 054	*Bufotes viridis*	neg	0	21.04.16	NBI	f	Zeq/swab
WK 055	*Bufotes viridis*	pos	40.67	21.04.16	NBI	m	Zeq/swab
WK 056	*Bufotes viridis*	pos	57.73	21.04.16	NBI	m	Zeq/swab
WK 057	*Bufotes viridis*	neg	0	21.04.16	NBI	m	Zeq/swab
WK 058	*Bufotes viridis*	pos	10805.7	21.04.16	NBI	m	Zeq/swab
WK 059	*Bufotes viridis*	neg	0	21.04.16	NBI	m	Zeq/swab
WK 060	*Bufotes viridis*	pos	44.54	21.04.16	NBI	m	Zeq/swab
WK 061	*Bufotes viridis*	pos	23.85	21.04.16	NBI	m	Zeq/swab
WK 062	*Bufotes viridis*	pos	217.31	21.04.16	NBI	m	Zeq/swab
WK 063	*Bufotes viridis*	pos	11.56	21.04.16	NBI	m	Zeq/swab
WK 064	*Bufotes viridis*	neg	0	21.04.16	NBI	f	Zeq/swab
WK 065	*Bufotes viridis*	neg	0	21.04.16	NBI	f	Zeq/swab
WK 066	*Bufotes viridis*	pos	190.48	21.04.16	NBI	m	Zeq/swab
WK 067	*Bufotes viridis*	neg	0	23.04.16	R2.12	m	Zeq/swab
WK 068	*Lissotriton vulgaris*	neg	0	23.04.16	R2.12	m	Zeq/swab
WK 069	*Lissotriton vulgaris*	neg	0	23.04.16	R2.12	m	Zeq/swab
WK 070	*Lissotriton vulgaris*	neg	0	23.04.16	R2.12	m	Zeq/swab
WK 071	*Epidalea calamita*	pos	5.44	29.04.16	Ginsterpfad	m	Zeq/swab
WK 072	*Epidalea calamita*	neg	0	29.04.16	Ginsterpfad	m	Zeq/swab
WK 073	*Epidalea calamita*	pos	114.42	02.05.16	Ginsterpfad	m	Zeq/swab
WK 074	*Epidalea calamita*	pos	113.51	02.05.16	Ginsterpfad	m	Zeq/swab
WK 075	*Epidalea calamita*	pos	82.62	02.05.16	Ginsterpfad	m	Zeq/swab
WK 076	*Bufotes viridis*	neg	0	02.05.16	Ginsterpfad	m	Zeq/swab
WK 077	*Bufotes viridis*	pos	99.69	02.05.16	Ginsterpfad	m	Zeq/swab
WK 078	*Epidalea calamita*	pos	3.26	02.05.16	R2.12	m	Zeq/swab
WK 079	*Epidalea calamita*	pos	11.56	02.05.16	R2.12	m	Zeq/swab
WK 080	*Bufotes viridis*	neg	0	02.05.16	R2.12	m	Zeq/swab
WK 081	*Bufotes viridis*	pos	433.31	05.05.16	Ginsterpfad	m	Zeq/swab
WK 082	*Bufotes viridis*	pos	5.8	05.05.16	Ginsterpfad	m	Zeq/swab
WK 083	*Bufotes viridis*	pos	30202.46	05.05.16	Ginsterpfad	m	Zeq/swab
WK 084	*Bufotes viridis*	pos	431.81	05.05.16	Ginsterpfad	m	Zeq/swab
WK 085	*Bufotes viridis*	neg	0	05.05.16	Ginsterpfad	m	Zeq/swab
WK 086	*Bufotes viridis*	pos	7671.16	05.05.16	Ginsterpfad	m	Zeq/swab
WK 087	*Bufotes viridis*	pos	2.11	06.05.16	Ginsterpfad	m	Zeq/swab

Sample ID	Species	Bd (pos./neg.)	BdLoad	Date	SamplingSite	Sex	Type
WK 088	*Epidalea calamita*	pos	4.36	06.05.16	Ginsterpfad	m	Zeq/swab
WK 089	*Epidalea calamita*	neg	0	06.05.16	Ginsterpfad	m	Zeq/swab
WK 090	*Pelophylax* kl. *esculentus*	neg	0	06.05.16	Ginsterpfad	m	Zeq/swab
WK 091	*Epidalea calamita*	neg	0	06.05.16	Ginsterpfad	m	Zeq/swab
WK 092	*Bufotes viridis*	neg	0	06.05.16	Ginsterpfad	f	Zeq/swab
WK 093	*Pelophylax* kl. *esculentus*	pos	33.38	06.05.16	Ginsterpfad	m	Zeq/swab
WK 094	*Pelophylax* kl. *esculentus*	neg	0	06.05.16	Ginsterpfad	m	Zeq/swab
WK 095	*Bufo bufo*	pos	476.55	08.05.16	Westhovener Aue	f	Zeq/swab
WK 096	*Bufotes viridis*	pos	186.65	08.05.16	Westhovener Aue	m	Zeq/swab
WK 097	*Bufotes viridis*	neg	0	08.05.16	Westhovener Aue	m	Zeq/swab
WK 099	*Bufotes viridis*	pos	4.76	08.05.16	Westhovener Aue	m	Zeq/swab
WK 100	*Bufotes viridis*	pos	9.28	08.05.16	Westhovener Aue	m	Zeq/swab
WK 101	*Bufotes viridis*	neg	0	08.05.16	Westhovener Aue	m	Zeq/swab
WK 102	*Bufotes viridis*	neg	0	08.05.16	Westhovener Aue	m	Zeq/swab
WK 103	*Bufotes viridis*	neg	0	08.05.16	Westhovener Aue	m	Zeq/swab
WK 104	*Bufotes viridis*	neg	0	08.05.16	Porz Wahn	m	Zeq/swab
WK 105	*Bufotes viridis*	pos	5.73	08.05.16	Porz Wahn	m	Zeq/swab
WK 106	*Bufotes viridis*	neg	0	08.05.16	Porz Wahn	m	Zeq/swab
WK 107	*Bufotes viridis*	neg	0	08.05.16	Porz Wahn	m	Zeq/swab
WK 108	*Bufotes viridis*	neg	0	11.05.16	Kiesgrube Esser	m	Zeq/swab
WK 109	*Bufotes viridis*	neg	0	11.05.16	Kiesgrube Esser	m	Zeq/swab
WK 110	*Bufotes viridis*	pos	10.37	11.05.16	Kiesgrube Esser	m	Zeq/swab
WK 111	*Bufotes viridis*	neg	0	11.05.16	Kiesgrube Esser	m	Zeq/swab
WK 112	*Bufotes viridis*	neg	0	11.05.16	Kiesgrube Esser	m	Zeq/swab
WK 113	*Bufotes viridis*	neg	0	11.05.16	Kiesgrube Esser	m	Zeq/swab
WK 114	*Epidalea calamita*	pos	115.88	11.05.16	R2.12	m	Zeq/swab
WK 115	*Bufotes viridis*	neg	0	11.05.16	R2.12	m	Zeq/swab
WK 116	*Bufotes viridis*	neg	0	11.05.16	R2.12	m	Zeq/swab
WK 117	*Epidalea calamita*	pos	7.09	11.05.16	R2.12	f	Zeq/swab
WK 118	*Epidalea calamita*	neg	0	11.05.16	R2.12	f	Zeq/swab
WK 119	*Epidalea calamita*	neg	0	11.05.16	R2.12	m	Zeq/swab
WK 120	*Pelophylax* kl. *esculentus*	neg	0	18.05.16	Ginsterpfad	m	Zeq/swab
WK 121	*Epidalea calamita*	pos	377.11	18.05.16	Ginsterpfad	m	Zeq/swab
WK 122	*Epidalea calamita*	neg	0	18.05.16	Ginsterpfad	f	Zeq/swab
WK 123	*Epidalea calamita*	pos	4.31	18.05.16	Ginsterpfad	f	Zeq/swab
WK 125	*Epidalea calamita*	neg	0	18.05.16	Ginsterpfad	m	Zeq/swab
WK 126	*Bufotes viridis*	neg	0	20.05.16	Westhovener Aue	m	Zeq/swab
WK 127	*Bufotes viridis*	pos	7.7	20.05.16	Westhovener Aue	m	Zeq/swab
WK 128	*Bufotes viridis*	neg	0	20.05.16	Westhovener Aue	m	Zeq/swab
WK 129	*Bufotes viridis*	neg	0	21.05.16	Westhovener Aue	f	Zeq/swab
WK 130	*Bufotes viridis*	neg	0	20.05.16	Westhovener Aue	m	Zeq/swab
WK 131	*Bufotes viridis*	pos	9.87	20.05.16	Westhovener Aue	m	Zeq/swab
WK 132	*Bufotes viridis*	pos	19746.84	26.05.16	Ginsterpfad	m	Zeq/swab
WK 133	*Pelophylax* kl. *esculentus*	pos	100.93	26.05.16	Ginsterpfad	m	Zeq/swab
WK 134	*Epidalea calamita*	pos	22.1	26.05.16	Ginsterpfad	m	Zeq/swab
WK 135	*Bufotes viridis*	pos	113.63	02.06.16	Vogelacker	m	Zeq/swab

Sample ID	Species	Bd (pos./neg.)	BdLoad	Date	SamplingSite	Sex	Type
WK 136	*Bufotes viridis*	pos	6.67	02.06.16	Vogelacker	m	Zeq/swab
WK 137	*Bufotes viridis*	pos	76.04	15.06.16	NBI	m	Zeq/swab
KWK2019 1	*Lissotriton vulgaris*	neg	0	10.05.19	Ginsterpfad	f	ITS copies
KWK2019 2	*Lissotriton vulgaris*	neg	0	10.05.19	Ginsterpfad	m	ITS copies
KWK2019 3	*Lissotriton vulgaris*	neg	0	10.05.19	Ginsterpfad	f	ITS copies
KWK2019 4	*Lissotron vulgaris*	neg	0	10.05.19	Ginsterpfad	m	ITS copies
KWK2019 5	*Bufotes viridis*	neg	0	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 6	*Bufotes viridis*	pos	1649	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 7	*Bufotes viridis*	pos	4850	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 8	*Bufotes viridis*	neg	0	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 9	*Epidalea calamita*	neg	0	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 10	*Epidalea calamita*	neg	0	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 11	*Epidalea calamita*	neg	0	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 12	*Bufotes viridis*	neg	0	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 13	*Bufotes viridis*	neg	0	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 14	*Bufotes viridis*	neg	0	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 15	*Epidalea calamita*	pos	17680	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 16	*Epidalea calamita*	pos	4590	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 17	*Epidalea calamita*	neg	0	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 18	*Bufotes viridis*	pos	2073797	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 19	*Bufotes viridis*	pos	20474	10.05.19	Ginsterpfad	f	ITS copies
KWK2019 20	*Bufotes viridis*	pos	17285	10.05.19	Ginsterpfad	m	ITS copies
KWK2019 21	*Bufotes viridis*	pos	4905	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 22	*Pelophylax* kl. *esculentus*	pos	100489	10.05.19	Ginsterpfad	nA	ITS copies
KWK2019 23	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 24	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 25	*Bufoces viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 26	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 27	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	nA	ITS copies
KWK2019 28	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 29	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 30	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 31	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	f	ITS copies
KWK2019 32	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	nA	ITS copies
KWK2019 33	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 34	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 35	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 36	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 37	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 38	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies

Sample ID	Species	Bd (pos./neg.)	BdLoad	Date	SamplingSite	Sex	Type
KWK2019 39	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 40	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 41	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 42	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 43	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	nA	ITS copies
KWK2019 44	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 45	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	m	ITS copies
KWK2019 46	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	f	ITS copies
KWK2019 47	*Bufotes viridis*	neg	0	30.05.19	Westhovener Aue	nA	ITS copies
KWK2019 48	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 49	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 50	*Bufotes viridis*	pos	11725	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 51	*Bufotes viridis*	pos	3434	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 52	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 53	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 54	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 55	*Bufotes viridis*	pos	180634	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 56	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 57	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 58	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 59	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 60	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 61	*Bufotes viridis*	pos	159648	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 62	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 63	*Bufotes viridis*	pos	171175	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 64	*Bufotes viridis*	pos	5119	23.06.19	Westhovener Aue	f	ITS copies
KWK2019 65	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies
KWK2019 66	*Bufotes viridis*	neg	0	23.06.19	Westhovener Aue	nA	ITS copies

Supplementary document 2. Overview of *Bd* sampling in 2017 and 2018 sorted by sample ID, species, date, sampling location as well as cycle number of curve amplification and putative positives and negatives following two different approaches (conservative *Bd* positive = C_t 28 ± 3; inclusive-*Bd* positive = C_t 15–35).

Sample-ID	Species	Date	Sampling site	C_t	CONSERVATIVE	INCLUSIVE
WK201	*Lissotriton vulgaris*	10.05.2017	Ginsterpfad	0	no	no
WK202	*Lissotriton vulgaris*	10.05.2017	Ginsterpfad	0	no	no
WK203	*Lissotriton vulgaris*	10.05.2017	Ginsterpfad	0	no	no
WK204	*Lissotriton vulgaris*	10.05.2017	Ginsterpfad	0	no	no
WK205	*Lissotriton vulgaris*	10.05.2017	Ginsterpfad	0	no	no
WK206	*Bufo calamita*	10.05.2017	Ginsterpfad	36.51	no	no
WK207	*Bufo calamita*	10.05.2017	Ginsterpfad	0	no	no
WK208	*Bufotes viridis*	10.05.2017	Ginsterpfad	0	no	no
WK209	*Bufotes viridis*	10.05.2017	Ginsterpfad	0	no	no
WK210	*Bufotes viridis*	10.05.2017	Ginsterpfad	38.18	no	no
WK211	*Bufo calamita*	10.05.2017	Ginsterpfad	0	no	no
WK212	*Bufo calamita*	10.05.2017	Ginsterpfad	0	no	no
WK213	*Lissotriton vulgaris*	16.05.2017	Ginsterpfad	31.09	no	yes
WK214	*Bufotes viridis*	16.05.2017	Ginsterpfad	0	no	no
WK215	*Bufotes viridis*	16.05.2017	Ginsterpfad	0	no	no
WK216	*Bufotes viridis*	16.05.2017	Ginsterpfad	30.9	yes	yes
WK217	*Bufotes viridis*	16.05.2017	Ginsterpfad	38.29	no	no
WK218	*Bufotes viridis*	16.05.2017	Ginsterpfad	34.71	no	yes
WK219	*Bufotes viridis*	16.05.2017	Ginsterpfad	0	no	no
WK220	*Bufotes viridis*	16.05.2017	Ginsterpfad	39.42	no	no
WK221	*Bufotes viridis*	16.05.2017	Ginsterpfad	36.89	no	no
WK222	*Bufo bufo*	16.05.2017	Ginsterpfad	0	no	no
WK223	*Bufo bufo*	16.05.2017	Ginsterpfad	37.74	no	no
WK224	*Bufo bufo*	16.05.2017	Ginsterpfad	35.29	no	no
WK225	*Bufo bufo*	16.05.2017	Ginsterpfad	0	no	no
WK226	*Bufo bufo*	16.05.2017	Ginsterpfad	36.12	no	no
WK227	*Bufo bufo*	16.05.2017	Ginsterpfad	0	no	no
WK228	*Pelophylax* kl. esculentus	18.05.2017	Westhovener Aue	40.71	no	no
WK229	*Bufotes viridis*	18.05.2017	Westhovener Aue	0	no	no
WK230	*Bufotes viridis*	18.05.2017	Westhovener Aue	6.31	no	no
WK231	*Bufotes viridis*	18.05.2017	Westhovener Aue	0	no	no
WK232	*Bufotes viridis*	18.05.2017	Westhovener Aue	24.36	no	yes
WK233	*Bufotes viridis*	18.05.2017	Westhovener Aue	0	no	no
WK234	*Bufotes viridis*	18.05.2017	Westhovener Aue	0	no	no
WK235	*Bufotes viridis*	18.05.2017	Westhovener Aue	0	no	no
WK236	*Bufotes viridis*	18.05.2017	Westhovener Aue	0	no	no
WK237	*Bufotes viridis*	18.05.2017	Westhovener Aue	0	no	no
WK238	*Bufotes viridis*	18.05.2017	Westhovener Aue	38.89	no	no
WK239	*Bufotes viridis*	18.05.2017	Westhovener Aue	16.32	no	yes
WK240	*Bufotes viridis*	18.05.2017	Westhovener Aue	0	no	no
WK241	*Bufotes viridis*	18.05.2017	Westhovener Aue	0	no	no

Sample-ID	Species	Date	Sampling site	C_t	CONSERVATIVE	INCLUSIVE
WK242	*Bufotes viridis*	18.05.2017	Westhovener Aue	0	no	no
WK243	*Bufo bufo*	18.05.2017	Westhovener Aue	0	no	no
WK244	*Bufo bufo*	18.05.2017	Westhovener Aue	0	no	no
WK245	*Lissotriton vulgaris*	28.05.2017	Ginsterpfad	0	no	no
WK246	*Bufo bufo*	28.05.2017	Ginsterpfad	0	no	no
WK247	*Bufo bufo*	28.05.2017	Ginsterpfad	0	no	no
WK248	*Bufo bufo*	28.05.2017	Ginsterpfad	0	no	no
M-SA 001	*Bufo bufo*	29.03.2018	Ginsterpfad	0,63	no	no
M-SA 002	*Bufo Bufo*	29.03.2018	Ginsterpfad	9,11	no	no
M-SA 005	*Bufo Bufo*	29.03.2018	Ginsterpfad	32,2	no	yes
M-SA 007	*Bufo Bufo*	29.03.2018	Ginsterpfad	31,54	no	yes
M-SA 009	*Bufo Bufo*	29.03.2018	Ginsterpfad	15,11	no	yes
M-SA 011	*Bufo Bufo*	29.03.2018	Ginsterpfad	26,31	yes	yes
M-SA 013	*Bufo Bufo*	29.03.2018	Ginsterpfad	33,1	no	yes
M-SA 015	*Bufo Bufo*	29.03.2018	Ginsterpfad	0	no	no
M-SA 017	*Bufo Bufo*	29.03.2018	Ginsterpfad	19,65	no	yes
M-SA 019	*Bufo Bufo*	29.03.2018	Ginsterpfad	32,5	no	yes
M-SA 021	*Bufo Bufo*	04.04.2018	Baadenberger Senke	27,82	yes	yes
M-SA 022	*Bufo Bufo*	04.04.2018	Baadenberger Senke	4,03	no	no
M-SA 024	*Bufo Bufo*	04.04.2018	Baadenberger Senke	0	no	no
M-SA 026	*Bufo Bufo*	04.04.2018	Baadenberger Senke	32,92	no	yes
M-SA 029	*Bufo Bufo*	04.04.2018	Baadenberger Senke	36,06	no	no
M-SA 031	*Bufo Bufo*	04.04.2018	Baadenberger Senke	0	no	no
M-SA 033	*Bufo Bufo*	04.04.2018	Baadenberger Senke	0	no	no
M-SA 035	*Bufo Bufo*	04.04.2018	Baadenberger Senke	12,2	no	no
M-SA 037	*Bufo Bufo*	04.04.2018	Baadenberger Senke	24,29	no	yes
M-SA 039	*Bufo Bufo*	04.04.2018	Baadenberger Senke	27,6	yes	yes
M-SA 041	*Bufo Bufo*	04.04.2018	Baadenberger Senke	0	no	no
M-SA 042	*Bufo Bufo*	04.04.2018	Baadenberger Senke	0	no	no
M-SA 044	*Bufo Bufo*	04.04.2018	Baadenberger Senke	20,19	no	yes
M-SA 046	*Bufo Bufo*	06.04.2018	Ginsterpfad	27,54	yes	yes
M-SA 047	*Bufo Bufo*	06.04.2018	Ginsterpfad	0	no	no
M-SA 049	*Bufo Bufo*	06.04.2018	Ginsterpfad	0	no	no
M-SA 051	*Bufo Bufo*	06.04.2018	Ginsterpfad	0	no	no
M-SA 053	*Bufo Bufo*	06.04.2018	Ginsterpfad	0	no	no
M-SA 055	*Bufo Bufo*	06.04.2018	Ginsterpfad	37,41	no	no
M-SA 057	*Bufo Bufo*	06.04.2018	Ginsterpfad	36,81	no	no
M-SA 059	*Bufo calamita*	06.04.2018	Ginsterpfad	36,81	no	no
M-SA 061	*Bufo Bufo*	06.04.2018	Ginsterpfad	0	no	no
M-SA 063	*Bufo Bufo*	06.04.2018	Ginsterpfad	0	no	no
M-SA 065	*Bufo calamita*	06.04.2018	Ginsterpfad	0	no	no
M-SA 067	*Bufo calamita*	06.04.2018	Ginsterpfad	38,94	no	no
M-SA 069	*Bufo Bufo*	06.04.2018	Ginsterpfad	35,59	no	no
M-SA 071	*Bufotes viridis*	06.04.2018	Ginsterpfad	37,32	no	no

Supplementary document 3. Overview of amphibians sampled for the gut parasite metabarcoding study in 2018, sorted by sample ID, location, species, date and number of reads per sample for the respective organism.

Sample ID	Sampling site	Host species	Date	*Tritrichomonas augusta*	*Trichomitus batrachorum*	*Balantidium duodeni*	*Blastocystis* sp.	*Hexamita inflata*	*Nyctotherus cordiformis*	*Nyctotheroides hubeiensis*	*Rhabdias ranae*	*Batrachochytrium dendrobatidis*
SACHS020	Baadenberger Senke	*Bufo bufo*	04.04.18	1	0	0	0	0	0	0	0	0
SACHS023	Baadenberger Senke	*Bufo bufo*	04.04.18	1	0	0	0	0	0	0	0	0
SACHS025	Baadenberger Senke	*Bufo bufo*	04.04.18	4	0	1	0	0	0	0	0	0
SACHS028	Baadenberger Senke	*Bufo bufo*	04.04.18	5	0	1	0	0	0	0	1	0
SACHS030	Baadenberger Senke	*Bufo bufo*	04.04.18	3	0	2	0	0	0	0	0	0
SACHS32	Baadenberger Senke	*Bufo bufo*	04.04.18	21	0	3	0	0	0	0	0	0
SACHS034	Baadenberger Senke	*Bufo bufo*	04.04.18	5	0	3	0	0	0	0	0	0
SACHS036	Baadenberger Senke	*Bufo bufo*	04.04.18	18	0	2	1	0	0	0	2	0
SACHS038	Baadenberger Senke	*Bufo bufo*	04.04.18	2	0	3	0	0	0	0	1	0
SACHS040	Baadenberger Senke	*Bufo bufo*	04.04.18	0	0	0	0	0	0	0	0	0
SACHS043	Baadenberger Senke	*Bufo bufo*	04.04.18	0	0	0	0	0	0	0	0	0
SACHS050	Ginsterpfad	*Bufo bufo*	06.04.18	104	0	10	0	0	0	0	2	0
SACHS054	Ginsterpfad	*Bufo bufo*	06.04.18	4	0	0	0	0	0	0	0	0
SACHS056	Ginsterpfad	*Bufo bufo*	06.04.18	70	0	12	0	0	0	0	1	0
SACHS058	Ginsterpfad	*Epidalea calamita*	06.04.18	10	0	0	0	0	0	0	0	0
SACHS060	Ginsterpfad	*Bufo bufo*	06.04.18	68	0	20	0	0	0	0	2	0
SACHS062	Ginsterpfad	*Bufo bufo*	06.04.18	187	1	10	5	0	0	0	3	0
SACHS064	Ginsterpfad	*Epidalea calamita*	06.04.18	118	0	3	0	0	0	0	0	0
SACHS066	Ginsterpfad	*Epidalea calamita*	06.04.18	7	0	13	0	0	0	0	3	0
SACHS068	Ginsterpfad	*Bufo bufo*	06.04.18	13	0	26	0	0	0	0	2	0
SACHS070	Ginsterpfad	*Bufotes viridis*	06.04.18	27	0	4	0	0	0	0	2	0
SACHS072	Ginsterpfad	*Bufo bufo*	06.04.18	70	4	1	0	0	0	0	0	0
SACHS076	Am Vogelacker	*Epidalea calamita*	09.04.18	1279	0	0	0	0	0	0	0	0
SACHS078	Am Vogelacker	*Bufotes viridis*	09.04.18	1136	5	0	0	0	0	0	0	0

Sample ID	Sampling site	Host species	Date	*Tritrichomonas augusta*	*Trichomitus batrachorum*	*Balantidium duodeni*	*Blastocystis* sp.	*Hexamita inflata*	*Nyctotherus cordiformis*	*Nyctotheroides hubeiensis*	*Rhabdias ranae*	*Batrachochytrium dendrobatidis*
SACHS080	Am Vogelacker	*Epidalea calamita*	09.04.18	545	0	0	0	0	0	0	0	0
SACHS082	Am Vogelacker	*Bufo bufo*	09.04.18	394	2	122	0	0	0	0	15	0
SACHS084	Am Vogelacker	*Bufo bufo*	09.04.18	244	8	0	1	0	0	0	0	0
SACHS086	Am Vogelacker	*Bufo bufo*	09.04.18	9	0	6	0	0	0	0	2	0
SACHS088	Am Vogelacker	*Bufotes viridis*	09.04.18	24	3	12	0	0	0	0	2	0
SACHS090	Am Vogelacker	*Bufotes viridis*	09.04.18	0	0	0	0	0	0	0	0	0
SACHS093	Porz Wahn	*Bufotes viridis*	09.04.18	6	0	14	0	0	0	0	0	0
SACHS095	Porz Wahn	*Bufotes viridis*	09.04.18	58	1	3	0	0	0	0	4	0
SACHS097	Porz Wahn	*Bufotes viridis*	09.04.18	16	0	2	916	0	0	0	0	0
SACHS099	Porz Wahn	*Bufotes viridis*	09.04.18	32	2	1	0	0	0	0	0	0
SACHS101	Porz Wahn	*Bufotes viridis*	09.04.18	15	0	9	1	0	0	0	0	0
SACHS103	Porz Wahn	*Bufotes viridis*	09.04.18	3	0	1	0	0	0	0	0	0
SACHS105	Porz Wahn	*Bufotes viridis*	09.04.18	175	2	3	0	0	0	0	0	0
SACHS110	Ginsterpfad	*Epidalea calamita*	17.04.18	84	0	11	0	0	0	0	552	0
SACHS112	Ginsterpfad	*Epidalea calamita*	17.04.18	1619	0	3	0	0	0	0	1	0
SACHS114	Ginsterpfad	*Epidalea calamita*	17.04.18	196	0	77	0	0	0	0	8	0
SACHS116	Ginsterpfad	*Bufotes viridis*	17.04.18	23	0	1	0	0	0	0	0	0
SACHS118	Ginsterpfad	*Epidalea calamita*	17.04.18	1565	0	3	0	0	0	0	0	0
SACHS120	Ginsterpfad	*Epidalea calamita*	17.04.18	8	0	0	0	0	0	0	0	0
SACHS122	Ginsterpfad	*Epidalea calamita*	17.04.18	36	0	2	0	0	0	0	1	0
SACHS124	Ginsterpfad	*Epidalea calamita*	17.04.18	378	0	180	0	0	0	0	21	0
SACHS126	Ginsterpfad	*Bufo bufo*	17.04.18	1	0	0	0	0	0	0	0	0
SACHS128	Ginsterpfad	*Epidalea calamita*	17.04.18	322	0	15	0	0	0	0	324	0
SACHS130	Ginsterpfad	*Epidalea calamita*	17.04.18	45	0	93	0	0	0	0	12	0
SACHS132	Ginsterpfad	*Epidalea calamita*	17.04.18	1623	0	1	0	0	0	0	0	0
SACHS134	Ginsterpfad	*Epidalea calamita*	17.04.18	224	1	1	0	0	0	0	0	0
SACHS136	Ginsterpfad	*Epidalea calamita*	17.04.18	27	0	11	0	0	0	0	1	0
SACHS138	Ginsterpfad	*Epidalea calamita*	17.04.18	755	0	1	0	0	0	0	0	0
SACHS140	Ginsterpfad	*Epidalea calamita*	17.04.18	21	0	4	0	0	0	0	0	0
SACHS142	Ginsterpfad	*Epidalea calamita*	17.04.18	728	0	2	0	0	0	0	0	0

Sample ID	Sampling site	Host species	Date	*Tritrichomonas augusta*	*Trichomitus batrachorum*	*Balantidium duodeni*	*Blastocystis* sp.	*Hexamita inflata*	*Nyctotherus cordiformis*	*Nyctotheroides hubeiensis*	*Rhabdias ranae*	*Batrachochytrium dendrobatidis*
SACHS144	Westhovener Aue	*Bufotes viridis*	23.04.18	23	0	0	0	0	0	0	165	0
SACHS146	Westhovener Aue	*Bufotes viridis*	23.04.18	6	2	1	0	0	0	0	19	0
SACHS148	Westhovener Aue	*Bufotes viridis*	23.04.18	983	0	1	0	0	0	0	8	0
SACHS150	Westhovener Aue	*Bufotes viridis*	23.04.18	5	0	4	0	0	0	0	1372	0
SACHS152	Westhovener Aue	*Bufotes viridis*	23.04.18	30	0	3	1	0	0	0	285	0
SACHS154	Westhovener Aue	*Bufotes viridis*	23.04.18	15	0	1	2	0	0	0	43	0
SACHS156	Westhovener Aue	*Bufotes viridis*	23.04.18	81	0	88	0	0	0	0	590	0
SACHS158	Westhovener Aue	*Bufotes viridis*	23.04.18	0	35	0	0	0	0	0	880	0
SACHS160	Westhovener Aue	*Bufotes viridis*	23.04.18	10	0	0	0	0	0	0	1110	0
SACHS162	Westhovener Aue	*Bufotes viridis*	23.04.18	512	17	2	0	0	0	0	1	0
SACHS164	Westhovener Aue	*Bufotes viridis*	23.04.18	23	0	30	0	0	0	0	664	0
SACHS166	Westhovener Aue	*Bufotes viridis*	23.04.18	1	0	8	0	0	0	0	1126	0
SACHS168	Westhovener Aue	*Bufotes viridis*	23.04.18	182	0	156	0	0	0	0	111	0
SACHS170	Westhovener Aue	*Bufotes viridis*	23.04.18	0	0	0	0	0	0	0	554	0
SACHS171	Westhovener Aue	*Bufotes viridis*	23.04.18	19	0	36	1	0	0	0	697	0
SACHS173	Westhovener Aue	*Bufotes viridis*	23.04.18	20	0	4	0	0	0	0	424	0
SACHS175	Westhovener Aue	*Bufotes viridis*	23.04.18	8	0	0	0	0	0	0	234	0
SACHS177	Westhovener Aue	*Bufotes viridis*	23.04.18	6	0	51	0	0	0	0	112	0
SACHS179	Westhovener Aue	*Bufotes viridis*	23.04.18	41	1	17	0	0	0	0	3	0
SACHS181	Westhovener Aue	*Bufotes viridis*	23.04.18	1381	28	1	0	0	0	0	0	0
SACHS183	Westhovener Aue	*Bufotes viridis*	23.04.18	95	0	106	0	0	0	0	10	0
SACHS185	Westhovener Aue	*Bufotes viridis*	23.04.18	205	3	9	0	0	0	0	0	0
SACHS187	Westhovener Aue	*Bufotes viridis*	23.04.18	2	1	0	0	0	0	0	13	0
SACHS189	Westhovener Aue	*Bufotes viridis*	23.04.18	77	4	4	0	0	0	0	2	0
SACHS191	Westhovener Aue	*Bufotes viridis*	23.04.18	2	8	5	0	0	0	0	57	0
SACHS193	Westhovener Aue	*Bufotes viridis*	23.04.18	12	0	7	0	0	0	0	231	0
SACHS195	Westhovener Aue	*Bufotes viridis*	23.04.18	6	0	0	0	0	0	0	292	0
SACHS197	Am Vogelacker	*Pelophylax kl. esculentus*	07.05.18	4	0	0	0	0	0	0	0	0
SACHS199	Am Vogelacker	*Pelophylax kl. esculentus*	07.05.18	4	0	1797	2	0	0	0	0	0

Sample ID	Sampling site	Host species	Date	*Tritrichomonas augusta*	*Trichomitus batrachorum*	*Balantidium duodeni*	*Blastocystis* sp.	*Hexamita inflata*	*Nyctotherus cordiformis*	*Nyctotheroides hubeiensis*	*Rhabdias ranae*	*Batrachochytrium dendrobatidis*
SACHS201	Am Vogelacker	*Epidalea calamita*	07.05.18	139	21	0	0	0	0	0	0	0
SACHS203	Am Vogelacker	*Bufo bufo*	07.05.18	172	19	0	0	0	0	0	85	0
SACHS205	Am Vogelacker	*Epidalea calamita*	07.05.18	1934	0	0	0	0	0	0	1	0
SACHS207	Am Vogelacker	*Bufo bufo*	07.05.18	1772	0	4	0	0	0	0	2	0
SACHS209	Am Vogelacker	*Bufo bufo*	07.05.18	1143	189	0	0	45	0	0	18	0
SACHS211	Am Vogelacker	*Bufo bufo*	07.05.18	18	1087	3	0	0	0	0	4	0
SACHS213	Am Vogelacker	*Pelophylax kl. esculentus*	07.05.18	5	0	1727	0	0	0	0	0	0
SACHS215	Am Vogelacker	*Epidalea calamita*	07.05.18	12	0	5	0	0	0	0	0	0
SACHS217	Am Vogelacker	*Epidalea calamita*	07.05.18	688	0	0	0	0	0	0	0	0
SACHS219	Am Vogelacker	*Bufotes viridis*	07.05.18	235	149	10	367	0	0	0	2	0
SACHS221	Am Vogelacker	*Bufotes viridis*	07.05.18	837	0	0	0	0	0	0	0	0
SACHS223	Am Vogelacker	*Epidalea calamita*	07.05.18	14	0	0	0	0	0	0	0	0
SACHS225	Am Vogelacker	*Epidalea calamita*	07.05.18	212	28	1	0	0	0	0	0	0
SACHS227	Am Vogelacker	*Epidalea calamita*	07.05.18	41	0	0	0	0	0	0	0	0
SACHS229	Am Vogelacker	*Bufotes viridis*	07.05.18	336	0	0	0	0	0	0	1	0
SACHS231	Am Vogelacker	*Epidalea calamita*	07.05.18	99	23	0	0	0	0	0	1	0
SACHS233	NBI	*Bufo bufo*	07.05.18	13	0	3	0	0	0	0	0	0
SACHS235	NBI	*Pelophylax kl. esculentus*	07.05.18	1	0	448	8	0	279	73	0	0
SACHS237	NBI	*Bufotes viridis*	07.05.18	1674	10	0	0	0	0	0	50	0
SACHS238	NBI	*Bufotes viridis*	07.05.18	656	23	4	0	0	0	0	0	0
SACHS241	Am Vogelacker	*B. viridis tadpoles*	07.05.18	13	0	23	0	2	0	0	0	0
SACHS242	Am Vogelacker	*B. viridis tadpoles*	07.05.18	11	0	13	0	0	0	0	0	0
SACHS243	Porz Wahn	*Bufotes viridis*	08.05.18	10	1	1	0	0	0	0	0	0
SACHS244	Porz Wahn	*Bufotes viridis*	08.05.18	144	3	0	0	0	0	0	7	0
SACHS247	Porz Wahn	*Bufotes viridis*	08.05.18	17	0	0	550	0	0	0	0	0
SACHS249	Porz Wahn	*Bufotes viridis*	08.05.18	1310	59	0	0	0	0	0	0	0
SACHS251	Porz Wahn	*Bufotes viridis*	08.05.18	9	0	4	0	0	0	0	70	0
SACHS253	Porz Wahn	*Bufotes viridis*	08.05.18	605	17	1	769	0	0	0	1	0
SACHS255	Porz Wahn	*Bufotes viridis*	08.05.18	935	1	0	381	0	0	0	6	0

Sample ID	Sampling site	Host species	Date	*Tritrichomonas augusta*	*Trichomitus batrachorum*	*Balantidium duodeni*	*Blastocystis* sp.	*Hexamita inflata*	*Nyctotherus cordiformis*	*Nyctotheroides hubeiensis*	*Rhabdias ranae*	*Batrachochytrium dendrobatidis*
SACHS257	Porz Wahn	*Bufotes viridis*	08.05.18		53	1	1	0	0	0	0	1
SACHS259	Porz Wahn	*Bufotes viridis*	08.05.18		98	0	0	2	0	0	0	0
SACHS261	Porz Wahn	*Bufotes viridis*	08.05.18		486	0	13	36	0	0	0	14
SACHS263	Porz Wahn	*Bufotes viridis*	08.05.18		409	7	0	0	0	0	0	0
SACHS265	Porz Wahn	*Bufotes viridis*	08.05.18		32	0	0	828	0	0	0	2
SACHS267	Porz Wahn	*Bufotes viridis*	08.05.18		901	1	10	0	0	0	0	0
SACHS268	Porz Wahn	*Bufotes viridis*	08.05.18		26	7	0	1300	0	0	0	0
SACHS271	Porz Wahn	*Bufotes viridis*	08.05.18		83	0	0	0	0	0	0	0
SACHS273	Porz Wahn	*Bufotes viridis*	08.05.18		9	5	0	39	0	0	0	0
SACHS275	Porz Wahn	*Bufotes viridis*	08.05.18		16	0	0	28	0	0	0	0
SACHS277	Porz Wahn	*Bufotes viridis*	08.05.18		1249	1	27	0	0	0	0	7
SACHS279	Porz Wahn	*Bufotes viridis*	08.05.18		54	8	4	0	0	0	0	6
SACHS281	Porz Wahn	*Bufotes viridis*	08.05.18		63	3	1	2	0	0	0	0
SACHS283	Ginsterpfad	*Epidalea calamita*	15.05.18		232	0	0	1	0	0	0	0
SACHS285	Ginsterpfad	*Epidalea calamita*	15.05.18		1363	0	0	0	0	0	0	0
SACHS288	Ginsterpfad	*Epidalea calamita*	15.05.18		1094	0	26	0	0	0	0	29
SACHS291	Ginsterpfad	*Epidalea calamita*	15.05.18		1963	0	0	0	0	0	0	0
SACHS293	Ginsterpfad	*Epidalea calamita*	15.05.18		1104	0	0	0	0	0	0	0
SACHS295	Ginsterpfad	*Epidalea calamita*	15.05.18		35	0	28	0	0	0	0	2
SACHS297	Ginsterpfad	*Epidalea calamita*	15.05.18		39	0	2	0	0	0	0	101
SACHS299	Ginsterpfad	*Epidalea calamita*	15.05.18		1471	0	1	0	0	0	0	0
SACHS302	Ginsterpfad	*Epidalea calamita*	15.05.18		513	0	0	246	0	0	0	1
SACHS304	Ginsterpfad	*Epidalea calamita*	15.05.18		1163	0	1	0	0	0	0	15
SACHS306	Ginsterpfad	*Epidalea calamita*	15.05.18		1826	0	0	0	0	0	0	0
SACHS308	Ginsterpfad	*Bufotes viridis*	15.05.18		1125	0	1	164	0	0	0	0
SACHS310	Ginsterpfad	*Epidalea calamita*	15.05.18		751	0	0	0	0	0	0	0
SACHS312	Ginsterpfad	*Epidalea calamita*	15.05.18		1224	0	0	0	0	0	0	7

Sample ID	Sampling site	Host species	Date	*Tritrichomonas augusta*	*Trichomitus batrachorum*	*Balantidium duodeni*	*Blastocystis* sp.	*Hexamita inflata*	*Nyctotherus cordiformis*	*Nyctotheroides hubeiensis*	*Rhabdias ranae*	*Batrachochytrium dendrobatidis*
SACHS321	Ginsterpfad	*Epidalea calamita*	15.05.18	1287	0	4	0	0	0	0	170	0
SACHS323	Ginsterpfad	*Bufotes viridis*	15.05.18	1852	0	8	0	0	0	0	0	0
SACHS325	Ginsterpfad	*Epidalea calamita*	15.05.18	1668	0	6	0	0	0	0	8	0
SACHS327	Ginsterpfad	*Epidalea calamita*	15.05.18	8	0	0	0	0	0	0	0	0
SACHS329	Ginsterpfad	*Epidalea calamita*	15.05.18	1768	2	2	0	0	0	0	0	0
SACHS331	Ginsterpfad	*Epidalea calamita*	15.05.18	7	0	46	0	0	0	0	195	0
SACHS333	Ginsterpfad	*Pelophylax kl. esculentus*	15.05.18	23	0	4	0	0	0	0	0	0
SACHS335	Ginsterpfad	*Pelophylax kl. esculentus*	15.05.18	15	0	234	0	0	0	0	4	0
SACHS337	Ginsterpfad	*Epidalea calamita*	15.05.18	999	0	1	0	0	0	0	12	0
SACHS339	Ginsterpfad	*Epidalea calamita*	15.05.18	39	0	1	0	0	0	0	4	0
SACHS341	Ginsterpfad	*Epidalea calamita*	15.05.18	94	0	26	1	0	0	0	121	0
SACHS343	Ginsterpfad	*Epidalea calamita*	15.05.18	1229	0	5	0	0	0	0	1	0
SACHS345	Ginsterpfad	*Epidalea calamita*	15.05.18	1710	2	0	0	0	0	0	0	0
SACHS347	Ginsterpfad	*Epidalea calamita*	15.05.18	1374	0	2	0	0	0	0	1	0
SACHS349	Ginsterpfad	*Epidalea calamita*	15.05.18	1289	0	0	0	0	0	0	14	0

Supplementary document 4. Sampling sites of environmental samples in 2018, sorted by site and organism, and referring reads per sample (OTU = operational taxonomic unit).

OTU	NBI	Porz Wahn	Ginsterpfad	Westhovener Aue
Trichomitus sp.	0	1	1433	0
Tritrichomonas augusta	0	55	342	0
Leptolegnia caudata	292	0	0	0
Anurofeca sp.	0	57	0	0
Hexamita inflata	0	0	0	51
Blastocystis sp.	18	0	0	0
Saprolegnia sp.	2	1	3	5
Myxobolus sp.	1	2	1	1