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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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-
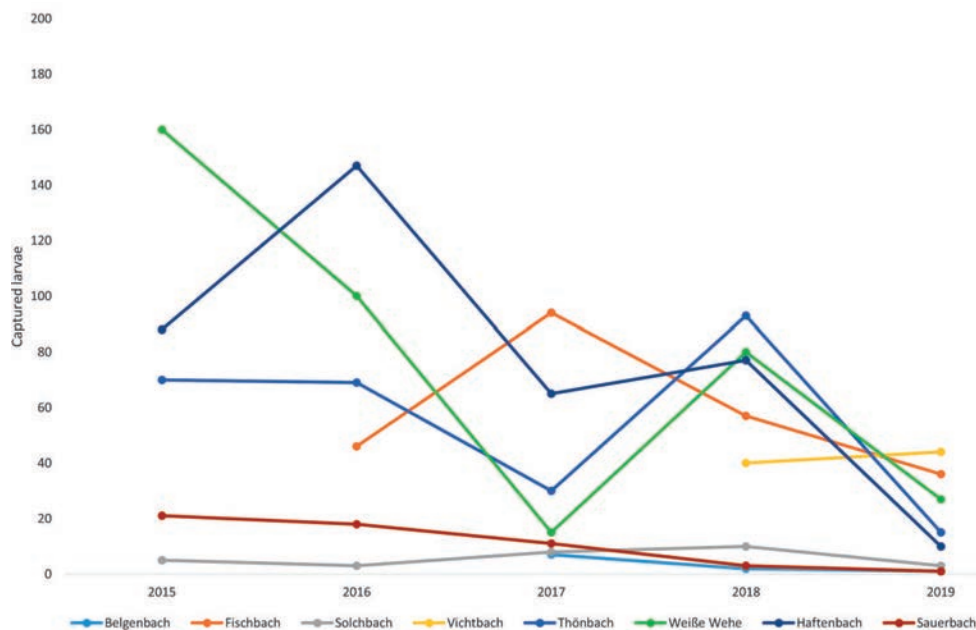

Figure 1. Captured salamander larvae in the *Bsal*-infected sites from the Northern Eifel Mountains. In most creeks, *Bsal* was present from the beginning of the monitoring in 2015, but it was first detected in the Haftenbach and Sauerbach in 2017, and in the Thönbach in 2019.
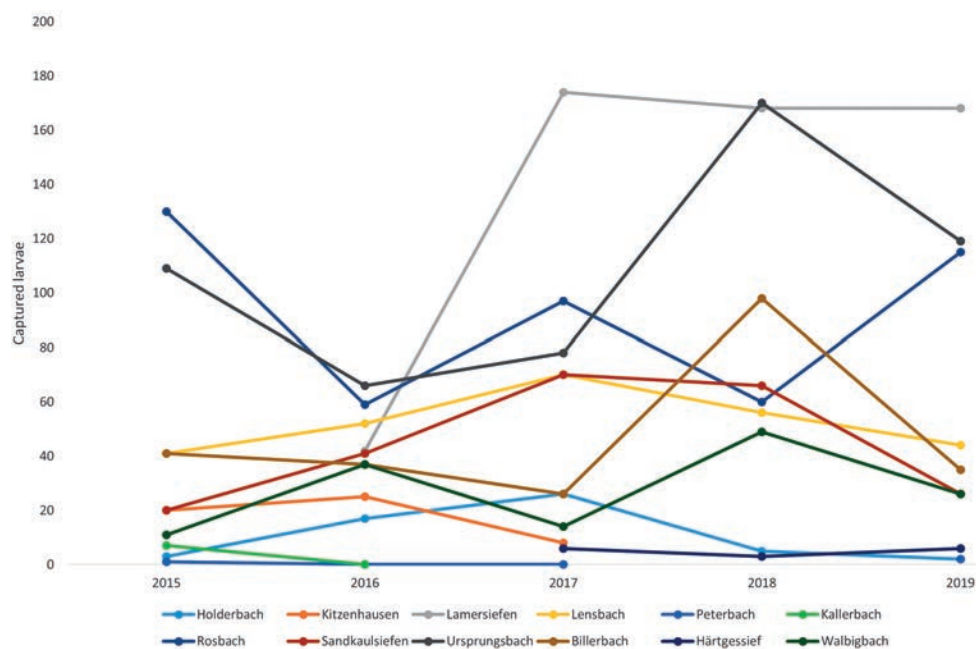

Figure 2. Captured salamander larvae in the 12 sites from the Northern Eifel Mountains, where *Bsal* was not detected.

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 occa-

sion 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-
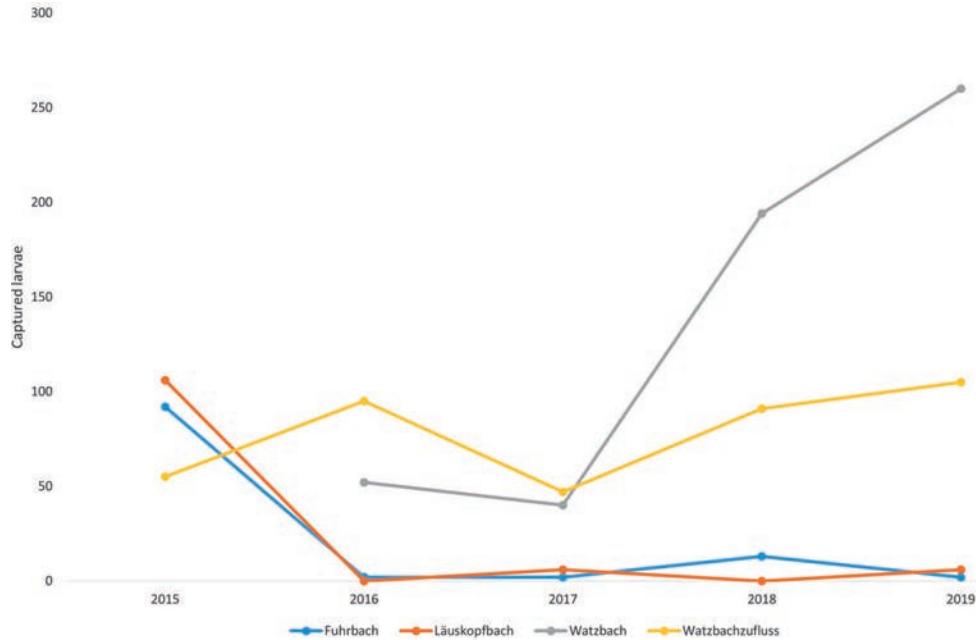

Figure 3. Captured salamander larvae in the *Bsal*-infected sites from the Southern Eifel Mountains. In the Fuhrbach and Läuskopfbach, *Bsal* was first detected in 2017, and in the Watzbach and Watzbachzufluss in 2019.
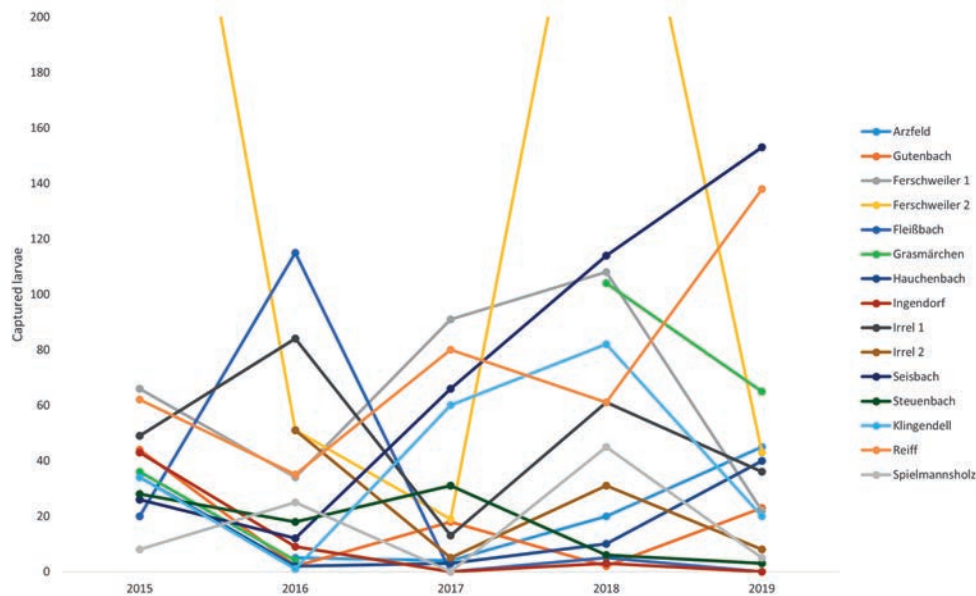

Figure 4. Captured salamander larvae in the 15 sites from the Southern Eifel Mountains, where *Bsal* was not detected.

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, R² = 0.1). The same accounts for the mean estimated larvae from 2015–2019 (P > 0.05, R² = 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, R² = 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

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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.