Quantitative larval monitoring of *Salamandra salamandra* as an early warning system for *Batrachochytrium salamandrivorans* detection in Luxembourg

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Abstract. With the further expansion of the invasive amphibian chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) in recent years, the nearest known infected populations to Luxembourg are located at approximately 15 km from the border with Germany and 30 km from the border with Belgium. As Luxembourg still is considered untouched by *Bsal* and the expansion of this pathogen towards the country is ever more likely, we conducted a removal sampling of the European fire salamander larvae, one of the most affected species, at 25 populations. The goal was to establish a baseline for population size estimations and monitoring of population trends in successive years, as part of an early detection system for *Bsal* in the European fire salamander. In addition, 57 skin samples from metamorphosed European fire salamanders were collected from five populations in regions with low larval presence. Although the pathogen was not detected, low sample sizes prevent us from drawing final conclusions on its absence. However, a baseline has been established for recognizing population declines in European fire salamander populations at an early stage, allowing a targeted *Bsal* screening in case of sudden declines. Furthermore, we suggest the need of implementing a *Bsal* monitoring system as a cornerstone of a national action plan for *Bsal* in Luxemburg, which in particular should include additional screening in newt species.

Keywords. Emerging infectious disease, chytrid fungus, salamander plague, *Bsal,* population decline, national action plan, removal sampling, Caudata.

1. Introduction

As one of the most threatened vertebrate classes (Mendelson et al. 2006, Stuart et al. 2008, Wake & Vredenburg 2008), amphibians are facing multiple threats leading to declines on a global scale (Grant et al. 2020). Among these, the impact of Emerging Infectious Diseases (EIDs), such as ranaviruses and amphibian chytrid fungi, has been linked to local mass mortalities and population or even species extinctions across a wide range of taxa (Berger et al. 1999, Lötters et al. 2009, Allain & Duffus 2019, Scheele et al.

2019, Fisher & Garner 2020). In Central and Western Europe, a new threat to urodelan amphibian diversity arose in recent decades: the salamander fungus *Batrachochytrium salamandrivorans* (*Bsal*). The invasive pathogen, apparently introduced from Asia via the pet trade, has a devastating impact on infested populations of the European fire salamander (*S. salamandra*) and is affecting newts of the genera *Ichthyosaura, Lissotriton* and *Triturus* (e. g. Martel et al. 2014, Spitzen-van der Sluijs et al. 2016, Stegen et al. 2017, Fitzpatrick et al. 2018, Lötters et al. 2020a, Schmeller et al. 2020). *Bsal* is increasingly spreading, even over large distances and possibly through novel introductions, and has led to infections of urodelans in the wild in Germany, Belgium, the Netherlands and Spain (Spitzen van der Sluijs et al. 2016, Dalbeck et al. 2018, Lötters et al. 2020a, Schulz et al. 2020, Martel et al. 2020).

The European fire salamander (*Salamandra salamandra*) is considered a sentinel species, being highly susceptible to *Bsal* infection (Martel et al. 2014). Declines in European fire salamander populations due to *Bsal* mostly happen undetected and can easily be overseen, mainly due to the lack of long-term monitoring programmes and the difficulty to detect the pathogen, especially once a population decline or local extinction of the species has occurred (Spitzen-van der Sluijs 2018, Wagner et al. 2020a, Lötters et al. 2020a, Sandvoß et al. 2020). Moreover, the pathogen poses a threat to other urodelan species, as *Bsal* infected individuals of all newt species (*Ichthyosaura alprestris*, *Lissotriton helvetivus*, *L. vulgaris*, *Triturus cristatus*) were found during surveys, partially outside the range of European fire salamander populations (Dalbeck et al. 2018).

Salamander and newt populations in Luxemburg are stated as naïve to the pathogen with a high potential to get in contact – and being afterwards infected with the pathogen. The reason is that the known edge of the *Bsal* ranges in Germany and Belgium are located 15 km to the northeast and 30 km to the north of Luxembourg, respectively (Fig. 1; Spitzen van der Sluijs et al. 2016, Sandvoß et al. 2020, Wagner et al. 2020a).

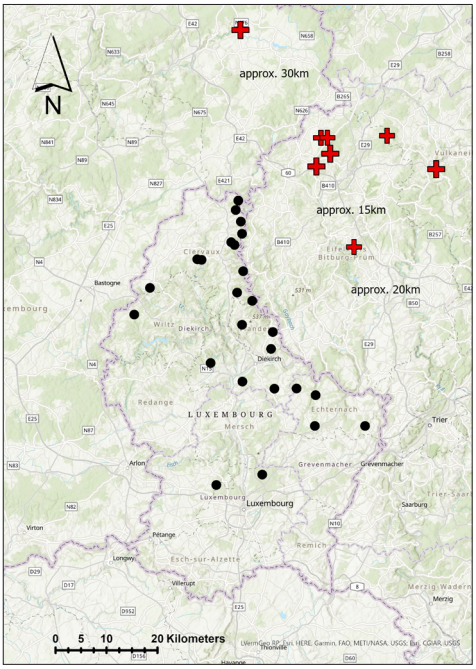


Fig. 1. Overview of larvae monitoring sites (black dots) and *Bsal* outbreaks (red crosses) in Western Europe (after Spitzen-van der Sluijs et al. 2016, Lötters et al. 2020a).

Luxembourg is home to five species of the family Salamandridae, of which only *Triturus cristatus* figures on Annexes II and IV of the EU Habitats Directive, requiring specific action. On the national level, all five species are listed as Least Concern (*sensu* Proess et al. 2016) and all are protected by national law. Given the current conservation status of these amphibians, so far no *Bsal* screening has been performed in Luxembourg. Besides the national species protection plan for *T. cristatus* (Proess & Schneider 2018) and a long-term monitoring action of one *S. salamandra* population in the west of the country (Willems 2019), no other long-term monitoring data exist for the urodelan species in Luxembourg, increasing the potential risk of overseeing population declines. However, at least a preliminary presence/absence monitoring of larvae confirmed the presence of *S. salamandra* throughout most studied sites in Luxembourg (Proess 2016, 2017).

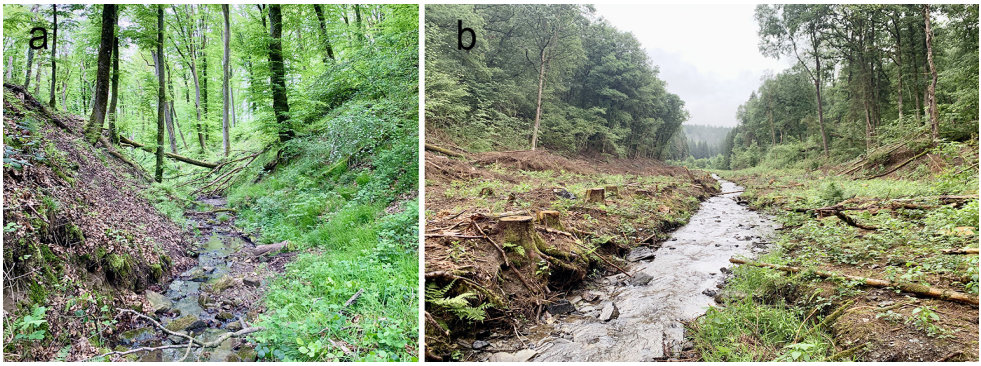


Fig. 2, a: Suitable habitat in Schieren; b: unsuitable habitat in Rambrouch. Photos: J. Muller.

According to the recommendations of the European *Bsal* Action Plan (*see* Gilbert et al. 2020), there is thus an urgent need of implementing a national *Bsal* action plan for Luxemburg. Among others, this includes an early warning system, as a tool for local authorities to detect infected populations in an early stage.

In the present study, we aim to establish a baseline for population size estimations and successive monitoring of population trends as part of an early detection for *Bsal* in the European fire salamander. We conducted a quantitative larval removal-sampling at selected sites in the north, center and east of the country which are so far the geographically closest populations to known *Bsal* outbreak sites (i. e. outside Luxembourg). In addition, we collected skin swabs – a standardized method for *Bsal* screening (e. g. Thomas et al. 2018, Lötters et al 2020a) – in some European fire salamander populations in regions where first results of larval monitoring indicated low abundances. Our primary study is to be seen as a first step towards establishing both methods as an early warning system to be integrated in a national *Bsal* action plan.

2. Material and Methods

All field work followed the national biosecurity protocol as published on www.sos-salamandre.lu (Stassen & Lestang 2021). Measures included the disinfection of boots and field material with a fungicide solution of 1% VirkonS® before and after entering field sites and handling all sampled individuals with a separate pair of nitrile gloves. Through the National Museum of Natural History a permit was granted by the Ministry of Environment to the researchers, for the temporary capture, manipulation and transport of all urodelan species in Luxembourg.

2.1. Larval monitoring

Between the end of April 2021 and the beginning of July 2021, we visited selected sampling sites for a European fire salamander larvae monitoring (Table 1). The selection of suitable sites was based on records from the following data sources. First, we included data of Proess (2016, 2017) as well as observations from the iNaturalist® platform provided by the National Museum of Natural History (MNHN). Second, we included small, first-order streams surrounded by deciduous forest as potential habitats by manual review of high-resolution satellite images (available at www.geoportail.lu). Hence, we defined a total of 30 study sites as potential monitoring localities. Subsequently, we visited these sites to assess habitat suitability (forest cover, forest type, width, depth and number of pools in forest streams) and accessibility for our removal sampling approach.

With this, we listed 25 sites as monitoring sites while five sites were classified as unsuitable (Fig. 2). The majority were in the north-eastern and eastern parts of Luxembourg, due to the proximity with the nearest known

infected populations in Germany, the high density of species in this region (Proess et al. 2016) and the high suitability for the establishment of *Bsal* according to niche models in these areas (Beukema et al. 2021). The larvae monitoring - performed as a quantitative removal sampling method - followed the standardized and commonly applied study design of Schmidt et al. (2015). At each monitoring site, we defined a representative 75 m transect along a stream, divided into three 25 m sub-transects. We then recorded the exact coordinates of the starting position using a GPS unit (Garmin Etrex 32x). In all three sub-transects, 3 observers searched for and removed larvae simultaneously for 15 min. Then, observers rotated randomly (*sensu* Schmidt et al. 2015). In this way, every observer has searched for larvae in all three sub-transects. Larval counts as well as additional field parameters (see Table 1) were documented in the field protocol. The captured larvae were temporarily kept in plastic aquariums (type faunabox) and immediately released into the sub-transects of their capture after the end of the search action. The larval monitoring and the assessment of environmental parameters were conducted upstream of each site (Wagner et al. 2020a).

We used the software R (https://www.r-project.org/) for the statistical analysis of our data. We performed generalized multinomial mixture models (function "gmultmix ()") with the package "unmarked" to determine the detection probability and the larval abundance including environmental parameters as covariates (Fiske & Chandler 2011). All models were ranked by their information loss using the Akaike Information Criterion (AIC) as decribed by Mazerolle (2006) with the R-package "AICcmodav" (Mazerolle 2015, function "modsel ()"). Following Wagner et al. (2020a), we then defined all models with a $\Delta AIC \leq 2$ as eligible for our data. Finally, we estimated and compared population estimations (function "ranef ()") of all suitable models.

2.2. *Bsal* Sampling

Our sampling focused on areas close to the German border in 5 populations. This included three sites where low larval counts

Table 1. Total larvae counts and field parameters for the 25 sampling sites. (Day: Occasion 1-12; Turbity, Aquatic wood, Gammaridae, Terrestrial shelters: 1 = low, 2 = medium, 3 = high; Cloud cover from 1 = no clouds to 8 = full cloud cover; Pools: absolute counts; Substrate: 1 = gravel/stone, 2 = sand, 3 = mud; Forest type: 1 = Deciduous forest, 2 = Mixed forest, 3 = Coniferous forest).

ID	Date	Closest village	Creek	Start northing WGS84	Start easting WGS84	Total larvae count	Day	Turbidity	Cloud cover	Pools	Aquatic wood	Width [m]	Gammaridae	Slope [%]	Substrate	Terrestrial shelters	Forest type (100m Buffer)
1	30.04.21	Hersberg	Tributary of Härdbaach	6.23271	49.757155	66	1	1	1	11	1	1.5	3	35	1	3	1
2	30.04.21	Mompach	Tributary of Kléngelbaach	6.460194	49.756903	159	1	1	8	5	1	1.6	1	15	3	2	1
3	09.05.21	Lieler	Schelsbaach	6.121292	50.118357	22	2	1	1	5	1	0.9	3	34	1	2	1
4	09.05.21	Leithum	Schibech	6.113853	50.155501	0	2	1	1	10	1	0.6	1	23	1	2	2
5	09.05.21	Weiswampach	Tributary of Réibaach	6.107285	50.13871	91	2	1	1	8	1	0.5	1	32	1	1	3

ID	Date	Closest village	Creek	Start northing WGS84	Start easting WGS84	Total larvae count	Day	Turbidity	Cloud cover	Pools	Aquatic wood	Width [m]	Gammaridae	Slope [%]	Substrate	Terrestrial shelters	Forest type (100m Buffer)
6	09.05.21	Kalborn	Feierbech	6.124548	50.096722	90	2	1	8	11	3	1.1	1	17	1	3	2
7	13.05.21	Fischbach	Folkesbur	6.094895	50.081842	3	3	1	5	7	2	0.8	2	20	1	3	2
8	13.05.21	Clerf	Botteschbaach	6.013654	50.050657	104	3	1	7	10	1	0.7	1	13	1	2	2
9	13.05.21	Eselborn	Eselbaach	6.001857	50.051786	0	3	1	3	6	3	1.2	1	14	1	3	2
10	16.05.21	Fischbach	Stroumbaach	6.103869	50.076917	3	4	1	4	7	3	2	1	38	1	3	2
11	17.05.21	Rodershausen	Fallbech	6.127762	50.030779	25	5	2	8	6	3	1	1	37	1	3	2
12	17.05.21	Parc Hosingen	Bollertsbaach	6.110953	49.992792	3	5	2	5	7	3	1.2	1	27	1	3	1
13	23.05.21	Longsdorf	Ramgriecht	6.204143	49.893382	61	6	1	4	15	2	1.2	3	30	3	3	1
14	23.05.21	Stolzemburg	Stolzebuerger Akeschterbaach	6.152175	49.978342	77	6	1	8	8	2	2	1	27	1	3	2
15	23.05.21	Nachtmanderscheid	Hiermesbaach	6.12466	49.936052	34	6	1	2	3	1	2	1	30	1	2	2
16	24.05.21	Niederfeulen	Tributary of Wark	6.038868	49.868572	146	7	2	8	7	2	0.6	1	23	1	3	2
17	24.05.21	Schieren	Widebaach	6.125591	49.835423	293	7	1	8	7	3	2	3	50	1	3	2
18	26.05.21	Bettel	Schmitbaach	6.208882	49.923314	49	8	3	6	7	1	0.6	3	20	3	2	2
19	20.06.21	Berdorf	Tributary of Schwaarz Iernz	6.325323	49.811654	2	9	2	6	12	1	0.8	2	27	2	3	1
20	20.06.21	Haller	Tollbaach	6.273567	49.823287	0	9	1	4	2	1	0.8	1	30	2	2	2
21	27.06.21	Eisenborn	Schetzelbaach	6.17971	49.671464	0	10	1	4	7	1	1.2	2	9	1	3	2
22	27.06.21	Kopstal	Riedelbaach	6.054662	49.653132	0	10	1	4	7	3	0.8	3	20	1	3	1
23	29.06.21	Schleif	Tributary of Sunsbech	5.872395	50.000935	23	11	1	2	10	2	0.6	1	17	1	1	3
24	29.06.21	Tarchamps	Harambois	5.829824	49.953622	27	11	3	8	3	3	0.8	2	22	1	3	2
25	09.07.21	Ermsdorf	Deifebaach	6.212913	49.822991	80	12	1	5	4	2	1.2	1	30	3	3	2

were recorded during the removal sampling, with less than 5 larvae found in an otherwise optimal habitat, and two neighboring locations in order to extend the search to a broader area. Herein, we conducted nocturnal opportunistic visual encounter surveys for metamorphosed individuals, aiming to sample 30 individuals in each location. With this minimum sample size, it ought to be possible to detect at least one *Bsal*-positive European fire salamander with a probability of 95% assuming a prevalence of at least 10 % (following Cannon & Roe 1986). However, due to low abundances we did not reach that number of samples at any site. Our sampling method followed the standardized protocol used in earlier *Bsal* studies (e .g. Dalbeck et al. 2018, Lötters et al. 2020a): We used two swabs for each sampled individual. If the first sample shows a positive result, the second sample is used for verification in an independent laboratory. DNA was extracted using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's Animal Tissues protocol. *Bsal* detection was performed by amplifying the internal transcribed spacer (ITS) region 1 of rRNA (120 bp) with the quantitative polymerase chain reaction (qPCR, StepOnePlus™, ThermoFisher Scientific) method following the standard protocols of Blooi et al. (2013) and Standish et al. (2018). All samples were run at Trier University in duplicate with a *Bsal* standard as well as a negative control. The calculation of each site prevalence as well as 95% Bayesian credible intervals followed the procedure described in Lötters et al. (2012).

3. Results

3.1. Larval monitoring

In the year 2021 we found European fire salamander larvae in 20 of the 25 selected streams. A total of 1,358 larvae were caught. The mean value was 54.32 individuals. For a statistical analysis, we had to exclude Site 17 as outlier because of the high number of captured larvae. Among the localities with zero larvae counts, Leithum (ID4) was the northernmost sample site just 16.75 km away from the closest *Bsal* record in Germany. Furthermore, Eisenborn (ID21) and Kopstal (ID22), the southernmost sample sites located close to the capital, yielded no larvae counts. The other two localities without counts were Eselsbach (ID7) and Haller (ID20) both located in the northeast. The lowest abundance was detected in Berdorf (ID19) with only 2 larvae, and the highest abundance in Schieren (ID16) with 293 captures. At four sites (ID7, 10, 12, 19), the number of larvae was less than 5 individuals (*see* Fig. 3, Table 1).

Regarding larval counts in combination with the environmental parameters, the 10 best fit models (all with Δ AIC $\leq$ 2) differ in their explanatory factors in Abundance (λ) (Table 2). The larval abundance is either constant (i.e. no factor is influencing abundance, Model 1 and Model 3) or is affected by several covariates. The amount of dead wood in the water occurs twice (Model 4,

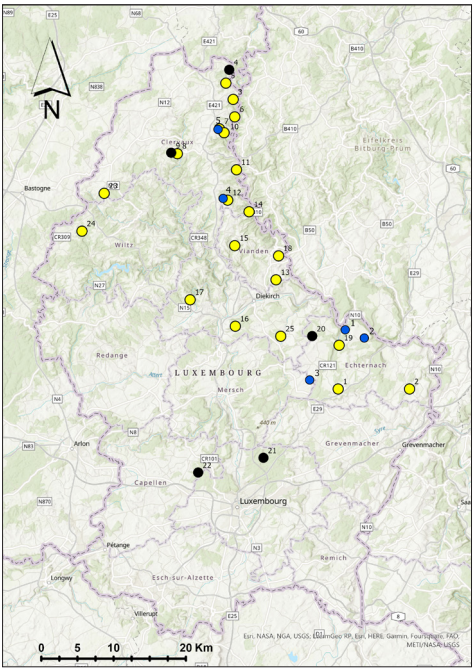


Fig. 3. Overview of larvae monitoring site and the *Bsal* sampling sites in Luxembourg for the year 2021. Yellow dots indicate sites with larval presence, black dots indicate sites where no larvae were found, blue dots indicate sites where skin swabs were collected. (Number = location ID).

Table 2. Suitable models ranked by the Akaike Information Criterion (AIC), λ = Abundance; p = Detection probability; ΔAIC = deltaic; AICwt = Model weight; (.) = constant; (~ Covariable) = dependency).

Model ID	Abundance	Detection	AIC	Δ AIC	AICwt
Model 1	***λ (.)***	*p(~ Width)*	-4776.338	0.000000	0.08885754
Model 2	***λ (~Day)***	*p(~ Width)*	-4775.211	1.126850	0.05058268
Model 3	***λ (.)***	*p(~ Clouds)*	-4775.113	1.224191	0.04817977
Model 4	***λ (~Wood)***	p(~ Width)	-4775.025	1.312795	0.04609188
Model 5	***λ (~Prey)***	*p(~ Width)*	-4774.820	1.517371	0.04161035
Model 6	*λ (~Hides)*	*p(~ Width)*	-4774.452	1.885635	0.03461257
Model 7	*λ (~Slope)*	*p(~ Width)*	-4774.448	1.890128	0.03453490
Model 8	***λ (~Wood)***	*p(~ Clouds)*	-4774.405	1.932699	0.03380758
Model 9	*λ (~Pools)*	*p(~ Width)*	-4774.401	1.936653	0.03374080
Model 10	*λ (~Forest)*	*p(~ Width)*	-4774.357	1.981051	0.03300005

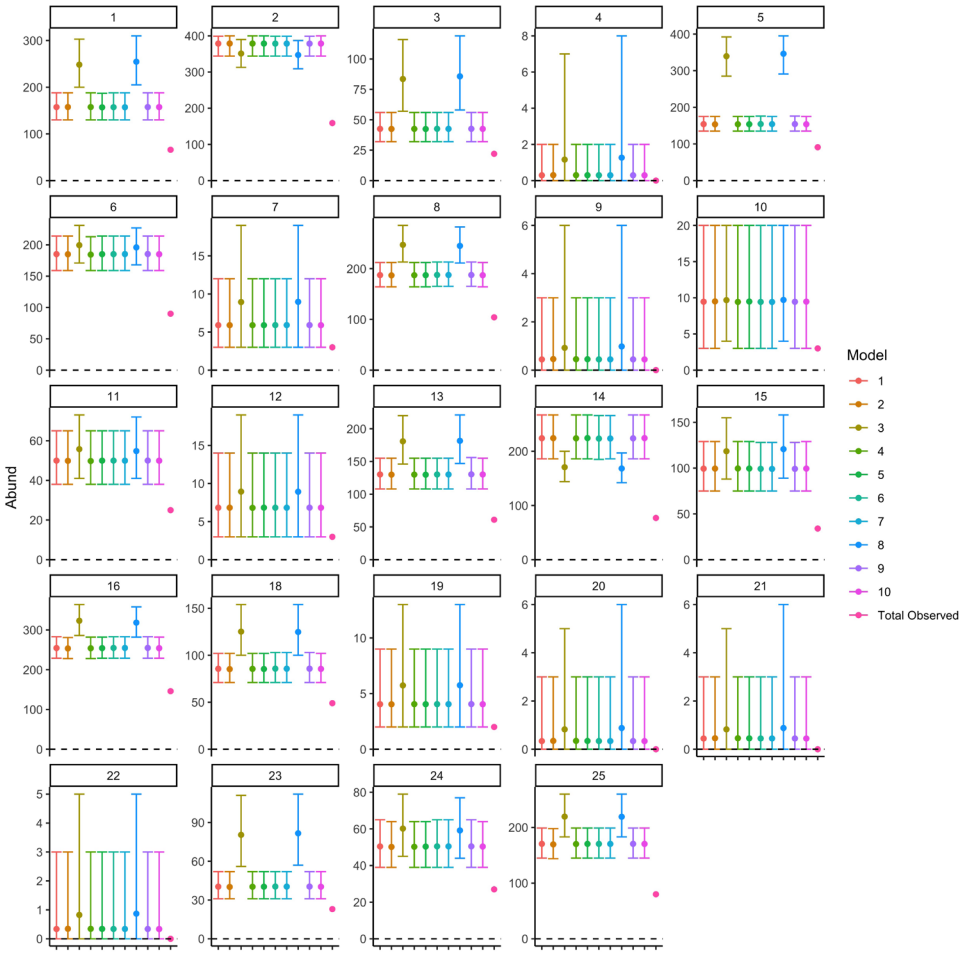


Fig. 4. Overview of estimated population sizes (with confidence intervals) of all eligible models per stream (Abund = larval abundance; 1-25 = sampling sites (17 not included)).

Table 3. Overview of 57 *S. salamandra Bsal* samples per locality including prevalence of each site for year 2021 (Bayesian credible Intervals (CI) are shown in parentheses).

ID	Locality	Coordinates	Number of samples	*Bsal* detected	Prevalence
1	Berdorf 1	6,335786°E 49,829849°N	3	none	21% (CI: 10%-61%)
2	Berdorf 2	6,372156°E 49,819665°N	9	none	9% (CI: 0%-30%)
3	Christnach	6,267146°E 49,767439°N	14	none	6% (CI: 0%-20%)
4	Fischbach	6,090757°E 50,079894°N	3	none	21% (CI: 10%-61%)
5	Parc Hosingen	6,100728°E 49,993934°N	28	none	3% (CI: 0%-12%)

Model 8) whereas sampling day (Model 2), the amount of prey in the stream (Model 5), the number of hides (Model 6), the slope of the stream (Model 7), the number of pools (Model 9) and the forest type (Model 10) are represented once (Table 2). In contrast, the detection probability (p) depended twice on cloud cover (Model 3) and in all others on the stream width.

At sites with larval counts, all models show higher larval population estimations than absolute larval observations (Fig. 4). In comparison, only the two models with the detection probability affected by cloud cover (Model 3 and Model 8) differ from all other models resulting in higher population estimations. At four sites (See locality 1, 3, 5, 23 in table 1), this difference is significant for both and additionally at site 16 it applies to Model 3 (Fig. 4). In contrast, at site 14, the estimation is lower (not significant) in these two models.

3.2. *Bsal* Sampling

In total, we analysed skin swabs of 57 metamorphosed European fire salamanders (range between sites: 3–28, Table 3). Thus, prevalence and credible intervals varied. All studied samples were *Bsal*-negative. However, taking into account sample size, only the sample site "Parc Hosingen" shows reliable credible intervals ranging from 0-12% and a mean prevalence of 3%.

4. Discussion

Although, the presence of *Bsal* could not be confirmed in this study, we have to remain vigilant and further monitoring is required, preferably within the framework of a comprehensive national *Bsal* action plan. By monitoring larval abundances in 25 populations of European fire salamanders, we have established a baseline for recognizing population declines at an early stage as part of an early warning system for *Bsal*. From this point of view, quantitative monitoring should be continued along the same transects over the long term. Furthermore, we suggest the addition of monitoring sites (for a potential selection see Methods) to comprehensively cover the species` distribution in Luxembourg. This allows a quick action mechanism in case of *Bsal* outbreaks. It further serves as an effective data source in conservation planning. This sampling design did not cover by far the totality of European fire salamander populations in Luxembourg. However, it allows us to continue based on a robust sample throughout the species range in the country. Combined with other active and passive monitoring actions, this should allow an early detection of a first *Bsal* infection within the country, as it has been implemented in other countries (e. g. Gilbert et al. 2020, Martel et al. 2020).

Luxembourg is considered a high-risk area for *Bsal* infection, not only due to its proximity with the nearest known German and Belgian outbreak sites, but also due to the high habitat suitability (*sensu* Beukema et

al. 2021). The pathogen is associated with humid and relatively cool, stable climates. Furthermore, the high pressure of outdoor recreational activities and trail density within some areas near *Bsal*-positive regions in neighboring countries poses an additional risk for human-mediated spread of the pathogen (*sensu* Beukema et al. 2021).

Our population estimations confirm that the European fire salamander still occurs in partially high abundances in the study area, as suggested by earlier findings (Proess 2016, 2017). However, no population trends can be estimated after just one year of removal sampling. The rapid detection of declines depends on continuity of the larvae monitoring. Our findings on the factors shaping our results go in line with those of Wagner et al. (2020b): We found significant relationships between the detection probability and the creek width as well as a negative correlation between the abundance and the sampling day. This is explicable with an increase of metamorphosis events and a possible drift (*sensu* Wagner et al. 2020a). The number of pools and the amount of wood in the water correlated also with the abundance of larvae, as both covariates shape the stream and increase microhabitats. Moreover, these findings are congruent with the results of Wagner et al. (2020a). Additionally, mixed forest as well as the slope of the terrain in the 100 m buffer area enhances the estimated population size, as both variables provide preferred habitats for adult salamanders (Thiesmeier 2004). Surprisingly, our results show an effect of cloud cover on the detection probability of European fire salamander larvae in streams. At most sites, where no (clouds =1) or few clouds (clouds =2) were detected during the larval sampling, fewer larvae were found (compare Table 1, Fig. 3). We conclude that with less sunlight available the detection probability of European fire salamander larvae increases. We assume, that low light conditions during the day enhance the larval activity and thus detection of otherwise strictly nocturnal salamander larvae (Thiesmeier 2004).

All sampled salamanders were free from *Bsal*. However, the broad confidence intervals prevent us from drawing conclusions on pathogen presence in the sampled localities. These are mainly caused by low sample size due to time restrictions – except the site Parc Hosingen. At the current stage, it is not possible to clearly state if we failed to detect *Bsal* or if the pathogen is yet not present in Luxembourg. Our sampling should be considered (1) as a first basis for further studies and (2) as a quick reaction to the results of the larval monitoring. Further investigations should therefore aim on higher sampling efforts (i.e. increase sample size and localities). Increasing sample size is possible with the inclusion of other urodeles (*cf.* Stegen et al. 2017). We moreover recommend that sampling designs with different sampling periods should be implemented in the future. Own investigations as well as other expert opinions (A. Spitzen van der Sluijs, Netherlands; Jürgen Thein, Germany) suggest, that *Bsal* detection in urodelan populations possibly fluctuates between seasons. However, we are aware, that expert opinions are to be seen as suggestions rather than solid information (Lambert et al. 2020).

Although most studies on the distribution and prevalence of the pathogen focused on the European fire salamander, further caution is required as severe declines in newts such as the great crested newt (*Triturus cristatus*) were recorded recently in Germany, indicating a high probability these species are equally affected by the pathogen (Lötters et al. 2020a). On the other hand, the palmate newt (*Lissotriton helveticus*) as well as the alpine newt (*Ichthyosaura alpestris*) are capable of surviving sublethal infections thus likely functioning as a reservoir and vector for *Bsal* (Martel et al. 2014, Stegen at al. 2017, Lötters et al. 2020a). In addition, Beninde et al. (2021) show a high connectivity between alpine newt populations in Rhineland-Palatinate implying that multiple pathogen translocations (i.e. *Bsal* range expansion) are likely. Thus, future *Bsal* screening should include these tolerant taxa simplifying *Bsal* detection.

To enable a quick response to observed mortalities and increase screening capacities within the country, we recommend an integrative approach combining several tools: molecular analysis with standardized methods

of qPCR, the recently launched portal at www.sos-salamandre.lu where actors in the field and the public can alert when dead salamanders are found and the implementation of strict biosecurity protocols. As *Bsal* detection in populations with low pathogen prevalence or host abundances is challenging, we recommend the addition of eDNA-based approaches in these cases (Spitzen-van der Sluijs et al. 2020).

This allows the efficient use of resources for mitigating a pathogen causing unnoticed population declines and deploy measures to minimize further spreading towards other *Bsal*-naïve populations.

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