



Population monitoring of European fire salamanders (*Salamandra salamandra*) with a new photo-recognition software

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Abstract. Population monitoring is a crucial method for conservation projects, especially for the highly endangered clade of amphibians which is threatened by habitat loss and emerging infectious diseases such as the chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*). *Bsal* has led to massive population declines of European fire salamanders in Belgium, the Netherlands and parts of Germany and population trends are decreasing. Thus, regular population monitoring is essential to keep track of population dynamics and detect potential *Bsal* outbreaks, especially since valid population estimates for many species and/or populations are scarce. In recent years, photographic mark-recapture studies have gained importance enabling researchers to keep track of individuals without the use of harmful marking techniques that might influence behaviour or survival. We monitored a European fire salamander population over four years in the Kottenforst forest, Germany, by combining a mark-recapture approach with a new photo-recognition software, the Amphibian and Reptile Wildbook. We investigated potential differences of two ecotypes, i.e., larval populations bred in ponds and streams. Furthermore, we compared the observed number of larvae and the estimated population sizes between two consecutive years. There was a year effect on the observed number of larvae, the percentage of injured larva and estimated population size. The habitat type affected apparent survival rates. There was also a habitat effect on the percentage of injured larvae, but this was only existent in some years. The mean larval size, water temperature and the superpopulation size were not affected by any of the aforementioned factors. Although the population was free from *Bsal*, the high variation in the population estimates emphasize the need for a regular and standardised monitoring to assess the current population status and detect early population declines that might otherwise remain overlooked.

Key words. Amphibian and Reptile Wildbook, Caudata, conservation, individual growth rates, individual recognition, photographic mark-recapture.

Introduction

The lack of reliable population estimates for most of the species is one of the major problems in conservation biology. We are in the midst of a global biodiversity crisis and population trends are decreasing globally (reviewed in SINGH 2002). The main threats of biodiversity are changes in land and sea use, species overexploitation, pollution, climate change, invasive species, and new diseases (SALAFSKY et al. 2008, WWF 2020). Almost one third of species worldwide are threatened with extinction (IUCN 2021) and studies indicate that we are close to a sixth mass extinction (e.g., WAKE & VREDENBURG 2008, BARNOSKY et al. 2011, MCCALLUM 2015).

Amphibians, with 41% endangered species (IUCN 2021), are the most endangered group (STUART et al. 2004), but have gained far less attention than other taxa (reviewed in BRITO 2008). The causes of amphibian population declines are complex, spatially different (SODHI et al. 2008, GRANT et al. 2016) and include multiple factors such as cli-

matic or environmental conditions and the spread of new diseases (e.g., COLLINS 2010, MANENTI et al. 2022).

Thus, monitoring efforts have increased over the last decades (reviewed in MOUSSY et al. 2022) for purposes such as status assessment, measuring the effectiveness of conservation approaches or the estimation of population sizes (e.g., DONNELLY & GUYER 1994, STEM et al. 2005). Population size estimation for open populations requires the individual identification of animals, which, in past studies, was often accomplished by capturing, invasive marking and recapturing (DONNELLY et al. 1994, DAVIS & OVASKA 2001, WILLIAMS et al. 2002). However, those invasive markings, e.g., toe-clipping in amphibians (PHILLOT et al. 2007) or passive integrated transponder tags in fishes (reviewed in MUSSELMAN et al. 2017), may influence the behaviour or survival rate of individuals (reviewed in WILSON & McMAHON 2006). For example, visible implant tags negatively affected European fire salamander larvae, leading to lower body condition indices of marked larvae than newly captured larvae (WAGNER et al. 2020a). In recent years, non-

invasive methods have gained importance to minimise the potential effects on the captured animals. Individual animals can be identified by genotyping DNA from faeces or hair samples (e.g., BERRY et al. 2014, KUBASIEWICZ et al. 2017), by acoustic surveys such as analysing the distinctive howls of Indian wolves (SADHUKHAN et al. 2021) or by photographs of individual colouration or patterns, e.g., with camera traps (reviewed in CHOO et al. 2020).

Past photographic mark-recapture studies often included “by-eye” matching of photographs and were performed by more than one observer, probably resulting in high error rates and variability among observers (e.g., TREILIBS et al. 2016). The development of computer-assisted systems for photographic mark-recapture studies has led to substantial decreases in error rates and between-observer variability (CRUICKSHANK & SCHMIDT 2017), allows the analysis of large datasets (BOLGER et al. 2012) and is applicable for different species (e.g., JACKSON et al. 2006, VERNES et al. 2009, DE GASPERIS et al. 2017, RENET et al. 2019). A recent study has shown that photographic recognition softwares can also be applied to the less suspicious European fire salamander larvae (FAUL et al. 2022). The non-commercial, web-based Wildbook software even combines scientific research, crowdsourcing and citizen science and includes specialised platforms for a wide range of animals such as zebras, sea turtles, whales and giraffes (BERGER-WOLF et al. 2018). Another recently launched platform, the Amphibian and Reptile Wildbook (available from www.amphibian-reptile.wildbook.org), can currently be used for the photographic identification of amphibians such as adult yellow-bellied toads (*Bombina variegata*) and adult and larval European and Near eastern fire salamanders (*Salamandra salamandra* and *S. inframaculata*).

The European fire salamander occurs across central, west and south Europe and is categorised as least concerned along this range, although the population trend is decreasing (KUZMIN et al. 2009). In Germany, which holds about 10% of the species range, the status of the European fire salamander has recently been updated to be on the pre-warning list (SCHLÜPMANN & VEITH 2020). The population decline is probably related to increased human impacts in forest areas, spring droughts (SCHLÜPMANN & VEITH 2020) and the introduction of *Batrachochytrium salamandrivorans* (*Bsal*, MARTEL et al. 2013). *Bsal* is a pathogenic chytrid fungus that causes the erosive skin disease chytridiomycosis in European fire salamanders (MARTEL et al. 2013) and has led to population collapses in the Netherlands, Belgium and parts of Germany (SPITZEN-VAN DER SLUIJS et al. 2016, STEGEN et al. 2017, WAGNER et al. 2019, LÖTTERS et al. 2020, SCHULZ et al. 2020). Thus, population monitoring is crucial to detect potential disease outbreaks, prevent the spread of *Bsal* and maintain stable populations.

In this study we used the Amphibian and Reptile Wildbook to estimate the population size and investigate the individual growth of larval European fire salamanders based on count data from regular monitorings including photographic marking in the Kottenforst, a broadleaf forest near Bonn, Germany. Furthermore, we tested for the presence

of *Bsal* to confirm its absence since the last *Bsal*-negative reports in the study area (LÖTTERS et al. 2018). Using linear mixed effect models, we investigated potential effects of the larval habitat on population size (counts and estimations), mean larval size, individual growth, percentage of injured larvae, apparent survival and recapture rates. Although the Kottenforst salamander population has been subject to many studies investigating differences between the pond and stream habitat and potential speciation processes (e.g., STEINFARTZ et al. 2007, HENDRIX et al. 2017, SANCHEZ et al. 2017, SABINO-PINTO et al. 2019, OSWALD et al. 2020), their population size is unknown.

Methods

Study area and species

The European fire salamander is a biphasic amphibian with aquatic larvae and terrestrial adults, but during the reproductive phase, adult females return to water bodies to deposit their larvae. Usually, females prefer first-order streams for larval deposition (THIESMEIER 2004), but they have also been observed to breed in ephemeral ponds and even in caves (e.g., STEINFARTZ et al. 2007, MANENTI & FICETOLA 2013, CASPERS et al. 2015, MANENTI et al. 2017). In our study area, the Kottenforst (50°39'38.9" N, 7°04'16.7" E), a forest near Bonn, Germany, females use both ponds and streams for reproduction and this behavioural differentiation corresponds to the differentiation into a pond and a stream genetic cluster (HENDRIX et al. 2017, STEINFARTZ et al. 2007).

From 2019 to 2022 we monitored European fire salamander larvae (*Salamandra salamandra*) and took *Bsal* samples via skin swabs of adult and larval salamanders in the Kottenforst. Initially, we chose 5 ponds and a section of 20 m in each of 5 streams. The exact location of the ponds and streams were marked via GPS-coordinates (Fig. 1) and the 20m section of each stream was marked at the beginning, the middle and the end. This enabled us to always monitor the same section of the stream.

The Amphibian and Reptile Wildbook

The Amphibian and Reptile Wildbook (available for free from <https://amphibian-reptile.wildbook.org/>) is a newly launched web-based software developed by Wild Me (<https://www.wildme.org>) and is based on computer vision algorithm and deep learning to reliably detect and identify individual animals from photos. So far, the software is trained for adult yellow-bellied toads and adult and larval European fire salamanders (SCHULTE et al. 2022). The software allows the upload of large amounts of photographs without prior processing. It is also possible to add metadata such as GPS coordinates, time and date stamps or the size of the animal. Furthermore, it combines citizen science and scientific research and can thus provide a huge database and valid basis for conservation projects (SCHULTE et al. 2022).

Larval monitoring

We performed the larval monitoring following a set protocol (see supplementary material) with the time period for a certain area of the sample site as the limiting factor for the monitoring duration. Each pond was monitored for 30 minutes by one person. Each additional researcher reduced the total monitoring time, since the monitoring area per person was reduced. Larval density in streams is generally lower (WEITERE et al. 2004) and the terrain around streams was more difficult to traverse. Therefore, the total monitoring time for a 20 m section for one person was set to one hour. Except for some occasions in 2019, most of the monitoring events were performed by two researchers per sample site.

In the first year, we performed 10 monitoring events of 5 ponds and 5 streams. We visited each pond or stream weekly during the first 8 monitoring events from March to May, one month later followed by two more monitoring events in June and in July. Due to the Corona pandemic, in 2020 we performed 3 monitoring events (once per month) of the same 5 streams and 4 ponds (one pond desiccated in 2019) in April, May and June. The monitoring period was elongated in 2021 to a total number of 15 occasions, aiming

to get year-round population estimates of the larval European fire salamander population. That year, the monitoring was performed weekly from March to May, then monthly from the end of May to beginning of December. In 2022, we started with a monthly monitoring in January and February. We then performed 9 weekly monitoring events during the field season from March to May and continued with monthly monitoring events from end of May until September. Due to the low amount of water, we excluded one stream (AB) from the monitoring. One pond location (TG) had to be replaced by another pond (WT), as it was already desiccated in the beginning of the monitoring period. The monitoring events during the four years are visualised in Fig. 2 (see also Supplementary Table S1).

During the monitoring of the ponds, we first checked visually whether there were larvae visible. Visible larvae were caught by gently moving a dip net underneath the larvae and then slowly moving it upwards to the water surface. If no larvae were seen, we systematically went through the water with dip nets to capture the European fire salamander larvae. In ponds, we moved the dip net from the centre of the pond towards the littoral zone, because larvae tended to hide under the leaves and branches or float

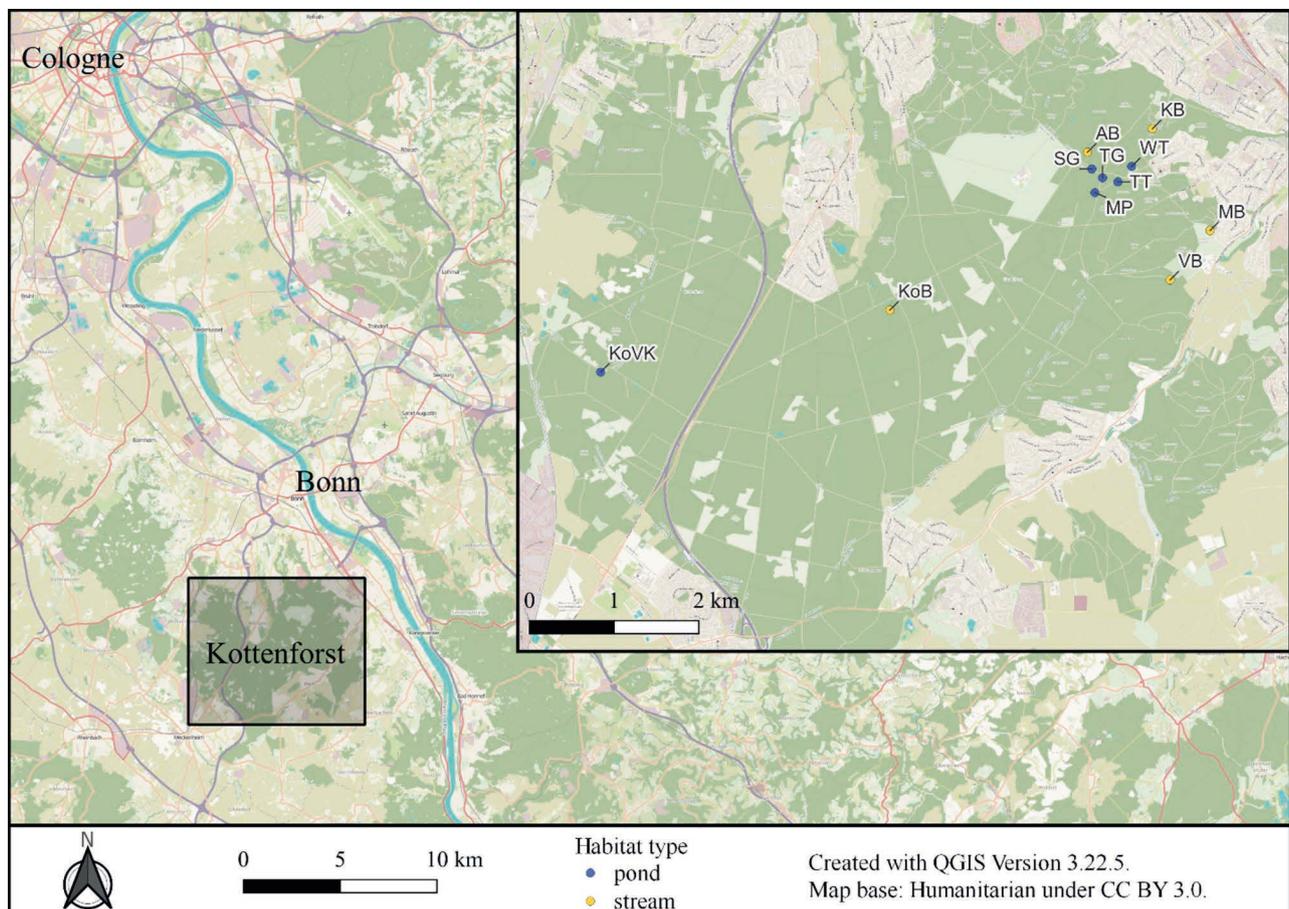


Figure 1. Map of the study area, the Kottenforst, near the city Bonn, Germany. The inset map of the Kottenforst on the upper right shows the ponds (blue circles) and streams (yellow circles) that were monitored regularly for four years.

near the surface in the littoral zone. Once, we could not capture any more larvae at one place (i.e., if several strokes with the dip net did not return any larvae), we moved on to the next part of the pond until the sampling time was over. In streams, we again first checked for larvae visually. Once detected, we positioned the dip net in an upright position downstream of the larvae and moved the dip net towards the larvae while simultaneously blocking the way upstream with one hand wearing nitrile gloves to prevent touching the sensitive skin of larva with our hands. If there was no visual detection of larvae, we moved the dip net upstream with small up and down movements near the ground, as most larvae were sitting on the ground instead of floating on the surface. We monitored a section of 1m for approximately 3 minutes and moved the dip net very carefully to not disturb the environment and organisms living in the stream. After about 3 minutes, we moved on to the next meter of the stream. We used an interval training App to stick to the time periods. For both ponds and streams, we put the captured larvae into an opaque 10 l bucket filled with water from the respective water body for a maximum time of one and a half hours. Afterwards, we transferred each larva into a Petri dish with millimetre paper as a scale underneath to measure the total body length (from snout to tail, in cm, according to KRAUSE et al 2011). Then, we transferred it into a small transparent tank with tap water (to reduce turbidity of water) to take a photograph from the right side of each larva with a digital camera (Sony Cybershot DSC WX220) or a mobile phone camera (Xiaomi Redmi Note 8 Pro, Fairphone 3). We noted specific features such as missing legs or tails. The handling time (size measurement and photograph) took maximal three minutes per individual. Afterwards, we collected the larvae in an enclosure separate from the bucket to prevent repeated sampling. We released all larvae to their capture site immediately, when the sampling was finished. In addition, we randomly sampled larvae from each location each year for *Bsal* (Supplementary Table S2). *Bsal* was not present at our study site (OSWALD et al. unpublished).

Population analyses

We uploaded all photographs of the larvae to the Amphibian and Reptile Wildbook via bulk import clustered for each sample site and monitoring event. We added information about the sampling site, date, id, observer and larval size (as snout-to-tail length). The website uses a specific algorithm to detect and match European fire salamander

larvae a, if they have been captured in former monitoring events. Wildbook does not require any processing of the images prior to the detection and matching process. We ran the matching process to analyse the number of recaptures and used the Wildbook output (capture history in binary format, e.g., 01101, where 0 = no capture and 1 = capture) for population analyses using the Jolly-Seber model (POPAN formulation) for open populations as described in WAGNER et al. (2020a). This model calculates survival probabilities, capture probabilities, the probability of new entries (e.g., via birth) and populations sizes. The model also allows for emigration by drift and metamorphosis and accounts for the prolonged breeding season of amphibians (WAGNER et al. 2020a). We used the R (R Core Team 2020) packages dplyr (WICKHAM et al. 2022) and magrittr (BACHE & WICKHAM 2022) for data processing and R2ucare (GIMENEZ et al. 2018) to test for goodness of fit. Since some models based on monthly monitoring data lacked goodness of fit for some sample sites, we calculated the apparent survival rate, the superpopulation size and the estimated population size including standard errors based on weekly monitoring data (March to May) with the package RMark (LAAKE 2013). Monthly monitoring data was partly used for the boxplots, but not for further analyses or models, since we would lose datapoints from multiple weeks that were averaged for monthly estimates.

We calculated the individual daily growth rates (g) based on the data of recaptured larvae in 2021 (March to December) and 2022 (January to September) by dividing the change in size between two successive captures by the number of days between the two captures:

$$g = \frac{s_r - s_c}{t},$$

where s_r = snout-to-tail length at the recapture r , s_c = snout-to-tail length at previous capture c and t = number of days between the two captures. This formula was modified from a study on European fire salamanders by LIMONGI et al. (2015), who used the same formula but with weight data instead of the snout-to-tail length.

The recapture rates at a given monitoring (r_k) were calculated by dividing the number of recaptured individuals by the total number of captured individuals (e.g., JAMESPIRRI & COBB 2000, DEROBA et al. 2005) with the following formula:

$$r_k = \frac{m_k}{n_k},$$

where m_k = number of recaptured individuals at a sampling event k and n_k = total number of captured individuals at a sampling event k .

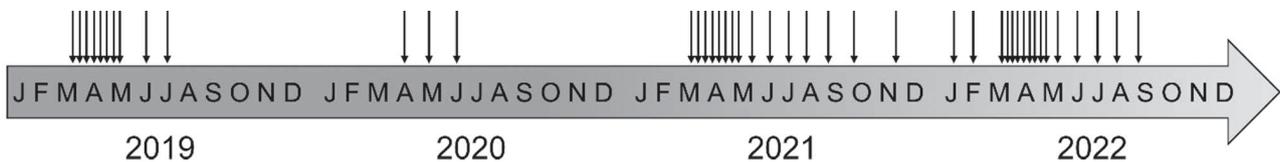


Figure 2. Overview of the monitoring events in the years 2019 to 2022. The abbreviations within the horizontal arrow indicate the months January to December. Small black arrows indicate a monitoring event.

Table 1. Linear (mixed) models used for the analysis with the response variable, random effects, fixed effects and their corresponding p-values. Significant values are in bold. LME = Linear mixed effects model, LM = Linear model. Due to non-normal distribution some variables were transformed. The respective transformation is stated underneath each response variable in parentheses.

Model	Response variable	Random effect	Fixed effect	p-value
LME1	Number of observed larvae (ordernorm transformation)	Sample site/year	Year	p = 0.007
LME2	Water temperature (ordernorm transformation)	Sample site/year	Habitat type	p = 0.603
LME3	Mean larval size (ordernorm transformation)	Sample site Session/year	Habitat type Water temperature Year	p = 0.172 p = 0.928 p = 0.150
LME4	Individual daily growth rate (ordernorm transformation)	ID Timespan	Habitat type Year Water temperature	p = 0.907 p = 0.382 p = 0.506
LM5	Recapture rate	–	Habitat	p = 0.653
LME6	Number of estimated larvae	Sample site Session/year	Year Habitat type	p = 0.019 p = 0.474
LM7	Estimated superpopulation size	–	Year	p = 0.570

The percentage of injured larvae (i_k) was calculated by dividing the number of injured larvae (e.g., missing limbs or tailfin, bite marks) by the total number of captured individuals:

$$i_k = \frac{b_k}{n_k},$$

with b_k = number of injured larvae at sampling event k and n_k = total number of captured larvae at sampling event k .

Plots and figures were created with the packages `ggplot2` (WICKHAM 2016), `ggpubr` (KASSAMBARA 2020), `grid` (MURRELL 2002) and `gridExtra` (AUGUIE 2017) in R.

Statistical models

We ran linear models with the packages `lme4` (BATES et al. 2015) and `lmerTest` (KUZNETSOVA et al. 2017) in R (see Table 1) to analyse which factors (e.g., habitat type, year) affect the mean larval size, the individual growth rate, the percentage of injured larvae, the recapture rate, the apparent survival rate and the estimated number of larvae. We also ran a linear model to check whether both habitats, pond and stream, differ in their temperature regime, as amphibians are ectotherm and thus highly susceptible to external temperatures. The exact models will be described in more detail below. Variables based on population analyses with the recapture data from Wildbook were calculated for the year 2021 and 2022, while the number of captured larvae, mean larval size and the percentage of injured larvae was based on raw data from 2019–2022. For comparability between years, we only used data from March to mid-May. The stream KoB was excluded from the models, since it revealed characteristics of both, ponds and streams, and could thus not be clearly assigned to any of the two habitat types.

Prior to model selection, we inspected the histogram of the variable of interest. We further performed a Shapiro Wilk test and variance test (F test for normal distributed data or Fligner test in case of non-normal distribution) to check for normality and homogeneity of variances (Supplementary Table S3). In case of non-normal data distribution, we used the `bestNormalize` package (PETERSON & CAVANAUGH 2020, PETERSON 2021) to choose the most suitable transformation for the data which in most cases was done with `ordernorm` transformation. Afterwards, we again performed a Shapiro Wilk and variance test. For model selection, we set up models with the variables of interest including different fixed and random effects and chose the model based on the lowest AIC or best model performance score as implemented in the `performance` package (LÜDECKE et al. 2021) in R. To validate the suitability of the chosen model, we inspected the diagnostic plots with a focus on the normality of residuals of the model with the `performance` package. If the transformed data did not normalise the data, we set up models with the non-transformed data using normal and other distributions (e.g., Poisson). We investigated the model residuals with diagnostic plots and checked for dispersion with the `DHARMA` package (HARTIG 2020) in R. If the diagnostic plots and model checks confirmed the underlying assumptions (e.g., normality of residuals), we used the most supported model. In case the assumptions were not confirmed we performed a simple non-parametric Wilcoxon test (also Wilcoxon rank sum test or Mann-Whitney U test) for a comparison of means to investigate potential differences between larvae from the two habitats (pond and stream).

After data inspection and model checks, we ran separate linear mixed (effect) models (LM and LME) for the dependent variables water temperature, mean larval size, individual daily growth rates and recapture rates. For detailed information on the models see Table 1. The percent-

age of injured larvae and apparent survival rates were compared between habitats with a Wilcoxon test, because the model assumptions were not met.

Table 2. Number of monitoring events and number of captured larvae from 2019 to 2022. The mean number of captured larvae (and its standard deviation = SD) is calculated across all sample sites per monitoring event and year.

Year	Number of monitoring events	Number of captured larvae	Mean number of captured larvae (\pm SD)
2019	10	2822	32.67 (\pm 21.64)
2020	3	406	27.77 (\pm 18.13)
2021	15	1439	20.36 (\pm 13.72)
2022	15	2017	26.30 (\pm 32.00)

Results
Number of captured larvae

The total number of captured larvae per year ranged from 406 to 2822 larvae and differed between single monitoring events within the years, between the years and between sample sites (Fig. 3, Table 2, Supplementary Table S1). For all sample sites, the number of captured larvae reached its maximum between mid-April to mid-May and then decreased until August. Due to desiccation, not all water bodies were monitored the same number of times. We excluded the stream (AB) from the monitoring due to the absence of water. One pond (TG) was desiccated after only two monitorings and we thus replaced it by another pond (WT).

Using the dataset including all years and sample sites (except for KoB), there was a significant influence of the year on the observed number of larvae (LME1, $p = 0.007$). The overall number of larvae decreased from 2019 to 2021, but there was a slight increase in 2022 (Supplementary Fig. S1).

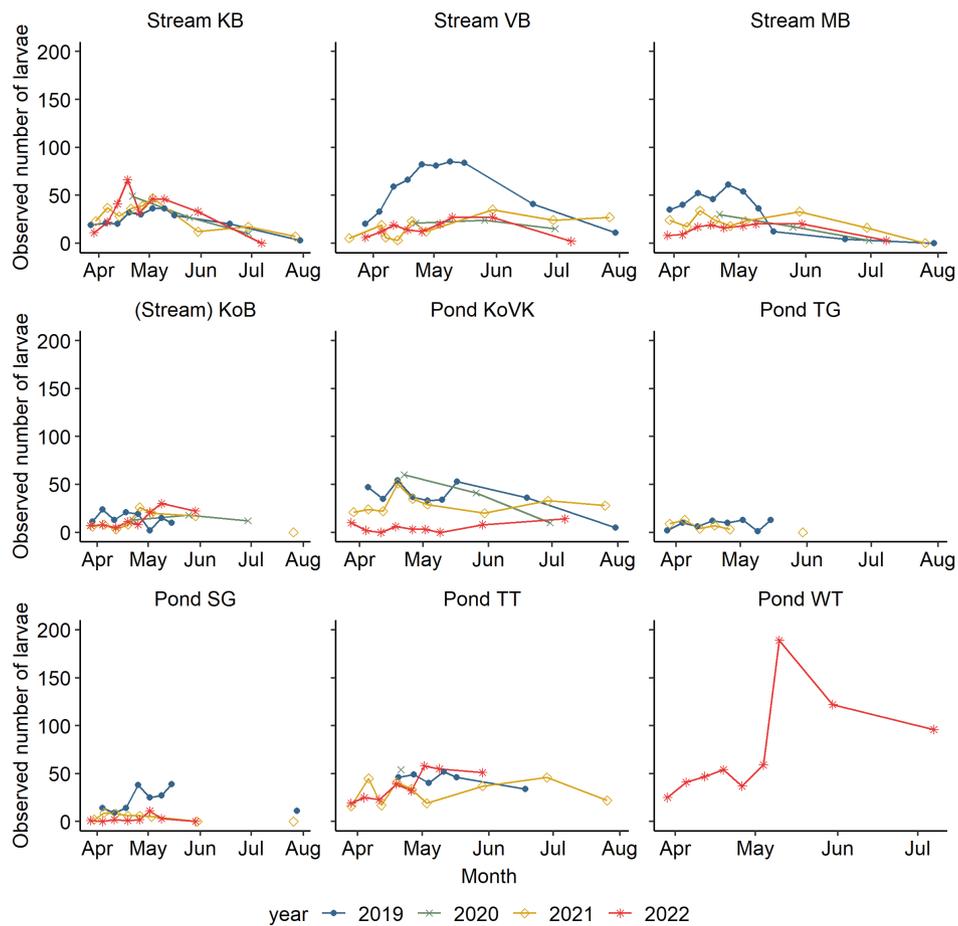


Figure 3. Comparison of the number of larvae per sample site that have been captured during the monitoring events in 2019, 2020, 2021 and 2022. Shown here are only monitoring events that were conducted during similar time periods in the three years from end of March until end of July. Due to the low number of monitoring events in 2020, there are maximum three data points in April, May and June per sample site. Interrupted lines or lines that do not reach until August have been desiccated. The pond TG was replaced by the pond WT in 2022, since it was completely desiccated.

Table 3. Range of mean larval sizes (in cm) of larvae captured from ponds and streams for the years 2019 to 2022. The range includes the minimum and maximum larval size per year and habitat type. The mean larval size (and its standard deviation = SD) is averaged across all sample sites per habitat type and year.

Year	Larval size range in ponds (cm)	Mean larval size (\pm SD) in ponds (cm)	Larval size range in streams (cm)	Mean larval size (\pm SD) in streams
2019	2.82–4.76	3.61 (\pm 0.44)	2.75–4.78	3.57 (\pm 0.46)
2020	3.83–4.11	3.96 (\pm 0.14)	3.23–4.08	3.66 (\pm 0.34)
2021	2.96–4.40	3.63 (\pm 0.39)	2.93–4.61	3.51 (\pm 0.45)
2022	3.13–5.01	3.69 (\pm 0.38)	3.00–5.13	3.62 (\pm 0.54)

Mean larval sizes

Larval sizes varied among monitoring events, sample sites and years and ranged from 2.75 to 5.01 cm (Fig. 4, Table 3). The mean larval size increased over the course of the monitoring period with larvae captured during later monitoring events being larger (Fig. 4, Fig. 5). The habitat type (LME₃, $p = 0.172$), water temperature (LME₃, $p = 0.928$) and year (LME₃, $p = 0.150$) did not influence the mean larval size

(Table 1). The water temperature did not differ between habitats (LME₃, $p = 0.603$, Supplementary Fig. S3).

Individual daily growth rate

The individual daily growth rate (from first to last capture) was calculated for the larvae captured in 2021 and 2022 and ranged from -12.86 to 15.83% (Table 4). Using

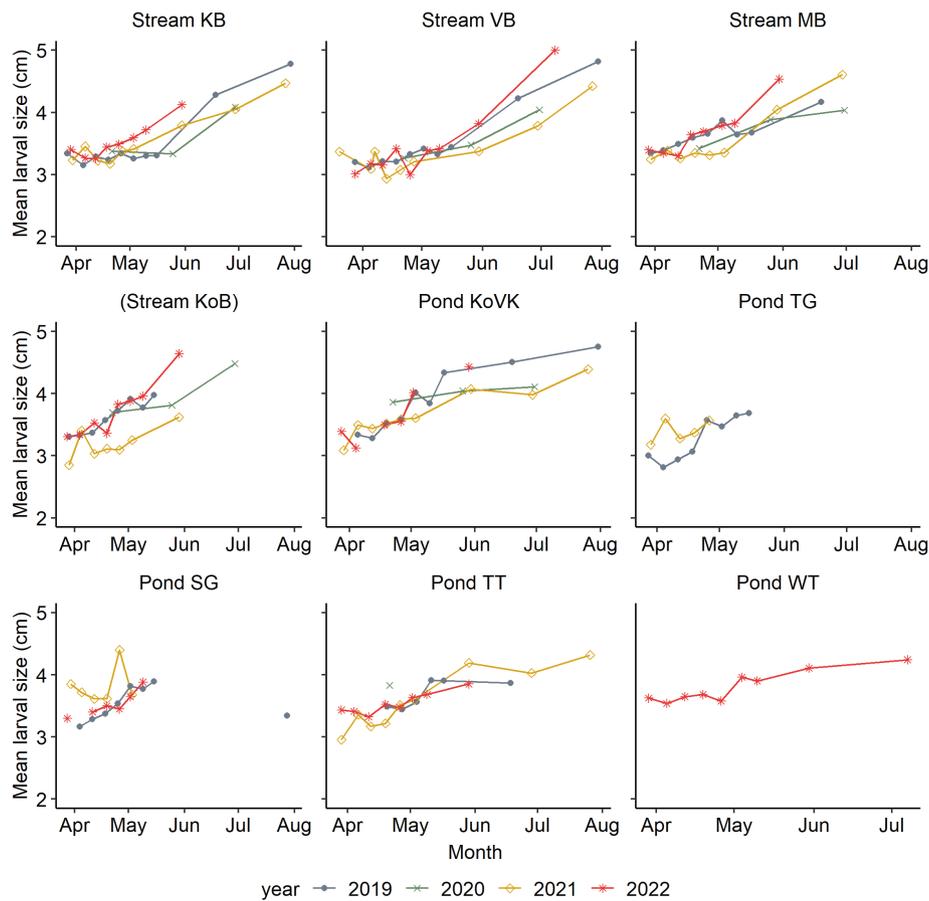


Figure 4. The mean larval sizes per sample site during the monitoring events in 2019, 2020, 2021 and 2022. Shown here are only monitoring events that were conducted during similar time periods in the three years from end of March until end of July. Due to the low number of monitoring events in 2020, there are maximum three data points in April, May and June per sample site. Interrupted lines or lines that do not reach until August have been desiccated. The pond TG was replaced by the pond WT in 2022, since it was completely desiccated.

Table 4. Range of individual daily growth rate (in %) of larvae captured from ponds and streams for the years 2021 and 2022. The range describes the minimum and maximum individual daily growth rate. The mean (and its standard deviation = SD) is calculated across all sample sites per habitat type and year.

Year	Range of growth rates (%) in pond larvae	Mean growth rate (%) in pond larvae (\pm SD)	Range of growth rates (%) in stream larvae	Mean growth rate (%) in stream larvae (\pm SD)
2021	-0.18–7.14	1.52 (\pm 1.21)	- 2.14–4.29	1.01 (\pm 1.23)
2022	-4.00–11.67	1.28 (\pm 1.80)	-12.86–15.83	1.19 (\pm 3.41)

the full dataset, there was no effect of the larval habitat type (LME4, $p = 0.907$), the year (LME4, $p = 0.382$) or the water temperature (LME4, $p = 0.506$) on daily growth (Fig. 5).

Percentage of injured larvae

The percentage of injured larvae (limbs or tailfin missing, bite marks) across all years ranged from 0 to 100% in pond larvae (mean 11.84%) and 0 to 37.5% in stream larvae (mean 9.80%). There was no significant difference between the two habitat types (Wilcoxon, $p = 0.250$, Fig. 6), but a significant difference between the years (Kruskal-Wallis, $p < 0.001$). When we split the dataset into the single years

(Fig. 7, Table 5), there was still no significant difference in the percentage of injured larvae from the two habitat types in 2019 (Wilcoxon, $p = 0.069$) and 2020 (LM5, $p = 0.653$), but there was a significance effect of the habitat type in the two following years. In 2021, there was a higher percentage of injured larvae in streams (Wilcoxon, $p = 0.016$). In 2022, ponds exhibited a higher number of injured larvae (Wilcoxon, $p = 0.037$).

Recapture rates

Recapture rates were based on the photographic recaptures during the monitoring events from March to May in 2021 and 2022. The recapture rates varied between sam-

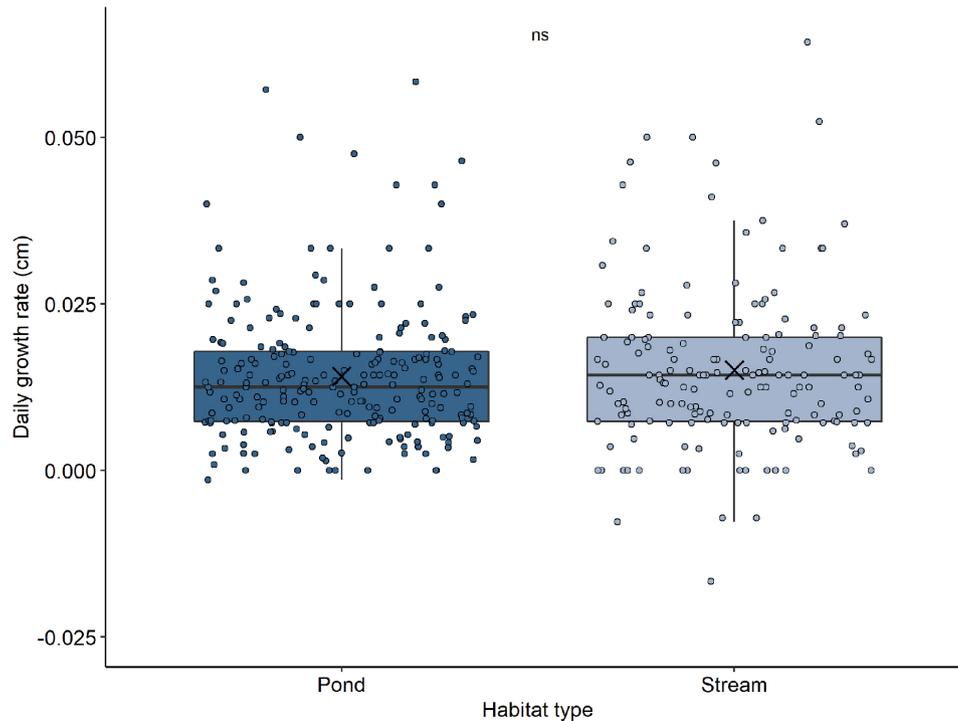


Figure 5. Individual daily growth rate per habitat type across all sample sites (excluding KoB) based on the monitoring events in 2021 and 2022. The box plots are representing the upper and lower quartile (upper/lower edge of the box), the median (black horizontal line within the box), and the maximum and minimum values (end of upper/lower whisker) lying within 1.5 times the interquartile range. Data points that are > 1.5 times the interquartile range (upper quartile–lower quartile) are defined as outliers. The annotation above the box plot represents possible differences of individual daily growth rates between fire salamander larvae from the two habitat types based on p -values: ns = non-significant.

Fire salamander population analyses via photo-recognition software

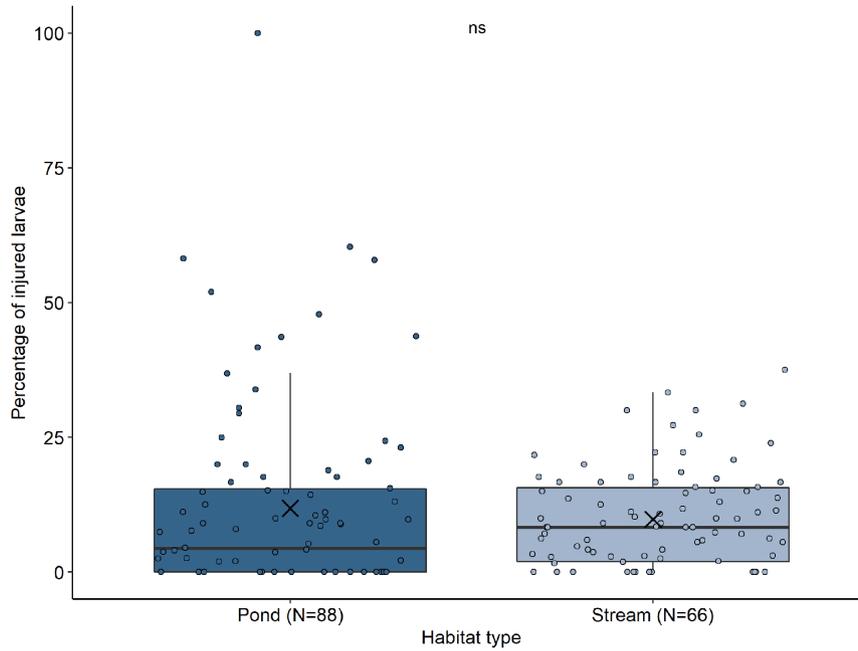


Figure 6. Percentage of injured larvae per habitat type across all sample sites (excluding KoB) during the monitoring events in 2019, 2020, 2021 and 2022. The box plots are representing the upper and lower quartile (upper/lower edge of the box), the median (black horizontal line within the box), and the maximum and minimum values (end of upper/lower whisker) lying within 1.5 times the interquartile range. Data points that are > 1.5 times the interquartile range (upper quartile–lower quartile) are defined as outliers. The annotation above the box plot represents possible differences of the percentage of injured larvae between the two habitat types based on p-values; ns = non-significant.

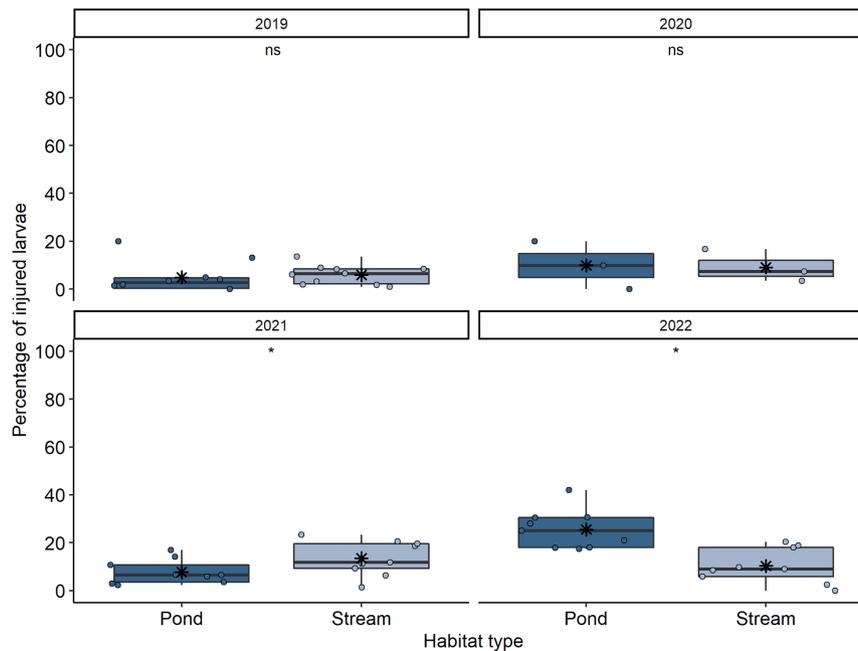


Figure 7. Percentage of injured larvae per habitat type across all sample sites (excluding KoB) per year during the monitoring events in 2019, 2020, 2021 and 2022. The box plots are representing the upper and lower quartile (upper/lower edge of the box), the median (black horizontal line within the box), and the maximum and minimum values (end of upper/lower whisker) lying within 1.5 times the interquartile range. Data points that are > 1.5 times the interquartile range (upper quartile–lower quartile) are defined as outliers. The annotation above the box plot represents possible differences in the recapture rate between the two habitat types based on p-values: ns = non-significant, * = $p < .05$

Table 5. Range of injured larvae (in %) from ponds and streams in the years 2019 to 2022. The range describes the minimum and maximum percentage of injured larvae in ponds and streams. The mean (and its standard deviation = SD) is calculated across all sample sites per habitat type and year.

Year	Range of the number of injured larvae in ponds (%)	Mean number of injured larvae (%) in ponds (\pm SD)	Range of the number of injured larvae in streams (%)	Mean number of injured larvae (%) in streams (\pm SD)
2019	0–20.59	3.52 (\pm 5.43)	0–27.27	5.68 (\pm 6.31)
2020	0–20	7.43 (\pm 9.55)	0–30	9.11 (\pm 10.73)
2021	0–23.08	7.18 (\pm 7.48)	0–37.50	13.70 (\pm 10.21)
2022	0–100	25.51 (\pm 24.87)	0–30	10.71 (\pm 8.71)

Table 6. Range of recapture rates (in %) for larvae from ponds and streams in the years 2021 and 2022. The range describes the minimum and maximum recapture rates (in %). The mean recapture rate (and its standard deviation = SD) is calculated across all sample sites per habitat type and year.

Year	Range of recapture rates in ponds (%)	Mean recapture rates (%) in ponds (\pm SD)	Range of recapture rates in streams (%)	Mean recapture rates (%) in streams (\pm SD)
2021	0–50	11.65 (\pm 14.30)	0–100	8.01 (\pm 9.06)
2022	0–50	9.51 (\pm 11.87)	0–58.78	10.47 (\pm 12.14)

ple sites and monitoring events (Supplementary Table S4, S5). In 2021, the highest recapture rate of 100% (in a pond) was found during a monitoring event with a very low sample size of only one individual. During monitoring events with sample sizes higher than ten, recapture rates were still high and reached up to 24.14% in ponds and 34.04%

in streams. In 2022, the recapture rates for monitoring events with sample sizes higher than ten were up to 37.70% in ponds and 58.70% in streams (Table 6). The recapture rate (including both years) did not differ between habitats (Wilcoxon, $p = 0.780$, Fig. 8) or years (Wilcoxon, $p = 0.898$).

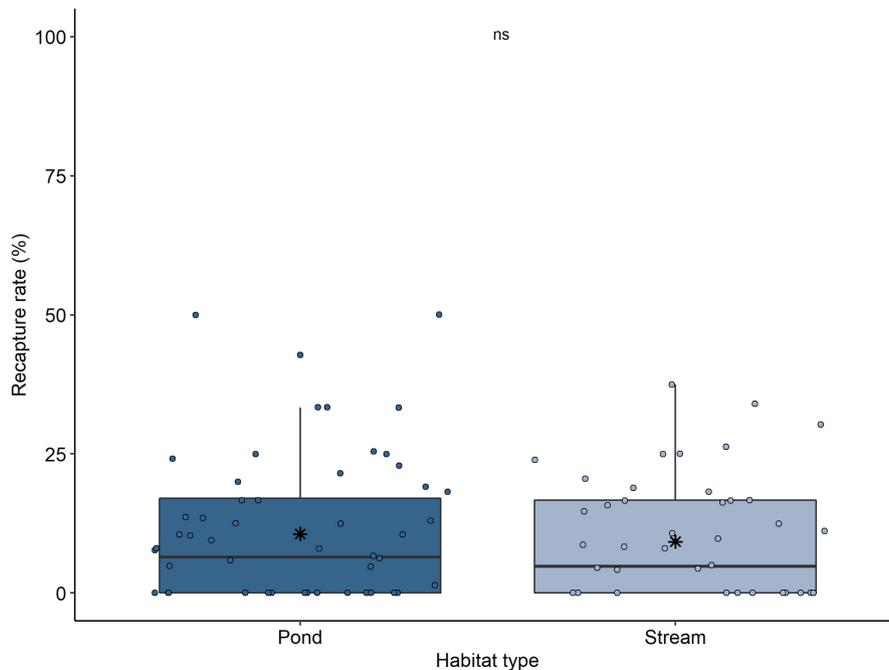


Figure 8. Recapture rate per habitat type across all sample sites (excluding KoB) during the weekly monitoring events from March to May in 2021 and 2022. The box plots are representing the upper and lower quartile (upper/lower edge of the box), the median (black horizontal line within the box), and the maximum and minimum values (end of upper/lower whisker) lying within 1.5 times the interquartile range. Data points that are > 1.5 times the interquartile range (upper quartile–lower quartile) are defined as outliers. The annotation above the box plot represents possible differences in the recapture rate between the two habitat types based on p -values: ns = non-significant.

Table 7. Range of the apparent survival (in %) of larvae from ponds and streams in the years 2021 and 2022. The range describes the minimum and maximum survival rates (in %). The mean (and its standard deviation = SD) is calculated across all sample sites per habitat type and year.

Year	Range of the apparent survival in ponds (%)	Mean apparent survival (%) in ponds (\pm SD)	Range of the apparent survival in streams (%)	Mean apparent survival (%) in streams (\pm SD)
2021	48.81–93.67	76.58 (\pm 17.45)	79.01–100	89.24 (\pm 8.79)
2022	0–100	57.89 (\pm 44.87)	0–100	64.77 (\pm 43.06)

Survival rates

The apparent survival rates were based on the photographic recapture data during the weekly monitorings from March to May in 2021 and 2022 and ranged from 0 to 100% (Table 7). There was no influence of the year on the apparent survival (Wilcoxon, $p = 0.502$), but the apparent survival differed significantly between habitat types (Wilcoxon, $p = 0.034$, Fig. 9).

Population size estimation

The estimated population size was based on the photographic recapture data from 2021 and 2022 and was high-

ly variable between sample sites and monitoring events (Fig. 10). Weekly and monthly estimates were similar in 2021, but deviated in 2022 (Supplementary Fig. S3, S4). In the following, we will focus on weekly estimates, since the time periods between weeks were more regular and thus more suitable for population analyses. The estimated number of larvae in 2021 ranged from 43.75 to 1330.30 individuals in ponds and from 15.34 to 561.00 individuals in streams (Supplementary Table S6). In 2022, the estimated larval population size ranged from 0 to 972.92 individuals in ponds and from 0 to 647.97 individuals in streams (Table 8, Supplementary Table S7). The year (LME6, $p = 0.019$), but not the habitat type (LME6, $p = 0.474$) had a significant influence on the number of estimated larvae.

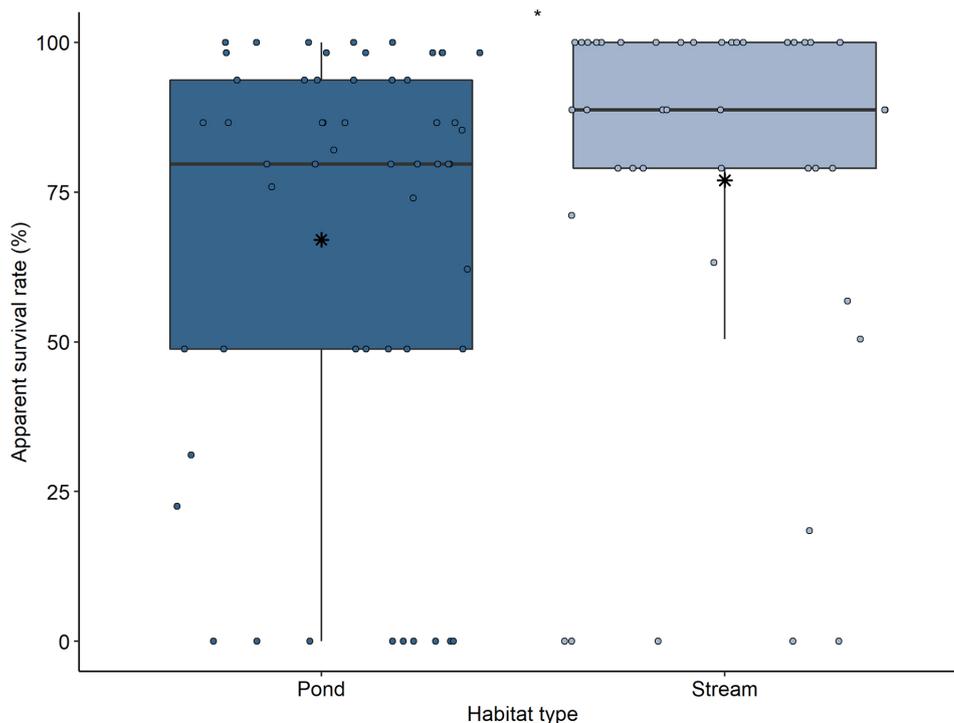


Figure 9. Apparent survival rate per habitat type across all sample sites (excluding KoB) during the weekly monitoring events from April to May in 2021 and 2022. The box plots are representing the upper and lower quartile (upper/lower edge of the box), the median (black horizontal line within the box), and the maximum and minimum values (end of upper/lower whisker) lying within 1.5 times the interquartile range. Data points that are > 1.5 times the interquartile range (upper quartile–lower quartile) are defined as outliers. The annotation above the box plot represents possible differences of the survival rate in the two habitat types based on p-values: ns = non-significant, * = $p < .05$.

Table 8. Range of the estimated larval population size. The range describes the minimum and maximum estimated number of larvae per habitat type and year (including the standard deviation = SD).

Year	Range of the estimated number of larvae in ponds (\pm SD)	Range of the estimated number of larvae in streams (\pm SD)
2021	43.75 (\pm 15.92) – 1330.30 (\pm 690.00)	15.34 (\pm 1.16) – 561.00 (\pm 174.90)
2022	0 (\pm 0) – 972.92 (\pm 35.93)	0 (\pm 0) – 647.97 (\pm 60.90)

The estimated size of the superpopulation in 2021 ranged from 172.94 (\pm 46.32) to 4495.61 (\pm 2084.50) individuals in ponds and from 383.32 (\pm 137.36) to 1013.93 (\pm 239.18) in streams. In 2022, the estimated superpopulation size ranged from 67.81 (\pm 11.83) to 1264.90 (\pm 13.05) in ponds and from 480.76 (\pm 0.00) to 16236.24 (\pm 2622.44) in streams (Supplementary Table S8). There was no significant difference in the estimated superpopulation size between the years 2021 and 2022 (LM7, $p = 0.570$, Fig. 11).

Discussion

In our study we investigated mean larval size, recapture rate, apparent survival, estimated larval population size, individual growth and the percentage of injured larvae of a European fire salamander population in Germany. We found high variation in those traits within and between sample sites and monitoring events. There was an effect of the year but not of the habitat type on the observed number

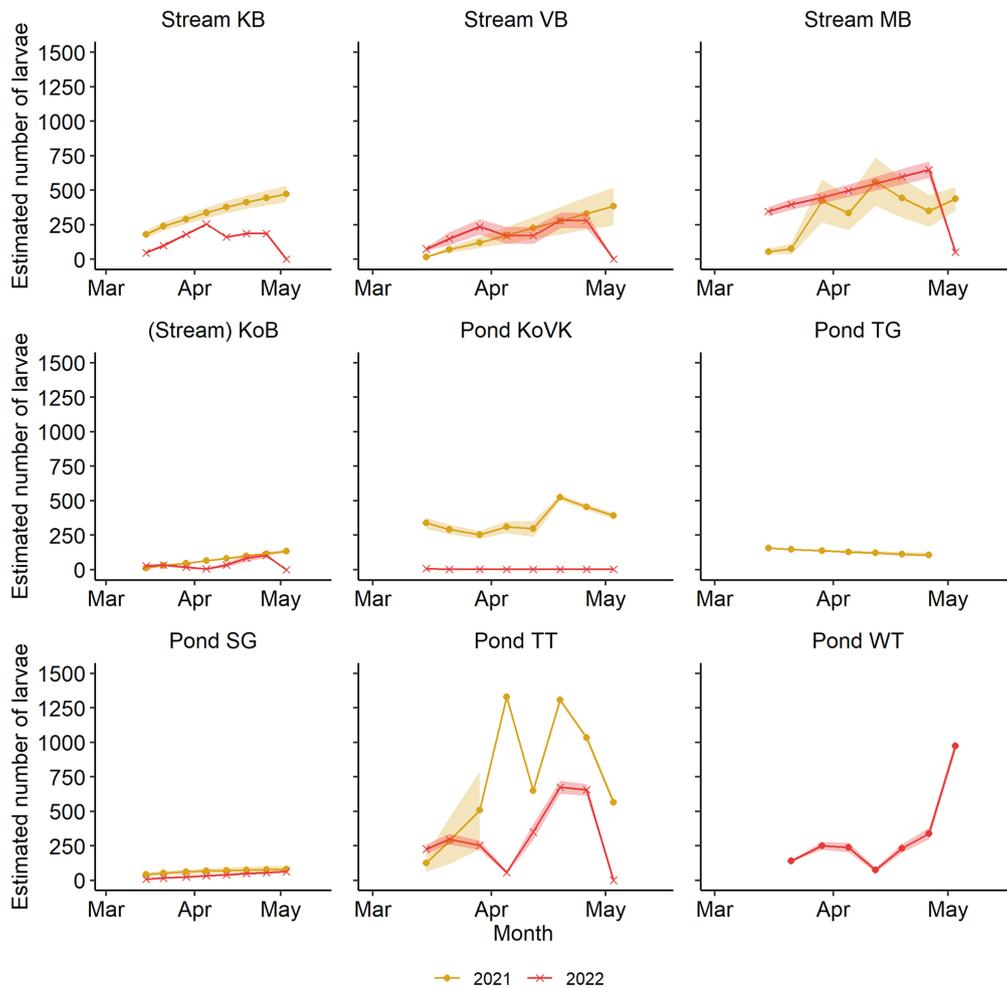


Figure 10. Larval population size estimates across all sample sites in the Kottenforst based on weekly monitoring events in 2021 and 2022. The upper row represents the estimated number of larvae for the streams, the bottom row represents estimated population sizes for the ponds. Stream KoB is in parentheses, since it has characteristics of both, pond and stream. In many cases, the monthly estimation of population size after July was not possible, since there were no more larvae found. Sample sites KoB, TG and SG desiccated after April and May, respectively.

of larvae, the percentage of injured larvae and the estimated population size. There was no difference in superpopulation size. When split into subsets for each year, the percentage of injured larvae differed between habitat types in 2021 (higher in streams) and 2022 (higher in ponds). There was no effect of the year or habitat type on recapture rates. Recapture rates, mean larval sizes and water temperature were independent of the habitat and year. The mean larval size was not influenced by the water temperature. The apparent survival rate and the daily growth rate differed significantly between habitats, although this effect was probably driven by year-specific differences. We found no cases of *Bsal* in our study area.

So far, only few studies have investigated European fire salamander population sizes (but see SEGEV et al. 2010, ÁLVAREZ et al. 2015, SINAI et al. 2020). According to two previous studies, population sizes were higher in permanent water bodies (SEGEV et al. 2010, SINAI et al. 2020) indicating permanent water bodies provide a more suitable habitat than temporary water bodies. However, in our study there was no significant difference between temporary ponds and permanent streams regarding the estimated population size. This discrepancy might result from different monitoring approaches. SEGEV et al. (2010) and SINAI et al. (2020) monitored adults in proximity to the water bodies, while we monitored larvae in the respective

habitats. Nevertheless, we found a higher apparent survival in streams than in ponds, indicating that streams, i.e., here the permanent water body, might indeed be advantageous.

We found no significant difference in the individual daily growth rate across the overall dataset with regard to the habitat type. These findings are in line with a previous study that did not find growth difference between larvae from the two habitat types (SABINO-PINTO et al. 2019). However, during a reciprocal transfer experiment that we conducted in 2019, we found higher growth rates before metamorphosis in larvae that were transferred to ponds (OSWALD & CASPERS, unpublished). Other studies found that larval growth was affected by temperature, desiccation events and food regime (e.g., ALCOBENDAS et al. 2004, KRAUSE et al. 2011). While low food abundance led to decreased growth rates in fire salamander larvae (e.g., ZAHN 2007, LIMONGI et al. 2015), desiccation risk accelerated larval development at the expense of reduced growth (RICHTER-BOIX et al. 2011, SZÉKELY et al. 2017). Although we did not find temperature differences between ponds and streams in our study, both habitats were different with regard to food abundance and desiccation risk (P. OSWALD, unpubl. obs.). As we did not find overall differences in growth, larvae seem to be able to compensate for disadvantageous conditions in the larval habitat.

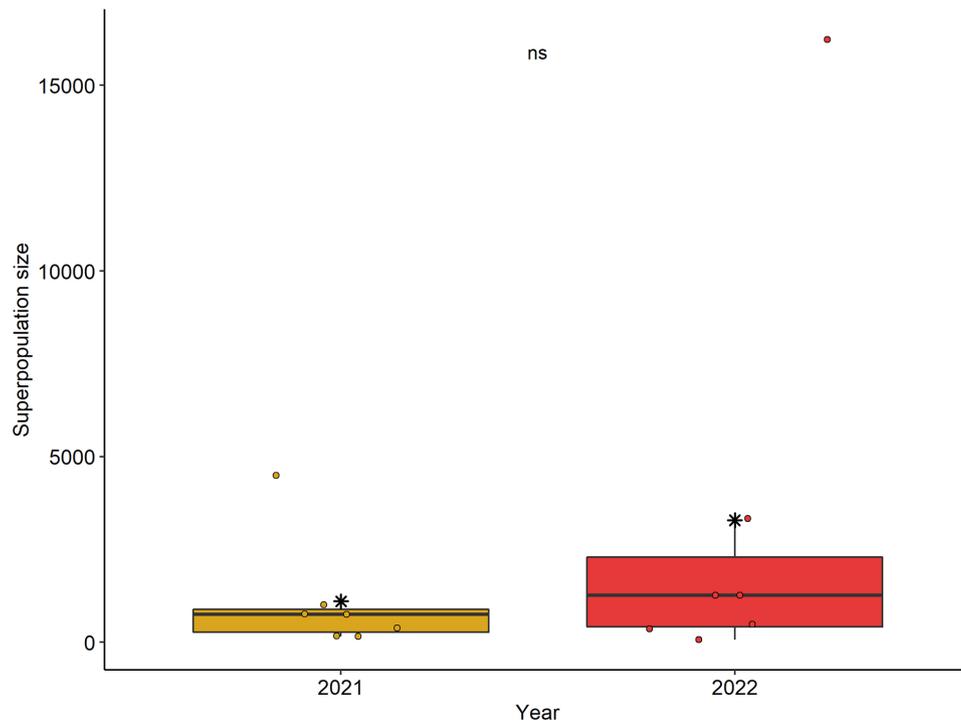


Figure 11. Larval superpopulation size per year across all sample sites (excluding KoB) based on the weekly monitoring data from March to May in 2021 and 2022. The box plots are representing the upper and lower quartile (upper/lower edge of the box), the median (black horizontal line within the box), and the maximum and minimum values (end of upper/lower whisker) lying within 1.5 times the interquartile range. Data points that are > 1.5 times the interquartile range (upper quartile–lower quartile) are defined as outliers. The annotation above the box plot represents possible differences in the estimated number of larvae between the two years based on p-values: ns = non-significant.

Previous studies often considered streams as the more suitable habitat for European fire salamander larvae (e.g., THIESMEIER 2004, REINHARDT 2014). In concordance with this, we found higher survival rates in streams. We have to consider that our approach calculates apparent survival, i.e., we cannot distinguish between emigration and death (LEBRETON et al. 1992). Apparent survival might thus be misleading and does not provide true survival estimates (often underestimated), since emigrated individuals might still survive outside of the investigated population (GILROY et al. 2012). As larval drift has a large influence on survival and catastrophic events such as strong precipitation decreases redetection probability (REINHARDT et al. 2018), downstream drift is likely to result in underestimated survival estimates in streams. In our study, the apparent survival for stream larvae was quite high indicating that either survival estimates were less underestimated than expected or survival was even higher. Since population estimates did not differ significantly between both habitats (but see comment above) and the apparent survival was quite high in both habitats, ponds and streams might provide equally suitable habitats.

Population estimates such as survival or population sizes are highly dependent on the used methods. For example, encounter surveys might best reflect relative abundances, while mark-recapture studies facilitate the estimation of population parameters such as survival and recapture rates (BOWER et al. 2014). According to SCHMIDT (2004), simple count data neglects potential differences in detection probabilities and does not reflect true population sizes. This was confirmed by a recent study comparing four different monitoring methods which found that count methods and removal sampling underestimated population sizes (WAGNER et al. 2020a). Furthermore, the estimated population size is dependent on the breeding system. In case of amphibians, which include “explosive” breeders and prolonged breeders (WELLS 1977), the superpopulation approach might be a useful tool to estimate population sizes in open populations where individuals occur asynchronously during the breeding season (PELLET et al. 2011).

In accordance with WAGNER et al. (2020b), our study emphasizes the need for regular population monitoring, as there was high variation between and within years and between different locations. Thus, sporadic monitoring does not always represent the actual population size and status. Especially, in the context of emerging infectious diseases such as *Bsal*, irregular monitoring events might fail the early detection of population declines. The choice of the appropriate monitoring method, depending on the objectives of the study and available resources, is crucial to get appropriate population estimates and detect potential declines (STORFER 2003). Alternatively, to our mark-capture-recapture approach, larval removal sampling, which does not require individual identification and can easily be conducted in the field, also reflects a less time consuming and inexpensive approach to detect population changes (SCHMIDT et al. 2015, WAGNER et al. 2020b). Although we did not find *Bsal* in our population, yet, other populations

have suffered from harsh declines due to this pathogenic fungus (e.g., SPITZEN-VAN DER SLUIJS et al. 2013, STEGEN et al. 2017, DALBECK et al. 2018). A recent study even found a new disease that threatens European fire salamander populations (MANENTI et al. 2022).

Thus, due to the multiple drivers of amphibian population declines that are often spatially different, local monitoring programs are key to amphibian conservation (GRANT et al. 2016). These programs often include capture-mark-recapture studies that come with a trade-off between the need for demographic estimates, logistic issues and potential harm to individuals during marking (HADDAD et al. 2008). For amphibians with distinctive colour patterns, there are several photographic identification softwares that vary in their efficacy to recognise and identify individuals (MATTHÉ et al. 2017). Monitoring efforts are often uncoordinated, unequally distributed among taxa and there is a general need for global open databases (MOUSSY et al. 2022). The Amphibian and Reptile Wildbook used in this study has the potential to become such a database for amphibians and reptiles, as it is easy to use, combines citizen science and scientific projects and even works for amphibian larvae. Larval monitoring has many advantages (easier to find, defined area), especially in species where terrestrial adults are difficult to survey, e.g., because they are cryptic or found in low densities (SKELLY & RICHARDSON 2009). The Amphibian and Reptile Wildbook is able to detect and identify both, larval and adult individuals of the European fire salamander (plus yellow-bellied toads and Near Eastern fire salamander). It is an open-source software and free to use, the software performs similarly or better than comparable photo-identification programs (L. SCHULTE et al. under study) and is robust against varying picture qualities (SCHULTE et al. 2022). It identifies 99.6% of larval recaptures from photos and proved to be a reliable tool in European fire salamander larvae research (L. SCHULTE et al., under study). Thus, the Amphibian and Reptile Wildbook might pave the way for future amphibian conservation projects based on both adult and larval monitoring including the opportunity of an open database and international collaborations.

Conclusion

We found quite high estimates that varied within and between sample sites and fluctuated over time. The population estimates decreased significantly from 2021 to 2022, but did not seem to differ between pond and stream habitats. However, we found a higher apparent survival rate in streams, compared to ponds. These results indicate that ponds and streams might be equally used as larval habitats, but streams might still be beneficial. It provides useful information for conservation projects such as the establishment of new breeding ponds or connectivity measures. Although the chytrid fungus *Bsal* is spreading, it has not yet reached the Kottenforst. However, the population should be monitored regularly to detect possible outbreaks. Fi-

nally, the larval monitoring and the new Amphibian and Reptile Wildbook software have proven to be efficient tools for population analyses of European fire salamanders. This method can probably be applied to other monitoring programs of amphibian adults and larvae, if the animals exhibit distinctive individual patterns and might be a first step towards a global database for amphibian and reptile monitoring data that supports (global) conservation efforts.

Data availability

Raw data and code are available at the online repositories GitHub and Open Science Framework (<https://doi.org/10.17605/OSF.IO/7BKQD>). The monitoring protocol for the European fire salamander larvae and additional tables can be found in the Supplementary material.

CRediT author statement

B.A.C, P.O. and B.A.T. contributed to study design and conception. B.A.T., P.O. and L.S. collected the data in the field. P.O. and L.S. processed the photographic data. P.O. analysed the data analysis and created the first draft of the manuscript. All authors refined previous versions of the manuscript and approved final submission.

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Supplementary data

The following data are available online:

Supplementary Table S1. Overview of the number of larvae captured during the monitoring events from 2019 to 2021.

Supplementary Table S2. Overview of the Bsal samples from 2019 to 2022.

Supplementary Table S3. Results of the tests for normal distribution and variance of homogeneity for all investigated variables.

Supplementary Table S4. Number of recaptures among captured animals and recapture rates per sample site based on the monitoring events in 2021.

Supplementary Table S5. Number of recaptures among captured animals and recapture rates per sample site based on the monitoring events in 2022.

Supplementary Table S6. Estimated larval population size (and standard error) per sample site based the weekly monitoring events in 2021.

Supplementary Table S7. Estimated larval population size per sample site based the weekly monitoring events in 2022.

Supplementary Table S8. Estimated larval superpopulation size per sample site based the weekly monitoring events in 2021 and 2022.

Supplementary Figure S1. Observed number of larvae across all sample sites during the monitoring events in 2019, 2020 and 2021 and 2022.

Supplementary Figure S2. Water temperature per habitat type across all sample sites during the monitoring events in 2019, 2020 and 2021 and 2022.

Supplementary Figure S3. Larval population size estimates across all sample sites in the Kottenforst based on weekly and monthly monitoring events in 2021.

Supplementary Figure S4. Larval population size estimates across all sample sites in the Kottenforst based on weekly and monthly monitoring events in 2022.