



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

NORMAN WAGNER, JOHANNES PFROMMER & MICHAEL VEITH

Trier University, Department of Biogeography, Universitätsring 15, 54296 Trier, Germany

Corresponding author: MICHAEL VEITH, e-mail: veith@uni-trier.de

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

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

### Introduction

Europe's urodelan diversity is severely threatened by the invasive and recently expanding chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) (MARTEL et al. 2013, 2014, SPITZEN-VAN DER SLUIJS et al. 2016, STEGEN et al. 2017). Especially, the European fire salamander (*Salamandra salamandra* (LINNAEUS, 1758)) can be classified as extremely sensitive towards this pathogene because usually infection leads to chytridiomycosis and individuals die within about two weeks (MARTEL et al. 2014, STEGEN et al. 2017). In European fire salamander populations in The Netherlands, Belgium and Germany (SPITZEN-VAN DER SLUIJS et al. 2013, 2016, DALBECK et al. 2018, STEGEN et al., 2017, LÖTTERS et al. 2020 in this issue) mass mortalities followed infection. In such cases, the rapid population collapse of adults should be followed by a strong downturn in numbers of larvae in the following years. The same was observed by BOSCH & MARTÍNEZ-SOLANO (2006), who found significantly decreasing larvae counts after a mass mortality event in a Spanish fire salamander population caused by the chytrid fungus *Batrachochytrium dendroba-*

*tidis* (*Bd*). Until today – after 18 years – this Spanish salamander population did not recover from this population decline (while other more resistant species in this amphibian community profited by the decline of the populations of susceptible species) (BOSCH et al. 2018). Similar strong effects of *Bsal* on salamander populations are described in Belgium by STEGEN et al. (2017) after mass mortality events.

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

The abundances of adult European fire salamanders are difficult to estimate (SCHMIDT et al. 2015), which, for

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

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

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

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

## Materials and methods

### Study region

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

### Field work

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

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

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

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

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

2.7 mm) to conduct a CMR study between 10/06/2017 and 19/07/2017 in the BB and between 15/06/2017 and 10/07/2017 in the EB. First, drift and upstream migration traps were checked for captured larvae every 2–3 days. Furthermore, larvae were captured in each 25 m subsection for 15 min

by opportunistic search and with the aid of a dip net. This corresponds to the first cycle of the hierarchical removal sampling models of SCHMIDT et al. (2015). Each larva was weighted to the nearest 0.001 g using a Kern EMB 200-3 field balance. Alpha-Tags were laterally injected using the

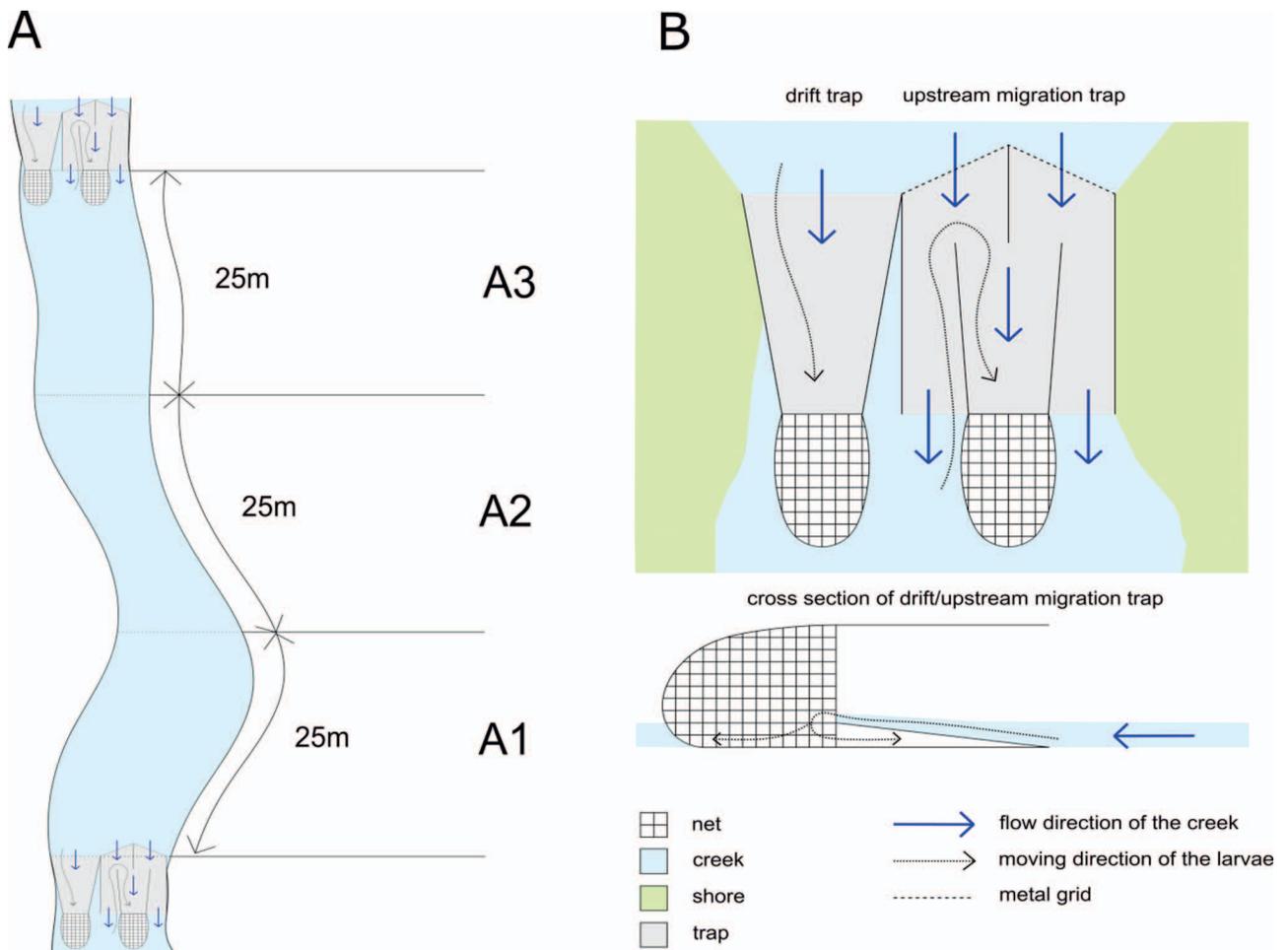


Figure 1. Schematic drawing of a study transect (A) and of a drift and upstream migration trap (B).

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

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

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

### Data analysis

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

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

CMR data were analysed using the program MARK (WHITE & BURNHAM 1999, COOCH & WHITE 2017). Death of individuals during the study time was possible and emigration by larval drift allowed (see above), also metamorphosing individuals at the end of the study period. Birth by newly deposited larvae is possible by single females during the whole study period (THIESMEIER 2004), furthermore immigration to the study sections by drift was also allowed. Hence, we choose the POPAN-approach for 'open populations' integrated in the program MARK (WHITE &

BURNHAM 1999, WAGNER et al. 2011, COOCH & WHITE 2017, SCHWARZ & ARNASON 2017). The POPAN-approach ('super-population approach') is a modified Jolly Seber model that not only estimates the population sizes at each occasion but also for the 'super-population' over the entire study period (WAGNER et al. 2011). We built 4–6 different models for each data set (i.e. section) by manipulating the parameters 'survival probability' ( $\phi$ ), 'detection probability' ( $p$ ), and the probability of an individual to enter the 'super-population' ( $b_i$ ) from 'time-varying' to 'constant'. Furthermore, we used the sinus or logit function for  $\phi$  and  $p$ . For  $b_i$ , we always used the Mlogit link function and for  $N$  the log-link function (as recommended by SCHWARZ & ARNASON 2017). Best fitting models were chosen by their small sample Akaike Information Criterion values (AICc: BURNHAM & ANDERSON 2002) and used for parameter estimation. Because both studied creeks were affected by dryness and subsequent flood between 21/06/2017 and 25/06/2017, we furthermore split the data sets at 25/06/2017 to estimate abundances also for a time period with relatively constant conditions.

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

the package ‘unmarked’ (function ‘ranef’: FISKE & CHANDLER 2011).

To compare the different methods, the estimates derived from CMR data were correlated with the results from the first time-count method and the estimates from using removal sampling data, and the latter estimates were furthermore correlated with the results of both time-count methods using IBM SPSS Statistics (Version 24.0). After testing for normal distribution (using SHAPIRO-WILKS tests) and variance homogeneity (using LEVENE tests), either a PEARSON or a SPEARMAN correlation coefficient was calculated. P-values were BONFERRONI-corrected for alpha adjustment (RUDOLF & KUHLISCH 2008).

## Results

### CMR study

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

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

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

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

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

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

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

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

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

### Removal sampling

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

### Comparison of the different methods

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

**Discussion**

The main goal of the present study was to compare the results of two different modelling approaches used for population size estimation. Their estimates differed by a factor of about 20. The large super-population estimates are in accordance to THIESMEIER & SOMMERHÄUSER (1995), which in an extreme case found up to 383 larvae/m<sup>2</sup>, with average densities of 18.1 and 19.0 larvae/m<sup>2</sup> in flowing parts and pools, respectively. The best fitting hierarchical model using removal sampling data suggests decreasing detection probability with increasing creek width (Fig. 3). Detection probability decreased from about 40% in creeks with < 1 m width to < 10% in creeks with 3 m width (Fig. 3). Hence,

also this ‘closed population approach’ suggests that many larvae are not found during capture occasions and thus indicates large larvae populations. This is in accordance with many studies on imperfect detection probabilities in wild animal populations (e.g., KÉRY 2002, SCHMIDT et al. 2002, KÉRY & SCHMIDT 2008, KÉRY et al. 2009, WAGNER et al. 2011), and the super-population estimates of partly > 2,000 larvae per study section seem plausible. THIESMEIER & SCHUHMACHER (1990) found that 83% of drifted larvae were newly deposited or in early developmental stages. Because our study took place in June and July, a large part of the drifted larvae remained unconsidered. Consequently, between March and May the larval populations could have been even larger.

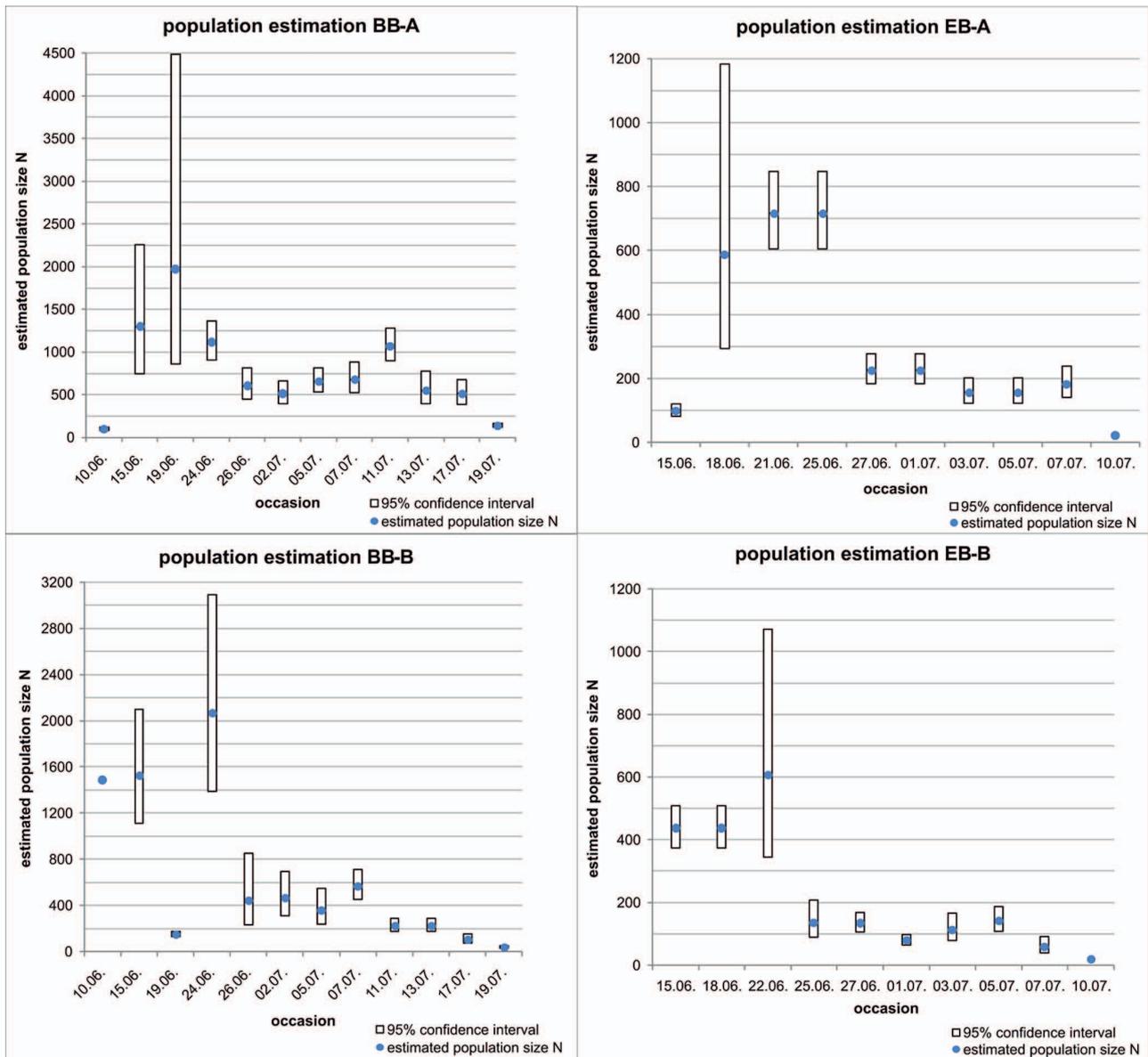


Figure 2. Population size estimates for each capture occasions and for each transect A and B in the BB and EB using CMR data of marked fire salamander larvae.

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

Regardless imperfect detection probabilities (they differ by a factor of about 40 to the POPAN-estimates), our strong correlation between CMR estimates and simple time-count methods suggest that the minimal time-consuming time-count method, as already applied by e.g.,

SOUND & VEITH (1994), can provide first insights into population size variation over time. They may even suffice the necessities of a long-term population monitoring programs that are conducted to detect drastic break-downs of larval populations, e.g., due to the currently range-expanding *Bsal* fungus (SPITZEN-VAN DER SLUIJS et al. 2016, WAGNER et al. 2017, 2019, LÖTTERS et al. 2020 in this issue). Nevertheless, we suggest to continue to apply the removal sampling with hierarchical modelling in cases where monitoring of fire salamander larvae has already started based on this approach. However, one should keep in mind that the modelled abundances using removal data apparently drastically underestimate true larval population sizes (as estimated based on the open population approach based on CMR data). This effect was shown by us to be even

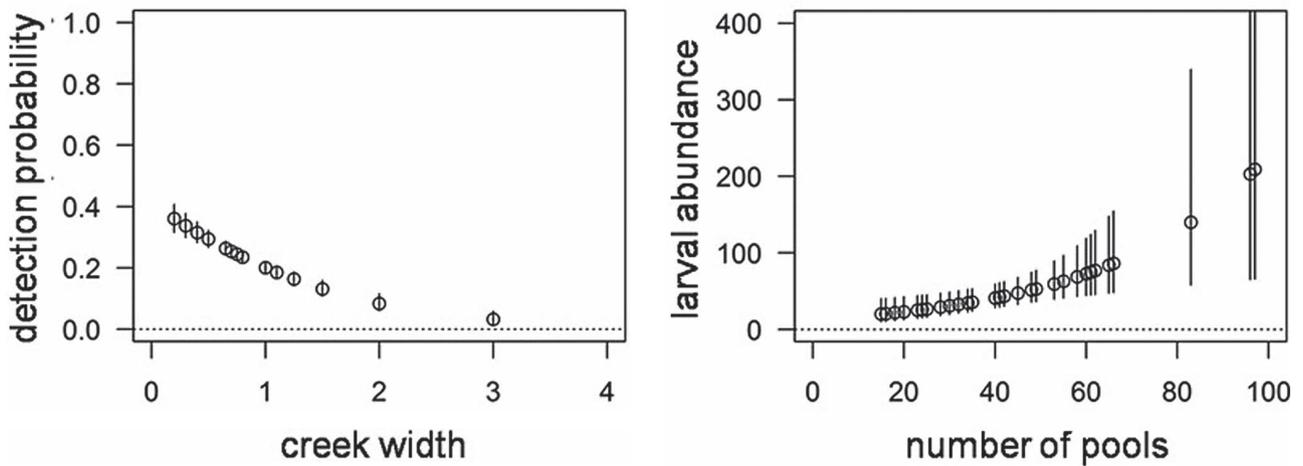


Figure 3. Decreasing detection probability with increasing creek width (m) and increasing larval abundance with increasing number of pools according to the best fitting hierarchical model using removal sampling data.

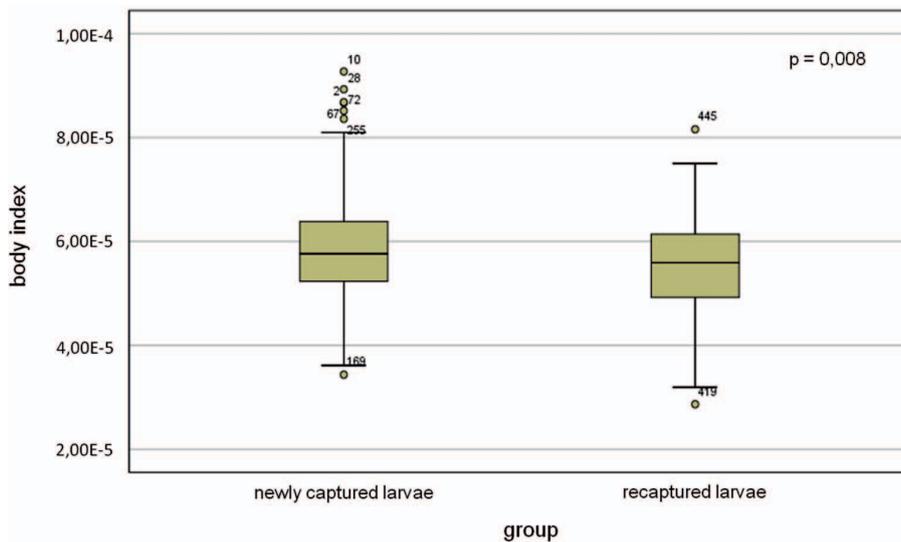


Figure 4. Comparison of means of the body index of newly captured and recaptured larvae suggest a significant impact of the Alpha-tags.

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

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#### References

- BAILEY, L. L., T. R. SIMONS & K. H. POLLOCK (2004): Comparing population size estimators for plethodontid salamanders. – *Journal of Herpetology*, **38**: 370–380.
- BORCHERS, D. L., S. T. BUCKLAND & W. ZUCCHINI (2002): Estimating animal abundance: closed populations. – Springer, New York.
- BOSCH, J. & I. MARTÍNEZ-SOLANO (2006): Chytrid fungus infection related to unusual mortalities of *Salamandra salamandra* and *Bufo bufo* in the Peñalara Natural Park, Spain. – *Oryx*, **40**: 84–89.
- BOSCH, J., S. FERNÁNDEZ-BEASKOETXEA, T. W. J. GARNER & L. M. CARRASCAL (2018): Long-term monitoring of an amphibian community after a climate change-and infectious disease-driven species extirpation. – *Global Change Biology*, **24**: 2622–2632.
- BURNHAM, K. P. & D. R. ANDERSON (2002): Model Selection and Multi-model Inference: A Practical Information-Theoretic Approach. 2<sup>nd</sup> Edition. – Springer, New York.
- COOCH, E. & G. WHITE (2017): Program Mark – A Gentle Introduction, 17<sup>th</sup> Edition. – <http://www.phidot.org/software/mark/docs/book/>.
- DALBECK, L., H. DÜSSEL-SIEBERT, A. KERRES, K. KIRST, A. KOCH, S. LÖTTERS, D. OHLHOFF, J. SABINO-PINTO, K. PREISSLER, U. SCHULTE, V. SCHULZ, S. STEINFARTZ, M. VEITH, M. VENCES, N. WAGNER & J. WEGGE (2018): Die Salamanderpest und ihr Erreger *Batrachochytrium salamandrivorans* (Bsal): aktueller Stand in Deutschland. – *Zeitschrift für Feldherpetologie*, **25**: 1–22.
- DORAZIO, R. M., H. L. JELKS & F. JORDAN (2005): Improving removal-based estimates of abundance by sampling a population of spatially distinct subpopulations. – *Biometrics*, **61**: 1093–1101.
- EITAM, A. & K. BLAUSTEIN (2002): Non-invasive individual identification of larval *Salamandra* using tailfin spot patterns. – *Amphibia-Reptilia*, **23**: 215–219.
- FELDMANN, R. (1971): Felduntersuchungen an westfälischen Populationen des Feuersalamanders, *Salamandra salamandra terrestris* Lacépède, 1788. – *Dortmunder Beiträge zur Landeskunde, Naturwissenschaftliche Mitteilungen*, **5**: 37–44.
- FISKE, I. J. & R. B. CHANDLER (2011): unmarked: An R package for fitting hierarchical models of wildlife occurrence and abundance. – *Journal of Statistical Software*, **43**: 1–23.
- GOEDMAKERS, A. (1980): Population dynamics of three gamma-rid species (Crustacea, Amphipoda) in a French chalk stream. – *Bijdragen tot de Dierkunde*, **50**: 1–34.
- HEMMER, H. & K. KADEL (1972): Gewichtszustand und Wachstumsverlauf bei der Kreuzkröte. – *forma et functio*, **5**: 113–120.
- KÉRY, M. (2002): Interfering the absence of a species – a case study of snakes. – *Journal of Wildlife Management*, **66**: 330–338.
- KÉRY, M. & B. R. SCHMIDT (2008): Imperfect detection and its consequences for monitoring for conservation. – *Community Ecology*, **9**: 207–216.
- KÉRY, M., R. M. DORAZIO, L. SOLDAAT, A. VAN STRIEN, A. ZUIDERWIJK & J. A. ROYLE (2009): Trend estimation in populations with imperfect detection. – *Journal of Applied Ecology*, **46**: 1163–1172.
- KOPP-HAMBERGER, M. A. J. A. (1998): Eine Methode zur individuellen Erkennung von Feuersalamandern (*Salamandra salamandra terrestris*) anhand des Zeichnungsmusters. – *Salamandra*, **34**: 239–244.
- LÖTTERS, S., N. WAGNER, G. ALBALADEJO, P. BÖNING, L. DALBECK, H. DÜSSEL, S. FELDMEIER, M. GUSCHAL, K. KIRST, D. OHLHOFF, K. PREISSLER, T. REINHARDT, M. SCHLÜPMANN, U. SCHULTE, V. SCHULZ, S. STEINFARTZ, S. TWIETMEYER, M.

- VEITH, M. VENCES & J. WEGGE (2020): The amphibian pathogen *Batrachochytrium salamandrivorans* in the hotspot of its European invasive range: past – present – future. – *Salamandra*, **56**: 173–188 [this issue].
- LUNGI, E. & M. VEITH (2017): Are Visual Implant Alpha tags adequate for individually marking European cave salamanders (genus *Hydromantes*)? – *Amphibia-Reptilia*, **53**: 541–544.
- MARTEL, A., A. SPITZEN-VAN DER SLUIJS, M. BLOOI, W. BERT, R. DUCATELLE, M. C. FISHER, A. WOELTJES, W. BOSMAN, K. CHIERS, F. BOSSUYT & F. PASMANS (2013): *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. – *Proceedings of the National Academy of Sciences of the United States of America*, **110**: 15325–15329.
- MARTEL, A., M. BLOOI, C. ADRIAENSEN, P. VAN ROOIJ, W. BEUKEMA, M. C. FISHER, R. A. FARRER, B. R. SCHMIDT, U. TOBLER, K. GOKA, K. R. LIPS, C. MULETZ, K. ZAMUDIO, J. BOSCH, S. LÖTTERS, E. WOMBWELL, T. W. J. GARNER, A. A. CUNNINGHAM, A. SPITZEN-VAN DER SLUIJS, S. SALVIDIO, R. DUCATELLE, K. NISHIKAWA, T. T. NGUYEN, J. E. KOLBY, I. VAN BOXCLAER, F. BOSSUYT & F. PASMANS (2014): Recent introduction of a chytrid fungus endangers Western Palaearctic salamanders. – *Science*, **346**: 630–631.
- MAY, R.M. (2004): Ethics and amphibians. – *Nature*, **431**: 403.
- MAZEROLLE, M. J. (2015): AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.0-2. – <http://CRAN.R-project.org/package=AICcmodavg>.
- R Development Core Team (2012): R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, <http://www.R-project.org>.
- REINHARDT, T., L. BALDAUF, M. ILIC & P. FINK (2018): Cast away: drift as the main determinant for larval survival in western fire salamanders (*Salamandra salamandra*) in headwater streams. – *Journal of Zoology*, **306**: 171–179.
- ROYLE, J. A. (2004): Generalized estimators of avian abundance from count survey data. – *Animal Biodiversity and Conservation*, **27**: 375–386.
- ROYLE, J. A. & R. M. DORAZIO (2006): Hierarchical models of animal abundance and occurrence. – *Journal of Agricultural, Biological and Environmental Statistics*, **11**: 249–263.
- RUDOLF, M. & W. KUHLISCH (2008): Biostatistik: Eine Einführung für Biowissenschaftler. – Pearson, München.
- RUFF, H. & G. MAIER (2000): Calcium carbonate deposits reduce predation pressure on *Gammarus fossarum* from salamander larvae. – *Freshwater Biology*, **43**: 99–105.
- SCHMIDT, B. R., M. SCHAUB & B. ANHOLT (2002): Why you should use capture-recapture methods when estimating survival and breeding probabilities: On bias, temporary emigration, overdispersion, and common toads. – *Amphibia-Reptilia*, **23**: 375–388.
- SCHMIDT, B. R., G. GSCHWEND, J. A. BACHMANN & P. DERMOND (2015): Use of removal sampling to estimate abundance of larval fire salamanders (*Salamandra salamandra*) in streams. – *Amphibia-Reptilia*, **36**: 87–92.
- SCHWARZ, C. J. & A. N. ARNASON (2017): Jolly Seber models in MARK. – pp. 402–454 in: COOCH, E. & G. WHITE (eds): Program Mark – A Gentle Introduction, 17<sup>th</sup> Edition. – <http://www.phidot.org/software/mark/docs/book/>.
- SOUND, P. & M. VEITH (1994): Zum Vorkommen der Larven des Feuersalamanders in Abhängigkeit von der Forellendichte sowie einiger abiotischer Faktoren. – *Zeitschrift für Feldherpetologie*, **1**: 89–101.
- SPITZEN-VAN DER SLUIJS, A., F. SPIKMANS, W. BOSMAN, M. DE ZEEUW, T. VAN DER MEIJ, E. GOVERSE, M. KIK, F. PASMANS & A. MARTEL (2013): Rapid enigmatic decline drives the fire salamander (*Salamandra salamandra*) to the edge of extinction in the Netherlands. – *Amphibia-Reptilia*, **34**: 233–239.
- SPITZEN-VAN DER SLUIJS, A., A. MARTEL, J. ASSELBERGHS, E. BALES, W. BEUKEMA, M. BLETZ, L. DALBECK, E. GOVERSE, A. KERRES, T. KINET, K. KIRST, A. LAUDELOUT, L. MARIN DA FONTE, A. NÖLLERT, D. OHLHOFF, J. SABINO-PINTO, B. R. SCHMIDT, J. SPEYBROECK, F. SPIKMANS, S. STEINFARTZ, M. VEITH, M. VENCES, N. WAGNER, F. PASMANS & S. LÖTTERS (2016): Expanding distribution of lethal amphibian fungus *Batrachochytrium salamandrivorans* in Europe. – *Emerging Infectious Diseases*, **22**: 1286–1288.
- STEGEN, G., F. PASMANS, B. R. SCHMIDT, L. O. ROUFFAER, S. VAN PRAET, M. SCHAUB, S. CANESSA, A. LAUDELOUT, T. KINET, C. ADRIAENSEN, F. HAESBROUCK, W. BERT, F. BOSSUYT & A. MARTEL (2017): Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. – *Nature*, **544**: 353–356.
- THIESMEIER B. & H. SCHUHMACHER (1990): Causes of larval drift of the fire salamander, *Salamandra salamandra terrestris*, and its effects on population dynamics. – *Oecologia*, **82**: 259–263.
- THIESMEIER, B. & M. SOMMERHÄUSER (1995): Larvalökologische Merkmale einer Feuersalamanderpopulation (*Salamandra salamandra terrestris*) eines temporären Fließgewässers im nordwestdeutschen Flachland. – *Zeitschrift für Feldherpetologie*, **2**: 23–36.
- THIESMEIER, B. (2004): Der Feuersalamander. – Laurenti Verlag, Bielefeld.
- VAN ROOIJ, P., F. PASMANS, Y. COEN & A. MARTEL (2017): Efficacy of chemical disinfectants for the containment of the salamander chytrid fungus *Batrachochytrium salamandrivorans*. – *PLoS ONE*, **12**: e0186269.
- VEITH, M. (1992): The fire salamander, *Salamandra salamandra* L., in Central Europe: Subspecies distribution and intergradation. – *Amphibia-Reptilia*, **13**: 297–313.
- VEITH, M., M. BAUBKUS, S. KUGEL, K. KULPA, T. REIFENRATH, M. SCHAFFT & N. WAGNER (2019): Drift compensation in larval fire salamanders (*Salamandra salamandra*)? – *Hydrobiologia*, **828**: 315–325.
- WAGNER, N., J. PELLET, S. LÖTTERS, B. R. SCHMIDT & T. SCHMITT (2011): The superpopulation approach for estimating the population size of ‘prolonged’ breeding amphibians: Examples from Europe. – *Amphibia-Reptilia*, **32**: 323–332.
- WAGNER, N., S. LÖTTERS, S. D. BAUER, J. BENINDE, J. EWEN, G. FICHERA, L. FEILER, S. FELDMEIER, B. FONTAINE, D. GÖB, L. GUSSONE, W. HARMS, M. HARZHEIM, L. HASENJÜRGEN, C. KOLWELTER, S. KRONE, A. LAMBING, L. F. MARIN DA FONTE, A. MARTENS, J. PETERS, L. PFEIFER, J. PFROMMER, N. REINHARDT, M. SANDVOSS, M. SCHAFFT, U. SCHULTE, F. TULL, K. UNTERBERG & M. VEITH (2017): Zur Verbreitung des Gebärderten Feuersalamanders (*Salamandra salamandra terrestris* Lacépède, 1788) im Naturpark Südeifel sowie im rheinland-pfälzischen Teil des Naturparks Nordeifel (Amphibia Urodela Salamandridae). – *Dendrocopos*, **44**: 7–20.
- WAGNER, N., V. SCHULZ, S. STEINFARTZ, T. REINHARDT, M. VENCES, S. LÖTTERS, L. DALBECK, H. DÜSSEL-SIEBERT, M. GU-

- SCHAL, K. KIRST, D. OHLHOFF, J. WEGGE & M. VEITH (2019): Aktuelle Erkenntnisse zum Status der Salamanderpest in Deutschland. – *Natur und Landschaft*, **94**: 463–471.
- WAGNER, N., S. LÖTTTERS, L. DALBECK, H. DÜSSEL, M. GUSCHAL, K. KIRST, D. OHLHOFF, J. WEGGE, T. REINHARDT & M. VEITH (2020): Long-term monitoring of European fire salamander populations (*Salamandra salamandra*) in the Eifel Mountains (Germany): five years of removal sampling of larvae. – *Salamandra*, **56**: 243–253 [this issue].
- WERNER, P., S. LÖTTTERS & B. R. SCHMIDT (2014): Analysis of habitat determinants in contact zones of parapatric European salamanders. – *Journal of Zoology*, **292**: 31–38.
- WHITE, G. C. & K. P. BURNHAM (1999): Program MARK: survival estimation from populations of marked animals. – *Bird Study*, **46**: 120–139.

### Supplementary data

The following data are available online:

- Supplementary Table S1. Model selection table and estimates for survival, capture and entry to the super-population) probabilities.
- Supplementary Figure S1. Population size estimates for a shortened capture period and for each transect.
- Supplementary Figure S2. Correlations between results gained from the two model approaches and the two count methods.