Master Thesis

Influence of water quality on the larval abundance of the stream-breeding fire salamander

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March 2020

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Abstract

Pesticide pollution in streams can be substantial. Pesticides are thought to be one of the drivers of the global decline of amphibians. As there is not much known about the pesticide exposure amphibians face in the wild, I conducted a field study to investigate how water quality in terms of pesticide and nutrient levels relates to the local abundance of fire salamander larvae (*Salamandra salamandra*). The stream breading fire salamander has been strongly declining in northern Switzerland since the last decades, but the reasons are unknown. Pesticide pollution may be a reason because pesticide concentrations in Swiss streams can be high and numerous laboratory studies showed negative effects of pesticides on amphibian species. I estimated larval abundances in 48 streams in northern Switzerland using N-mixture models and related estimated abundances to two indices of water quality: the IBCH and SPEAR index. These indices are based on the macroinvertebrate communities in the streams and inform about organic and pesticide pollution. I also measured additional habitat characteristics to account for other sources of habitat suitability. High concentrations of oxygen and higher nutrient levels (low IBCH values) increased the larval abundance. Also, larval abundance and occupancy were associated with low levels of pesticides (high SPEAR index values). Furthermore, larval abundance was positively associated with the percentage of forest cover around the stream, the amount of pools, and the absence of fish predators. These results indicate that fire salamander larvae are sensitive to pesticide pollution but not to organic pollution, at least up to a certain level. Therefore, conservation efforts should focus on preserving fishless forest streams from pesticide pollution. In this study, I showed that negative effects of pesticides can be measured in wild amphibian populations.

Zusammenfassung

Bäche können erhebliche Verschmutzungen durch Pestizide aufweisen. Es wird vermutet, dass Verschmutzung durch Pestizide eine entscheidende Rolle im globalen Amphibiensterben spielt. Da jedoch nicht viel bekannt ist über die tatsächliche Pestizidexposition, der Amphibien in freier Wildbahn ausgesetzt sind, habe ich eine Feldstudie durchgeführt, um zu untersuchen, welche Auswirkung Wasserqualität bezüglich Verschmutzung durch Pestizide und Nährstoffgehalt auf die lokale Abundanz von Feuersalamanderlarven (Salamandra salamandra) hat. Der Feuersalamander war ursprünglich weit verbreitet, zeigte aber während der letzten Jahrzehnte rückläufige Populationszahlen im Norden der Schweiz. Gründe für diesen Rückgang sind nur begrenzt bekannt. Pestizide könnten jedoch ein Grund sein, da viele Laborstudien negative Einflüsse von Pestiziden auf Amphibien zeigen konnten. Mit N-Mixture Modellen habe ich die Abundanz von Feuersalamanderlarven geschätzt und den Zusammenhang mit zwei Indizes für Wasserqualität geprüft. Dafür habe ich die zwei Makroinvertebratenindizes SPEAR Index und IBCH benutzt, welche aufgrund von Makroinvertebratengemeinschaften der Bäche Rückschlüsse auf Verschmutzung durch Pestizide oder organische Verschmutzung zulassen. Zusätzlich habe ich weitere Habitatsmerkmale gemessen, um weitere Quellen der Eignung eines Habitats zu erfassen. Hohe Sauerstoffkonzentration, sowie hohe Nähstoffwerte des Wassers (tiefer IBCH) erhöhten die Abundanz der Larven. Des Weiteren waren eine hohe Abundanz, sowie die Bewohnung eines Flusses, positiv assoziiert mit tiefen Pestizidkonzentrationen (hoher SPEAR Index). Die Abundanz der Larven war auch positiv assoziiert mit dem Anteil waldbedeckter Fläche rund um einen Bach, der Menge an Kolke und dem Fehlen von Fischen. Diese Resultate zeigen, dass Feuersalamanderlarven sensitiv auf Verschmutzung durch Pestizide sind, bis zu einem gewissen Level jedoch nicht auf organische Verschmutzung. Programme zum Schutz des Feuersalamanders sollten daher versuchen, fischlose Waldbäche vor Pestizidbelastungen zu bewahren. In dieser Studie konnte ich zeigen, dass negative Effekte von Pestiziden in Amphibienpopulation auch in freier Wildbahn messbar sind.

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Introduction

Habitat destruction is one of the main threats to species diversity (Pimm and Raven 2000, Sala et al. 2000); it is a main threat to 85% of all threatened and endangered species listed on the IUCN Red List (IUCN, 2019). Habitat destruction is the process by which a habitat becomes unsuitable of supporting its native species community. Habitat destruction includes various processes such as degradation, fragmentation, pollution, and combinations thereof (Laurance 2010). Pollution is of particular concern for aquatic habitats because aquatic habitats are not only affected by pollutants deposited into the water, but also by pollutants deposited on surrounding terrestrial habitats (Preston et al. 2011). Streams and rivers receive direct organic input, interact with groundwater over large areas, and collect surface water runoff in the catchment area (Biddulph 2015, Peralta-Maraver et al. 2018). Therefore, pesticides and fertilizers released by agriculture can pollute nearby streams and rivers. Indeed, a recent study found increased pesticide concentrations after rainfalls in streams surrounded by agricultural land (Hutter et al. 2019).

Pesticide pollution can lead to reduced biodiversity in stream invertebrates (Beketov et al. 2013, Burdon et al. 2019) and at higher trophic levels (Geiger et al. 2010); it can directly or indirectly harm organisms inhabiting streams, such as algae, fish, and amphibians (Isenring 2010, Ockleford et al. 2018). Recent studies in Switzerland showed that pesticide pollution in streams can be substantial. In Switzerland, agriculture uses more than 2 000 tons of pesticides per year (Bundesamt für Landwirtschaft 2019); part of which ends up in aquatic habitats. Recent studies focussing on the pollution of small streams in intensively used agricultural areas in Switzerland found that all streams tested were polluted by several pesticides and fertilizers over long periods. Twenty-five per cent of all kilometres of running water in Switzerland flow through intensively used agricultural land and are therefore at high risk of pesticide pollution (Junghans et al. 2019, Spycher et al. 2019).

Amphibian populations have been declining for decades (Houlahan et al. 2000, Stuart et al. 2004). Pesticides are thought to be one of the drivers of the global decline of amphibians (Blaustein et al. 2003). Even generalist and once widely distributed amphibian species have experienced pronounced population declines. An example of such a species is the stream-breeding fire salamander (*Salamandra salamandra*); that has recently experienced rapid population declines. The larvae of this ovoviviparous species develop in small headwater streams until metamorphosis (Baumgartner et al. 1999). Even though fire salamanders are widely distributed in central, southern, and southeastern Europe (Kuzmin et al. 2009), they are strongly declining locally (Schmidt et al. 2005, Kuzmin et al. 2009, Spitzen-van der Sluijs et al. 2013). Therefore, the fire salamander was classified as vulnerable (VU) in the most recent update of the Swiss Red List of endangered species from 2005 (Schmidt and Zumbach 2005). Since then, the decline has been ongoing (Bänziger 2017).

Previous studies on the decline of fire salamanders in northern Switzerland focused on stream morphology, presence of fish predators, and the surrounding terrestrial habitat. These studies suggest that population size is negatively affected by road density and the release of fry in formerly fishless streams (Schmidt and Zumbach 2005, Dosch 2017). While the role of such biotic and abiotic drivers of the decline is relatively well understood, the role of water quality - pesticides and fertilizers in particular - remains poorly understood. Previous laboratory research showed that certain pesticides can have negative effects on salamander species on the individual level. For example, pesticides can alter gonaduct growth (Clark et al. 1998), disturb the skin microbiome (Bletz et al. 2018), reduce larval survival (Metts et al. 2005), or extend the larval period and decrease body size at metamorphosis (Rohr et al. 2004). Together, these studies suggest that pesticides might negatively influence fire salamanders in Switzerland as well. The risk a pesticide poses to a species is a combination of the toxicity and the exposure. However, very few data are available on the pesticide exposure amphibians encounter in Switzerland (Aldrich et al. 2016). Additionally, the effects of pesticides on individuals are not always translatable into effects on populations (Forbes and Calow 2002). As a result, it is unsure whether the negative effects of pesticides observed in laboratory studies are significant enough to be seen in wild amphibian populations (Aldrich et al. 2016).

Here, I investigate in a field study how water quality in terms of pesticide and nutrient levels relates to the local abundance of fire salamander larvae. To answer these questions, I estimated the abundance of fire salamander larvae in 48 streams with different water qualities in northern Switzerland and measured additional variables of the aquatic and the surrounding terrestrial habitat, to control for other factors of habitat suitability.

Knowledge about what environmental variables influence species distribution is crucial to protect suitable habitats. As the ongoing decline of fire salamander in northern Switzerland is still not fully understood, this study will contribute towards successful conservation strategies and help extend our knowledge about pesticide exposure on amphibians in the wild.

Methods

Study species

Fire salamanders (*Salamandra salamandra*) are terrestrial, ovoviviparous amphibians with an aquatic larval stage. During the adult stage, they live in deciduous or mixed forests (Werner et al. 2016). Mating occurs on land during late summer. In spring, females migrate to streams to give birth to 20 to 60 larvae. Fire salamander larvae are clearly distinguishable by characteristic bright spots between all limbs and their body (Info fauna CSCF 2020). Larvae undergo metamorphosis after a larval period which lasts approximately two to four months (Kopp and Baur 2000). Larvae are mostly found in small first- and second-order streams with low flow speed. High flow speed, turbulences and floods can cause hydraulic stress and downstream drift of fire salamander larvae (Baumgartner et al. 1999). Presence of predators, such as fish (Thiesmeier 1994) and potentially crayfish (Ficetola et al. 2011) reduces larval densities.

Macroinvertebrate indices

A common way to assess the water quality of a stream is by investigating its macroinvertebrate community (Stucki 2010). Since certain species are more sensitive to pesticide or organic pollution, macroinvertebrate indices can be calculated from the species community composition of a stream (Liess and Ohe 2005). These indices provide information about the state of a stream over a long period and can capture synergistic and antagonistic effects of all substances present in the water (Gunkel 1996). In contrast, a chemical analysis of a water sample would only provide information about substances it is tested for and which were present in the very moment the sample was collected. The macroinvertebrate index "index biologique Suisse" (IBCH) is a measure for the biological condition and nutrient content of a stream based on the diversity of its macroinvertebrate community and the sensitivity of certain taxa to abiotic impacts (Stucki 2010). The values of the IBCH range from zero to 20 and are organised in five quality classes. Low values (0-4 "bad" and 5-8 "dissatisfactory") represent streams with high organic pollution. Values between 9-12 are classified as "mediocre". High values (13-16 "good" and 17-20 "very good") represent streams with low organic pollution. The Species at Risk (SPEAR) index reflects pesticide pollution based on the community of macroinvertebrates with a focus on pesticide sensitive species (Hutter et al. 2019). The SPEAR index is calculated as the relative abundance of sensitive species (Beketov et al. 2009). Low values represent high pesticide concentrations. High values represent low pesticide concentrations.

Study sites and design

I visited streams with varying water qualities in the Swiss cantons of Zurich and Aargau. I selected streams for which two macroinvertebrate indices were available: the IBCH and the SPEAR index. I further refined the selection of streams to accessible streams with a slow to medium flow velocity. This led to a sample of 48 streams (Figure 1). I sampled every stream once and collected counts of fire salamander larvae. Additionally, I recorded the coordinates and a set of environmental variables.

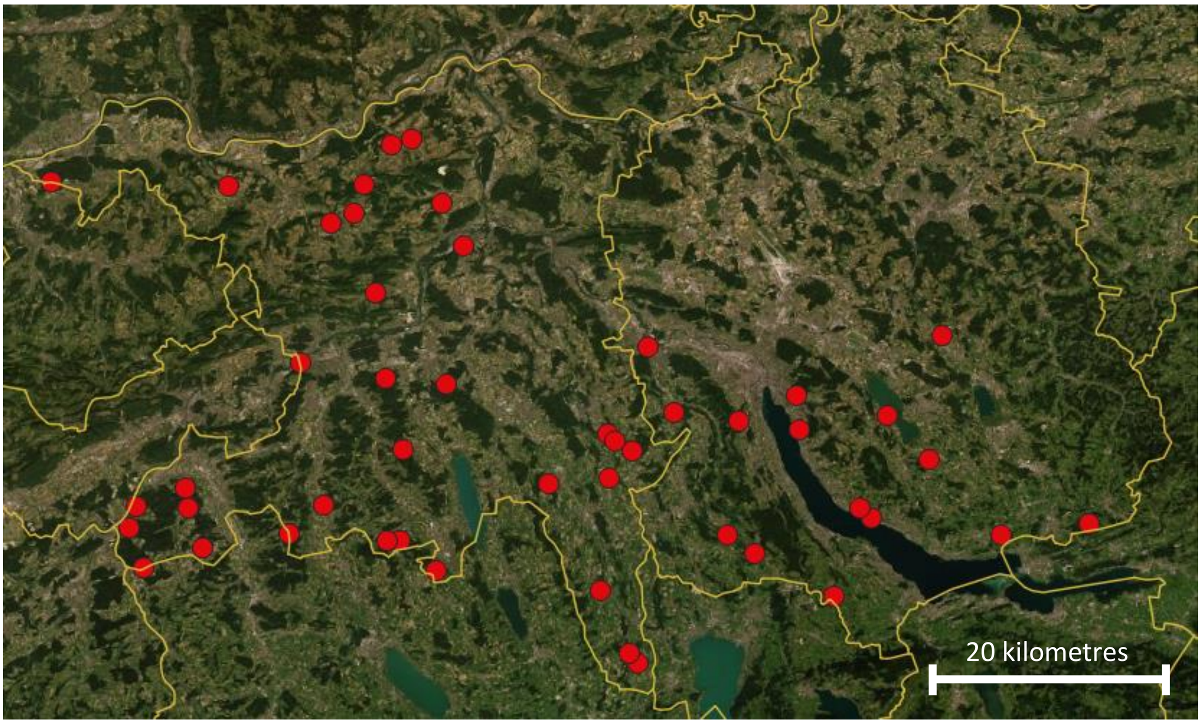

Figure 1: Map of the 48 sampled streams in the cantons of Aargau and Zurich, Switzerland.

Data collection

I conducted my fieldwork between April and June 2019. To avoid low detection probability caused by turbidity, I excluded rainy days and days after heavy rainfall. For each stream, I selected a 25-meter section located as closely as possible to the coordinate where the macroinvertebrate indices were determined. I collected three consecutive temporal replicated counts of fire salamander larvae with a five minutes break in between to allow any possible turbidity to clarify again. Each replicate was limited to 15 minutes of effort during which I actively searched larvae under stones, leaf litter, and branches. I used click counters and I always started at the downstream end and worked towards the upstream end of the section to maintain water clarity.

I measured the following environmental variables, that previously have been shown to influence detection probability or abundance of fire salamander larva. These variables partially served as a control to account for variation in habitat suitability.

- **Mean width:** Because fire salamander larvae are more common in small streams (Baumgartner et al. 1999), I measured the stream width every 5 meters of the section and calculated the mean.

- **Total length of pools:** Larvae prefer to stay in calm water and use pools as a refuge. Pools are defined as quiet, naturally dammed sections where flow velocity and turbulence are reduced (Baumgartner et al. 1999). I measured the total length of pools in the section using a yardstick.
- **Oxygen concentration:** Oxygen depletion can cause mortality in salamander larvae (Reinhardt et al. 2013). I measured the oxygen concentration [%] of the water once per stream in the middle of the section with an HQ 30D Flexi Multi-Meter.
- **Mean number of fish:** The presence of predatory fish is associated with decreasing densities (Thiesmeier 1994) and increased extinction probabilities of fire salamander larvae (Bänziger 2017). While searching through the section for fire salamander larvae, I counted every fish I encountered using a click counter. This resulted in three counts of which I calculated the mean.

Additionally, from external sources, I collected the following explanatory variables.

- **Macroinvertebrate indices (IBCH and SPEAR index):** Macroinvertebrate index values for the sampled streams were provided by the Departement Bau, Verkehr und Umwelt of the canton of Aargau and from the Amt für Abfall, Wasser, Energie und Luft of the canton of Zurich.
- **Rainfall:** Rainfall causes water levels to rise, accelerates flow speed and increases turbidity. These factors might decrease detection probability. Floods might also decrease abundance by downstream drift (Baumgartner et al. 1999). I recorded the amount of rain [mm] during three days before sampling using data from MeteoSwiss from the nearest weather station to the stream section.
- **Forest cover**: Forests are the most important habitat of adult fire salamanders (Werner et al. 2016). Therefore, I calculated the percentage of forest cover in a 200-meter buffer around the centre of the stream section using the World Imagery satellite map (Esri 2019) in QGIS (QGIS Development Team 2019).

Data analysis

To estimate abundances of fire salamander larvae while taking imperfect detection probability into account, I used N-mixture models. This hierarchical model enables the estimation of detection probability and abundance from repeated count data without individual identification (Royle 2004). I used the function *pcount* in the package *unmarked* (Fiske and Chandler 2011) in R (R Core Team 2019) to fit the N-mixture model to the data. The model consists of two levels, an observational level for detection probability and a biological level for abundance. The counts *n* in a location *i* and replicate *t* can be viewed as binomial random variables $n_{it} \sim \text{Binomial}(N_i, p)$ where N_i is the abundance in location *i* and *p* is the detection probability. The abundance is often described by a Poisson distribution. In cases of overdispersion zero-inflated Poisson or negative binomial distribution can yield a better model fit.

Estimates can heavily depend on the choice of abundance distribution (Kéry et al. 2005, Joseph et al. 2009). Therefore, I performed several goodness of fit tests with the global model (Table 1, Nr. 1) to test which distribution fitted the data the best. I calculated the overdispersion parameter c-hat with bootstrapping using the function *Nmix.gof.test* in the package *AICcmodavg* (Mazerolle 2019). Additionally, I calculated marginal and site-sum c-hat values, checked QQ-plots from randomized quantile residuals, and checked for stable estimates with increased limits for the upper bound of integration K (Knape et al. 2017). I proceeded with a negative binomial model because this distribution had the best model fit in all tests. I created 14 different candidate models (Table 1). Starting from the null model, I first added the variables pools, width, fish, and forest, because these were previously shown to influence fire salamander abundance. I then increased the number of variables with the water quality variables IBCH, SPEAR and oxygen concentrtion. To enable comparisons between explanatory variables, I normalized all explanatory variables by subtracting the sampled mean from each measurement and then divided by the standard deviation. To identify the best fitting model, the candidate models were ranked according to decreasing Akaike criterion (AIC) values. Additionally, I calculated delta AIC (ΔAIC) and Akaike weights (Franklin et al. 2001) to compare the candidate models.

Table 1: Candidate models. Mixture = negative binomial, upper limit of integration K = 500.

Nr.	detection	abundance
1	p(~ width +pools +fish +rain3d)	λ(~width +pools +fish +forestperc +O2perc + SPEAR +IBCH)
2	p(~ 1)	λ(~width +pools +fish +forestperc +O2perc + SPEAR +IBCH)
3	p(~ width +pools +fish +rain3d)	λ(~1)
4	p(~ 1)	λ(~1)
5	p(~ 1)	λ(~ pools +fish)
6	p(~ 1)	λ(~width +pools +fish +forestperc)
7	p(~ width)	λ(~width +pools +fish +forestperc)
8	p(~ width +pools)	λ(~ pools +fish +forestperc)
9	p(~ width)	λ(~ pools +fish +forestperc +SPEAR +IBCH)
10	p(~ width +fish)	λ(~ pools +fish +forestperc +SPEAR +IBCH)
11	p(~ width)	λ(~ pools +fish +forestperc +O2perc)
12	p(~ width +pools)	λ(~width +pools +fish +forestperc +O2perc +SPEAR +IBCH)
13	p(~ width +pools +fish)	λ(~width +pools +fish +forestperc +O2perc +SPEAR +IBCH)
14	p(~ fish)	λ(~ pools +fish +forestperc +SPEAR)

Note: pools, total length of pools; width, width of the stream; rain3d, rainfall during the last three days; fish, average number of fish; O2perc, oxygen concentration; depth, depth of the stream.

To investigate whether a different set of variables significantly influenced the abundance and detection probability in occupied streams compared to all sampled streams I analysed a subset of the data. I only looked at streams in which I found at least one fire salamander larvae. With this subset of occupied streams, I followed the same analysis of goodness of fit tests. Likewise, the negative binomial distribution yielded the best model fit and consequently was used for further analysis. I checked the same set of candidate models (Table 1) and ranked them according to decreasing AIC values and calculated delta AIC (ΔAIC) and Akaike weights (Franklin et al. 2001).

The results from the N-mixture model of the subset indicated that the variables measured in this study were less suitable to describe abundance in only occupied streams. As they seemed to contain information about the empty streams as well, I fitted an occupancy model to the data of the full set of streams. I used the function *occu* in the package *unmarked* (Fiske and Chandler 2011). I used the top-ranking candidate model from the N-mixture model analysis of the full set of streams, with no variables for detection probability and the full set of variables for abundance.

Results

In total, I detected 147 fire salamander larvae, which occupied 22 of the 48 sampled streams. The local maximum count of larvae in a stream was 22 individuals. The global N-mixture model contained four variables explaining detection and seven variables explaining larval abundance (Table 1, candidate model Nr. 1).

N-mixture model of the full set of streams

To measure model fit for different distributions, I performed goodness of fit tests on the global model. The lowest AIC value was achieved with the negative binomial distribution. Also, the overdispersion parameter c-hat was the closest to one with the negative binomial distribution, regardless of the method used to compute the value (Table 2). Consensually, the QQ-plot for the three different distributions showed the best fit for the negative binomial distribution (Figure 2).

Table 2: Goodness of fit test. The best fit for each category is indicated in bold. AIC, Akaike criterion; c-hat bootstrapping, parametric bootstrapping with 100 samples; c-hat marginal, following Knape et al. 2017; c-hat value site-sum, following Knape et al. 2017.

Distribution	AIC	c-hat bootstrapping	c-hat marginal	c-hat site-sum
Poisson	392.16	2.80	2.65	4.06
ZIP	362.38	1.58	1.36	1.81
NB	**312.78**	**1.26**	**0.79**	**0.96**

Note: Poisson, Poisson distribution; ZIP, zero-inflated Poisson distribution; NB, negative binomial distribution.

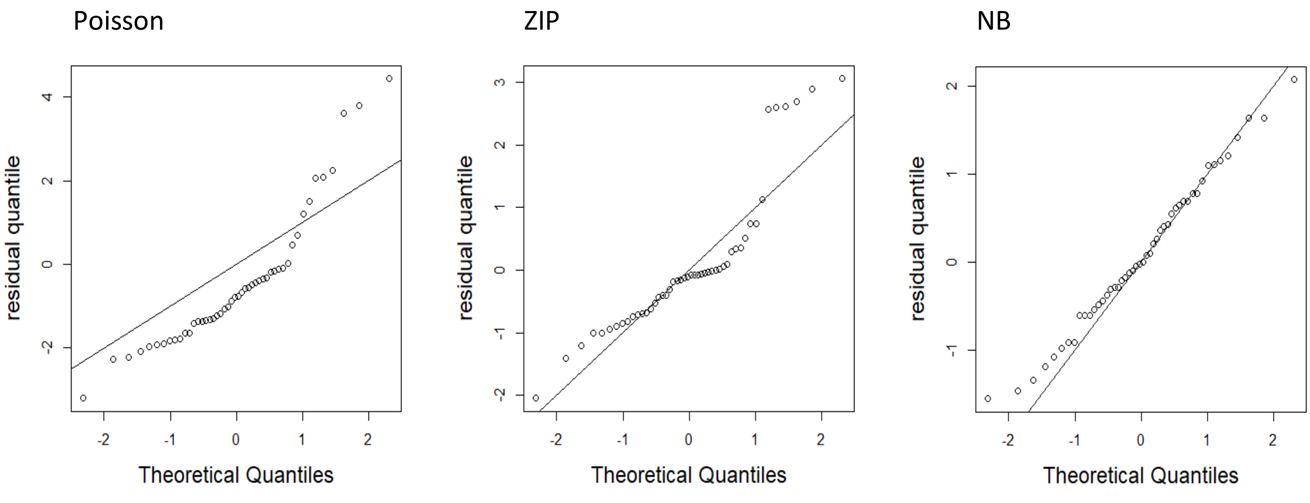

Figure 2: Residual QQ Plots for the three global models with different distributions for abundance. Poisson, Poisson distribution; ZIP, zero-inflated Poisson distribution; NB, negative binomial distribution.

The 14 candidate models were ranked according to AIC values. The top-ranking model contained no variables explaining detection probability and seven variables explaining abundance (Table 3, Akaike weight 0.47). In this model, detection probability was estimated to be 0.965 (Table 4, standard error 0.288).

The abundance of fire salamander larvae increased with SPEAR index, oxygen concentration, total pool length and percentage of forest cover (Table 4, Figure 3). However, larval abundance decreased with IBCH and the average number of fish (Table 4, Figure 3). Higher values in the SPEAR index and the IBCH both reflect higher habitat quality in terms of low pesticide concentrations and low nutrient content, respectively. The SPEAR index increased larval abundance significantly. Contrarily, the IBCH decreased larval abundance. Also, larval abundance decreased with the average number of fish per stream. Width did not have a significant effect on larval abundance. Additionally, larval abundance was increased by the percentage of forest in a 200-meter buffer around the stream and by the total length of pools. These results are consistent with previous studies indicating higher larval abundances in calm and fishless streams (Baumgartner et al. 1999).

Inclusion of measurements of water quality strongly improved the model likelihood. Specifically, the model in rank nine contained all explanatory variables of the top-ranking model except the IBCH, SPEAR index and oxygen concentration. This model showed a ΔAIC of 8.54 and an AIC weight of 0.01 and therefore is 47 times less likely than the top-ranking model (Table 3). The six top-ranking models all include the two macroinvertebrate indices IBCH and SPEAR index. These six models have a cumulative AIC weight of 0.93 (Table 3). The three top-ranking models all contain the same set of variables for abundance and have a cumulative AIC weight of 0.73.

Table 3: Candidate models with different explanatory variables for detection probability p and abundance λ. The full model (Nr.) is presented in bold. K, number of parameters estimated; AIC, Akaike's Information Criterion; ΔAIC, difference between the AIC and the smallest AIC; ω, Akaike weight; cu. ω, cumulative Akaike weight.

Nr.	N-Mixture Model	*K*	rank	AIC	ΔAIC	*ω*	cu. *ω*
2	p(~ 1) λ(~ width +pools +fish +forestperc +O2perc +SPEAR +IBCH)	10	1	309.99	0.00	0.47	0.47
13	p(~ width +pools +fish) λ(~ width +pools +fish +forestperc +O2perc +SPEAR +IBCH)	13	2	312.47	2.48	0.14	0.61
1	**p(~ width +pools +fish +rain3d)** **λ(~ width +pools +fish +forestperc +O2perc +SPEAR +IBCH)**	14	3	312.78	2.79	0.12	0.73
10	p(~ width +fish) λ(~ pools +fish +forestperc +SPEAR +IBCH)	10	4	313.44	3.45	0.08	0.81
12	p(~ width +pools) λ(~ width +pools +fish +forestperc +O2perc +SPEAR +IBCH)	12	5	313.75	3.76	0.07	0.88
9	p(~ width) λ(~ pools +fish +forestperc +SPEAR +IBCH)	9	6	314.60	4.61	0.05	0.93
14	p(~ fish) λ(~ pools +fish +forestperc +SPEAR)	8	7	315.42	5.42	0.03	0.96
11	p(~ width) λ(~ pools +fish +forestperc +O2perc)	8	8	315.92	5.93	0.02	0.99
6	p(~ 1) λ(~ width +pools +fish +forestperc)	7	9	318.53	8.54	0.01	0.99
8	p(~ width +pools) λ(~ pools +fish +forestperc)	8	10	320.39	10.40	0.00	1.00
7	p(~ width) λ(~ width +pools +fish +forestperc)	8	11	320.53	10.54	0.00	1.00
5	p(~ 1) λ(~ pools +fish)	5	12	322.79	12.80	0.00	1.00
4	p(~ 1) λ(~ 1)	3	13	330.14	20.15	0.00	1.00
3	p(~ width +pools +fish +rain3d) λ(~1)	7	14	332.63	22.64	0.00	1.00

Note: pools, total length of pools; width, width of the stream; rain3d, rainfall during the last three days; fish, average number of fish; O2perc, oxygen concentration; depth, depth of the stream.

Table 4: Effect size estimates of the explanatory variables of the top-ranking model number two with detection probability p(~1) and abundance λ(~ width +pools +fish +forestperc +O2perc +SPEAR +IBCH). Mixture = negative binomial, upper limit of integration K = 500.

	Estimate	SE	Z	P(>|z|)
Abundance				
Intercept	-0.223	0.385	-0.578	0.563
width	-0.241	0.328	-0.736	0.462
pools	0.974	0.309	3.150	0.002
fish	-1.904	0.889	-2.143	0.032
forestperc	0.653	0.287	2.276	0.023
O2perc	0.866	0.335	2.583	0.010
IBCH	-0.728	0.260	-2.800	0.005
SPEAR	0.713	0.362	1.971	0.049
Detection				
	0.965	0.288	3.350	0.001
Dispersion				
	-0.334	0.351	-0.951	0.342

Note: SE, standard error; Z, z-score.

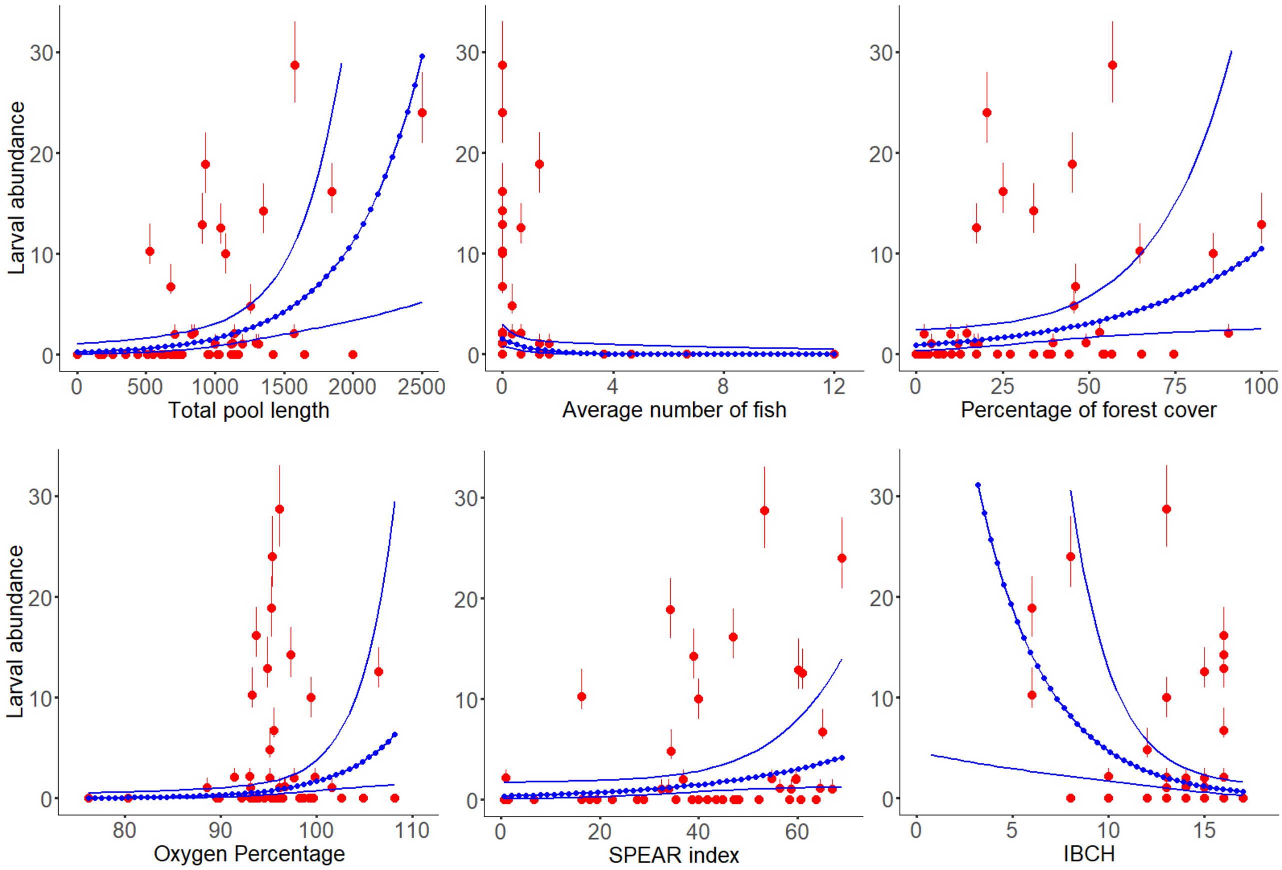

Figure 3: Top-ranking N-mixture model, estimated abundances per stream in red (ranef function) with 95% confidence intervals and the predicted effect (predict function) of each significant variable in blue (dots) with 95% confidence interval (lines).

N-mixture models of the subset of occupied streams

With the subset of only occupied streams, the distribution yielding the best model fit was also achieved with the negative binomial distribution (Appendix 1). The negative binomial distribution also showed the best fit in the QQ-plots (Appendix 2). Same as in the N-mixture model of the full set of streams, the top-ranking model did not contain variables for detection probability (Appendix 3, Akaike weight 0.25). This model gave an estimate for detection probability of 0.94 (Appendix 4, standard error 0.305). The top-ranking model contained four variables explaining abundance; width, total pool length, percentage of forest cover and average number of fish (Appendix 3). None of these variables significantly influenced larval abundance (Appendix 4 & 5). The top-ranking model had an AIC weight of 0.25 and was 2.5 times more likely to be the best model compared to the second-best ranking model. The next best models contained different variables for detection probability and abundance without consistency. These results indicate that the measured variables in this study are more suitable to explain abundance in occupied and unoccupied streams rather than in only occupied streams.

Occupancy model

The occupancy model contained no variables for detection probability and seven variables for occupancy (Table 5). Occupancy was positively influenced by the total pool length and the SPEAR index. Also, oxygen concentration showed a trend in positively influencing occupancy. Stream width and the percentage of forest cover were not significantly associated with occupancy. However, the average amount of fish negatively influenced occupancy and IBCH also showed a trend in negatively influencing occupancy.

Table 5: Effect size estimates of the explanatory variables of the top-ranking model number two with detection probability p(~1) and abundance λ(~ width +pools +fish +forestperc +O2perc +SPEAR +IBCH). Mixture = negative binomial, upper limit of integration K = 500.

	Estimate	SE	*Z*	P(>|z|)
Occupancy				
Intercept	-0.8381	0.552	-1.519	0.1287
width	-0.0676	0.468	-0.145	0.8851
pools	1.4222	0.596	2.385	0.0171
fish	-3.0354	1.357	-2.237	0.0253
forestperc	0.6990	0.484	1.444	0.1489
O2perc	1.0477	0.586	1.787	0.0740
IBCH	-1.1962	0.641	-1.867	0.0619
SPEAR	1.6523	0.742	2.226	0.0260
Detection				
	2.500	0.467	5.350	<0.000

Note: SE, standard error; Z, z-score.

Discussion

The aim of this study was to investigate whether the negative effects of pesticide exposure can be observed in wild fire salamander populations. In this study, I was able to identify several environmental variables that influenced the abundance of fire salamander larvae in streams in my study area in the Swiss cantons of Aargau and Zurich. Pesticide concentrations were associated with lower abundances of fire salamander larvae and I found that the inclusion of measurements of water quality improved models about larval abundance.

The top-three candidate N-mixture models of the full set of streams consistently included the full set of variables that were tested to influence abundance (Table 3, cumulative Akaike weight 0.73). All top-six candidate models contained both macroinvertebrate indices (Table 3, cumulative Akaike weight 0.93) and all top-eight candidate models contained at least one measurement of water quality (Table 3, cumulative Akaike weight 0.99). This indicates that macroinvertebrate indices, and therefore water quality, indeed influence the abundance of fire salamander larvae. To see whether a different set of variables describe the abundance of fire salamander larvae exclusively in occupied streams, I performed N-mixture models with the subset of occupied streams. In comparison, the top-three candidate N-mixture models of the subset all included a different number and combination of variables for abundance and detection probability. Additionally, the top-ranking model was supported by a much lower AIC weight and did not include any variables with significant estimated effects. Because measurements of water quality did not appear consistently in the top-ranked models of the subset but did so in the top-ranked candidate models of the full set, they could contain information about the occupancy of a stream. The occupancy model confirmed that the SPEAR index, as well as the total number of pools and the average number of fish, indeed influence occupancy. The direction of the estimates was consistent with the results from the N-mixture model of the full set of streams.

In the N-mixture model of the full set of streams, the estimated effects of the variables, which previously were shown to influence fire salamander larvae, all showed the expected direction. The abundance of fire salamander larvae was strongly decreased by the average number of fish predators per stream like previously observed (Baumgartner et al. 1999). Contrarily, the larval abundance increased with the percentage of forest cover and total pool length like previously observed (Dosch 2017). These results indicate that fire salamander larvae in northern Switzerland are more common in fishless streams with pools and surrounding forest. These findings are consistent with previous knowledge about fire salamander habitat preferences in Switzerland (Schmidt and Zumbach 2005) and northern Italy (Manenti et al. 2009).

Larval abundance was significantly positively correlated with the oxygen concentration of the water. Likewise, the occupancy model showed a trend of a positive correlation between occupancy and oxygen concentration. However, the oxygen concentration was only measured once per stream and might not represent the oxygen concentration throughout a longer period. Oxygen depletion can cause mortality in fire salamander when oxygen concentrations are below 10% (Reinhardt et al. 2013). Therefore, the observed values of oxygen concentration in this study are unlikely to cause mortality. As many macroinvertebrates prefer high oxygen concentrations (Connolly et al. 2004, Jacobsen 2008), the correlation of fire salamander larvae and oxygen concentration could occur due to the indirect effect of enhanced food availability.

The IBCH was negatively related to larval abundance. Low IBCH values indicate higher levels of organic pollution and increased suspended solids (Stucki 2010). The IBCH can range from zero to 20. However, the streams I sampled all showed IBCH values between six and 17 and ranged from the classification "mediocre" to "very good" (Stucki 2010). I could not sample any streams with low IBCH values. Therefore, the negative correlation might not be representative of streams classified as "poor".

I found that larval abundance and occupancy was significantly positively correlated with the SPEAR index. Since the SPEAR index is an inverse measure of pesticide load (Liess and Ohe 2005) this result suggests that higher pesticide levels lead to lower abundance of fire salamander larvae. Contrarily to the IBCH, I was able to sample streams with a wide range of SPEAR index value, from strongly polluted to pristine streams. Pesticides can directly or indirectly harm the larvae (Gibbons et al. 2015). Direct harm includes lethal or sublethal toxic effects. Contrarily, indirect harm could be caused by limited food availability, such as a decrease in macroinvertebrates upon which salamander larvae prey. Pesticide sensitive macroinvertebrates already disappear from streams with low pesticide levels, but more robust species only disappear at rather high pesticide levels (Hutter et al. 2019). Fire salamander larvae feed on aquatic organisms, mostly crustacea, which do not belong to very pesticide sensitive macroinvertebrates (Bressi et al. 1996, Nery and Schmera 2016). Therefore, I expect indirect effects to only play a role in strongly polluted streams. However, direct negative effects are likely to occur given the amount of laboratory and mesocosm studies about amphibians suffering from the direct negative effects of pesticides (Clark et al. 1998, Rohr et al. 2004, Metts et al. 2005, Bletz et al. 2018). Also, a recent in-situ field study found that common pesticide exposure in agricultural sites led to reduced survival and mobility of tadpoles of four amphibian species, suggesting direct effects in wild amphibian populations (Agostini et al. 2020).

I restricted the analysis to streams where the cantonal offices already evaluated the macroinvertebrate indices IBCH and SPEAR index. Although, the number of streams was limited the results clearly show the existence of significant indicators for the abundance of salamander larvae and occupancy depending on the water quality, especially the amount of pesticides. Due to the limited sample size, I had to exclude several variables to reduce the risk of overparameterization. I excluded additional water measurements which were the least informative because of narrow observed ranges of values, namely pH and salinity. Additionally, I excluded the water temperature because of a failure of the loggers.

Negative effects of pesticides on amphibians on the individual level have been shown in numerous laboratory studies (Egea-Serrano et al. 2012). My finding of the positive correlation of SPEAR index and the abundance and occupancy of fire salamander larvae suggests that fire salamanders in northern Switzerland can face high pesticide exposure during the larval stage. These findings indicate that the effects of pesticides can be strong enough to translate into negative effects in wild amphibian population during the life cycle stage of the exposure.

In conclusion, the abundance of fire salamander larvae in northern Switzerland is strongly associated with the absence of predatory fish, the quantity of pools, and higher water quality in terms of low pesticide levels and high oxygen concentration. Based on these findings, I suggest that fire salamander conservation efforts include water quality as a criterion to assess habitat suitability. Specifically, conservation efforts should protect fishless forest streams from pesticide pollution. Even though many streams flow through intensively used agricultural sites and are therefore at risk of pesticide pollution, long-term reduction for pesticides and larger buffer zones would help preserve aquatic habitats.

Acknowledgements

I would like to thank Dr. Benedikt Schmidt and Prof. Dr. Arpat Ozgul for their supervision, support, and for making this thesis possible. Also, I would like to thank the members of the Population Ecology Research Group for the discussions, insights, and great memories throughout the year. Special thanks to Svenja Bächli and Dr. Torsten Haibach for proofreading. I thank the Departement Bau, Verkehr und Umwelt of the canton of Aargau and the Amt für Abfall, Wasser, Energie und Luft of the canton of Zurich for the access to the macroinvertebrate data of the streams.

Statement of Authorship:

I declare that I have used no other sources and aids other than those indicated. All passages quoted from publications or paraphrased from these sources are indicated as such, i.e. cited and/or attributed. This thesis was not submitted in any form for another degree or diploma at any university or other institution of tertiary education.

G. Haibach

Zurich, 03.03.2020

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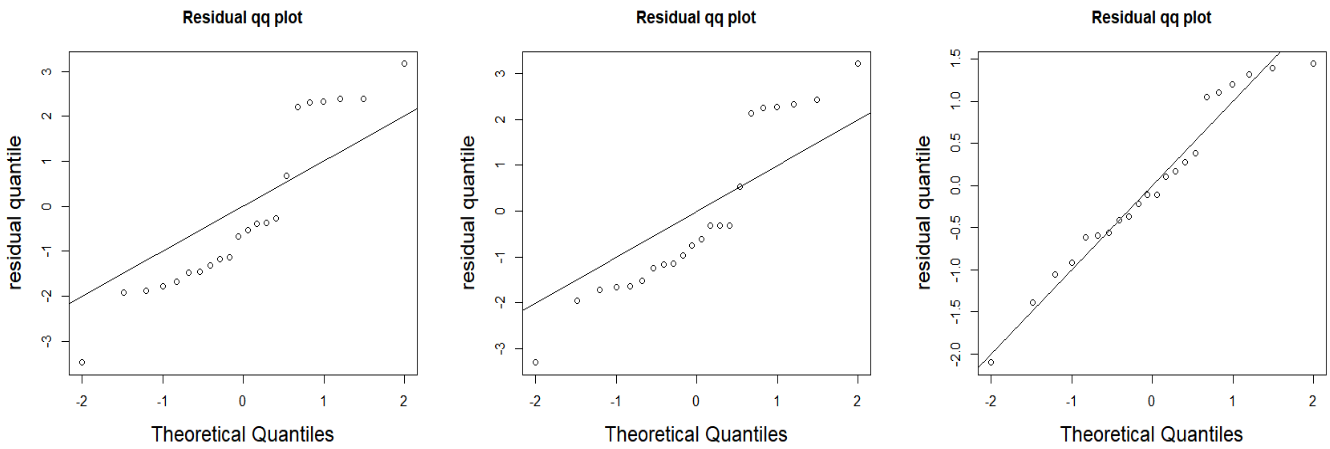
Appendix

Appendix 1: Goodness of fit test with a subset of only occupied streams. The best fit for each category is indicated in bold. AIC, Akaike criterion; c-hat bootstrapping, parametric bootstrapping with 100 samples; c-hat marginal, following Knape et al. 2017; c-hat value site-sum, following Knape et al. 2017

Distribution	AIC	c-hat bootstrapping	c-hat marginal	c-hat site-sum
Poisson	302.36	4.39	3.59	8.25
ZIP	304.36	4.08	3.66	9.27
NB	**272.94**	**1.85**	**1.03**	**2.17**

Note: Poisson, Poisson distribution; ZIP, zero-inflated Poisson distribution; NB, negative binomial distribution.

Poisson ZIP NB



Appendix 2: Subset QQ-Plots, Residual QQ Plots for the three global models with different distributions for abundance. Poisson, Poisson distribution; ZIP, zero-inflated Poisson distribution; NB, negative binomial distribution.

Appendix 3: Subset of occupied streams, candidate models with different explanatory variables for detection probability p and abundance λ. The full model is presented in bold. K, number of parameters estimated; AIC, Akaike's Information Criterion; ΔAIC, difference between the AIC and the smallest AIC; ω, Akaike weight; cu. ω, cumulative Akaike weight.

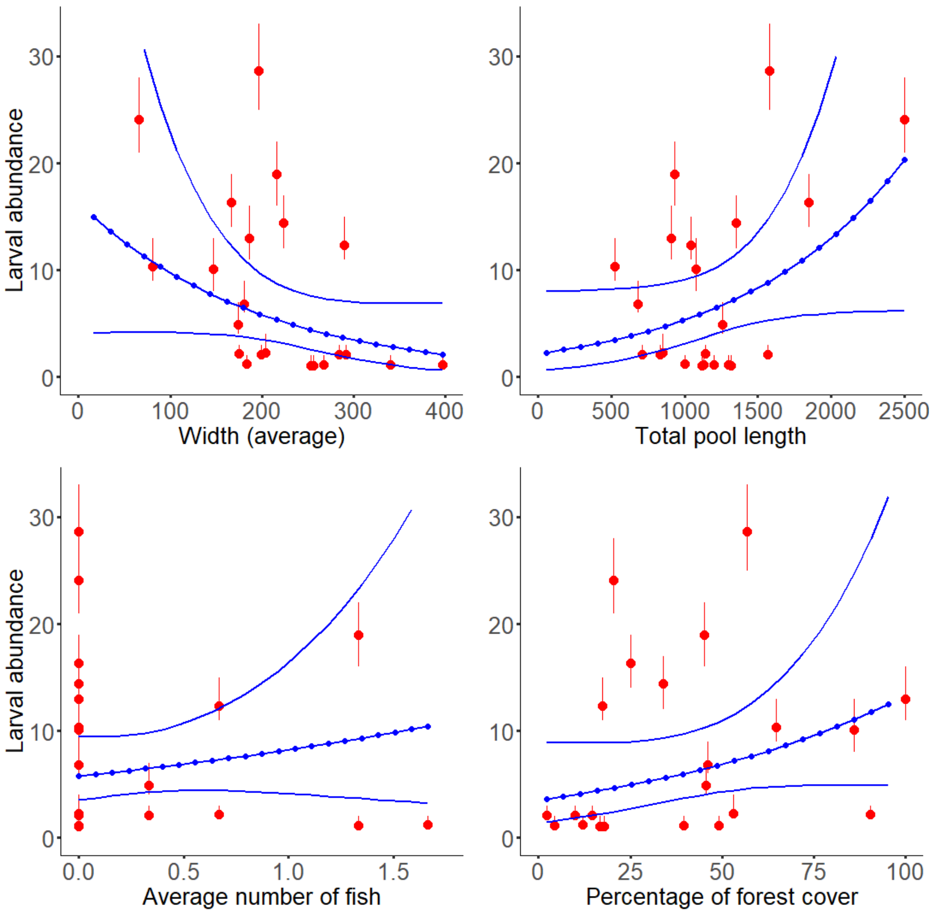
Nr.	N-Mixture Model	*K*	Rank	AIC	ΔAIC	*ω*	cu. *ω*
6	p(~ 1) λ(~ width +pools +fish +forestperc)	7	1	270.52	0.00	0.25	0.25
10	p(~ width +fish) λ(~ pools +fish +forestperc +SPEAR +IBCH)	10	2	272.37	1.86	0.10	0.35
2	p(~ 1) λ(~ width +pools +fish +forestperc +O2perc +SPEAR +IBCH)	10	3	272.40	1.88	0.10	0.45
7	p(~ width) λ(~ width +pools +fish +forestperc)	8	4	272.50	1.98	0.09	0.54
14	p(~ fish) λ(~ pools +fish +forestperc +SPEAR)	8	5	272.59	2.08	0.09	0.63
4	p(~ 1) λ(~ 1)	3	6	272.94	2.42	0.07	0.70
1	**p(~ width +pools +fish +rain3d)** **λ(~ width +pools +fish +forestperc +O2perc +SPEAR +IBCH)**	14	7	272.94	2.43	0.07	0.77
11	p(~ width) λ(~ pools +fish +forestperc +O2perc)	8	8	273.80	3.28	0.05	0.82
5	p(~ 1) λ(~ pools +fish)	5	9	273.99	3.47	0.04	0.87
3	p(~ width +pools +fish +rain3d) λ(~1)	7	10	274.31	3.79	0.04	0.90
13	p(~ width +pools +fish) λ(~ width +pools +fish +forestperc +O2perc +SPEAR +IBCH)	13	11	274.50	3.98	0.03	0.94
8	p(~ width +pools) λ(~ pools +fish +forestperc)	8	12	274.83	4.31	0.03	0.97
9	p(~ width) λ(~ pools +fish +forestperc +SPEAR +IBCH)	9	13	275.90	5.38	0.02	0.98
12	p(~ width +pools) λ(~ width +pools +fish +forestperc +O2perc +SPEAR +IBCH)	12	14	275.96	5.44	0.02	1.00

Note: pools, total length of pools; width, width of the stream; rain3d, rainfall during the last three days; fish, average number of fish; O2perc, percentage of oxygen concentration; depth, depth of the stream.

Appendix 4: Subset of occupied streams. Effect size estimates of the explanatory variables of the top-ranking model with detection probability p(~1) and abundance λ(~ width +pools +fish +forestperc +O2perc +SPEAR +IBCH).

	Estimate	SE	Z	P(>|z|)
Abundance				
Intercept	1.877	0.199	9.458	<0.001
width	0.384	0.203	1.889	0.059
pools	0.184	0.216	0.851	0.395
fish	0.372	0.244	1.527	0.127
forestperc	-0.397	0.235	-1.687	0.092
Detection				
Estimate	0.940	0.305	3.080	0.002
Dispersion				
Estimate	0.586	0.390	1.500	0.133

Note: SE, standard error; Z, z-score.



Appendix 5: Subset of occupied streams. Top-ranking N-mixture model, estimated abundances per stream in red (ranef function) with 95% confidence intervals and the predicted effect (predict function) of each significant variable in blue (dots) with 95% confidence interval (lines).