



## Increased bat hunting at polluted streams suggests chemical exposure rather than prey shortage

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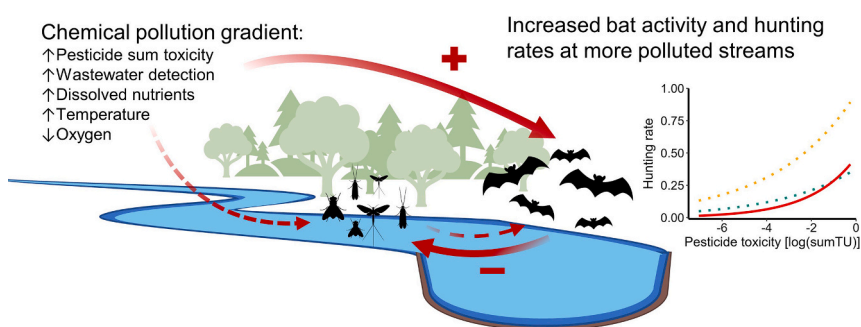
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### HIGHLIGHTS

- Water quality may negatively impact insectivorous bats hunting in riparian areas.
- We measured 77 pesticides and 4 wastewater indicators in 14 forested streams.
- The abundance of emergent insect prey was not reduced by stream pollution.
- Hunting rate and activity of *Myotis* bats were highest at more polluted streams.
- Bats may be exposed to stream pollutants through consumption of contaminated prey.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Streams and their riparian areas are important habitats and foraging sites for bats feeding on emergent aquatic insects. Chemical pollutants entering freshwater streams from agricultural and wastewater sources have been shown to alter aquatic insect emergence, yet little is known about how this impacts insectivorous bats in riparian areas. In this study, we investigate the relationships between the presence of wastewater effluent, in-stream pesticide toxicity, the number of emergent and flying aquatic insects, and the activity and hunting behaviour of bats at 14 streams in southwestern Germany. Stream sites were located in riparian forests, sheltered from direct exposure to pollutants from agricultural and urban areas. We focused on three bat species associated with riparian areas: *Myotis daubentonii*, *M. cf. brandtii*, and *Pipistrellus pipistrellus*. We found that streams with higher pesticide toxicity and more frequent detection of wastewater also tended to be warmer and have higher nutrient and lower oxygen concentrations. We did not observe a reduction of insect emergence, bat activity or hunting rates in association with pesticide toxicity and wastewater detections. Instead, the activity and hunting rates of *Myotis* spp. were higher at more polluted sites. The observed increase in bat hunting at more polluted streams suggests that instead of reduced prey availability, chemical pollution at the levels measured in the present study could expose bats to pollutants transported from the stream by emergent aquatic insects.

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## 1. Introduction

Bats are known to be vulnerable to disturbances and stressors in their ecosystems, and require high-quality food sources to maintain their energy-intensive lifestyle (Jones et al., 2009). Many European populations have suffered declines in the past as a result of habitat degradation, human disturbance and chemical pollution, among other stressors (Browning et al., 2021; Jones et al., 2009). In response, all European bat species are protected under the 1991 EUROBATS agreement and the Habitats Directive (Council Directive 92/43/EEC). Although some populations appear to be recovering, bats remain at risk from a plethora of threats, many of which are poorly understood (Browning et al., 2021; Frick et al., 2020). In particular, chemical pollutants and water pollution have been identified as significant, yet understudied, threats to European bats (Browning et al., 2021; EFSA et al., 2019).

Streams and surrounding riparian areas represent important habitats for many bat species, either as flight paths, sources of water or foraging areas (Grindal et al., 1999; Scott et al., 2010). Emergent aquatic insects, which spend their larval stages in streams before emerging as flying adults, represent an important, high-quality prey source for bats (Guo et al., 2017; Hixson et al., 2015). Some bat species specialise in hunting over water surfaces and mainly consume aquatic insects, such as Daubenton's bat (*Myotis daubentonii*, Kuhl 1817; Nissen et al., 2013; Vestterinen et al., 2018). However, even less-specialised species take advantage of riparian areas (Bellamy et al., 2013; Stahlschmidt et al., 2012), and streams have been found to be “hotspots” of bat activity in forests, especially in areas of high aquatic insect emergence (Fukui et al., 2006; Power et al., 2004). Ensuring good habitat quality of streams, and the aquatic prey they provide, benefits many bats (Bellamy et al., 2013).

Chemical pollution is a major challenge for stream quality and freshwater ecosystems (Malaj et al., 2014), as well as for bat conservation (Frick et al., 2020). This stressor has the potential to affect large stream stretches, as pollutants can be transported to otherwise unexposed areas downstream (Barber et al., 2013; Wolfram et al., 2023). Chemical pollutants enter streams from point and non-point sources, such as effluent from a wastewater treatment plant (WWTP), and runoff from agricultural fields, respectively (Neumann et al., 2002). Once in the stream, they can negatively affect organisms, altering the stream community with potential food web effects in riparian areas (Burdon et al., 2019; Graf et al., 2017; Manning and Sullivan, 2021). Both pesticides and wastewater effluent found in streams have been associated with decreases in insect emergence (Kalcounis-Rueppell et al., 2007; Kraus et al., 2021; Marshall et al., 2022; Roodt et al., 2023a). Thus, chemical pollution in streams could reduce the availability of high-quality aquatic prey for riparian bats.

Few studies have evaluated how effects of chemical stream pollution propagate into the riparian food web, especially in the context of bats. Some have observed changes in bat activity and hunting behaviour in connection with altered insect emergence around WWTPs, with varying responses (Abbott et al., 2009; Kalcounis-Rueppell et al., 2007; Vaughan et al., 1996). On the other hand, we are not aware of any studies evaluating indirect food web effects of current-use pesticides in streams on bats, though several reviews have stressed the importance of this knowledge gap (Browning et al., 2021; Torquetti et al., 2020; Voigt and Kingston, 2016). Pesticide toxicity in streams has been associated with a reduction in the number of riparian spiders preying on emergent insects and changes in the riparian spider community (Graf et al., 2019), and similar effects can be expected for bats hunting in riparian areas. An additional consideration is that emergent insects can take up pollutants and transport them from the stream into the terrestrial ecosystem (Kraus et al., 2021; Previšić et al., 2021; Richmond et al., 2018), potentially leading to negative impacts on bats through dietary exposure. Although studies have looked at effects of historical pollutants on bats, little is known about effects of chemicals used today (Torquetti et al., 2020).

Our aim was to investigate potential indirect effects of chemical

pollution in streams on the activity and hunting rate of riparian bats. To do this, we conducted an 11-week field study at 14 streams along a pollution gradient in southwestern Germany. We analysed 77 pesticides and 4 wastewater indicators in addition to measuring nutrient concentrations and other physicochemical stream parameters, collecting emerging and flying insects, and recording bat activity and hunting behaviour at the sites. We focused on three bat species known to forage at streams with different degrees of specialisation: the common pipistrelle (*Pipistrellus pipistrellus*, Schreber 1774), Brandt's bat (*Myotis cf. brandtii*, Eversmann 1845), and Daubenton's bat (*M. daubentonii*). We hypothesised that stream pollution would negatively affect bat foraging behaviour by reducing the available emergent insect prey. Specifically, we predicted that sites with a higher pesticide sum toxicity and wastewater detection would have fewer emergent aquatic insects. We also predicted that bat activity and hunting rates would be lower at sites with more stream pollution, due to a reduction of the available emergent insect prey. We predicted that effects would be strongest for Daubenton's bat, the species most specialised in hunting at streams.

## 2. Materials and methods

### 2.1. Study area and stream sites

The field study was conducted at 14 streams located in southern Rhineland-Palatinate, Germany. The study area is bordered by the Palatinate Forest, a UNESCO Biosphere reserve, to the west, and the Rhine river to the east (Fig. S1). It is characterised by second and third order streams running west to east through forest then vineyards and agricultural land mixed with urban settlements and forested areas.

Forty-metre-long stream sections were selected to represent a gradient of chemical pollution while maintaining a homogeneous and natural habitat structure. We chose sections classified as no more than “moderately altered” according to the stream structural quality classification from <https://wasserportal.rlp-umwelt.de/servlet/is/2025/> (accessed March 2020), to avoid the influence of anthropogenic alterations of stream structure in the study (Table S1). Stream sections were generally calmly-flowing with a dominance of smooth surfaces, to match the preferred foraging habitat of *Myotis daubentonii* (Warren et al., 2000). We attempted to keep stream size and pollution as independent as possible by including streams with low and high potential pollution levels across the range of sizes selected for the study. All sites were located in deciduous or mixed forest to standardise the riparian habitat, and were sheltered from direct exposure to agricultural and urban areas. The percentage of land cover types within a 100 m buffer around the sites, derived from aerial photographs (40 cm ground resolution, Map: WMS RP DOP40 v.2023-02-25 ©GeoBasis-DE/LVermGeoRP, 2023, dl-de/by-2-0, <http://www.lvermgeo.rlp.de>; QGIS version 3.12; QGIS Development Team, 2023) are provided in Table S1.

Sites were visited weekly over 11 weeks (April 21st 2020 to July 1st 2020) to sample water, stream physicochemical characteristics, emergent and flying insects (i.e. available prey for bats), and ultrasonic bat calls. In addition, the riparian vegetation near the streams was characterised and high-water event samples were collected on one occasion each.

### 2.2. Physicochemical stream characteristics

Stream width and depth were recorded several times throughout the study period. Dissolved nutrients nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), phosphate ( $\text{PO}_4^{3-}$ ) and sulphate ( $\text{SO}_4^{2-}$ ) were measured in-stream using a nutrient analysis kit (VISOCOLOR® ECO reagents with PF-12 Spectrophotometer; Macherey-Nagel GmbH, Germany). Nutrient concentrations below the level of detection (LOD) were reported as half of the LOD. Water temperature, dissolved oxygen (mg/L), conductivity ( $\mu\text{S}/\text{cm}$ ) and pH were measured with a multi-parameter meter (Multi 3620 IDS or Multi 340i, WTW Xylem Analytics GmbH, Germany).

Additionally, we placed two temperature loggers (HOBO Pendant® Temperature/Light 8K Data Logger #UA-002-08, Onset) 10 m apart on the stream shore to measure hourly air temperature on nights when bat detectors were recording. All physicochemical parameter measurements were averaged for each site over the study period (Table S2).

The vegetation of the riparian areas was characterised at each site on one occasion (June 23rd 2020). Canopy cover, shrubs, and vegetation obstructing the stream surface have been shown to affect the activity and behaviour of bats along streams (Biscardi et al., 2007; Boonman et al., 1998; Ober and Hayes, 2008) and the insect emergence due to changes in stream productivity (Marshall et al., 2022). The percent canopy cover was calculated as the average of three pictures taken of the tree canopy from the upper, middle, and downstream sections of each site. The pictures were taken mid-stream, 1 m above the water surface facing directly upwards. They were converted to blue-channel greyscale and then analysed in black and white pixels using ImageJ 1.53e (Ecological forester, 2011; Schneider et al., 2012). The distance between shrubs (shrub separation; Coulloudon et al., 1999) in the riparian area was classified on a scale for both stream banks, then averaged (Table S3). The percentage of the stream water surface interrupted or covered by clutter (i.e., vegetation disrupting the water surface or blocking a bat's flightpath) was also classified on a scale (Table S4) for the upper, middle and downstream sections of the site, then averaged. The height of clutter above the stream surface was measured along the sampling site and averaged. The vegetation surveys were conducted by the same observer at all sites.

### 2.3. Quantification of chemical stream pollution

Each week, 1 L water grab samples were taken for the analysis of chemical pollution by filling clean amber glass bottles mid-stream, below the water surface. In addition to grab samples, high-water event samples were collected during rain events. Run-off triggered by rain washes chemicals from agricultural fields and other surfaces into streams and can lead to peak concentrations of chemical pollutants, which may be missed by regular grab sampling (Rabiet et al., 2010). Event samplers consisting of two upright 1 L amber glass bottles with a small opening between the bottle and lid (Fig. S2) were attached to a stake and placed in the streams. The lowest bottle was 2–3 cm and the highest approximately 10 cm above the normal water line. We checked samplers during rain events and collected any full event bottles. If the bottles were not filled, a grab sample was taken. Event samples from one occasion were included for each site, taken during a rain event which occurred at all streams during the study (June 5th – 7th, 2020). All water samples were kept on ice during transport to the laboratory and then stored at 4 °C for 24–48 h to allow for settling of sediment prior to extraction and analysis of chemical pollutants.

#### 2.3.1. Extraction of analytes

Chemical pollution analytes were extracted from 10 weekly grab samples and one event sample per site using solid phase extraction (SPE), following the method of Machado et al. (2016). At least one blank sample of 1 L ultra-pure water (18.2 MΩ-cm, D3750 2 μm endfilter, Barnstead™/Werner Reinstwassersystem, Thermo Fischer Scientific, Waltham, MA, USA) was included with each weekly extraction ( $n = 14$ ), as well as solvent blanks run during the analyte elution ( $n = 5$ ). Further details are presented in Section S1.1 of the Supplementary Information.

#### 2.3.2. Concentration measurements

High-performance liquid chromatography tandem to triple-quadrupole mass spectrometry by electrospray ionization (HPLC-ESI-MS/MS) was used to analyse the samples for 77 currently used pesticides and 4 established wastewater indicators (Table S5). Measurements were performed with an Agilent 1260 Infinity II HPLC system tandem to an Agilent 6495 triple quadrupole mass spectrometer (MS/MS; Agilent Technologies, Inc., Santa Clara, CA, USA). A ZORBAX Eclipse Plus C18

HPLC column (3 × 150 mm, particle size 2.7 μm; Agilent Technologies, Inc., Santa Clara, CA, USA) kept at 45 °C was used to achieve chromatographic separation. The sample injection volume was 10 μL with a flow rate of 0.45 mL/min. At least two multiple reaction monitoring (MRM) transitions were used per compound to confirm the identity of and quantify the selected analytes, except for proquinazid, which only had one transition (Table S6). Processing of the HPLC-ESI-MS/MS data was performed with the Agilent MassHunter Workstation (Quantitative analysis for QQQ v10, Agilent Technologies, Inc., Santa Clara CA, USA).

#### 2.3.3. Analytical quality assurance and data analysis

Analytical standards were prepared for the calculation of the limits of detection (LODs) and limits of quantification (LOQs) based on calibration curves (Table S5). In addition, the accuracy and reproducibility of the extraction method were evaluated with five ultrapure water samples containing a mixture of all analytes at a known concentration. Analytes with recoveries between 70 and 120 % and relative standard deviations between replicates (RSD) of 15 % or less were quantified in the samples (Table S5). Any analytes ( $n = 23$ ) which did not meet these standards were only considered qualitatively and not included in the toxicity calculations. However, fipronil, which had a recovery of 50 %, was quantified as an exception due to its high ecotoxicological relevance and frequent occurrence in analysed samples.

Measured concentrations of each chemical pollutant analyte were normalised to the actual volume of water used for the SPE of each sample and for HPLC-ESI-MS/MS analysis (Section S1.2). Next, any concentration below the LOD was set to zero and concentrations between the LOD and LOQ were set as half of the LOQ (George et al., 2021). The LOQs of boscalid and caffeine were adjusted to account for a quantifiable background signal in blank samples (Table S12). Further details are provided in Section S1.2 of the supplementary information.

### 2.4. Presence of wastewater

Four of the measured analytes had been selected to indicate the presence of wastewater effluent in the streams. Caffeine is highly abundant in global freshwaters but is effectively removed with wastewater treatment (Li et al., 2020), allowing it to be used as an indicator of untreated wastewater. Carbamazepine, diclofenac, and sulfamethoxazole are three common pharmaceuticals present in surface waters but which are not effectively removed by treatment in WWTPs (Čelić et al., 2019). Concentrations of wastewater effluent and some pharmaceuticals have been shown to vary throughout the day (Nelson et al., 2011; Paíga et al., 2019). As we could not visit all stream sites at similar times of day, we avoided potential bias by only considering whether each indicator was detected (i.e. >LOD) in a sample, and calculated the total number of detections during the study period for each stream site.

### 2.5. Pesticide sum toxicity calculation

We used the logarithmic sum of toxic units (sumTU; Schäfer et al., 2013) to quantify the potential sum toxicity of the pesticide mixture measured in the stream samples:

$$\text{sumTU} = \log_{10} \left( \frac{C_i}{EC_{50_i}} \right) \quad (1)$$

where  $C_i$  is the normalised concentration of pesticide  $i$ , and  $EC_{50_i}$  is the concentration affecting 50 % ( $EC_{50}$ ) of organisms in an acute test with pesticide  $i$ . Because we were interested in the direct effects of pesticides on emergent aquatic insect larvae in the streams, we calculated the sumTU for freshwater invertebrates. We used the  $EC_{50}$  for the most sensitive freshwater invertebrate from acute toxicity tests (24–96 h) for each analyte, based on available data (Table S5) mainly obtained from the USEPA ECOTOX database (U.S. EPA, 2021) using the Standartox package for R (Scharmüller et al., 2020), or the Pesticide Properties

Database (Lewis et al., 2016). The sumTU was then calculated for each sample (10 grab and 1 event), and averaged for each stream to obtain the average pesticide sum toxicity. A larger sumTU indicates a higher sum toxicity, whereas a more negative sumTU indicates a lower sum toxicity. We assigned a sumTU of  $-9.4$ , a factor of ten smaller than the lowest calculated sumTU, to three individual samples without detections of pesticides used in the sumTU calculation.

## 2.6. Measuring available emergent and flying insect prey

We used a combination of traps to approximate 1) the production of emergent aquatic insects and 2) the abundance of flying terrestrial and aquatic insect prey available for bats at each stream. Each site had two pyramid-shaped emergence traps with  $0.25 \text{ m}^2$  surface area, based on Cadmus et al. (2016), in place on the water surface throughout the entire study period to continuously sample adult insects emerging from the stream. Traps were placed at least 10 m apart, when possible, in different parts of the stream channel, and had 125 mL of propylene glycol trapping medium (33 % propane-1,2-diol, 66 % water, 1 mL/L dish soap and 10 mg/L denatonium benzoate for deterring larger animals) in 500 mL collection bottles. Captured emergent insects were collected from the bottles weekly throughout the study. Flying terrestrial and aquatic insects were sampled at all sites on four occasions (May 12/13, May 19/20, June 2/3, June 9/10) using SLAM-style Malaise traps (McCravy, 2018; MegaView Science Co., Ltd., Taichung, Taiwan; Table S7). Each site had one SLAM trap suspended 1 m directly above the stream shoreline, secured so that the open sides were parallel to the stream. The trap bottles contained 125 mL of propylene glycol trapping medium (as for emergence traps) and were collected after one week.

Both flying (i.e. from SLAM traps) and emergent insect samples were kept on ice for transport to the lab, where they were removed from the trapping medium and stored in 80 % ethanol at  $4^\circ\text{C}$ . Emergent insects were identified to order level (Brohmer et al., 2009; Chinery, 2012), and flying insects were identified to family level for orders with aquatic and terrestrial families (Brohmer et al., 2009; Köhler, 2015). The total number of individuals was used to estimate the abundance of emergence and flying insect prey at each site over the study period. The total number of emergent insects was corrected to account for differing trap numbers (Table S7) due to losses of some samples during storms.

## 2.7. Recording bat activity and hunting success

Bats emit echolocation calls during flight, which can be used to assess their overall activity and specific behaviours with bioacoustic methods. We deployed full-spectrum ultrasonic bat detectors (Audiomoth v1.1.0 with Firmware v1.2.2, Open Acoustic Devices; Hill et al., 2019) at each site for one night per week to automatically record bat calls. Detectors were wrapped in one layer of household cling film to protect them from moisture and dirt, and were taped to the trunks of trees approximately 40 m apart, at a height of 1.5 m approximately 1 m away from the shoreline. The microphones faced the stream and were unobstructed by vegetation. Recording was programmed to begin one hour before sunset and end one hour after sunrise, with a sample rate of 192 kHz, medium gain and continuous 1-h recording periods. Bats were only recorded on nights without precipitation and high wind speeds.

Audio recordings were processed with Kaleidoscope Pro (version 5.6, Bats of Europe 5.4, Wildlife Acoustics, Inc.), which split the recordings into 60-s-long files and filtered out noise files (i.e., without recognized ultrasonic signal detections). We used the default signal parameters in “Bat analysis mode” and the Auto-ID function with sensitivity set to “Balanced” to produce an initial species classification for each recorded minute, grouping minutes with similar calls for later manual identification. Only the 22 species known to occur in Rhineland-Palatinate (Lindermann, 2017) were included in the Auto-ID list.

Because automatic identification software is not yet fully reliable (Rydell et al., 2017), each minute was manually identified (sonogram

settings FFT size 128, WIN size 64 in Kaleidoscope) after the initial classification by Auto-ID. As bats at or in close proximity to the stream would be within a few metres of the microphone, we assumed that all species using the streams would be detectable by the bat detectors (Barataud, 2020). We only considered those sequences containing at least one call recognized by Kaleidoscope (i.e., surpassing Kaleidoscope's noise threshold, with visual zero-crossing points) and excluded noise files, where any calls were likely too quiet or of too poor quality. There may have been some loss of calls as noise due to interference from the water surface, though we ensured that the detector placement was similar to keep this likelihood equal for all sites.

Five nights per site (May 5/6, May 18/19, June 2, June 12, June 23/24) were included. Specific procedures and details for manual identification are provided in Section S2 of the Supplementary Information. We counted the number of minutes containing bat calls of each species (“active minutes”) as a proxy for bat activity. Bats emit special call types directly before prey capture, known as feeding buzzes. We counted the number of feeding buzzes in each minute, which we differentiated from drinking buzzes (Griffiths, 2013; Russo et al., 2016), as described in Section S2. The hunting rate, or the number of feeding buzzes per active minute, could then be calculated following (2):

$$\text{Hunting rate}_i = \frac{n_{\text{feeding buzz}_i}}{n_{\text{active minutes}_i} + 1} \quad (2)$$

where  $n_{\text{feeding buzz}_i}$  is the number of feeding buzzes recorded and  $n_{\text{active minutes}_i}$  the number of active minutes of bat species  $i$ .

We focused on three bat species for this study: the common pipistrelle, *Pipistrellus pipistrellus*, Daubenton's bat, *Myotis daubentonii*, and Brandt's bat, *M. cf. brandtii*, as they were the most common across the study area, present at all stream sites, and are all known to forage at forested streams (Roswag et al., 2019; Todd and Williamson, 2019; Warren et al., 2000). The calls of Brandt's bat are almost indistinguishable from the whiskered bat, *M. mystacinus* (Kuhl, 1817) (Russ, 2021). However, both species share overlapping ecological niches (Roswag et al., 2019), with Brandt's bat more restricted to woodlands. Thus, we assumed that most calls were likely to be Brandt's bat, though whiskered bats may have been included. The soprano pipistrelle, *P. pygmaeus* (Leach, 1825) was also common at some sites, but was excluded since it does not normally occur in the Palatinate forest (Lindermann, 2017).

## 2.8. Statistical analysis

Statistical analyses were conducted with R (version 4.2.2; R Core Team, 2022). All variables were summarised for each site over the entire study period, either as a total or an average value. To avoid correlation between variables, a correlation matrix was constructed with Spearman's rank correlation. Out of highly correlated variables ( $\rho > 0.8$ ), only those with the highest expected relevance for bats were retained in the analysis.

A principal component analysis (PCA) was conducted based on the environmental variables measured (VEGAN; Oksanen et al., 2022). We then added the number of both emerged and flying aquatic Diptera and Ephemeroptera, Plecoptera, and Trichoptera (EPT), the number of all flying insects, and the activity and hunting rate of the three bat species to the biplot as passive variables, using “predict” in R to calculate their positions on the first two principal component axes.

Next, three series of generalized linear models (GLMs) were fitted. We used automated model selection and model averaging for each GLM to test the relationships between: 1) the number of emergent and flying aquatic insects explained by the environmental variables, 2) bat activity and hunting rates explained by the environmental variables and 3) bat activity and hunting rates explained by the number of emergent and flying insects (prey availability). Average pesticide sum toxicity (sumTU), stream width, and tree canopy cover were used as proxies of the various groups of correlated environmental variables and PCA axes:



water pollution/water quality, stream size, and vegetation, respectively.

For each GLM, a global model containing all variables was fitted (Table S10). The error distribution family and link functions were selected to match the distribution of the dependent variable (linear and gamma distributions fitted with “glm” in R, negative binomial with “glm.nb”; LME4; Bates et al., 2015; tweedie with “glmmTMB”; GLMMTMB; Brooks et al., 2017). The hunting rate of the common pipistrelle required log transformation for one GLM. Model assumptions were checked (“check\_model”, PERFORMANCE; Lüdecke et al., 2021) and a VIF <3 was deemed acceptable. Each model was tested for spatial autocorrelation using Moran's I test (SPDEP; Bivand et al., 2013) and inspected with variograms (GSTAT; Pebesma, 2004). In the case of significant spatial patterns, the AICc values of the original model was compared to models containing spatial correlation structures to choose the best-fitting model (Zuur et al., 2009).

The “dredge” function (MuMIN; Bartoń, 2022) was used to compute all possible models from the global model and rank them by AICc, with a maximum of two explanatory variables allowed per model due to the small number of sites. An average model (MuMIN; Bartoń, 2022) was then calculated from all models within 4 points of difference in AICc from the best model (Burnham and Anderson, 2002). We considered the output of the conditional average model. Results of all GLMs are presented in Table S10. We also conducted generalized linear mixed effect models (GLMMs) with time as a fixed and site as a random effect to evaluate the temporal dynamics in the relationship between bats and insects. As these results are not directly related to our main hypotheses, they are presented in Table S14. A significant result was defined as  $p < 0.05$ . Plots were created with GGLOT2 (Wickham, 2016) and GGPUBR (Kassambara, 2022).

### 3. Results

#### 3.1. Chemical stream pollution

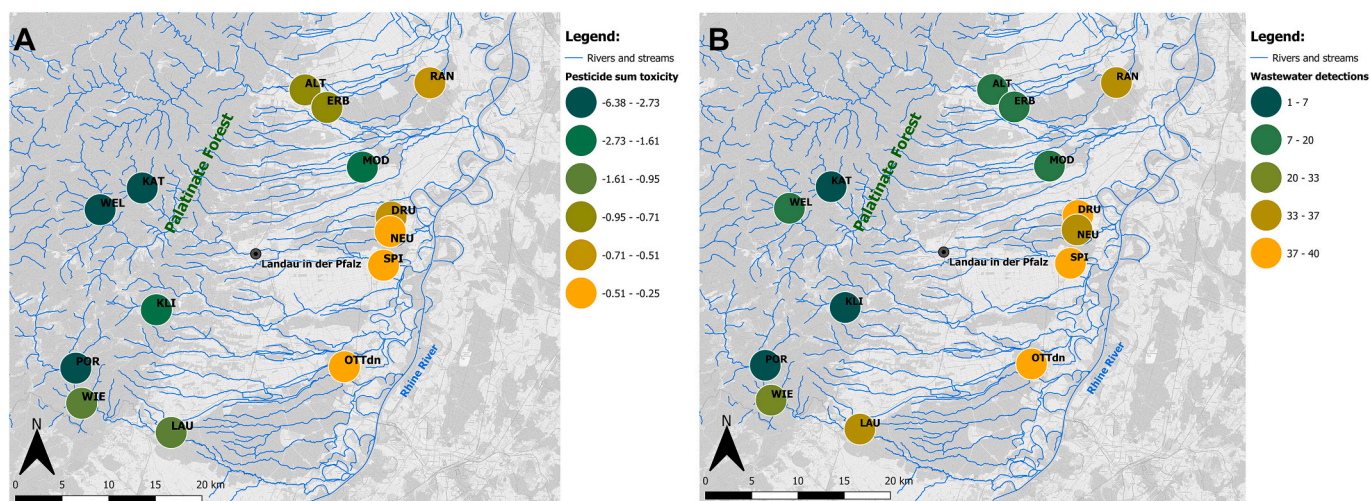
We found differing profiles in pesticide sum toxicity (average sumTU) and wastewater pollution (total number of detections) across stream sites (Fig. 1). In addition, average pesticide toxicity, wastewater indicator detections, and count of pesticide detections were highly correlated, and also highly correlated with nitrogen and sulphate concentrations measured in the streams (Table 1).

Of the 81 measured analytes, we detected 69 pesticides and all 4 wastewater indicators in at least one water sample (Table S8). An average of 17.9 (standard deviation  $\pm 13.5$ ) pesticides and 2.1 (standard deviation  $\pm 1.4$ ) wastewater indicators were detected per sample, with a maximum of 50 pesticides detected in a single sample. At least one chemical pollutant was detected in every water sample but two. The insecticide fipronil was detected in 83 % of samples, followed by mecoprop and metholachlor-S (herbicides, 75 %), and 2,4-D (herbicide, 73 %; Table S8). In addition, diclofenac was the most commonly detected wastewater indicator, present in 77 % of samples (Table S8). There were no strong temporal changes in pesticide toxicity nor wastewater detections throughout the study period (Fig. S3).

There was a wide range in pesticide toxicity across streams, driven by few, toxic compounds. The average sumTU per site had a large range which was skewed towards higher toxicity (Table 1) and the maximum measured sumTU in an individual water sample was  $-0.061$  (SPI, week 6). Fipronil, a non-agricultural insecticide, drove the sum toxicity for most sites due to its ubiquitous presence and high toxicity, whereas herbicides generally had the highest concentrations in the samples (Table S8). There was no strong peak in pesticide toxicity detected by high-water event samples, so they were considered together with weekly grab samples.

#### 3.2. Relationships between water quality, stream size, vegetation, emergent and flying aquatic insects and bats

The streams were characterised by two independent environmental gradients (Fig. 2). Variables reflecting water quality and chemical pollution were grouped along the first axis, and explained most of the variation between sites. Streams with higher pesticide toxicity and more pesticide detections were warmer, had more wastewater detections, and higher nitrogen, sulphate and phosphate concentrations, but less dissolved oxygen. Oxygen levels were never measured below 7.07 mg/L during the study period. The second axis mainly represented stream size and vegetation characteristics. Wide streams tended to be deeper and to have less canopy cover and a lower surface vegetation clutter score than narrow streams. In terms of variation within streams over the study period, water temperature tended to increase and dissolved oxygen to decrease over time, while most other variables were either consistent or varied with no clear temporal trend (Table S13). Ranges within sites are reported in Table S2.



**Fig. 1.** Chemical pollution measured at 14 stream sites in southwestern Germany over 11 weeks. Dark grey fields are forested areas and vineyards, while light grey represents other agricultural and urban land. The Rhine river and Palatinate forest are labelled, as well as the city of Landau in der Pfalz. A) Average pesticide sum toxicity of the streams, measured as the logarithmic sum toxic unit (sumTU), is represented by the colours of the circles (range of average sumTU:  $-6.4$  to  $-0.2$ ). B) Total number of wastewater indicator detections in streams are represented by the colours of the circles (range 10 to 44 total detections). Note that the sumTU is on a logarithmic scale. The basemap is OpenStreetMap, available under the Open Database Licence (CC BY-SA 2.0). The stream layer “Gewässernetz 2017”, available from WWV RLP (CC BY 4.0). The maps were created in QGIS 3.12.1-București.

**Table 1**

Chemical pollution, physicochemical and vegetation measurements per stream from 14 stream sites. Physicochemical variables are averages of measurements conducted over an 11-week field study, whereas vegetation characteristics were recorded on one occasion. The ranges and median values of each variable from the 14 streams are stated. In addition, the correlation of each variable to the average pesticide mixture toxicity (sumTU) given by Spearman's  $\rho$ .

Variable	Unit	Type	Range	Median	Correlation ( $\rho$ ) with average sumTU
Pesticide sum toxicity	sumTU	Average over study period	-7.05 to -0.25	-0.95	-
Pesticide detections	Count	Average over study period	1.6–38.0	11.3	0.86
Wastewater detections	Count	Total over study period	1–40	25.5	0.87
Caffeine detections	Count	Total over study period	0–10	3.5	0.52
Pharmaceutical detections	Count	Total over study period	0–33	20	0.89
Width	m	Average over study period	2.1–8.8	3.9	0.11
Depth	cm	Average over study period	9–94	16	0.18
pH	-	Average over study period	6.7–8.1	7.6	0.44
Night air temperature	°C	Average over study period	10.1–13.9	12.7	0.65
Water temperature	°C	Average over study period	10.9–15.7	14.1	0.50
Conductivity	$\mu\text{S}/\text{cm}^2$	Average over study period	64.6–603.9	201.6	0.64
Dissolved O <sub>2</sub>	mg/L	Average over study period	7.9–10.9	9.5	-0.55
Dissolved Nitrogen combined	mg/L	Average over study period, summed NO <sub>2</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , NH <sub>4</sub> <sup>+</sup>	0.6–2.7	1.5	0.86
Dissolved PO <sub>4</sub> <sup>3-</sup>	mg/L	Average over study period	0.1–0.2	0.1	0.57
Dissolved SO <sub>4</sub> <sup>2-</sup>	mg/L	Average over study period	10.0–32.8	13.9	0.72
Shrub separation score	-	Average of both banks	0–2	0.25	-0.23
Surface clutter score	-	Average of three locations on stream	0–2.5	1	-0.20
Surface clutter height	cm	Average over 40 m of stream	30–150	75	-0.082
Canopy cover	%	Average of three locations over stream	44.9–86.9	80.5	0.23

### 3.3. Insect emergence and flying insects

The PCA showed that more polluted streams tended to have higher numbers of emerging Diptera and EPT (Fig. 2). However, none of the relationships between the number of emerging insects and the stream toxicity, canopy cover, or stream width were significant (Fig. 3, Table S10). Contrary to the emergence pattern, the number of flying aquatic Diptera, EPT, and of all flying terrestrial and aquatic insects tended to either be higher at less polluted sites or have no clear relationship with stream pollution as shown in the PCA, again with no significant relationships (Fig. 2, Fig. 3, Table S10).

### 3.4. Bat activity and hunting rate

Streams with more pollution were associated with higher hunting rates of all three bat species and higher activity of both *Myotis* species, similar to emergent Diptera and EPT in the PCA (Fig. 2). The activity of Daubenton's bat ( $z = 2.0, p = 0.04$ , Fig. 4A) and hunting rate of Brandt's bat ( $z = 2.2, p = 0.03$ , Fig. 4B) were significantly higher at streams with higher pesticide toxicity (Table S10). Though the hunting rate of Daubenton's bat tended to be higher at more polluted sites, this was not significant (Fig. 2, Fig. 4B, Table S10). The activity of the common pipistrelle showed no clear increase with stream toxicity (Fig. 4A). The common pipistrelle was the only bat whose activity was related to stream vegetation and structure: they were significantly more active at sites with less canopy coverage ( $z = 2.2, p = 0.03$ , Table S10) and tended to prefer larger, more open streams (Fig. 2).

Overall relationships between bats and insects were highly variable. The activity of both *Myotis* species and hunting rate of all three bat species were similarly positioned to the number of emergent insects in the PCA (Fig. 2). The overall comparison of bats and insect abundance between streams revealed only few clear relationships (Table S10). While the activity of Brandt's bat was significantly higher at streams with a higher number of emerging Diptera ( $z = 2.1, p = 0.004$ ; Fig. 5B), there was no relationship between their hunting rate and the number emerging or flying insects. The activity ( $z = 2.9, p = 0.003$ ) and hunting rate ( $z = 2.6, p = 0.01$ ) of Daubenton's bat were both negatively related to the abundance of flying EPT (Fig. 2, Fig. 5AC). There were no significant relationships between the hunting rate of Daubenton's bat and emerging and flying Diptera, though they showed a positive trend (Fig. 5D, Fig. 2). There were no strong relationships between the number of insects and the activity nor hunting rate of the common pipistrelle.

When including temporal variation by analysing the data of each sampling period, the foraging behaviour of all three bat species increased with the number of insects (Table S14). In particular, the hunting rates of all bats increased significantly with both the number of emerging and flying Diptera at the stream sites.

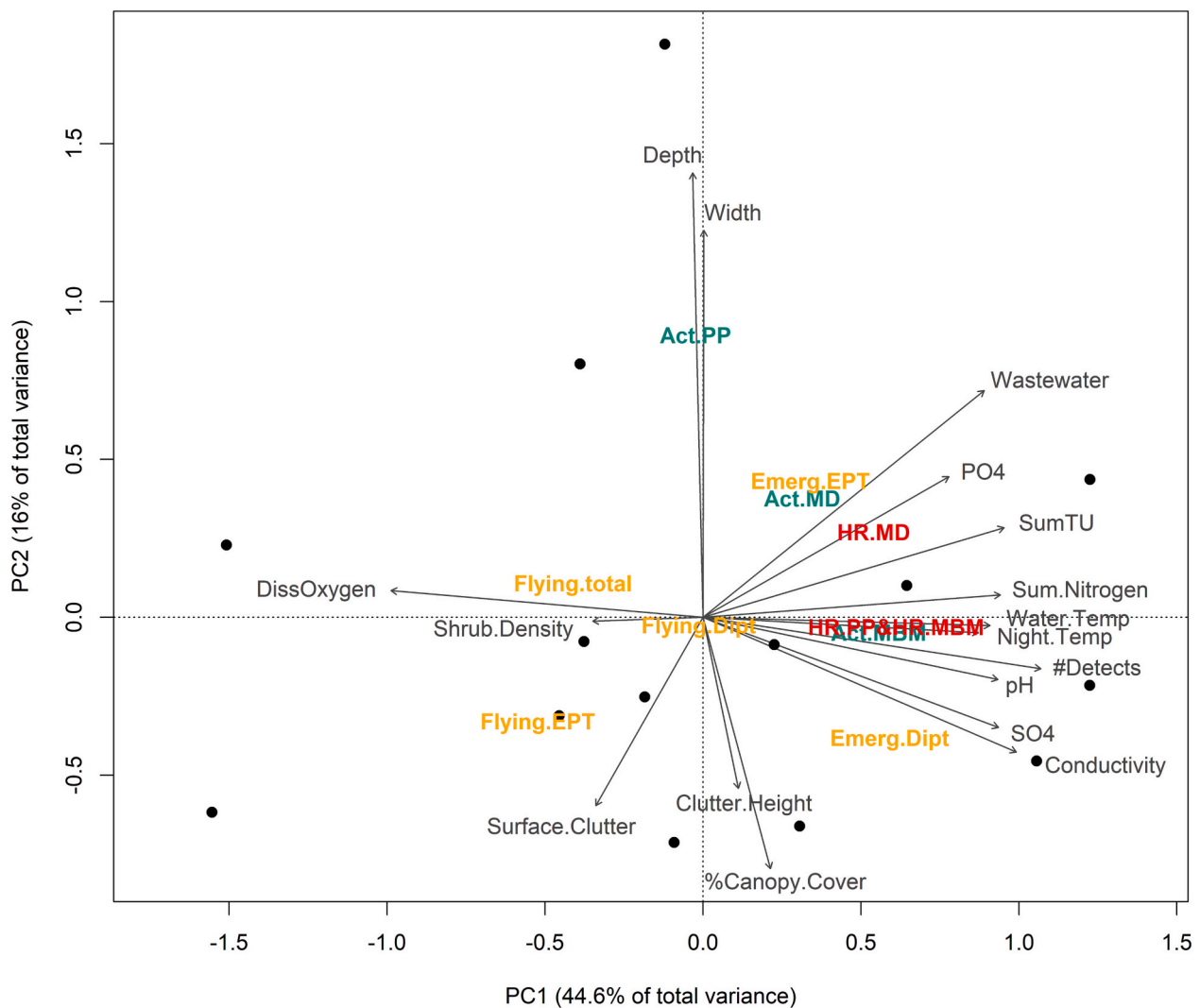
## 4. Discussion

### 4.1. Chemical stream pollution

We found a clear gradient of stream pollution across our sites in terms of pesticide, wastewater and nutrient load. Measurements of pesticides and wastewater were highly correlated with other water-quality parameters, such as decreased oxygen, increased pH, water temperature, and nutrient concentrations (Fig. 2). Therefore, polluted streams in our study tended to be more polluted overall, and not due to specific sources of pollution. In addition, pollution was not related to stream width or depth, as shown in the PCA (Fig. 2), confirming that our selection of streams across a pollution gradient was not strongly biased by stream size.

The pesticide pollution measured in our study is comparable to levels measured in similar German streams. The average and maximum number of pesticides detected per sample as well as the toxicity range in our streams are similar to those measured in the "Kleingewässermonitoring" (KGM), a Germany-wide stream monitoring programme conducted in 2018–2019 (Liess et al., 2021; Weisner et al., 2021). Moreover, the pesticide sum toxicity and number of detected pesticides we measured is similar to that measured in streams in the same area in 2019 (Schneeweiss et al., 2022). Schneeweiss et al. (2022) compared pesticide toxicity and its effects between unpolluted upstream sections in the Palatinate forest and polluted stream sections adjacent to agricultural areas. Our stream sites were all in forested areas, with 12 sites located in protected areas and three sites in nature conservation areas (Table S1). Although none of our sites were adjacent to agricultural or urban areas, almost all had a pesticide profile similar to that measured in agricultural stream sections by Schneeweiss et al. (2022). The fact that we measured similar pollution levels at stream sites downstream from pollutant sources demonstrates the potential for streams to import pollutants into otherwise unexposed natural areas (Wolfram et al., 2023), which are hotspots for bat activity and foraging (Fukui et al., 2006; Stahlschmidt et al., 2012).

While our focus was on using the pesticide sum toxicity as a general



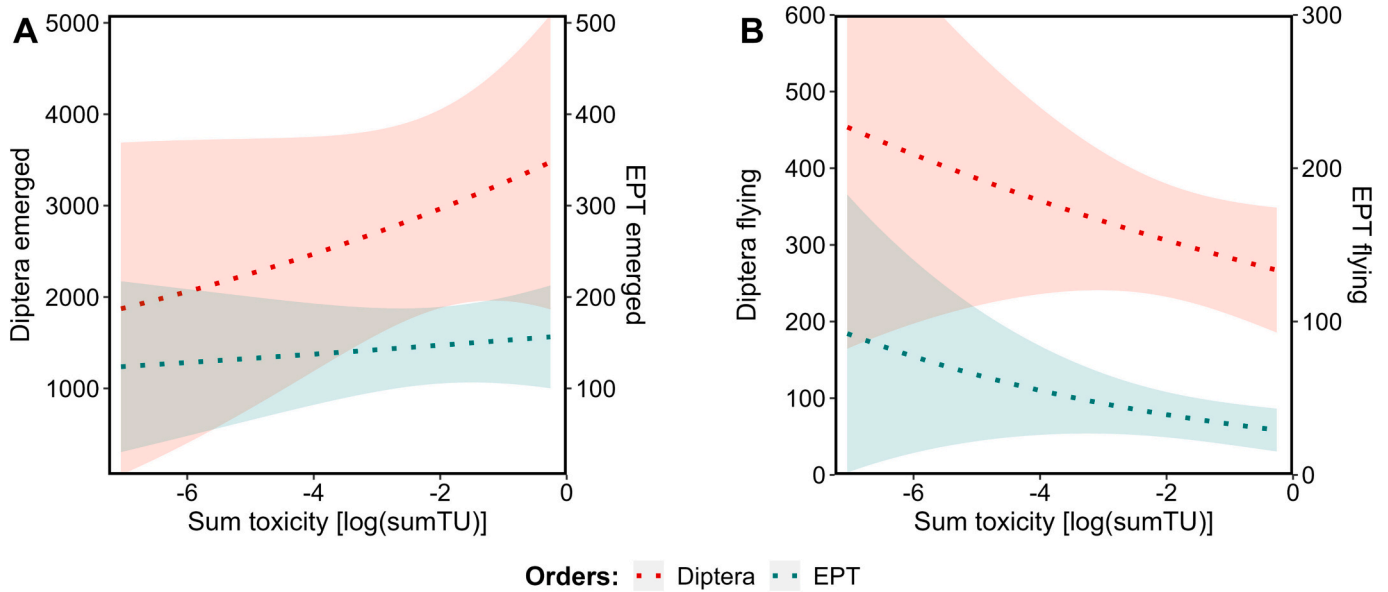
**Fig. 2.** Principal component analysis biplot showing the main environmental gradients among different stream sites (black points) explained by measured environmental variables (grey labelled arrows). Bat activity (blue) and hunting rate (red) of three bat species (MBM: Brandt's bat *Myotis cf. brandtii*, MD: Daubenton's bat *M. daubentonii*, PP: common pipistrelle *Pipistrellus pipistrellus*), as well as the number of flying and emergent aquatic insects (yellow; Dipt: Diptera, EPT: Ephemeroptera, Plecoptera and Trichoptera, Total: all flying insects) are included as passive variables in the biplot. Abbreviations: Clutter.Height is the average height of vegetation clutter on the stream surface, %Canopy.cover is the percentage of tree canopy cover, Conductivity is the average water conductivity, SO4 is the average concentration of dissolved sulphate, #Detects is the average number of pesticides detected, pH is the average pH of the stream water, Sum.Nitrogen is the average concentration of dissolved nitrate, nitrite and ammonium combined, Night.Temp is the average air temperature on nights when bat calls were recorded, Water.Temp is the average water temperature, SumTU is the average sum toxicity of pesticides for freshwater invertebrates, Wastewater is the total number of wastewater indicators detected in the streams, PO4 is the average concentration of dissolved phosphate, Width is the stream width, Depth is the water depth, Shrub.Density is the density score of shrubs along the stream shore, DissOxygen is the average concentration of dissolved oxygen, Surface.Clutter is the average score of water surface coverage by vegetation clutter. Note that the hunting rates of *M. cf. brandtii* and *P. pipistrellus*, "HR.PP&HR.MBM", overlaps the activity of *M. cf. brandtii*, "Act.MBM" in the centre of the right quadrants.

indication of the level of pesticide pollution in our streams, there was one substance of concern. Fipronil, an insecticide which is still used as a veterinary drug against ectoparasites (CVMP, 2023) and for indoor pest control (EC, 2011), was banned for agricultural use in the European Union in 2017 (EC, 2016). However, it was the most detected pesticide in our study. Fipronil also drove the sumTU in most streams due to its high toxicity for freshwater invertebrates (Miller et al., 2020; Weston and Lydy, 2014). The presence of fipronil in surface waters is a widespread issue and has been attributed to use on household pets and entry via wastewater effluent (CVMP, 2023; Bradley et al., 2017; Miller et al., 2020; Teerlink et al., 2017). This may also explain its frequent occurrence in our study. Due to its high potential ecological risk, the presence and implications of fipronil in streams merit further investigation.

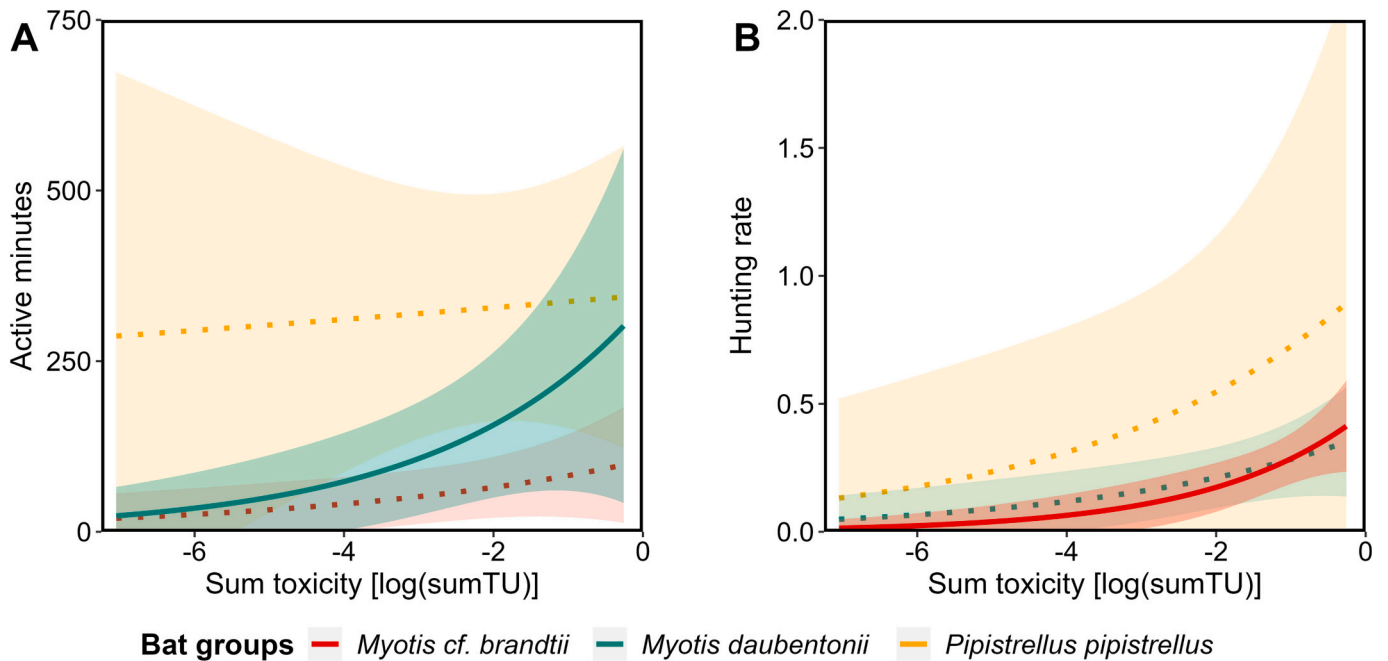
#### 4.2. Response of emerging and flying insects to pollution

While we predicted that fewer aquatic insects would emerge from streams with higher pesticide toxicity, we did not observe negative responses of insect emergence to stream pollution. We also did not find any drivers clearly explaining the differences in the numbers of emerging nor flying aquatic insects at different stream sites, other than a tendency for more insects emerging at more polluted sites (Fig. 2). Previous studies have documented a reduction in insect emergence due to pesticide toxicity and wastewater effluent in streams (Kalcounis-Rueppell et al., 2007; Kraus et al., 2021; Marshall et al., 2022; Miller et al., 2020). The average sum toxicity measured in most of our streams was relatively high and had the potential to negatively affect sensitive





**Fig. 3.** Relationships between the number of A) emergent and B) flying insects captured at streams with varying degrees of pesticide toxicity, measured in logarithmic sum toxic units (sumTU). The order group EPT is the combination of Ephemeroptera, Plecoptera, and Trichoptera individuals, and is on a separate axis from Diptera. No relationship is statistically significant. The relationships were calculated using generalized linear models based on measurements from 14 stream sites, and the 95 % confidence intervals are shown by the shaded areas surrounding the model lines.



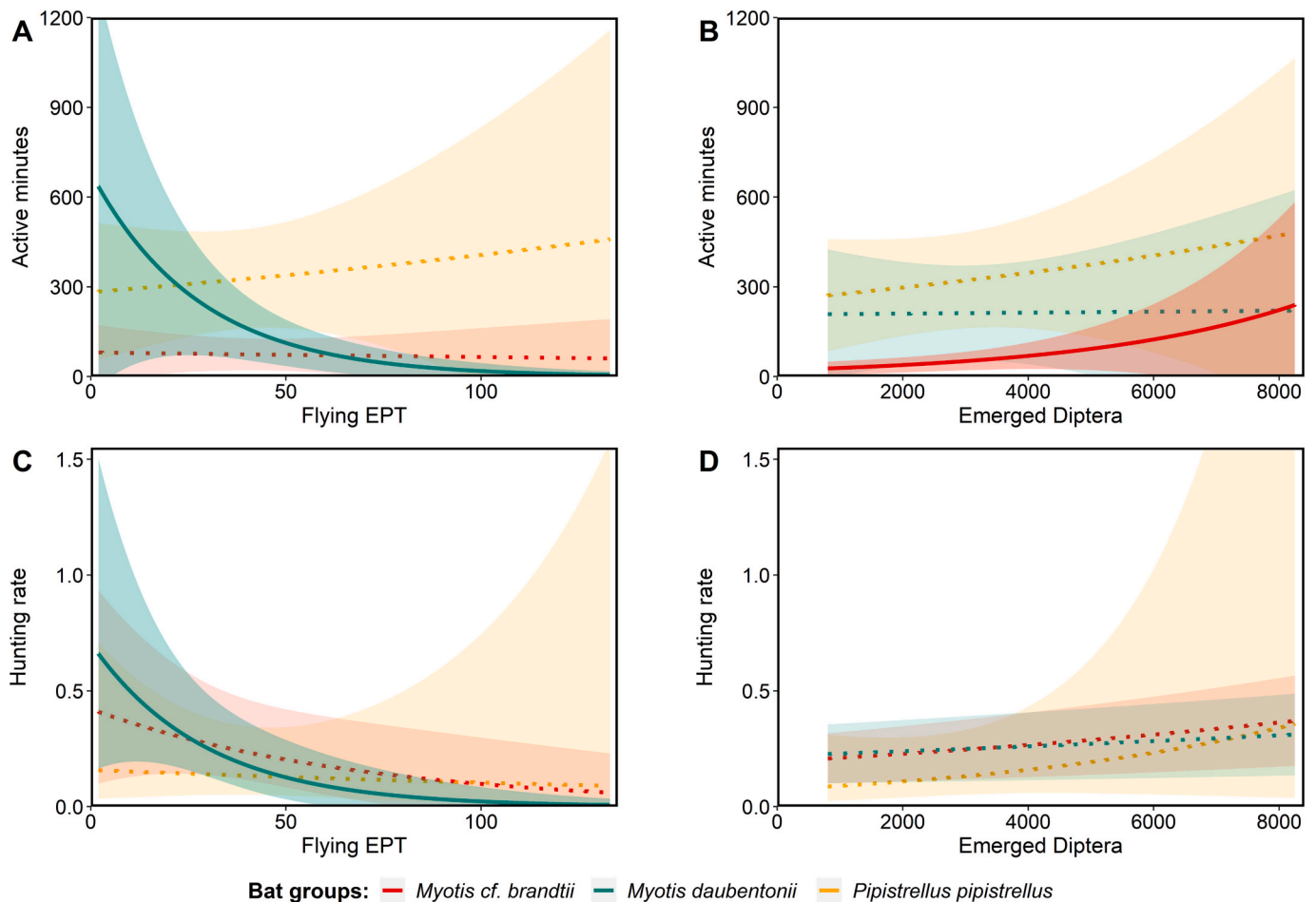
**Fig. 4.** Relationships between the (A) activity and (B) hunting rates of three bat species and the toxicity of pesticide mixtures measured in forested streams. Bat activity was measured as the number of active minutes, i.e., the number of minutes that a species was recorded calling. The hunting rate is the number of “feeding buzz” hunting calls per active minute. The stream toxicity was calculated as the log sum toxic unit (sumTU) obtained from the measurement of 77 pesticides in the stream water. The relationships were calculated using generalized linear models based on measurements from 14 stream sites. Significant relationships ( $p < 0.05$ ) are shown by solid lines and the 95 % confidence intervals are shown in the shaded areas surrounding the model lines.

stream insects (Liess and Von der Ohe, 2005; Liess et al., 2021; Miller et al., 2020).

Rather than a reduction of all insect emergence, the communities at our stream sites may have shifted to more tolerant species at polluted sites and more sensitive species at less-polluted sites, as has been observed in other studies (Burdon et al., 2016; Ohler et al., 2023; Schneeweiss et al., 2022). Liess et al. (2021) calculated a maximum sumTU of  $-3.27$  as a threshold for maintaining a good in-stream

ecological quality for invertebrates at 95 % of streams based on their field study. The average sumTU for all but two of our sites and maximum sumTU for all sites were above  $-3$  (Table S11) and, by this definition, not protective for sensitive species when considering that our streams are similar to those of Liess et al. (2021). Furthermore, the lack of competition from more sensitive species in situations of constant pesticide exposure could lead to higher success of tolerant species (Liess et al., 2013). For example, Ohler et al. (2023) recorded higher biomass





**Fig. 5.** Relationships between the activity and hunting rates of three bat species and A/C) the number of Diptera emerging from forested streams, and B/D) the number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) flying at the stream banks. A&B show bat activity, in terms of active minutes, while C&D show bat hunting rates. The hunting rate is the number of “feeding buzz” hunting calls recorded per active minute. Significant relationships ( $p < 0.05$ ) are presented as solid lines. The relationships were calculated using generalized linear models based on measurements from 14 stream sites and the 95 % confidence intervals are shown in the shaded areas surrounding the model lines.

and abundance of tolerant emergent insects, as well as a temporal shift in emergence from agricultural streams compared to forested streams, which may also have occurred at more polluted sites in our study. We found that pesticide toxicity and wastewater presence were highly correlated with nutrient load and higher temperatures (Fig. 2, Table 1), and tolerant taxa could have taken advantage of higher productivity in polluted streams (Abbott et al., 2009; Raitif et al., 2018). Although we cannot confirm a shift to more tolerant taxa in the prey community at our streams, studies sampling the benthic invertebrate community or including finer taxonomic scales using methods such as DNA metabarcoding of insect samples or the bat diet would be capable of revealing such effects of stream pollution.

#### 4.3. Bat activity and hunting rate

Bat activity and hunting rates were either unrelated to pollution or higher at more polluted sites (Fig. 4). The activity of Daubenton's bat, as well as the hunting rate of Brandt's bat were higher at sites with more pesticide pollution (Fig. 2). However, we did not observe strong relationships of bat foraging behaviour and the number of aquatic insects, although bats are known to track insect emergence at streams (Fukui et al., 2006). Only the activity of Brandt's bat showed a positive relationship with the abundance of emergent Diptera in the overall between-stream comparison (Table S10). However, all bats tracked the number of

Diptera in the temporally-resolved dataset (Table S14). This is expected, as Diptera make up the majority of their diet (Galan et al., 2018; Vesterinen et al., 2018), though the small size of the numerically-dominant Chironomidae could mean that they are less valuable in terms of nutritional quality than the larger EPT.

While we did not see clear relationships between bats and the number of insects along the pollution gradient, results of previous studies may help to explain the higher *Myotis* spp. activity and hunting rates at polluted sites. For example, studies investigating bat activity upstream and downstream from wastewater treatment plants found that, in some cases, bats were more active downstream, which was explained by a higher insect emergence due to a suspected increase in dissolved nutrients (Abbott et al., 2009; Vaughan et al., 1996). Likewise, positive effects of increased nutrients and temperature on emergent insects in our study could have negated toxic effects of pollution. Higher prey abundances may have encouraged bats to spend more time foraging at these sites. We saw that the sites with more emergent insects and bat foraging behaviour also tended to be more polluted, warmer, and have higher concentrations of dissolved nutrients (Fig. 2). Additional factors such as proximity to roosts may also have contributed to the numbers of bats spending time at certain streams, but we were not able to control for this in our study. Furthermore, although there was no significant spatial autocorrelation in our study (Table S10), we cannot exclude some influence of spatial patterns inevitably present in our study area on the

stream habitats at the sites. Future experimental field studies are needed to clarify the effect of different drivers leading to increased bat activity at polluted sites.

While we expected riparian bats to respond positively to the number of aquatic insects present at the streams, we observed a negative relationship between the activity and hunting rate of Daubenton's bat and flying EPT abundance (Fig. 5). As bats are attracted by high prey densities, there must be other reasons for the negative correlations between Daubenton's bat and flying EPT. One explanation could be opposing habitat preferences. Daubenton's bats prefer to hunt over calm and open water surfaces (Boonman et al., 1998; Todd and Williamson, 2019; Warren et al., 2000), while most EPT prefer fast-flowing streams (Beermann et al., 2018). Some EPT such as Plecoptera, which were more frequently sampled by the malaise traps, are highly sensitive to stream pollution and poor water quality (Chang et al., 2014) and may have been less common at polluted sites preferred by bats, though we cannot confirm this with our results. Alternatively, negative correlations between prey and predator abundances could indicate top-down regulation (Polis et al., 1997). Top-down regulation of insect densities by bats has been suggested for agricultural systems (Tuneu-Corral et al., 2023) and documented in urban parks (Villarroya-Villalba et al., 2021), and a forest experiment (Beilke and O'Keefe, 2023). Thirdly, bats may need to exert less hunting effort in areas with more abundant prey due to more rapid satiation. However, the negative correlation between lower hunting rates at sites with high EPT abundance suggests that this was not the case. Exploring potential explanations for the negative relationship between Daubenton's bat and flying EPT abundance would require further study, possibly including dietary analysis, and is out of the scope of the current investigation.

In accordance with our predictions, the relationships that we observed between bats and stream-specific variables (insects and pollution) were strongest for the two *Myotis* species (Figs. 4 and 5), which are more associated with streams than the common pipistrelle. Daubenton's bat is a specialised riparian species, often hunting directly above the water surface (Kalko and Schnitzler, 1989). Brandt's bat is also associated with riparian areas, though to a lesser degree (Roswag et al., 2019). Thus, it is not surprising that they had the strongest relationships to insect emergence and pollution. On the other hand, the common pipistrelle is a generalist bat and is widely distributed in a variety of habitats, including riparian areas (Lundy and Montgomery, 2009). The common pipistrelle was the most common species in our study. It was also the only species that responded to structural characteristics around the stream sites, confirming its generalist habitat choice. The semi-open conditions for such an edge-space forager are best met by the streams with a relatively open canopy (Kusch et al., 2004), as seen in our results.

#### 4.4. Implications of bat response to pollution

We observed higher activity and hunting rates of riparian bats at streams with more pollution and poorer water quality. Though the correlative nature of our study does not allow for the establishment of a mechanistic relationship, foraging at polluted sites may lead to detrimental effects for bats. Emergent insects are known to take up pesticides and pharmaceuticals from the water, and can transport them into the terrestrial ecosystem (Kraus et al., 2021; Previšić et al., 2021; Roodt et al., 2023b). Kraus (2019) describes the balance between pollutant toxicity and insect emergence, where fewer insects emerge due to negative effects at higher toxicity levels, but the higher emergence at lower levels can lead to a higher pollutant flux from the stream, depending on the accumulation potential of the pollutants. This may also apply to pesticides if they are retained into the adult stage of emergent insects. Although Kraus (2019) suggests that current-use pesticides are more likely to reduce emergence flux via mortality rather than to accumulate in and be transported by the insects, Roodt et al. (2022, 2023a) experimentally demonstrated that certain pesticides, including insecticides such as neonicotinoids, are retained by

chironomids through metamorphosis. In addition, a study by Roodt et al. (2023b) conducted in the same area as our study confirmed that certain pesticides are transferred by emergent insects, especially dipterans, and bioaccumulate in spiders feeding on stream emergence. Combined with this knowledge, our results suggest that, at the observed concentrations of chemical pollutants in our streams, the unaffected numbers of emergent insects and higher bat hunting rates at polluted sites led to a dietary exposure of bats to chemical pollutants from streams.

Many pesticides and other contaminants have already been reported in bats, for example across Germany (Schanzer et al., 2022), but the contribution and significance of stream pollution to this is not yet known. In addition, although dietary exposure to contaminants in streams may negatively affect bats, a lack of research in this area makes specific consequences difficult to predict (Torquetti et al., 2020). Changes in the microbiome of bats after hunting near WWTPs have been reported, likely due to pharmaceuticals in the water and emergent insects (Mehl et al., 2021). This could also occur at some streams in this study, as sulfamethoxazole, an antibiotic, was measured in the water. In addition, Roodt et al. (2023b) reported the bioaccumulation and biomagnification of neonicotinoid pesticides in spiders at our streams via emergent insects, which can also apply to bats. A detailed review and risk assessment by Mineau and Callaghan (2018) suggest that exposure of bats to neonicotinoids may lead to immunological, behavioural, reproductive and mortality effects, though few studies have tested bats directly.

In terms of sublethal effects of contaminants such as pesticides, neurological effects leading to poorer hunting efficiency or migration performance, effects on metabolism reducing survivability of hibernation or reductions in reproductive success would have detrimental consequences for bat populations (Amaral et al., 2012; Eidels et al., 2016; Hsiao et al., 2016). Bats may be particularly vulnerable to effects of chemical exposure as they require high amounts of energy for flight and hibernation, have a long lifespan and produce few offspring (Jones et al., 2009). Thus, it remains imperative to evaluate the risks of chemical pollutant exposure to bats, including the role played by streams.

Bats face a plethora of threats globally (Browning et al., 2021). Both indirect effects of pollution through prey loss and direct effects from contaminant uptake put bats at risk. We have only included chemical stream pollution in this study, which is a globally relevant stressor (Stehle and Schulz, 2015), but it is also important to consider interactions with other stressors affecting bats such as habitat loss, climate change, and disease (Frick et al., 2020). For example, increased contaminant uptake with effects on immune functions may reduce bats' ability to cope with diseases such as white-nose syndrome (Cable et al., 2022; Korine et al., 2017), or parasites (Pilosof et al., 2014). Any effect of pollutants on bat survival or reproduction adds to that of other stressors causing high mortality in bats, and this pressure is expected to increase in the future with climate change (O'Shea et al., 2016). This can have serious implications for the recovery and conservation of vulnerable bat populations. Furthermore, the potential threat of consuming insects from polluted freshwater may be exacerbated by the insect decline recorded over the last decades (Hallmann et al., 2017). The stronger decline in terrestrial than aquatic insect species could further increase the reliance of bats on insects from freshwater ecosystems (Van Klink et al., 2020).

#### 4.5. Conclusion

There was no net negative effect of wastewater or pesticide pollution on the abundance of emergent aquatic insects. Thus, the pollution levels measured at our stream sites did not appear to reduce prey availability for bats. The higher foraging rates of bats at polluted sites may instead have resulted in increased pesticide exposure. However, negative effects of pesticides on insect emergence and prey availability can be expected in systems with higher levels of pesticide and wastewater pollution. It is

also concerning that streams in our study transported micropollutants into protected areas. Given the sensitivity of bats to stressors in their habitats and the lack of knowledge associated with emerging contaminants and bats, chemical pollution in streams remains a topic of concern, especially in the context of multiple stressors that bats are facing globally. Thus, we encourage further ecotoxicological investigation for the conservation of these important and vulnerable mammals.

#### CRedit authorship contribution statement

**Maïke Huszarik:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Alexis P. Roodt:** Methodology, Validation, Investigation, Writing – review & editing. **Teagan Wernicke:** Methodology, Investigation, Writing – review & editing. **Fernanda Chávez:** Investigation, Writing – review & editing. **Annika Metz:** Methodology, Investigation, Writing – review & editing. **Moritz Link:** Methodology, Investigation, Writing – review & editing. **Eva Lima-Fernandes:** Methodology, Investigation, Writing – review & editing. **Ralf Schulz:** Supervision, Funding acquisition, Writing – review & editing. **Martin H. Entling:** Conceptualization, Formal analysis, Supervision, Funding acquisition, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

The raw data for this study are available in the supplementary information and upon request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.167080>.

## References

- Abbott, I., Sleeman, D., Harrison, S., 2009. Bat activity affected by sewage effluent in Irish rivers. *Biol. Conserv.* 142, 2904–2914. <https://doi.org/10.1016/j.biocon.2009.07.012>.
- Amaral, T.S., Carvalho, T.F., Silva, M.C., Goulart, L.S., Barros, M.S., Picanço, M.C., Neves, C.A., Freitas, M.B., 2012. Metabolic and histopathological alterations in the fruit-eating bat *Artibeus lituratus* induced by the organophosphorus pesticide fenthion. *Acta Chiropterol.* 14 (1), 225–232. <https://doi.org/10.3161/150811012X654420>.
- Barataud, M., 2020. Acoustic ecology of European bats: species identification, study of their habitats and foraging behaviour, 2nd ed. In: *Biotope éditions, Méze - Muséum nationale d'Histoire naturelle, Paris*.
- Barber, L.B., Keefe, S.H., Brown, G.K., Furlong, E.T., Gray, J.L., Kolpin, D.W., Meyer, M.T., Sandstrom, M.W., Zaugg, S.D., 2013. Persistence and potential effects of complex organic contaminant mixtures in wastewater-impacted streams. *Environ. Sci. Technol.* 47 (5), 2177–2188. <https://doi.org/10.1021/es303720g>.
- Bartoni, K., 2022. MuMIn: Multi-Model Inference.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 6 (7), 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Beeremann, A.J., Elbrecht, V., Karnatz, S., Ma, L., Matthaei, C.D., Piggott, J.J., Leese, F., 2018. Multiple-stressor effects on stream macroinvertebrate communities: a mesocosm experiment manipulating salinity, fine sediment and flow velocity. *Sci. Total Environ.* 610–611, 961–971. <https://doi.org/10.1016/j.scitotenv.2017.08.084>.
- Beilke, E.A., O'Keefe, J.M., 2023. Bats reduce insect density and defoliation in temperate forests: an exclusion experiment. *Ecology* 104 (2), e3903. <https://doi.org/10.1002/ecy.3903>.
- Bellamy, C., Scott, C., Altringham, J., 2013. Multiscale, presence-only habitat suitability models: fine-resolution maps for eight bat species. *J. Appl. Ecol.* 50 (4), 892–901. <https://doi.org/10.1111/1365-2664.12117>.
- Biscardi, S., Russo, D., Casciani, V., Cesarini, D., Mei, M., Boitani, L., 2007. Foraging requirements of the endangered long-fingered bat: the influence of micro-habitat structure, water quality and prey type. *J. Zool.* 273 (4), 372–381. <https://doi.org/10.1111/j.1469-7998.2007.00337.x>.
- Bivand, R.S., Pebesma, E., Gomez-Rubio, V., 2013. *Applied Spatial Data Analysis with R*, Second edition. Springer, NY.
- Boonman, A.M., Boonman, M., Bretschneider, F., van de Grind, W.A., 1998. Prey detection in trawling insectivorous bats: duckweed affects hunting behaviour in Daubenton's bat, *Myotis daubentonii*. *Behav. Ecol. Sociobiol.* 44 (2), 99–107. <https://www.jstor.org/stable/4601554>.
- Bradley, P.M., Journey, C.A., Romanok, K.M., Barber, L.B., Buxton, H.T., Foreman, W.T., Furlong, E.T., Glassmeyer, S.T., Hladik, M.L., Iwanowicz, L.R., Jones, D.K., Kolpin, D.W., Kuivila, K.M., Loftin, K.A., Mills, M.A., Meyer, M.T., Orlando, J.L., Reilly, T.J., Smalling, K.L., Villeneuve, D.L., 2017. Expanded target-chemical analysis reveals extensive mixed-organic-contaminant exposure in U.S. streams. *Environ. Sci. Technol.* 51 (9), 4792–4802. <https://doi.org/10.1021/acs.est.7b00012>.
- Brohmer, P., Schäfer, M., Ansoerge, H., 2009. *Brohmer, Fauna von Deutschland: Ein Bestimmungsbuch unserer heimischen Tierwelt*, 23rd ed. Quelle & Meyer Verlag GmbH & Co.
- Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* 9 (2), 378–400. <https://doi.org/10.32614/RJ-2017-066>.
- Browning, E., Barlow, K.E., Burns, F., Hawkins, C., Boughey, K., 2021. Drivers of European bat population change: a review reveals evidence gaps. *Mammal Rev.* 51 (3), 353–368. <https://doi.org/10.1111/mam.12239>.
- Burdon, F.J., Reyes, M., Alder, A.C., Joss, A., Ort, C., Räsänen, K., Jokela, J., Eggen, R.I., Stamm, C., 2016. Environmental context and magnitude of disturbance influence trait-mediated community responses to wastewater in streams. *Ecol. Evol.* 6 (12), 3923–3939. <https://doi.org/10.1002/ece3.2165>.
- Burdon, F.J., Munz, N.A., Reyes, M., Focks, A., Joss, A., Räsänen, K., Altermatt, F., Eggen, R.I.L., Stamm, C., 2019. Agriculture versus wastewater pollution as drivers of macroinvertebrate community structure in streams. *Sci. Total Environ.* 659, 1256–1265. <https://doi.org/10.1016/j.scitotenv.2018.12.372>.
- Burnham, K.P., Anderson, D.R., 2002. *Model Selection and Multimodel Inference: A Practical Information-theoretic Approach*, 2nd ed. Springer New York.
- Cable, A.B., Willcox, E.V., Leppanen, C., 2022. Contaminant exposure as an additional stressor to bats affected by white-nose syndrome: current evidence and knowledge gaps. *Ecotoxicology* 31 (1), 12–23. <https://doi.org/10.1007/s10646-021-02475-6>.
- Cadmus, P., Pomeranz, J.P., Kraus, J.M., 2016. Low-cost floating emergence net and bottle trap: comparison of two designs. *J. Freshw. Ecol.* 31 (4), 653–658. <https://doi.org/10.1080/02705060.2016.1217944>.
- Čelić, M., Gros, M., Farré, M., Barceló, D., Petrović, M., 2019. Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain). *Sci. Total Environ.* 652, 952–963. <https://doi.org/10.1016/j.scitotenv.2018.10.290>.
- Chang, F.-H., Lawrence, J.E., Rios-Touma, B., Resh, V.H., 2014. Tolerance values of benthic macroinvertebrates for stream biomonitoring: assessment of assumptions



- underlying scoring systems worldwide. *Environ. Monit. Assess.* 186, 2135–2149. <https://doi.org/10.1007/s10661-013-3523-6>.
- Chinery, M., 2012. *Pareys Buch der Insekten, über 2000 Insekten Europas*, 2nd ed. Kosmos, Kosmos-Naturführer.
- Committee for Veterinary Medicinal Products (CVMP), 2023. Reflection paper on the environmental risk assessment of ectoparasiticide veterinary medicinal products used in cats and dogs. In: European Medicines Agency. Consultant Draft, EMA/CVMP/ERA/31905/2021.
- Coulloudon, B., Eshelman, K., Gianola, J., Habich, N., Hughes, L., Johnson, C., Pellant, M.L., Podborny, P., Rasmussen, A., Robles, B., et al., 1999. Sampling vegetation attributes: interagency technical reference. In: U.S. Department of the Interior Bureau of Land Management. Technical Reference 1734-4.
- Ecological forester, 2011. Canopy Closure from Digital Photos Using ImageJ [WWW Document]. URL: <http://ecologicalforester.blogspot.com/2011/03/canopy-closure-from-digital-photos.html> (accessed 04.2021).
- EFSA Panel on Plant Protection Products and their Residues (EFSA), Hernández-Jerez, A., Adriaens, P., Aldrich, A., Berny, P., Coja, T., Duquesne, S., Gimsing, A., Marina, M., Millet, M., Pelkonen, O., Pieper, S., Tiktak, A., Tzoulaki, I., Widenfalk, A., Wolterink, G., Russo, D., Streissl, F., Topping, C., 2019. Scientific statement on the coverage of bats by the current pesticide risk assessment for birds and mammals. *EFSA J.* 17, e05758.
- Eidels, R.R., Sparks, D.W., Whitaker Jr., J.O., Sprague, C.A., 2016. Sub-lethal effects of chlorpyrifos on big brown bats (*Eptesicus fuscus*). *Arch. Environ. Contam. Toxicol.* 71, 322–335. <https://doi.org/10.1007/s00244-016-0307-3>.
- European Commission (EC), 2011. Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Assessment Report Fipronil Product-type PT18. European Chemicals Agency (ECHA).
- European Commission (EC), 2016. Commission Implementing Regulation (EU) 2016/2035 of 21 November 2016 amending Implementing Regulation (EU) No 540/2011 as regards the approval periods of the active substances fipronil and maneb. *J. Eur. Union L* 314 (7).
- Frick, W.F., Kingston, T., Flanders, J., 2020. A review of the major threats and challenges to global bat conservation. *Ann. N. Y. Acad. Sci.* 1469 (1), 5–25. <https://doi.org/10.1111/nyas.14045>.
- Fukui, D., Murakami, M., Nakano, S., Aoi, T., 2006. Effect of emergent aquatic insects on bat foraging in a riparian forest. *J. Anim. Ecol.* 75 (6), 1252–1258. <https://doi.org/10.1111/j.1365-2656.2006.01146.x>.
- Galan, M., Pons, J.-B., Tournayre, O., Pierre, É., Leuchtmann, M., Pontier, D., Charbonnel, N., 2018. Metabarcoding for the parallel identification of several hundred predators and their prey: application to bat species diet analysis. *Mol. Ecol. Resour.* 18 (3), 474–489. <https://doi.org/10.1111/1755-0998.12749>.
- George, B.J., Gains-Germain, L., Broms, K., Black, K., Furman, M., Hays, M.D., Thomas, K.W., Simmons, J.E., 2021. Censoring trace-level environmental data: statistical analysis considerations to limit bias. *Environ. Sci. Technol.* 55 (6), 3786–3795. <https://doi.org/10.1021/acs.est.0c02256>.
- Graf, N., Bucher, R., Schäfer, R.B., Entling, M.H., 2017. Contrasting effects of aquatic subsidies on a terrestrial trophic cascade. *Biol. Lett.* 13 (5), 20170129. <https://doi.org/10.1098/rsbl.2017.0129>.
- Graf, N., Batters, K.P., Cimpean, M., Dittrich, P., Entling, M.H., Link, M., Scharmüller, A., Schreiner, V.C., Szöcs, E., Schäfer, R.B., 2019. Do agricultural pesticides in streams influence riparian spiders? *Sci. Total Environ.* 660, 126–135. <https://doi.org/10.1016/j.scitotenv.2018.12.370>.
- Griffiths, S.R., 2013. Echolocating bats emit terminal phase buzz calls while drinking on the wing. *Behav. Process.* 98, 58–60. <https://doi.org/10.1016/j.beproc.2013.05.007>.
- Grindall, S.D., Morrisette, J.L., Brigham, R.M., 1999. Concentration of bat activity in riparian habitats over an elevational gradient. *Can. J. Zool.* 77 (6), 972–977. <https://doi.org/10.1139/z99-062>.
- Guo, F., Bunn, S.E., Brett, M.T., Kainz, M.J., 2017. Polyunsaturated fatty acids in stream food webs-high dissimilarity among producers and consumers. *Freshw. Biol.* 62 (8), 1325–1334. <https://doi.org/10.1111/fwb.12956>.
- Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., de Kroon, H., 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One* 12, 1–21. <https://doi.org/10.1371/journal.pone.0185809>.
- Hill, A.P., Prince, P., Snaddon, J.L., Doncaster, C.P., Rogers, A., 2019. AudioMoth: a low-cost acoustic device for monitoring biodiversity and the environment. *HardwareX* 6, e00073. <https://doi.org/10.1016/j.ohx.2019.e00073>.
- Hixson, S.M., Sharma, B., Kainz, M.J., Wacker, A., Arts, M.T., 2015. Production, distribution, and abundance of long-chain omega-3 polyunsaturated fatty acids: a fundamental dichotomy between freshwater and terrestrial ecosystems. *Environ. Rev.* 23 (4), 414–424. <https://doi.org/10.1139/er-2015-0029>.
- Hsiao, C.J., Lin, C.L., Lin, T.Y., Wang, S.E., Wu, C.H., 2016. Imidacloprid toxicity impairs spatial memory of echolocating bats through neural apoptosis in hippocampal CA1 and medial entorhinal cortex areas. *Neuroreport* 27 (6), 462–468. <https://doi.org/10.1097/WNR.0000000000000562>.
- Jones, G., Jacobs, D.S., Kunz, T.H., Willig, M.R., Racey, P.A., 2009. Carpe noctem: the importance of bats as bioindicators. *Endanger. Species Res.* 8, 93–115. <https://doi.org/10.3354/esr00182>.
- Kalcounis-Rueppell, M., Payne, V., Huff, S., Boyko, A., 2007. Effects of wastewater treatment plant effluent on bat foraging ecology in an urban stream system. *Biol. Conserv.* 138 (1–2), 120–130. <https://doi.org/10.1016/j.biocon.2007.04.009>.
- Kalko, E.K.V., Schnitzler, H.-U., 1989. The echolocation and hunting behavior of Daubenton's bat, *Myotis daubentoni*. *Behav. Ecol. Sociobiol.* 24, 225–238.
- Kassambara, A., 2022. ggpubr: “ggplot2” Based Publication Ready Plots.
- Köhler, G. (Ed.), 2015. *Müller/Bährmann Bestimmung wirbelloser Tiere - Bildtafeln für zoologische Bestimmungsübungen und Exkursionen*, 7th ed. Springer Spektrum; Springer Berlin.
- Korine, C., Pilosof, S., Gross, A., Morales-Malacara, J.B., Krasnov, B.R., 2017. The effect of water contamination and host-related factors on ectoparasite load in an insectivorous bat. *Parasitol. Res.* 116, 2517–2526. <https://doi.org/10.1007/s00436-017-5561-4>.
- Kraus, J.M., 2019. Contaminants in linked aquatic-terrestrial ecosystems: predicting effects of aquatic pollution on adult aquatic insects and terrestrial insectivores. *Freshw. Sci.* 38 (4), 919–927. <https://doi.org/10.1086/705997>.
- Kraus, J.M., Kuivila, K.M., Hladik, M.L., Shook, N., Mushet, D.M., Dowdy, K., Harrington, R., 2021. Cross-ecosystem fluxes of pesticides from prairie wetlands mediated by aquatic insect emergence: implications for terrestrial insectivores. *Environ. Toxicol. Chem.* 40 (8), 2282–2296. <https://doi.org/10.1002/etc.5111>.
- Kusch, J., Weber, C., Idelberger, S., Koob, T., 2004. Foraging habitat preferences of bats in relation to food supply and spatial vegetation structures in a western European low mountain range forest. *Folia Zool. Praha* 53 (2), 113–128.
- Lewis, K.A., Tzivilakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. *Hum. Ecol. Risk Assess. Int. J.* 22 (4), 1050–1064. <https://doi.org/10.1080/10807039.2015.1133242>.
- Li, S., Wen, J., He, B., Wang, J., Hu, X., Liu, J., 2020. Occurrence of caffeine in the freshwater environment: implications for ecopharmacovigilance. *Environ. Pollut.* 263 (B), 114371. <https://doi.org/10.1016/j.envpol.2020.114371>.
- Liess, M., Von Der Ohe, P.C., 2005. Analyzing effects of pesticides on invertebrate communities in streams. *Environ. Toxicol. Chem.* 24 (4), 954–965. <https://doi.org/10.1897/03-652.1>.
- Liess, M., Foit, K., Becker, A., Hassold, E., Dolciotti, I., Kattwinkel, M., Duquesne, S., 2013. Culmination of low-dose pesticide effects. *Environ. Sci. Technol.* 47 (15), 8862–8868. <https://doi.org/10.1021/es401346d>.
- Liess, M., Liebmann, L., Vormeyer, P., Weisner, O., Altenburger, R., Borchardt, D., Brack, W., Chatzinotas, A., Escher, B., Foit, K., Gunold, R., Henz, S., Hitzfeld, K.L., Schmitt-Jansen, M., Kamjunke, N., Kaske, O., Knillmann, S., Krauss, M., Küster, E., Link, M., Reemtsma, T., 2021. Pesticides are the dominant stressors for vulnerable insects in lowland streams. *Water Res.* 201, 117262. <https://doi.org/10.1016/j.watres.2021.117262>.
- Lindermann, C., 2017. *Bericht zur Verbreitung der Rheinland-Pfälzischen Fledermäuse im Rahmen des FFH-Monitorings 2016*. NABU Rheinland-Pfalz.
- Lüdecke, D., Ben-Shachar, M.S., Patil, I., Waggoner, P., Makowski, D., 2021. Performance: an R package for assessment, comparison and testing of statistical models. *J. Open Source Softw.* 6 (60), 3139. <https://doi.org/10.21105/joss.03139>.
- Lundy, M., Montgomery, L., 2009. Summer habitat associations of bats between riparian landscapes and within riparian areas. *Eur. J. Wildl. Biol.* 56, 385–394.
- Machado, K.C., Grassi, M.T., Vidal, C., Pescara, I.C., Jardim, W.F., Fernandes, A.N., Sodré, F.F., Almeida, F.V., Santana, J.S., Canela, M.C., Nunes, C.R.O., Bichinho, K.M., Severo, F.J.R., 2016. A preliminary nationwide survey of the presence of emerging contaminants in drinking and source waters in Brazil. *Sci. Total Environ.* 572, 138–146. <https://doi.org/10.1016/j.scitotenv.2016.07.210>.
- Malaj, E., von der Ohe, P.C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci. U. S. A.* 111 (26), 9549–9554. <https://doi.org/10.1073/pnas.1321082111>.
- Manning, D.W., Sullivan, S.M.P., 2021. Conservation across aquatic-terrestrial boundaries: linking continental-scale water quality to emergent aquatic insects and declining aerial insectivorous birds. *Front. Ecol. Evol.* 9, 68. <https://doi.org/10.3389/fevo.2021.633160>.
- Marshall, M.M., Metzner, G.K., McCluney, K.E., 2022. Caffeine and canopy cover interact to alter biofilm nutrient content, benthic invertebrates, and insect emergence. *River Res. Appl.* 38, 863–872. <https://doi.org/10.1002/rra.3954>.
- McCrary, K.W., 2018. A review of sampling and monitoring methods for beneficial arthropods in agroecosystems. *Insects* 9 (4), 170. <https://doi.org/10.3390/insects9040170>.
- Mehl, C., Schoeman, M.C., Sanko, T.J., Bezuidenhout, C., Mienie, C.M., Preiser, W., Vosloo, D., 2021. Wastewater treatment works change the intestinal microbiomes of insectivorous bats. *PLoS One* 16, e0247475. <https://doi.org/10.1371/journal.pone.0247475>.
- Miller, J.L., Schmidt, T.S., Van Metre, P.C., Mahler, B.J., Sandstrom, M.W., Nowell, L.H., Carlisle, D.M., Moran, P.W., 2020. Common insecticide disrupts aquatic communities: a mesocosm-to-field ecological risk assessment of fipronil and its degradates in US streams. *Sci. Adv.* 6 (43), eabc1299. <https://doi.org/10.1126/sciadv.abc1299>.
- Mineau, P., Callaghan, C., 2018. *Neonicotinoid Insecticides and Bats: An Assessment of the Direct and Indirect Risks* (Canadian Wildlife Federation).
- Nelson, E.D., Do, H., Lewis, R.S., Carr, S.A., 2011. Diurnal variability of pharmaceutical, personal care product, estrogen and alkylphenol concentrations in effluent from a tertiary wastewater treatment facility. *Environ. Sci. Technol.* 45 (4), 1228–1234. <https://doi.org/10.1021/es102452f>.
- Neumann, M., Schulz, R., Schäfer, K., Müller, W., Mannheller, W., Liess, M., 2002. The significance of entry routes as point and non-point sources of pesticides in small streams. *Water Res.* 36 (4), 835–842. [https://doi.org/10.1016/S0043-1354\(01\)00310-4](https://doi.org/10.1016/S0043-1354(01)00310-4).
- Nissen, H., Krüger, F., Fichtner, A., Sommer, R.S., 2013. Local variability in the diet of Daubenton's bat (*Myotis daubentonii*) in a lake landscape of northern Germany. *Folia Zool.* 62 (1), 36–41. <https://doi.org/10.25225/fozo.v62.i1.a5.2013>.
- Ober, H.K., Hayes, J.P., 2008. Influence of vegetation on bat use of riparian areas at multiple spatial scales. *J. Wildl. Manag.* 72 (2), 396–404. <https://doi.org/10.2193/2007-193>.



- Oehler, K., Schreiner, V.C., Link, M., Liess, M., Schäfer, R.B., 2023. Land use changes biomass and temporal patterns of insect cross-ecosystem flows. *Glob. Chang. Biol.* 29 (1), 81–96. <https://doi.org/10.1111/gcb.16462>.
- Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M.D., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McClintock, D., Ouellette, M.-H., Cunha, E.R., Smith, T., Stier, A., Braak, C.J.F.T., Weedon, J., 2022. *vegan: Community Ecology Package*.
- O'Shea, T.J., Cryan, P.M., Hayman, D.T.S., Plowright, R.K., Streicker, D.G., 2016. Multiple mortality events in bats: a global review. *Mammal Rev.* 46 (3), 175–190. <https://doi.org/10.1111/mam.12064>.
- Paíga, P., Correia, M., Fernandes, M.J., Silva, A., Carvalho, M., Vieira, J., Jorge, S., Silva, J.G., Freire, C., Delerue-Matos, C., 2019. Assessment of 83 pharmaceuticals in WWTP influent and effluent samples by UHPLC-MS/MS: hourly variation. *Sci. Total Environ.* 648, 582–600. <https://doi.org/10.1016/j.scitotenv.2018.08.129>.
- Pebesma, E.J., 2004. Multivariable geostatistics in S: the gstat package. *Comput. Geosci.* 30, 683–691.
- Pilosof, S., Korine, C., Moore, M.S., Krasnov, B.R., 2014. Effects of sewage-water contamination on the immune response of a desert bat. *Mamm. Biol.* 79 (3), 183–188. <https://doi.org/10.1016/j.mambio.2013.10.005>.
- Polis, G.A., Anderson, W.B., Holt, R.D., 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annu. Rev. Ecol. Syst.* 289–316.
- Power, M.E., Rainey, W.E., Parker, M.S., Sabo, J.L., Smyth, A., Khandwala, S., Finlay, J. C., McNeely, F.C., Marsee, K., Anderson, C., 2004. River-to-watershed subsidies in an old-growth conifer forest. In: Polis, Gary A and Power, Mary E and Huxel, Gary R (Eds.), *Food Webs at the Landscape Level*. University of Chicago Press: Chicago, IL, USA, pp. 217–240.
- Previšić, A., Vilenica, M., Vučković, N., Petrović, M., Rožman, M., 2021. Aquatic insects transfer pharmaceuticals and endocrine disruptors from aquatic to terrestrial ecosystems. *Environ. Sci. Technol.* 55, 3736–3746. <https://doi.org/10.1021/acs.est.0c07609>.
- QGIS Development Team, 2023. QGIS Geographic Information System. QGIS Association. <http://www.qgis.org>.
- R Core Team, 2022. *R: A Language and Environment for Statistical Computing*. Austria, Vienna.
- Rabiet, M., Margoum, C., Gouy, V., Carluier, N., Coquery, M., 2010. Assessing pesticide concentrations and fluxes in the stream of a small vineyard catchment-effect of sampling frequency. *Environ. Pollut.* 158 (3), 737–748. <https://doi.org/10.1016/j.envpol.2009.10.014>.
- Raitif, J., Plantegenest, M., Agator, O., Piscart, C., Roussel, J.-M., 2018. Seasonal and spatial variations of stream insect emergence in an intensive agricultural landscape. *Sci. Total Environ.* 644, 594–601. <https://doi.org/10.1016/j.scitotenv.2018.07.021>.
- Richmond, E.K., Rosi, E.J., Walters, D.M., Fick, J., Hamilton, S.K., Brodin, T., Sundelin, A., Grace, M.R., 2018. A diverse suite of pharmaceuticals contaminates stream and riparian food webs. *Nat. Commun.* 9, 4491. <https://doi.org/10.1038/s41467-018-06822-w>.
- Roodt, A.P., Röder, N., Pietz, S., Kolbenschlager, S., Manfrin, A., Schwenk, K., Bundschuh, M., Schulz, R., 2022. Emerging midges transport pesticides from aquatic to terrestrial ecosystems: importance of compound-and organism-specific parameters. *Environ. Sci. Technol.* 56 (9), 5478–5488. <https://doi.org/10.1021/acs.est.1c08079>.
- Roodt, A.P., Schaufelberger, S., Schulz, R., 2023a. Aquatic-terrestrial insecticide fluxes: midges as neonicotinoid vectors. *Environ. Toxicol. Chem.* 42 (1), 60–70. <https://doi.org/10.1002/etc.5495>.
- Roodt, A.P., Huszarik, M., Entling, M.H., Schulz, R., 2023b. Aquatic-terrestrial transfer of neonicotinoid insecticides in riparian food webs. *J. Hazard. Mater.* 455, 131635. <https://doi.org/10.1016/j.jhazmat.2023.131635>.
- Roswag, A., Becker, N.I., Drangusch, R., Kuhring, K., Ohlendorf, B., Encarnação, J.A., 2019. Teasing apart cryptic species groups: nutritional ecology and its implications for species-specific conservation of the *Myotis mystacinus* group. *Popul. Ecol.* 61 (1), 14–24. <https://doi.org/10.1002/1438-390X.1003>.
- Russ, J. (Ed.), 2021. *Bat Calls of Britain and Europe: A Guide to Species Identification*. Pelagic Publishing.
- Russo, D., Ancillotto, L., Cistrone, L., Korine, C., 2016. The buzz of drinking on the wing in echolocating bats. *Ethology* 122 (3), 226–235. <https://doi.org/10.1111/eth.12460>.
- Rydell, J., Nyman, S., Eklöf, J., Jones, G., Russo, D., 2017. Testing the performances of automated identification of bat echolocation calls: a request for prudence. *Ecol. Indic.* 78, 416–420. <https://doi.org/10.1016/j.ecolind.2017.03.023>.
- Schäfer, R.B., Gerner, N., Kefford, B.J., Rasmussen, J.J., Beketov, M.A., de Zwart, D., Liess, M., von der Ohe, P.C., 2013. How to characterize chemical exposure to predict ecologic effects on aquatic communities? *Environ. Sci. Technol.* 47 (14), 7996–8004. <https://doi.org/10.1021/es4014954>.
- Schanzer, S., Koch, M., Kiefer, A., Jentke, T., Veith, M., Bracher, F., Bracher, J., Müller, C., 2022. Analysis of pesticide and persistent organic pollutant residues in German bats. *Chemosphere* 305, 135342. <https://doi.org/10.1016/j.chemosphere.2022.135342>.
- Scharmüller, A., Schreiner, V.C., Schäfer, R.B., 2020. Standartox: standardizing toxicity data. *Data* 5 (6), 46. <https://doi.org/10.3390/data5020046>.
- Schneeweiss, A., Schreiner, V.C., Reemtsma, T., Liess, M., Schäfer, R.B., 2022. Potential propagation of agricultural pesticide exposures and effects to upstream sections in a biosphere reserve. *Sci. Total Environ.* 836, 155688. <https://doi.org/10.1016/j.scitotenv.2022.155688>.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675. <https://doi.org/10.1038/nmeth.2089>.
- Scott, S., McLaren, G., Jones, G., Harris, S., 2010. The impact of riparian habitat quality on the foraging and activity of pipistrelle bats (*Pipistrellus* spp.). *J. Zool.* 280 (4), 371–378. <https://doi.org/10.1111/j.1469-7998.2009.00670.x>.
- Stahlschmidt, P., Pätzold, A., Ressler, L., Schulz, R., Brühl, C.A., 2012. Constructed wetlands support bats in agricultural landscapes. *Basic Appl. Ecol.* 13 (2), 196–203. <https://doi.org/10.1016/j.baee.2012.02.001>.
- Stehle, S., Schulz, R., 2015. Agricultural insecticides threaten surface waters at the global scale. *Proc. Natl. Acad. Sci.* 112 (18), 5750–5755. <https://doi.org/10.1073/pnas.1500232112>.
- Teerlink, J., Hernandez, J., Budd, R., 2017. Fipronil washoff to municipal wastewater from dogs treated with spot-on products. *Sci. Total Environ.* 599, 960–966. <https://doi.org/10.1016/j.scitotenv.2017.04.219>.
- Todd, V.L., Williamson, L.D., 2019. Habitat usage of Daubenton's bat (*Myotis daubentonii*), common pipistrelle (*Pipistrellus pipistrellus*), and soprano pipistrelle (*Pipistrellus pygmaeus*) in a North Wales upland river catchment. *Ecol. Evol.* 9 (8), 4853–4863. <https://doi.org/10.1002/ece3.5085>.
- Torquetti, C.G., Guimarães, A.T.B., Soto-Blanco, B., 2020. Exposure to pesticides in bats. *Sci. Total Environ.* 755 (1), 142509. <https://doi.org/10.1016/j.scitotenv.2020.142509>.
- Tuneu-Corral, C., Puig-Montserrat, X., Riba-Bertolin, D., Russo, D., Rebelo, H., Cabeza, M., López-Baucells, A., 2023. Pest suppression by bats and management strategies to favour it: a global review. *Biol. Rev. (Early View)*. <https://doi.org/10.1111/brv.12967>.
- U.S. Environmental Protection Agency, 2021. ECOTOX User Guide: ECOTOXicology Knowledgebase System [WWW Document]. URL. <https://www.epa.gov/ecotox/> (accessed 04.2021).
- Van Klink, R., Bowler, D.E., Gongalsky, K.B., Swengel, A.B., Gentile, A., Chase, J.M., 2020. Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. *Science* 368, 417–420. <https://doi.org/10.1126/science.aax9931>.
- Vaughan, N., Jones, G., Harris, S., 1996. Effects of sewage effluent on the activity of bats (Chiroptera: Vespertilionidae) foraging along rivers. *Biol. Conserv.* 78 (3), 337–343. [https://doi.org/10.1016/S0006-3207\(96\)00009-2](https://doi.org/10.1016/S0006-3207(96)00009-2).
- Vesterinen, E.J., Puisto, A.I., Blomberg, A.S., Lilley, T.M., 2018. Table for five, please: dietary partitioning in boreal bats. *Ecol. Evol.* 8 (22), 10914–10937. <https://doi.org/10.1002/ece3.4559>.
- Villarroya-Villalba, L., Casanelles Abella, J., Moretti, M., Pinho, P., Samson, R., Van Mensel, A., Chiron, F., Zellweger, F., Obrist, M., 2021. Response of bats and nocturnal insects to urban green areas in Europe. *Basic Appl. Ecol.* 51, 59–70. <https://doi.org/10.1016/j.baee.2021.01.006>.
- Voigt, C.C., Kingston, T., 2016. *Bats in the Anthropocene: Conservation of Bats in a Changing World*. Springer Nature.
- Warren, R.D., Waters, D.A., Altringham, J.D., Bullock, D.J., 2000. The distribution of Daubenton's bats (*Myotis daubentonii*) and pipistrelle bats (*Pipistrellus pipistrellus*) (Vespertilionidae) in relation to small-scale variation in riverine habitat. *Biol. Conserv.* 92 (1), 85–91. [https://doi.org/10.1016/S0006-3207\(99\)00062-2](https://doi.org/10.1016/S0006-3207(99)00062-2).
- Weisner, O., Frische, T., Liebmann, L., Reemtsma, T., Roß-Nickoll, M., Schäfer, R.B., Schäfer, A., Scholz-Starke, B., Vormeier, P., Knillmann, S., Liess, M., 2021. Risk from pesticide mixtures: the gap between risk assessment and reality. *Sci. Total Environ.* 796, 149017. <https://doi.org/10.1016/j.scitotenv.2021.149017>.
- Weston, D.P., Lydy, M.J., 2014. Toxicity of the insecticide fipronil and its degradates to benthic macroinvertebrates of urban streams. *Environ. Sci. Technol.* 48 (2), 1290–1297. <https://doi.org/10.1021/es4045874>.
- Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
- Wolfram, J., Bub, S., Petschick, L.L., Schemmer, A., Stehle, S., Schulz, R., 2023. Pesticide occurrence in protected surface waters in nature conservation areas of Germany. *Sci. Total Environ.* 858 (3), 160074. <https://doi.org/10.1016/j.scitotenv.2022.160074>.
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., others, 2009. *Mixed Effects Models and Extensions in Ecology With R*. Springer.