STATISTICAL ECO(-TOXICO)LOGY

IMPROVING THE UTILISATION OF DATA FOR ECOLOGICAL RISK ASSESSMENT

by

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1 INTRODUCTION AND OBJECTIVES

THREATS TO FRESHWATER ECOSYSTEMS FROM CHEMICAL POLLUTION

Freshwater ecosystems, like streams, lakes and wetlands, make up only 0.01% of the World's water and cover only 0.8% of Earth's surface (Dudgeon et al., 2006), yet they host an important component of global biodiversity. Freshwaters are a habitat for more than 125,000 species, which represents 10% of global biodiversity and ⅓ of all vertebrate species (Balian et al., 2007; Strayer and Dudgeon, 2010) and provide essential services for human well-being (Aylward et al., 2005). Small water bodies are of particular importance, because of their high abundance (Downing et al., 2012), the high biodiversity they host (Davies et al., 2008) and the ecosystem services they provide (Biggs et al., 2016).

The earth is currently experiencing a functional change driven by human activities which are so far-reaching, that a new geological epoch "Anthropocene" has been proposed (Steffen et al., 2011; Waters et al., 2016). Consequently, these changes are also associated with biotic changes: 65% of rivers are currently at threat (Vörösmarty et al., 2010), 21% of 27,516 assessed freshwater species are currently threatened with extinction (IUCN, 2016) and freshwaters are the ecosystem experiencing the greatest losses of biodiversity (WWF, 2016). A multitude of stressors contribute to this deterioration of freshwater biodiversity including habitat loss and degradation, overexploitation, invasive species and pollution (Dudgeon et al., 2006; Vörösmarty et al., 2010; WWF, 2016). Studies investigating water pollution have mainly focused on nutrient loading, acidification and pollution by organic loading (Schäfer et al., 2016). However, chemicals have become ubiquitous throughout humankind. Currently, more than 100,000 chemicals are registered and in daily use (Schwarzenbach et al., 2010; Schwarzman and Wilson, 2009). These substances will ultimately end somewhere in the environment.

Despite their potential negative effects on biota and humans and their intentional release, pesticides have been neglected in the past by ecological studies investigating threats to freshwaters (Schäfer et al., 2016) and it is unknown how

much they contribute to biodiversity loss (Persson et al., 2013; Rockström et al., 2009). However, recent studies indicated that pollution by pesticides may be a frequent threat to freshwaters that might have been neglected by ecological studies in the past. Malaj et al. (2014) showed that almost half of European water bodies are at risk from pesticides. In the United States, Stone et al. (2014) showed that 61% of assessed agricultural streams exceed aquatic-life benchmarks. On a global scale, Stehle and Schulz (2015) found that 52.4% of detected insecticide concentrations (n = 11,300) exceeded risk thresholds. The high contact with adjacent land and low water volume of small streams make them particularly vulnerable to pesticide pollution (Biggs et al., 2016), however, there is currently a lack of data on pesticide pollution of small streams (Lorenz et al., 2016).

As a reaction to the degradation of freshwaters, several legal frameworks have been established to safeguard and improve the quality of freshwater ecosystems. In the European Union (EU), the Water Framework Directive (WFD) (European Union, 2000) regulates the protection of aquatic ecosystems and commits the member states to achieve a 'good' status of all water bodies. Knowing of the toxicity of pesticides and their intentional release into the environment, also the introduction and use of new pesticides are highly regulated. Sophisticated environmental risk assessment procedures have been developed and are requested by the EU (European Union, 2009) to ensure that the use of pesticides does not cause unacceptable effects to non-target organism, soil, air and water.

ENVIRONMENTAL RISK ASSESSMENT

Environmental risk assessment (ERA) tries to estimate risks to animals, populations or ecosystems. It investigates if a chemical can be used as intended without causing detrimental impacts to the environment. Moreover, ERA is used as a tool to support decision making under uncertainty (Newman, 2015). Environmental risk is defined as a combination of the severity and the probability of occurrence of a potential adverse effect on the environment (Suter, 2007). Therefore, ERA is based on two components: Effect- and exposure assessment. A combination of both is needed to characterise environmental risks.

Effect assessment characterises the strength of effects using laboratory and semi-field experiments. It establishes relationships between the concentration of a compound and the observed effects. In the European Union a tiered approach with increasing complexity and realism. Lower tier assessment is based on highly standardised single species laboratory experiments, whereas higher

tier assessment is refined by testing additional species, extended laboratory experiments or model ecosystem experiments (Brock et al., 2006). To address the various uncertainties in effect assessment (e.g. experimental variation, variation between species, variation in environmental conditions etc.) the retrieved toxicity values are divided by an assessment factor (AF) between 100 (lower tier assessment) and 2 (higher tier assessment) depending on data quality, which yields to a regulatory acceptable concentration (RAC) (Brock et al., 2006; EFSA, 2013).

Exposure Assessment for freshwaters aims to characterise the probability of an adverse effect by deriving a predicted environmental concentration (PEC) in surface waters and sediments (Newman, 2015). It is mainly based on modelling the fate of chemicals in the environment using computer simulations. In the European Union, the FOCUS models are used (EFSA, 2013; FOCUS, 2001). To calculate PECs these models need many compound specific input parameters like the molecular weight, water solubility, partitioning coefficients and dissipation time. Additionally, information on the application regime and crop type is needed. FOCUS models the concentration within edge-of-field streams of 1 meter width (corresponding a catchment size of approx. 7km², see Figure **??**) and 30 cm depth (Erlacher and Wang, 2011). Nevertheless, recent research showed that FOCUS models fail to predict measured field concentrations of pesticides (Knäbel et al., 2014; Knäbel et al., 2012).

The final step in ERA is risk characterisation. It puts together the information gained from effect and exposure assessment. Risk can be expressed in several ways, a quantitative way being the risk quotient approach: If the ratio PEC / RAC exceeds a value of one potential risks cannot be rebutted (EFSA, 2013; Solomon et al., 2000; Suter, 2007). Consequently, pesticides can be authorised only if the risk quotient is below one indicating that harmful effects are unlikely.

ENVIRONMENTAL MONITORING

Widespread anthropogenic activities and the induced environmental changes have resulted in concerns about the state of the environment and have led to the development of environmental monitoring programs worldwide (Nichols and Williams, 2006). After authorization, pesticides applied on agricultural fields may enter aquatic ecosystems via diffuse sources like spray-drift, surface runoff or drainage (Carter, 2000; Liess et al., 1999; Schulz, 2004; Stehle et al., 2013). These entered pesticides may have ecological effects and worsen the chemical

status, acting contrary to the goal of the WFD. For monitoring the progress towards the goal of a 'good' status and for assessment of the chemical status of surface waters the EU WFD established monitoring requirements for all European river basins (European Union, 2000). For chemical monitoring the WFD requires grab sampling and chemical analysis of 21 priority substances (of which 7 are pesticides) every third month and of 24 other pollutants (of which 12 are used as pesticides) every month and derived for these environmental quality standards (EQS) (European Union, 2013). Additionally, 14 substances (of which 8 are used as pesticides, including all Neonicotinoids) that may pose a significant risk, have an insufficient data basis and are candidates for future priority substances are currently monitored until 2019 (European Union, 2015). Nevertheless, monitoring programs on a national scale might monitor a broader spectrum of chemical substances, e.g. for investigative monitoring. Recent studies indicate that the current sampling and chemical analyses strategy greatly underestimate the pesticide exposure (Moschet et al., 2014; Stehle et al., 2013; Xing et al., 2013).

Environmental monitoring produces humongous amounts of data containing information on pesticide concentrations in the field on a large under many conditions. Therefore, it can be complementary to ERA (Suter, 2007). Moreover, data from long-term monitoring programs can be used to study hypotheses about spatial and temporal dynamics and interactions, that are not evident from short term and short scale studies (Gitzen, 2012) and provide insights modelling approaches. If the environmental risk assessment process captured all relevant sources of risk, no concentrations above the derived RAC should be observable in European rivers. Therefore, monitoring data could be used to provide feedback for ERA after approval (Knauer, 2016). The WFD has its main focus on large water bodies >10 km^2 catchmentsize (European Union, 2000), whereas ERA has its focus on small water bodies <10 km^2 (Brock et al., 2006; European Union, 2009). However, at present little is known on pesticide concentrations in small streams comparable to those assessed in ERA (Biggs et al., 2016; Lorenz et al., 2016).

STATISTICAL ECOTOXICOLOGY

Environmental effect assessment generates data on ecological effects using experiments. The produced datasets range from small univariate datasets (lower tier assessment) to medium sized multivariate datasets (higher tier assessment).

In order to extract usable information for assessment, these datasets are analysed using statistical techniques and therefore, statistics are crucial for effect assessment (Newman, 2012). Statistical ecotoxicology combines statistics with the specific needs and constraints of ecotoxicology. Ecotoxicologists deal generally with low replicated experiments, making statistical inference difficult (Van Der Hoeven, 1998). For example, a recent analysis of eleven mesocosm studies revealed that the sample sizes for these kind of experiments range between two and five. Statistical ecotoxicology aims to provide solutions to statistical challenges in ecotoxicology (Fox and Landis, 2016a), guidance on experimental designs (Johnson et al., 2015) and tools to integrate big data (Van den Brink et al., 2016). The ultimate goal is to improve the accuracy of ERA.

The relationships between the concentration of a compound and the observed effects are usually analysed using dose-response models, which can be used to derive an effective concentration for x% effect (EC_x) (Ritz, 2010). Nevertheless, such relationships cannot always be established from experimental data. For example, mesocosm experiments are conducted to characterise effects on whole biological communities. However, because of multivariate responses and potential indirect effects, there is no clear dose-response relationship and no models for this kind of data available. There are also examples were fitting dose-response models is problematic (Green, 2016). In such cases, there is usually a no-observed-effect concentration (NOEC) computed.

The NOEC is the highest tested concentration that does not lead to significant deviation from the control response and therefore relies on null hypothesis significance testing (NHST). However, the use of NOEC as a toxicity measure in environmental effect assessment has been heavily criticised in the past (Chapman et al., 1996; Fox et al., 2012; Fox and Landis, 2016b; Jager, 2012; Laskowski, 1995; Warne and van Dam, 2008). One such critic is the low statistical power for NHST in common ecotoxicological experiments (Van Der Hoeven, 1998). *A priori* power calculations can provide useful guidance for choosing experimental designs (Johnson et al., 2015), but are rarely used by ecotoxicologists (Newman, 2008).

Instead of conducting experiments, toxicity could be also predicted from molecular structures using quantitative structure-activity relationships (QSAR), which are usually calculated using machine-learning techniques (Cortes-Ciriano, 2016; Murrell et al., 2015). Nevertheless, in order to improve and validate these models to give sufficient prediction accuracy more data from experiments is needed (Kühne et al., 2013). Indeed, a large amount of data is available that

could be used for effect and exposure assessment. For example, the US EPA ECOTOX database (U.S. EPA, 2016), the Pesticides Properties Database (Lewis et al., 2016) and ETOX (Umweltbundesamt, 2016) provide toxicity data that could be used for effect assessment. Databases like Physprop (Howard and Meylan, 2016) and PubChem (Kim et al., 2016) provide chemical properties that are needed as input for exposure models. Monitoring data provides information on realised concentrations, could be used for validation of models and retrospective risk assessment. This "big data" can provide new information and opportunities for ERA (Dafforn et al., 2015). However, it needs to be harmonised, linked and easily accessible in order to be used effectively in ERA.

OBJECTIVES AND OUTLINE OF THE THESIS

The overall goal of this thesis was to contribute to the emerging field of statistical ecotoxicology, environmental risk assessment and environmental monitoring. The main objectives were (i) to scrutinise new methods in statistical ecotoxicology, (ii) explore available monitoring data and (iii) provide tools to deal with big data. Figure 1.1 provides a conceptual overview on ERA and environmental monitoring as outlined in the previous sections, as well as the parts considered in this thesis and the relations between them.

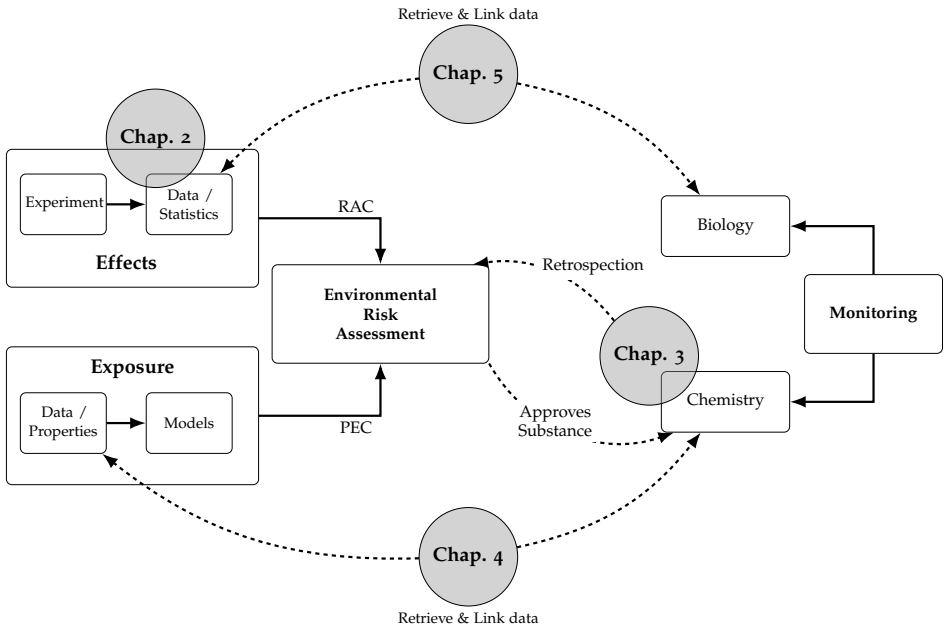

Figure 1.1: Conceptual overview on environmental risk assessment, environmental monitoring and the parts addressed by this thesis.

The thesis starts with a comparison of statistical methods to analyse ecotoxicological experiments using NHST in effect assessment (Chapter **??**). Specific questions addressed were:

- Are newer statistical methods, explicitly considering the type of analysed data, more powerful than currently used methods for NHST?
- How much statistical power do current experimental designs in ecotoxicology exhibit?

Risk assessment procedures in the European Union has it main focus on small waterbodies adjacent to agricultural fields where plant protection products are applied. Therefore, chapter 2 focuses on measured large-scale environmental concentrations in small streams, the drivers thereof and comparison with RACs derived from ERA. Specific goals of this study were:

- Compile monitoring data on pesticides in small streams in Germany and check if the available data is suitable to inform ERA.
- Explore the relationship between agricultural land use and stream size and RAC exceedances.
- Scrutinise the annual dynamics of pesticide exposure, as well as the influence of precipitation on measured pesticide concentrations.
- We use RACs derived from ERA to assess the current pollution in German streams and identify pesticides exhibiting currently a risk to freshwaters.

The compilation of monitoring data from different data sources in Chapter 2, resulted in a big inhomogeneous amount of data. Moreover, Biologists, Chemists and ecotoxicologists face similar problems with the need to identify and harmonise their biological and chemical data. Chapters **??** (chemical data) and **??** (biological data) describe software solutions to simplify and accelerate the workflow of:

- validating and harmonising chemical and taxonomic data
- linking datasets from different databases
- retrieving properties and identifiers

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2 LARGE SCALE RISKS FROM PESTICIDES IN SMALL STREAMS

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ABSTRACT

Small streams are important refugia for biodiversity. In agricultural areas they may be at high risk from pesticide pollution. However, most related studies have been limited to a few streams on the regional level, hampering extrapolation to larger scales. We used data from German governmental water quality monitoring to quantify the drivers of pesticide risk and to assess pesticide risk in small streams on a large scale. The data set comprised of 1,766,104 measurements of 478 pesticides (including metabolites) related to 24,743 samples from 2,301 sampling sites. We investigated the influence of agricultural land use, catchment size, as well as precipitation and seasonal dynamics on pesticide risk using new statistical modelling techniques that explicitly consider the limit of quantification. Agricultural land use lead to a 3.7-fold increase in exceedance of risk thresholds when the proportion of agriculture in a catchment exceeded 28 percent. Precipitation increased pesticide risk by 36% and risk was the highest during summer months. Risk thresholds were exceeded in 26% of streams, with the highest risk related to neonicotinoid insecticides. We conclude that pesticides from agricultural land use are a major threat to small streams and their biodiversity and that a realistic pesticide sampling would be driven by precipitation events.

INTRODUCTION

More than 50% of the total land area in Germany is used by agriculture (Statistisches Bundesamt, 2014). In the year 2014 more than 45,000 tonnes of 776 authorised plant protection products were sold for application on this area (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2015). The applied pesticides may enter surface waters via spray-drift, edge-of-field run-off or drainage (Liess et al., 1999; Schulz, 2001; Stehle et al., 2013). Once entered the surface waters they may have adverse effects on biota and ecosystem functioning (Schäfer et al., 2012). Although it is known that pesticide pollution and its ecological effects increase with the fraction of agricultural land use in the catchment (Schulz, 2004), the shape of the relationship is unknown and studies on potential thresholds are lacking.

Two recent studies indicate that pesticides might threaten freshwater biodiversity in the European union. Malaj et al. (2014) analysed data supplied to the European Union (EU) in the context of the Water Framework Directive (WFD)

and showed that almost half of European water bodies are at risk from pesticides. Stehle and Schulz (2015b) compiled 1,566 measured concentrations of 23 insecticides in the EU from scientific publications. They found that many of these measurements exceed regulatory acceptable concentrations (RAC). However, these studies reflect only a small amount of potentially available data (173 sites in predominantly mid-sized and large rivers in Malaj et al. (2014) and 138 measurements in Stehle and Schulz (2015b)), and it is unclear how representative they are for Germany. Much more comprehensive data on thousands of sites are available from national monitoring programs that are setup for the surveillance of water quality, which is done independently by the federal states in Germany in compliance with the WFD (Quevauviller et al., 2008) and additional state-specific needs. Despite that these data are providing the opportunity to study pesticide risks and other research questions on a large scale with high spatial density, to date these data have not been compiled and related analyses are lacking.

Small streams comprise a major fraction of streams (Nadeau and Rains, 2007), accommodate a higher proportion of biodiversity compared to larger freshwater systems (Biggs et al., 2014; Davies et al., 2008) and play an important role in the recolonization of disturbed downstream reaches (Liess and von der Ohe, 2005; Orlinskiy et al., 2015). Nevertheless, a clear definition of small streams in terms of catchment or stream size is currently lacking (Lorenz et al., 2016). For example, the WFD defines small streams with a catchment size between 10 and 100 km², without further categorisation of streams <10km² and Lorenz et al. (2016) defines small streams with catchment size <10km². Moreover, small streams might particularly be at high risk of pesticide contamination in case of adjacent agricultural areas given their low dilution potential (Liess et al., 1999; Schulz, 2004). Indeed, meta-analyses using data from studies with a few sites reported higher pesticide pollution in smaller streams compared to bigger streams (Schulz, 2004; Stehle and Schulz, 2015b). Despite their ecological relevance and potentially higher pesticide exposure, a recent analysis of pesticide studies showed that a disproportionally small fraction of studies was conducted in small water bodies, and these were largely limited to a few sites (Lorenz et al., 2016). Consequently, knowledge on the pesticide pollution of small streams on larger scales is scant. In European law, the Directive 2009/128/EC (European Union, 2009) places an obligation on the EU Member States to adopt National Action Plans (NAP) for the Sustainable Use of Plant Protection Products and the

German NAP also addresses the knowledge gap concerning pesticide impact on small streams, specifically including those with catchment size <10km².

In this study, we compiled and analysed large-scale chemical monitoring data from small streams in Germany. First, we analysed the shape of the relationship between pesticide risk, agricultural land use, and catchment size and examined whether related thresholds for pesticide risks can be derived. Second, we investigated the influence of precipitation and seasonal dynamics on pesticide detections, given that precipitation proved an important driver of pesticide exposure in several small-scale studies (Schulz, 2004; Wittmer et al., 2010), but it is unknown whether a precipitation signal prevails on large scales. Finally, we quantified the current risks from pesticides in small streams in Germany and the compounds accountable for the risk.

METHODS

Data compilation

We queried pesticide monitoring data from sampling sites that can be classified as small streams (catchment sizes < 100 km² according to the WFD) from all 13 non-city federal states of Germany (see Supplemental Table S1 for the abbreviations of federal state names) for 2005 to 2015. We homogenised and unified all data provided by the federal states into a database and implemented a robust data-cleaning workflow (see Supplemental Figure S1 for details) (Poisot, 2015).

We identified precipitation at sampling sites by a spatio-temporal intersection of sampling events with gridded daily precipitation data (60×30 arcsec resolution) available from the German Meteorological Service (DWD). This data spatially interpolates daily precipitation values from local weather stations (Rauthe et al., 2013). We performed the intersection for the actual sampling date and the day before and extracted precipitation during and up to 48 hours before sampling.

Characterization of catchments

We compiled a total of 2,369 sampling sites in small streams with pesticide measurements. Alongside, we also queried catchment sizes and agricultural land use within the catchment for the sampling sites from the federal states. Catch-

ment size was provided for 59% of sites. Additionally, we delineated upstream catchments for each of the sampling sites using (i) a digital elevation model (DEM) (EEA, 2013) and the multiple flow direction algorithm (Holmgren, 1994) as implemented in GRASS GIS 7 (Neteler et al., 2012) and (ii) from drainage basins provided by the Federal Institute of Hydrology (BfG). Delineated catchments were visually checked for accuracy by comparison with state stream networks and derived information amalgamated with existing data. Thus, catchment size information was available for 99% of all sites (59% from authorities, 24% from DEM and 16% from drainage basins).

For each derived catchment (either from DEM or drainage basins) we calculated the % agricultural land-use within the catchment based on the Authoritative Topographic-Cartographic Information System (ATKIS) of the land survey authorities (AdV, 2016). Thus, agricultural land use information was available for 98% of all sites (24% from authorities, 52% from DEM and 22% from drainage basins). 68 sites (3%) that lacked catchment size or land use information were omitted from the analysis, resulting in 2301 sites used in the analyses outlined below.

Characterization of pesticide pollution

We characterised pesticide pollution using regulatory acceptable concentrations (RAC) (Brock et al., 2010). RACs are derived during pesticide authorisation as part of the ecological risk assessment. No unacceptable ecological effects are expected if the environmental concentration remains below this concentration. Stehle and Schulz (2015b) showed that RAC exceedances reflect a decrease in biodiversity and from this perspective are ecologically relevant indicators. The German Environment Agency (UBA) provided RACs for 107 compounds, including those with the highest detection rates (Supplemental Table S2). Based on theses RACs, we calculated Risk Quotients (RQ):

$$RQ_i = \frac{C_i}{RAC_i} \tag{2.1}$$

where C_i is the concentration of a compound i in a sample and RAC_i the respective RAC.

Statistical analyses

All data-processing and analyses were performed using R (R Core Team, 2016). To display differences in the spectra of analysed compounds between federal states we used Multidimensional Scaling (MDS) based on Jaccard dissimilarity in conjunction with complete linkage hierarchical clustering using the vegan package (Oksanen et al., 2016). We determined the optimum number of clusters using the average silhouette width (Rousseeuw, 1987).

We expected non-linear responses to agriculture and catchment size and therefore, used generalised additive models (GAM) to establish relationships (Fewster et al., 2000). We modelled the number of RAC exceedances (RQ >1) at a site as:

$$\begin{aligned} No(RQ > 1)_i &\sim NB(\mu_i, \kappa) \\ \log(\mu_i) &= \beta_0 + f_1(agri_i) + f_2(size_i) + \log(n_i) \end{aligned} \tag{2.2}$$

where $No(RQ > 1)_i$ is the observed number of RAC exceedances at site i. We modelled $No(RQ > 1)_i$ as resulting from a negative binomial distribution (NB) with mean μ_i and a quadratic mean-variance-relationship ($Var(No(RQ > 1)_i) = \mu_i + \frac{\mu_i^2}{\kappa}$). The proportion of agriculture within the catchment ($agri_i$) and the catchment size of the site ($size_i$) were used as predictors of the number of RAC exceedances. β_0 is the intercept and f_1 and f_2 are smoothing functions using penalized cubic regression splines (Wood, 2006). The degree of smoothness was estimated using restricted maximum likelihood (REML) during the model fitting process (Wood, 2011). The number of measurements per site (n_i) was used as an offset to account for differences in sampling efforts (sampling interval and analysed compound spectrum) at a site and is equivalent to modelling the rate of exceedances. We used point-wise 95% Confidence Intervals (CI) of the first derivative of the fitted smooth to identify regions of statistically significant changes. GAMs were fitted using the mgcv package (Wood, 2011).

To assess the influence of precipitation and seasonality, we modelled the RQ of individual compounds as the response variable. RQ and concentrations show a skewed distribution with an excess of zeros (no pesticides detected and quantified). Therefore, we modelled these as two processes (one generating values below the limit of quantification (LOQ) and one generating values above LOQ) using a Zero-Adjusted Gamma (ZAGA) distribution (Rigby and Stasinopoulos, 2005; Stasinopoulos et al., 2016) (Equation 2.3). These two processes can be in-

terpreted as changes in the mean value of RQ (change in μ) and changes in the probability of exceeding LOQ and showing any risk (change in ν).

$$RQ_i \sim ZAGA(\mu_i, \sigma, \nu_i) = \begin{cases} (1 - \nu_i) & \text{if } y < LOQ \\ \nu_i \times f_{Gamma}(\mu_i, \sigma) & \text{if } y \geqslant LOQ \end{cases} \tag{2.3}$$

ν_i denotes the probability of a measurement i being above LOQ and f_{Gamma} denotes the gamma function and is used for values equal to or greater LOQ, with μ being the mean and σ the standard deviation of RQ. We used the $\log(x + 0.05)$ transformed precipitation at sampling date ($\log prec_0$) and the day before ($\log prec_{-1}$), as well as quarters of the year ($Q1 - Q4$) as linear predictors for μ and ν. We used appropriate link functions for μ and ν and assumed σ to be constant. Equation 2.4 summarises the deterministic part of the model for a measurement i.

$$\begin{aligned} \log(\mu_i) &= \log prec_{0i} + \log prec_{-1i} + Q1_i + Q2_i + Q3_i + Q4_i \\ logit(\nu_i) &= \log prec_{0i} + \log prec_{-1i} + Q1_i + Q2_i + Q3_i + Q4_i \end{aligned} \tag{2.4}$$

To account for temporal autocorrelation and differences between federal states we used $site$ nested within $state$ as random intercepts. We implemented this model using the gamlss package (Stasinopoulos and Rigby, 2007).

We fitted this model separately to each compound with a RAC, measured in at least 1000 samples and with more than 5% of values above LOQ (n = 22 compounds, see Supplemental Table S3 for a list of compounds). To summarise the coefficients across the 22 modelled compounds we used a random effect meta-analysis for each model coefficient separately (Harrison, 2011), resulting in an averaged effect of the 22 compounds. The results of individual compounds are provided in the Supplemental Table S4 and Figure S7. The meta-analysis was performed using the metafor package (Viechtbauer, 2010).

RESULTS

Overview of the compiled data

The compiled dataset used for analysis comprised 1,766,104 pesticide measurements in 24,743 samples from 2,301 sampling sites in small streams. These samples were all taken via grab sampling. We found large differences between federal states in the number of sampling sites and their spatial distribution (Figure 2.1 and Supplemental Table S1). The number of small stream sampling sites per state ranged from 1 (Lower Saxonia, NI) to 1139 (North Rhine-Westphalia, NW). No data were available from Brandenburg.

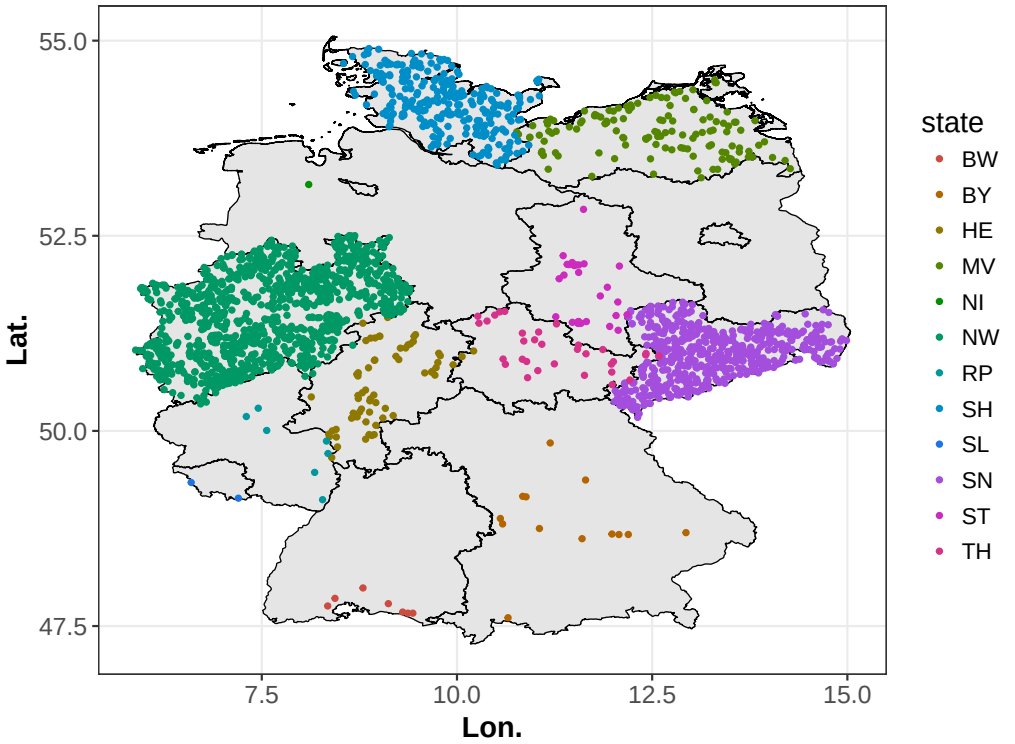

Figure 2.1: Spatial distribution of the 2,301 small stream sampling sites. Colour codes different federal states (see Supplemental Table S1 for abbreviations).

In total 478 different compounds used as pesticides and their metabolites were measured at least once (Supplemental Table S2). Most of the compounds were herbicides (179), followed by insecticides (117) and fungicides (109). Most samples were taken in the months April till October, while fewer samples were taken during winter (see Supplemental Figure S2). We found substantial differences in the spectra of analysed pesticides between federal states (Figure 2.2). The number of different pesticides per state ranged from 57 (SL) to 236 (RP) (Supplemental Table S1). Hierarchical clustering revealed that RP and NI analysed distinct compound spectra compared to the cluster of other states. However, it has to be noted that both states surveyed these distinct spectra in special monitoring programs from only a few sites. Although there was high variability within the remaining cluster, this could not be further split (Figure 2.2, also Supplemental Figures S3 and S4). 4% (=71,113) of all measurements were concentrations above LOQ.

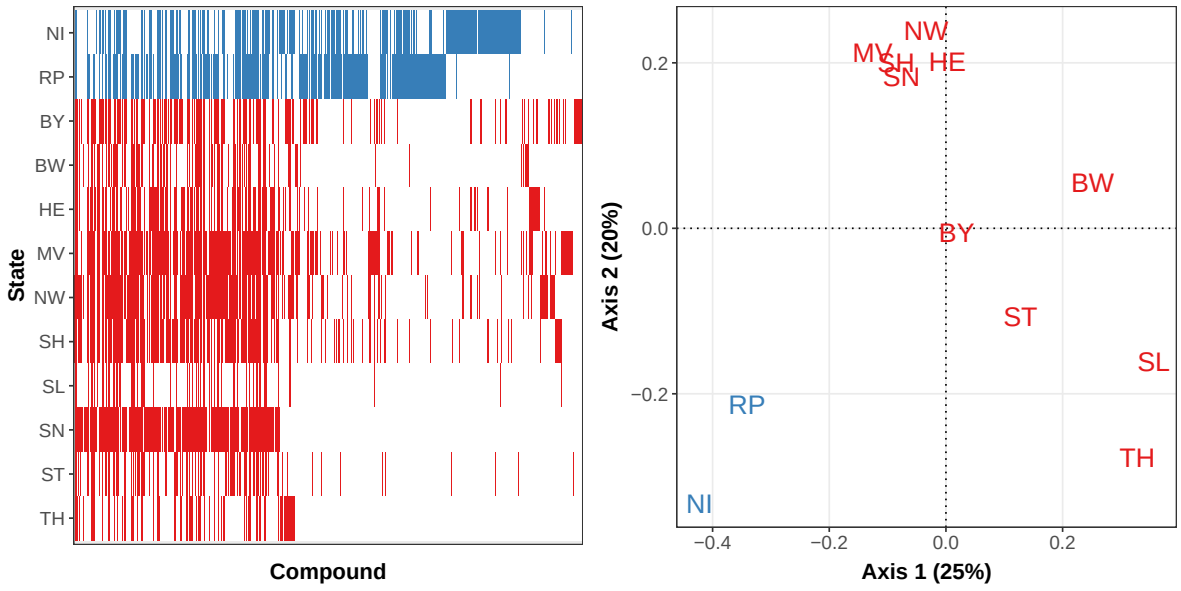

Figure 2.2: Compound spectra of the different federal states. Left: Barcode plot - each vertical line is an analysed compound. Right: MDS ordination. Colors according to two clusters determined by hierarchical clustering (see Supplemental Figure S3 and S4).

The distribution of sampling sites across catchment sizes indicated a disproportionally low number of sites with catchments below 10 km^2, with most sampling sites having catchment sizes between 10 and 25 km^2 (Figure 2.3).

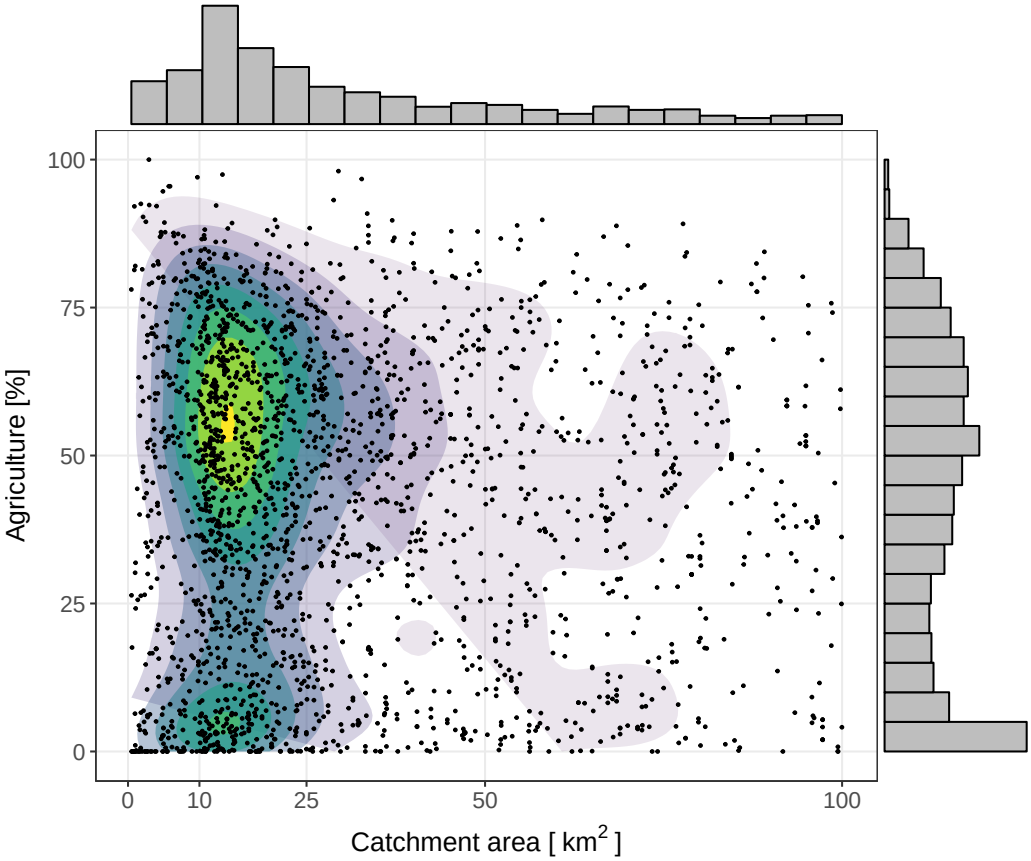

Figure 2.3: Distribution of catchment area and agriculture within the catchment area across the sampling sites. Colour codes the 2-dimensional density of points.

Influence of agricultural land use and catchment size

The number of RAC exceedances increased strongly and statistically significant up to 28% agriculture within the catchment. The mean number of RAC exceedances per site increased 3.7-fold from 0.10 (no agriculture) to 0.39 (28% agriculture within the catchment). Above this threshold the exceedances levelled. Above 75% agriculture within the catchment the number of exceedances further increased, but the increase was not statistically significant (Figure 2.4,

left). Catchment size had no statistically significant effect on the number of RAC exceedances (Figure 2.4, right). We also could not detect a statistically significant interaction between catchment size and agriculture.

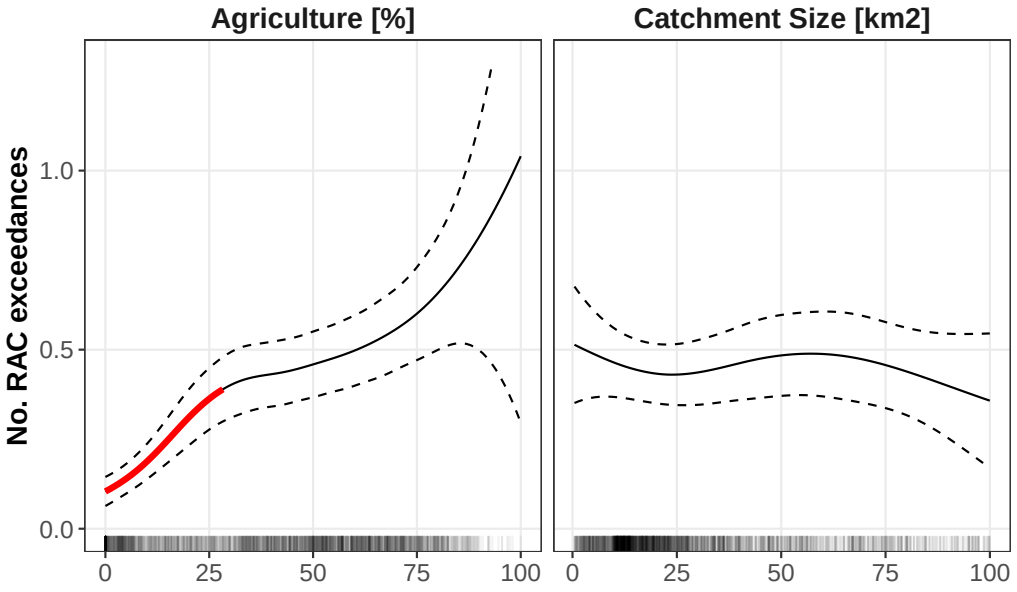

Figure 2.4: Effect of percent agriculture within the catchment (left) and catchment size (right) on the number of RAC exceedances. Red line marks statistically significant changes. Dashed lines denote 95% point-wise Confidence Intervals.

Effect of precipitation on pesticide risk

The spatio-temporal intersection revealed that most samples were taken during periods of low precipitation. For example, only 5% of the samples were taken at or after days with rainfall events greater than 10mm / day that may lead to run-off (Supplemental Figure S6).

$prec_0$ and $prec_{-1}$ increased the probability of exceeding LOQ and RQ. In Q2 an increase from 0.1 mm to 10 mm of precipitation before sampling ($prec_{-1}$) lead on average to a 36% higher mean RQ of 0.05. The probability to exceed LOQ increases 1.6-fold from 8.7% to 13.5% (Figure 2.5, top). Effects differed between individual compounds and are provided in the Supplemental Table S4. Precipitation before sampling ($prec_{-1}$) had a stronger effect than precipitation

during sampling ($prec_0$) on the probability of exceeding LOQ. This difference was less pronounced for the mean value of RQ (Figure 2.5, top).

The first quarter showed the lowest RQ and probability of exceeding LOQ. Both increased during summer months and decreased towards the end of the year. There was a 2.5-fold higher probability of exceeding LOQ in Q2 (10.6%) than in Q1 (4.6%). The differences were less pronounced for the mean value of RQ and with less precision (Figure 2.5, bottom). Individual compounds showed different temporal patterns (see Supplemental Table S4).

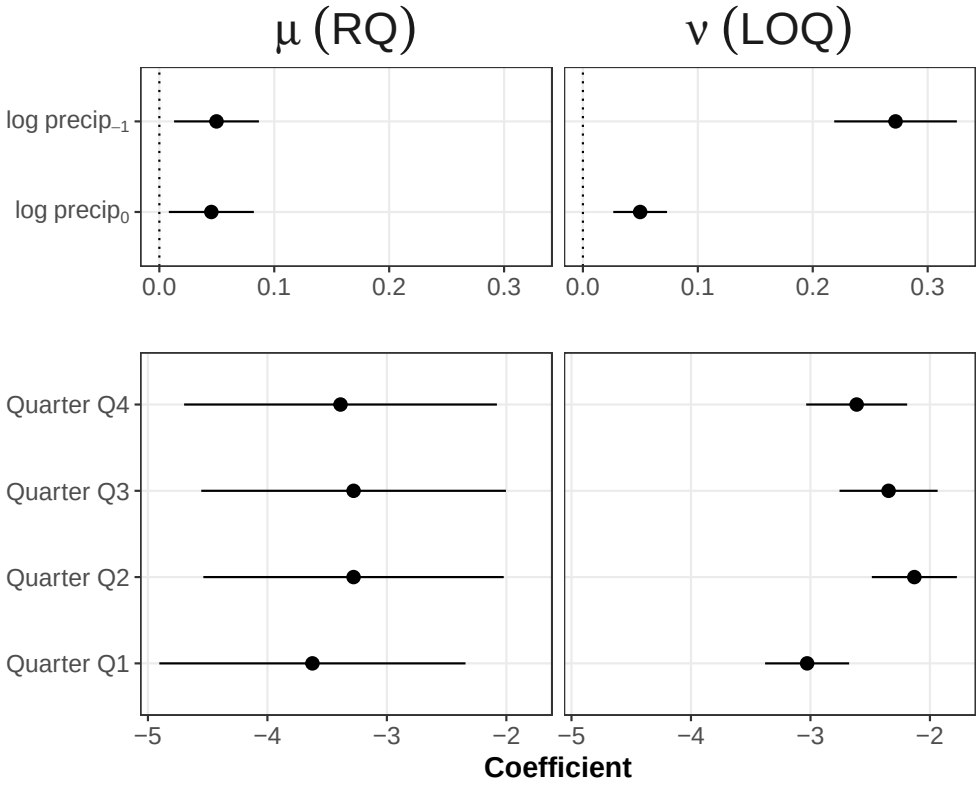

Figure 2.5: Summarised coefficients (and their 95% CI) for precipitation (top row) and quarter (bottom row) from a meta-analysis of the 22 modelled compounds. Left: coefficients for mean RQ (μ), right: coefficients for probability of exceeding LOQ (ν). Coefficients are shown on the link scale (see Eq. 2.4). Single compound coefficients are provided in Supplemental Table S4 and Figure S7).

Pesticide risk in small streams

We found RAC exceedances in 25.5% of sampling sites and RQ > 0.1 in 54% of sites. In 23% of sites none of the chemicals, for which RACs were available, were detected (see also Supplemental Figure S8). Neonicotinoid insecticides and Chlorpyrifos showed the highest RQ (Figure 2.6). For Thiacloprid and Chlorpyrifos the RAC was equal or less than LOQ, therefore, all detections have a RQ $\geqslant$ 1. The herbicides Nicosulfuron and Diflufenican, as well as the fungicide Dimoxystrobin also showed high exceedances of RQ (26.7, 14.1 and 21.1 % of measurements > LOQ), see also Supplemental Table S5). RAC exceedances were found in 14% of samples with concentrations >LOQ (and 7.3% of all samples).

The highest RQs were observed for Chlorpyrifos (max(RQ) = 220), Clothianidin (max(RQ) = 157), Dimoxystrobin(max(RQ) = 117) and Isoproturon (max(RQ) = 80). Where analysed, metabolites exhibited the highest detection rates (for example, Metazachlor sulfonic acid was detected in 84% of all samples where it was analysed (n = 3038, see also Supplemental Figure S9). Glyphosate was the compound with the highest detection rates (41%, n = 3557 samples), followed by Boscalid (23%, n = 9886) and Isoproturon (22%, n = 19112). However, only the latter showed RAC exceedances (Figure 2.6). In 45.9% of samples more than one compound was quantified, with a maximum of 54 different compounds in one sample (Supplemental Figure S10).

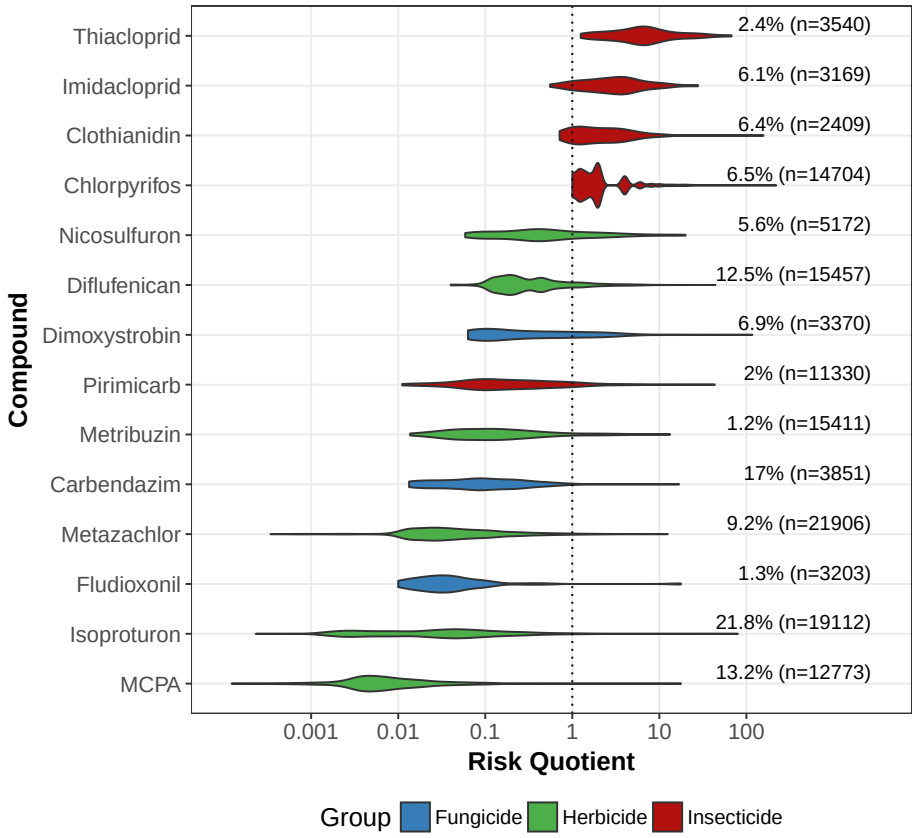

Figure 2.6: 15 compounds with the highest risk quotients in small streams. Non-detects are not shown due to the logarithmic axis. Numbers on the right give the percentage of values >LOQ and the total number of samples were the compound was analysed.

DISCUSSION

Overview on the compiled dataset

The compiled dataset of governmental monitoring data, with a particular focus on small streams, represents currently the most comprehensive available for Germany. Similar nationwide datasets have been compiled for the Netherlands (Vijver et al., 2008), Switzerland (Munz and Leu, 2011) and the United States (Stone et al., 2014). While the compilations from Europe are of similar quantity and quality to the data compiled and analysed here, the compilation used in

Stone et al. (2014) is much smaller, though these data may be complemented by more data in future analyses.

A nationwide assessment of pesticide pollution is hampered by inhomogeneous data across federal states: Beside large differences in the spatial distribution and quantity of sampling sites (Figure 2.1), the spectrum of analysed compounds (Figure 2.2) and the quality of chemical analyses differed between states. Despite the outlined differences between states, all ecoregions occurring in Germany (Abell et al., 2008; Illies, 1978) were covered by the presented dataset and thus it might nonetheless represent a sample covering all types of small streams in Germany. For Thiacloprid and Chlorpyrifos the LOQs were above the RAC, which means that exceedances are likely underestimated. For these compounds a lowering of LOQ through an improvement of chemical analysis is essential for reliable assessment. Moreover, a nationwide assessment would benefit from a harmonised spectrum of analysed compounds between federal states.

Given their high abundance in the landscape (Nadeau and Rains, 2007) small streams below 10 km² are disproportionally less sampled in current monitoring (Figure 2.3), which may be attributed to the missing categorisation in the WFD. Clearly, there is currently a lack of knowledge on stressor effects on small streams. We analysed only data from small streams, however, for lentic small water bodies this lack might be even greater (Lorenz et al., 2016).

Influence of agricultural land use and catchment size

We found a strong influence of agriculture on the pollution of streams. Above 25% agriculture within a catchment, it is likely that a RAC will be exceeded, with a further increase in entirely agricultural catchments (above 75 % agriculture). To our knowledge, this is the first study investigating such thresholds of pesticide risk. Previous studies examined thresholds for the percentage of agricultural land use with respect to the response of biological communities, integrating different agricultural stressors. Feld (2013) found change points of biological community metrics at 40% agricultural land use in lowland streams in Europe. Similarly, Waite (2014) found a threshold for aquatic diatoms at 40% agricultural land use in wadeable streams in the United States. Our results coincide with these thresholds and suggest that pesticides might contribute to the observed biological changes.

We did not find a statistically significant relationship between pesticide pollution and catchment size. However, previous studies showed that small streams

are more polluted than bigger streams (Knauer, 2016; Schulz, 2004; Stehle and Schulz, 2015b). This can be explained by the relatively short gradient of catchment sizes in our dataset, with most of the streams with catchments above 10 km^2 and below 100 km^2 (Figure 2.3, top). For example, the gradient of Schulz (2004) covered 6 orders of magnitude.

Effect of precipitation on pesticide risk

Our results revealed that pesticide sampling for chemical monitoring in Germany is mainly performed when no precipitation occurs. Nevertheless, we found a 36% higher RQ if samples were taken after rainfall events. Samples taken on the day of a rainfall event showed less risk than samples taken one day after a rainfall event. This could be explained by the sampling preceding the rainfall event and the delay between the start of a rain event and the peak in discharge or runoff. The effects of precipitation were more pronounced for the probability to exceed LOQ, with smaller effect sizes for the absolute value of RQ. This may be explained by a higher variability of absolute concentrations. Overall, our results indicate that current pesticide monitoring relying on grab sampling, largely disconnected from precipitation events, underestimates pesticide risks. Automatic event-driven samplers (Stehle et al., 2013) and passive samplers (Fernández et al., 2014; Moschet et al., 2015) may help overcome these shortcomings and provide a better representation of risks, especially for small water bodies (Lorenz et al., 2016).

We found the highest the probability of exceeding LOQ during summer (10% for Q2) and lowest in the first quarter of the year (4%, Figure 2.5, bottom right). This annual pattern coincides with the main application season for pesticides in Central Europe. Nevertheless, there are compound-specific differences in the annual pattern, which explains the wide CI for the absolute RQ (Figure 2.5, bottom left). For example, the herbicide Diflufenican showed the highest RQ and the highest probability of exceeding LOQ during the winter quarters Q1 and Q4 (Supplemental Table S4), which coincides with the application period it is registered for in Germany (BVL, 2016). Our study suggests that pesticide risks display compound specific spatio-temporal dynamics. Currently, little is known about these and further research on those might provide useful information for future ecological risk assessment. For example, the sensitivity of organisms is often life stage dependent (Hutchinson et al., 1998) and knowledge on temporal dynamics could inform on concurrent exposure to multiple pesticides, as well

as assist to parameterise toxicokinetic and toxicodynamic models (Ashauer et al., 2016). Moreover, our results show that analysing absolute concentrations and probabilities of LOQ together might deliver valuable insights into risk dynamics.

Pesticides in small streams

Our results suggest that small streams are frequently exposed to ecologically relevant pesticide concentrations. In one-quarter of small streams RACs were exceeded at least once. Stehle and Schulz (2015b) found the highest percentage of RAC exceedances for organophosphate insecticides. By contrast, we found that neonicotinoid insecticides have highest exceedances of RACs, followed by the organophosphate chlorpyrifos. This difference can be attributed to the low sample size for neonicotinoid insecticides in their study (n = 33) compared to the dataset presented here (for example 3,540 samples of Thiacloprid, Figure 2.6). Overall, our results suggest that neonicotinoids may currently pose a high risk to freshwater ecosystems. Moreover, our results add further evidence to the growing literature on the risks arising from neonicotinoids for aquatic (Morrissey et al., 2015) and terrestrial (Pisa et al., 2015) ecosystems.

Compared to Stehle and Schulz (2015b) we found higher rates of RAC exceedances for insecticides. They found exceedances in 37.1% of insecticide measurements >LOQ (n = 1352, 23 insecticides), whereas, we found exceedances in 67% of insecticide measurements with RACs >LOQ (n = 1855, 22 insecticides). This could be attributed to different insecticides considered and different underlying RACs. Our study has only 7 insecticides with RACs in common with the insecticides investigated by Stehle and Schulz (2015b). Moreover, all RACs were lower in our study (average difference = -0.71 µg/L, range = [-2.757; -0.005]). Nevertheless, it must be noted that the dataset compiled here comprised only samples from grab sampling, which may considerably underestimate pesticide exposure (Stehle et al., 2013; Xing et al., 2013).

By contrast, Knauer (2016) found exceedances from monitoring data mainly for herbicides and fungicides and only one insecticide Chlorpyrifos-methyl. Moreover, RAC exceedances in Switzerland were generally lower and less abundant (for example 6 exceedances (=0.2%) for Isoproturon with a maximum RQ of 2) compared to our results for Germany. This might reflect differences in pesticide use between countries, ecoregions and RACs used. From the definition of RAC it follows that if the concentration of a compound exceeds its RAC

ecological effects are expected. Indeed, Stehle and Schulz (2015a) found that the biological diversity of stream invertebrates was significantly reduced by 30% at RQ = 1.12 and by 10% at 1/10 of RAC. We found RQ values greater than 1.12 in 25% of small streams and RQ at 1/10 of RAC in 54% of small streams. Consequently, we conclude that agricultural pesticides are on a large scale a major threat to small streams, the biodiversity they host and the services they provide. This threat may exacerbate because pesticides often occur in mixtures (Schreiner et al., 2016) and may co-occur with other stressors (Schäfer et al., 2016).

Monitoring data, despite the outlined limitations, provides an opportunity to study large-scale environmental occurrence patterns of pesticides. Furthermore, such nationwide compilations, may not only be used for governmental surveillance, but also to answer other questions, like validation of exposure modelling (Knäbel et al., 2014), retrospective evaluation of regulatory risk assessment (Knauer, 2016; Stehle and Schulz, 2015b)or occurrences of pesticide mixtures (Schreiner et al., 2016). However, the sampling design needs to account for precipitation events to provide robust data. Our results suggest that exceedances of RACs are landscape dependent and therefore, pesticide regulation should account for landscape features. Moreover, the high exceedances of RACs indicate that greater efforts are needed to describe causal links, which may lead to further developments of the current authorisation procedure.

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3 GENERAL DISCUSSION AND OUTLOOK

TOPICS IN STATISTICAL ECOTOXICOLOGY

The simulation study performed in chapter **??** clearly showed that common experimental designs in ecotoxicology exhibit unacceptably low statistical power (Szöcs and Schäfer, 2016; Van Der Hoeven, 1998). This underpins the criticism accumulated over the last 30 years towards the usage of NOEC as an endpoint for ERA (Fox and Landis, 2016). Nevertheless, the NOEC is still one of the standard endpoints of mesocosm experiments in higher tier risk assessment (EFSA, 2013). Therefore, further advances in the statistical evalation of mesocosm experiments is needed.

Recently, *a posteriori* calculations of statistical power have been proposed to counteract these limitations and aid the interpretation treatment-related effects in model ecosystems (Brock et al., 2015). The "minimum detectable difference" (MDD) estimates the difference between two means that must exist in order to produce a statistically significant result ($p < 0.05$ (Gelman and Stern, 2006)) and could be used to ínterpret NOEC. However, *a posteriori* calculations have been shown to have logical flaws when used for interpretation of non-significant results (Hoenig and Heisey, 2001; Nakagawa and Foster, 2004). However, conducting and reporting of *a priori* power calculations, as performed in chapter **??**, might provide researchers important information to optimise their study designs, ensuring that their experimental designs have appropriate power and can lead to interpretable results (Johnson et al., 2015).

Moreover, similar simulations could not only be used to study factorial but also regression designs. Indeed, simulations could be used to determine the optimal experimental design for dose-response models and EC_x determination, balancing precision and usage of resources. Regression designs are generally more powerful and provide more information than factorial designs (Cottingham et al., 2005). In mesocosm experiments, such designs, assigning the replicates to more tested concentrations, might also provide additional insights. Currently, statistical tools to analyse a community dose-response relationship are not well explored and no equivalent $EC_{x,mesocosm}$ can be derived. On pos-

sibility could be to fit separate dose-response models to each species, leading to a EC_x for each species in a mesocosm study. Subsequently, these EC_x values could be combined and summarised using Species Sensitivity Distributions (Posthuma et al., 2002), providing a single measure for the community response, e.g. a hazardous concentration ($HC_{x,mesocosm}$) for x % of species affected in mesocosms (Maltby et al., 2005). Another possibility would be to use a logistic type of ordination (van den Brink et al., 2003). Reduced-Rank vector generalised linear models (RR-VGLM) could be used to fit such type of models (Yee, 2015; Yee and Hastie, 2003), but they have not been applied in ecotoxicology yet.

In a similar vein, community ecology is currently experiencing a shift towards a new class of multivariate methods, incorporating statistical models for abundances across many taxa simultaneously (ter Braak and Šmilauer, 2015; Warton et al., 2015a; Warton et al., 2015b; Warton et al., 2012). However, these methods have not been applied frequently and their applicability to ecotoxicological data is currently unclear (Szöcs et al., 2015). All these models have in common, that the choice of statistical model is primarily based on the grounds of data properties. In chapter **??** we showed, that using statistical models that fit the type of data analysed, can provide higher statistical power. Simultaneously, Ives (2015) published a study reaching contradictory conclusions (*"For testing the significance of regression coefficients, go ahead and log-transform count data"*). It must be noted, that the simulation designs differed significantly between both studies: We used a low-replicated factorials design, whereas Ives (2015) simulated a well-replicated regression designs with two predictors. We both found that the negative-binomial GLMs were surprisingly prone to Type I errors, although the assumptions of this model closely matched the data. Nevertheless, as we show in chapter **??**, the parametric bootstrap might provide a solution to this problem, but is computationally intensive and not widely used. The parametric bootstrap is akin to Bayesian methods (Gelman et al., 2014), which might provide an alternative inference method for inference. The main point, leading Ives (2015) to his conclusions, was that GLM showed undesirable Type I errors in case of correlated predictors, a case not commonly encountered in ecotoxicology and not studied in chapter **??**. Recently, the current state-of-the-art was discussed by Warton et al. (2016): i) choose the statistical model based on the grounds of data properties; ii) fix Type I errors using parametric bootstrap or resampling; iii) take mean-variance relationship into account, which is in line with the findings of chapter **??**. However, there are still open questions regarding the use of GLMs for count data (see e.g. raised by Prof. John Main-

donald, http://uni-ko-ld.de/fb). To diagnose issues such as overdispersion and excess of zeros in count data models new tools like the recently developed *"Rootograms"* (Kleiber and Zeileis, 2016) provide useful additions.

In chapter 2 we applied new statistical modelling techniques that explicitly consider the limit of quantification. The currently most often used methods to deal with such censored data is to omit or substitute non-detects. Censoring is very common when dealing with chemical and ecological datasets, but is rarely taken into account (Fox et al., 2015). Recent examples from ecotoxicology and environmental chemistry show that omission (Hansen et al., 2015), randomization (Goulson, 2015) or substitution by a fixed value (Helsel, 2010; Helsel, 2006) can lead to biased results. Hansen et al. (2015) used a Tobit regression (Tobin, 1958) that takes the amount of censored data into account, assuming a (log-) normal distribution of concentrations. In chapter 2 we used a slightly different approach, using a zero adjusted gamma model (ZAGA). We modelled measured concentrations as two separate processes, generating i) zero values and ii) non-zero values assuming a gamma distribution of concentrations. In ecological statistics this type models is also known as *hurdle* models (Martin et al., 2005). The log-normal Tobit model has no probability mass at zero, whereas ZAGA model has a probability at zero. Generally, the difference between Tobit and two-part models are small (Min and Agresti, 2002) and the same holds for differences between the log-normal and Gamma distribution. Indeed, a Tobit-like model could be also fitted assuming a Gamma distribution (Sigrist and Stahel, 2010).

Grab sampling likely underestimates chemical concentrations because of short term peak concentrations (Stehle et al., 2013; Xing et al., 2013). Although this leads to an increased variation in chemical measurements, we still can learn from the process generating values above LOQ, even if the absolute value is subject to error. This is also highlighted by the results of chapter 2, with estimated coefficients for the absolute concentration showing much larger uncertainty than coefficients for the probability of exceeding LOQ (Figure 2.5). Currently, models explicitly taking the censored nature of chemical monitoring data are not well explored and seldom applied. Further research on those is needed and might provide useful information for analysing monitoring data, assessing the chemical status and trends thereof.

LEVERAGING MONITORING DATA FOR ECOLOGICAL RISK ASSESSMENT

In chapter 2 we compiled and analysed monitoring data leading to the currently most extensive dataset on pesticide exposure available for Germany. We demonstrated, that within the current monitoring scheme small streams below 10 km^2 are underrepresented (Figure 2.3, top). Given their importance, we must admit that we currently do not have much knowledge about these and their threats (Biggs et al., 2016; Lorenz et al., 2016). To fill these gaps, monitoring networks need to be adapted to give a better representation of small streams.

We analysed only data from small streams, however, data on lentic systems is even more scarce. Although, more the 95% of German standing waters are lentic small waterbodies a recent meta-analysis revealed that only 5% of studies investigating pesticides in freshwaters were performed on lentic small water bodies Lorenz et al. (2016). The data compiled in chapter 2 comprised also lentic small water bodies. However, the query to the federal states revealed that there are currently no such monitoring data available (Brinke et al., 2016). This highlights the urgent need to adapt monitoring schemes to also include small standing waters.

Our results revealed that chemical monitoring schemes within Germany differed largely in terms of spatio-temporal coverage and compound spectra between federal states. Similarly, Malaj et al. (2014) showed big differences between European countries. Overall, a homogenisation and standardisation of chemical monitoring programs would enhance the comparability and the possibility for a large-scale assessment.

We found that the signal from agricultural pesticides can be detected down to a small percentage of agriculture within the catchment (Figure 2.4). Thus, we can conclude that if there is agriculture with a catchment, it is very likely that pesticides will be applied, enter the streams and are detected. This has implications for selection of reference sites for environmental monitoring, that need to have no agricultural influence. We studied only the influence of agricultural non-point sources, however, point-sources like wastewater treatment plants can also contribute to pesticide pollution of streams (Bunzel et al., 2014).

We were able to detect a small, but distinct increase of risks after precipitation events. This is in line with findings that pesticides mainly enter surface waters via edge-of-field runoff (Schulz, 2001). Moreover, our results suggest that absolute measured concentrations are subject to a high error due to the sampling pro-

cess and adds evidence that current monitoring schemes, largely unconnected from precipitation events, underestimate pesticide risks (Stehle et al., 2013; Xing et al., 2013). Automatic event-driven samplers in small streams could provide knowledge on pesticide risk dynamics that are currently unknown.

Monitoring data can provide an opportunity to inform ERA after authorization and could possibly trigger a refinement of the assessment (Knauer, 2016). However, current monitoring mainly addresses streams bigger than those considered in ERA. Our results indicated that small streams are frequently exposed to high risks from pesticides. Moreover, to provide a suitable feedback for ERA small agricultural streams must be integrated into environmental monitoring schemes. As the measurements within the current monitoring schemes provide an underestimation, all exceedances of risk thresholds represent an unacceptable risk and indicate that current ERA might miss potential risks and further enhancements of the current authorisation procedure are needed.

Risk thresholds in chapter 2 were especially exceeded for the organophosphate Chlorpyrifos and neonicotinoid substances. This adds to the existing evidence that this particular class of insecticides poses currently a high threat to freshwaters and stricter regulations are warranted (Morrissey et al., 2015). The high number of exceedances shows that ERA for these substances was not accurate enough and lead to risks for the environment. Recent studies investigating large-scale pesticide risks did not consider neonicotinoid insecticides (Malaj et al., 2014; Stehle and Schulz, 2015) and therefore likely underestimated the risks to freshwaters. However, this also shows that the analysed spectrum is an important driver of detected compounds (Malaj et al., 2014; Schreiner et al., 2016) and must be taken into account when evaluating monitoring data for risk assessment. The WFD currently considers on a few, relatively well-known substances (European Union, 2013) and a status assessment based on EQS likely missed the actual chemical pollution (Moschet et al., 2014). Compared to the data presented in chapter 2 the WFD considers currently only 4% of the pesticides (19 out of 478 pesticides). Recently, neonicotinoid substances have been incorporated in the watch list of substances for Union-wide monitoring (European Union, 2015), so that more information on the environmental fate of compound group will be available. Monitoring data provide also valuable information for the priorisation of emerging pollutants and future monitoring with the WFD (Brack et al., 2017) and the compiled data we compiled could be a valueable input for such a priorisation.

Monitoring under the WFD is also performed for biological components of freshwaters and a combination with pesticide exposure data might provide valuable insights into large-scale field effects of chemical substances (Schipper et al., 2014). Currently, chemical and biological monitoring are not synchronised. On a continental scale, Malaj et al. (2014) was able to compile ecological status data only for 5% of sites with chemical measurements. For the dataset presented in chapter 2 we found a spatial match with biological monitoring for 60% of sites (Brinke et al., 2016). However, as biological data in Germany is sampled at lower frequencies (often less than once per year) a spatio-temporal match would result in much less accordance. Synchronising these samplings in a future monitoring would possibly enable to assess large-scale post-authorization field effects of chemical substances.

CHALLENGES UTILISING 'BIG DATA' IN ECOLOGICAL RISK ASSESSMENT

Effect assessment and environmental monitoring produce huge amounts of data. However, the profoundness of ecological risk assessment often determined by the available data (Van den Brink et al., 2016). Useful data for ERA is currently spread over several largely unconnected databases. E.g. ecotoxicity data is spread over database maintained by the U.S. EPA (ECOTOX, U.S. EPA (2016)), the University of Hertfordshire (PPDB, Lewis et al. (2016)), the German Environment Agency (ETOX, Umweltbundesamt (2016)) and others. Chemical information is similarly spread over several databases, like PubChem (Kim et al., 2016) or Chemspider (Pence and Williams, 2010). Additional complications arise because these databases use different identifiers for chemical substances. The U.S. EPA (U.S. EPA, 2016) uses solely the CAS-Number for identification, whereas other databases uses SMILES (Weininger, 1990) or InChI and InChIKeys (Heller et al., 2015). Integrating these databases is currently a challenge in ERA, which is complicated by ambiguous identifiers, e.g. should different salts be considered separately for aquatic risk assessment? Projects like the NORMAN EMPODAT database (Brack et al., 2012) or the STOFF-IDENT (Huckele and Track 2013, `http://uni-ko-ld.de/fc`) are first attempts for such an integration. Integration monitoring data and risk assessment data is a mandatory requirement for landscape level ecotoxicology and risk assessment (Focks, 2014) and needed for an improved model development and validation (Brock et al., 2006; Knäbel et al.,

2012). Chapter 2 is an example for such an integration, but represents only a preliminary assessment and spatial-temporal risk dynamics should be further investigated.

The webchem package, presented in chapter **??**, can foster such an integration. However, data must also be accessible in order to be retrievable by webchem. Unfortunately, major parts of data produce for environmental risk assessment are not available (Schäfer et al., 2013). Recently, it has been demonstrated that data from the European Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) database can be used to improve the characterisation of ecotoxicity in life cycle assessment (LCA) (Müller et al., 2016). Although this database hosts humongous amounts of data used risk assessment, it is currently not available in a convenient way. Indeed, a systematic data collection contravenes the legal usage of the REACH database (`http://uni-ko-ld.de/fd`). This may be also the reason, why the quality of chemical property data submitted this database is currently unknown (Müller et al., 2016; Stieger et al., 2014). Webchem currently can retrieve data from 11 data sources. However many other data sources are available and implementation of more than 15 other data sources is in the pipeline will be implemented in the future (`http://uni-ko-ld.de/fi`) and collaborators are welcome.

The software tools described in chapters **??** and **??** assist researchers handling and cleaning their data. Aggregating taxonomic data to a higher taxonomic level is a common task when analysing data from mesocosm experiments or from field sampling. Taxize facilitates the retrieval of taxonomic classification, which is the basis also for more sophisticated aggregation methods (Cuffney et al., 2007). Today, taxize has been used in more than thirty scientific publications. Recent applications of the webchem package, have been demonstrated by Münch and Galizia (2016) and Ranke (2016): Münch and Galizia (2016) compiled a database for odorant responses of *Drosophila melanogaster* and webchem *"likely saved [him] hundreds of working hours"*. Ranke (2016) is using webchem to compile and store chemical information for further analyses. The analyses performed in chapter 2 needed to integrate monitoring, chemical and risk assessment data which would not have been difficult without the webchem package. These examples show that researchers have been missing such tools in the past. If they can reduce their time spend on data retrieval and handling, they could focus more on the quintessence of their research.

CONCLUSIONS

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