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) and freshwaters are 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 (L. M. 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. 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 multiplied by an assessment factor between 0.01 (lower tier assessment) and 0.5 (higher tier assessment) depending on data quality, which yields to a regulatory acceptable concentration (RAC) (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. 7km^2, 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: A PEC / RAC ratio greater than one indicating potential risks (Amiard-Triquet, 2015; EFSA, 2013; 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 B. Williams, 2006). After authorization, pesticides applied on agricultural fields may enter aquatic ecosystems via diffuse sources like spray-drift, surface run-off or drainage (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 (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 environmental risk assessment (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). 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). 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 (Schipper et al., 2014).

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.

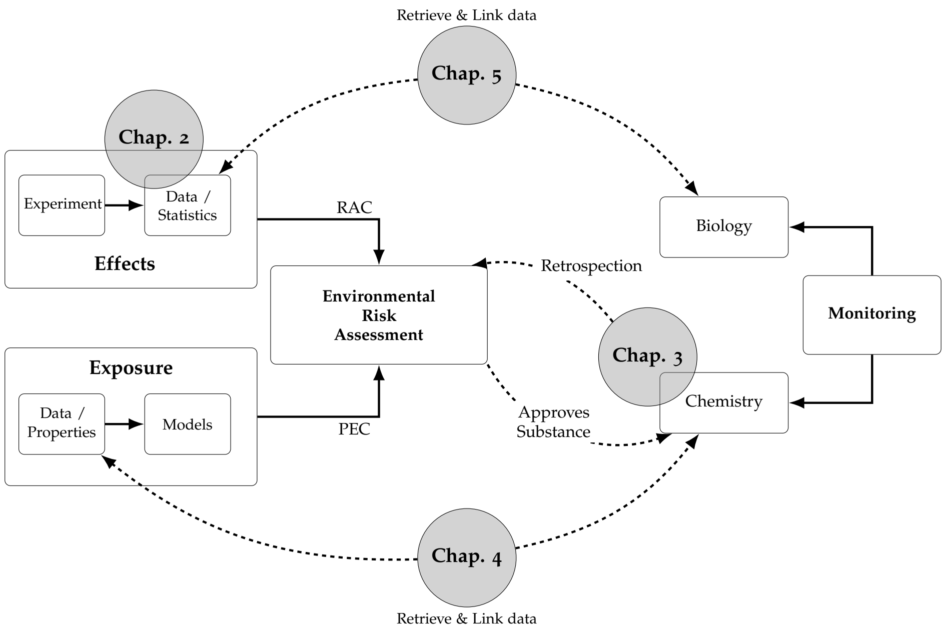

Figure 1.1: Conceptual overview on environmental risk assessment, environmental monitoring and the parts addressed by this thesis.

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?

Exposure assessment aims at predicting chemical concentrations in small streams. Chapter **??** focuses on measured large-scale environmental concentrations in small streams and the drivers thereof. 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 **??**, 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

REFERENCES

Amiard-Triquet, C. (2015). *Aquatic ecotoxicology: advancing tools for dealing with emerging risks*. Boston, MA: Elsevier.

Aylward, B., J. Bandyopadhyay, J.-C. Belausteguigotia, P. Borkey, A. Z. Cassar, L. Meadors, L. Saade, M. Siebentritt, R. Stein, S. Tognetti, et al. (2005). "Freshwater ecosystem services". *Ecosystems and human well-being: policy responses* 3, 213–256.

Balian, E. V., H. Segers, C. Lévèque, and K. Martens (2007). "The Freshwater Animal Diversity Assessment: an overview of the results". *Hydrobiologia* 595 (1), 627–637.

Biggs, J., S. von Fumetti, and M. Kelly-Quinn (2016). "The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers". *Hydrobiologia*.

Chapman, P., P. Chapman, and R. Caldwell (1996). "A warning: NOECs are inappropriate for regulatory use". *Environmental Toxicology and Chemistry* 15 (2), 77–79.

Cortes-Ciriano, I. (2016). "Bioalerts: a python library for the derivation of structural alerts from bioactivity and toxicity data sets". *Journal of Cheminformatics* 8 (1).

Dafforn, K. A., E. L. Johnston, A. Ferguson, C. Humphrey, W. Monk, S. J. Nichols, S. L. Simpson, M. G. Tulbure, and D. J. Baird (2015). "Big data opportunities and challenges for assessing multiple stressors across scales in aquatic ecosystems." *Marine and Freshwater Research*.

Davies, B., J. Biggs, P. Williams, M. Whitfield, P. Nicolet, D. Sear, S. Bray, and S. Maund (2008). "Comparative biodiversity of aquatic habitats in the European agricultural landscape". *Agriculture, Ecosystems & Environment* 125 (1-4), 1–8.

Downing, J. A., J. J. Cole, C. A. Duarte, J. J. Middelburg, J. M. Melack, Y. T. Prairie, P. Kortelainen, R. G. Striegl, W. H. McDowell, and L. J. Tranvik (2012). "Global abundance and size distribution of streams and rivers". *Inland waters* 2 (4), 229–236.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Leveque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan (2006). "Freshwater biodiversity: importance, threats, status and conservation challenges". *Biological Reviews* 81 (2), 163–182.

EFSA (2013). "Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters". *EFSA Journal* 11 (7), 3290.

Erlacher, E. and M. Wang (2011). "Regulation (EC) No. 1107/2009 and upcoming challenges for exposure assessment of plant protection products – Harmonisation or national modelling approaches?" *Environmental Pollution* 159 (12), 3357–3363.

European Union (2000). "Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy". *Official Journal of the European Union* L 327, 1–73.

European Union (2009). "Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC". *Official Journal of the European Union* L 309, 1–50.

European Union (2013). "Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy". *Official Journal of the European Union* L226, 1–17.

European Union (2015). "Commission Implementing Decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council (notified under document C(2015) 1756)". *Official Journal of the European Union* L28, 40–42.

FOCUS (2001). *FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC.* EC Document Reference SANCO/4802/2001-rev.2.

Fox, D. R., E. Billoir, S. Charles, M. L. Delignette-Muller, and C. Lopes (2012). "What to do with NOECS/NOELS—prohibition or innovation?" *Integrated Environmental Assessment and Management* 8 (4), 764–766.

Fox, D. R. and W. G. Landis (2016a). "Comment on ET&C perspectives, November 2015-A holistic view". *Environmental Toxicology and Chemistry* 35 (6), 1337–1339.

Fox, D. R. and W. G. Landis (2016b). "Don't be fooled-A no-observed-effect concentration is no substitute for a poor concentration-response experiment: NOEC and a poor concentration-response experiment". *Environmental Toxicology and Chemistry* 35 (9), 2141–2148.

Gitzen, R. A., ed. (2012). *Design and analysis of long-term ecological monitoring studies*. Cambridge ; New York: Cambridge University Press.

Green, J. W. (2016). "Issues with using only regression models for ecotoxicity studies". *Integrated Environmental Assessment and Management* 12 (1), 198–199.

Howard, P. H. and W. Meylan (2016). *Physical and Chemical Property Database*. URL: http://www.srcinc.com/what-we-do/environmental/scientific-databases.html.

Jager, T. (2012). "Bad habits die hard: The NOEC's persistence reflects poorly on ecotoxicology". *Environmental Toxicology and Chemistry* 31 (2), 228–229.

Johnson, P. C. D., S. J. E. Barry, H. M. Ferguson, and P. Müller (2015). "Power analysis for generalized linear mixed models in ecology and evolution". *Methods in Ecology and Evolution* 6 (2), 133–142.

Kim, S., P. A. Thiessen, E. E. Bolton, J. Chen, G. Fu, A. Gindulyte, L. Han, J. He, S. He, B. A. Shoemaker, J. Wang, B. Yu, J. Zhang, and S. H. Bryant (2016). "PubChem Substance and Compound databases". *Nucleic Acids Research* 44 (D1), D1202–D1213.

Knäbel, A., K. Meyer, J. Rapp, and R. Schulz (2014). "Fungicide Field Concentrations Exceed FOCUS Surface Water Predictions: Urgent Need of Model Improvement". *Environmental Science & Technology* 48 (1), 455–463.

Knäbel, A., S. Stehle, R. B. Schäfer, and R. Schulz (2012). "Regulatory FOCUS Surface Water Models Fail to Predict Insecticide Concentrations in the Field". *Environmental Science & Technology* 46 (15), 8397–8404.

Knauer, K. (2016). "Pesticides in surface waters: a comparison with regulatory acceptable concentrations (RACs) determined in the authorization process and consideration for regulation". *Environmental Sciences Europe* 28 (13).

Kühne, R., R.-U. Ebert, P. C. von der Ohe, N. Ulrich, W. Brack, and G. Schüürmann (2013). "Read-Across Prediction of the Acute Toxicity of Organic Compounds toward the Water Flea Daphnia magna". *Molecular Informatics* 32 (1), 108–120.

Laskowski, R. (1995). "Some good reasons to ban the use of NOEC, LOEC and related concepts in ecotoxicology". *Oikos* 73 (1), 140–144.

Lewis, K. A., J. Tzilivakis, D. J. Warner, and A. Green (2016). "An international database for pesticide risk assessments and management". *Human and Ecological Risk Assessment: An International Journal* 22 (4), 1050–1064.

Liess, M., R. Schulz, M.-D. Liess, B. Rother, and R. Kreuzig (1999). "Determination of insecticide contamination in agricultural headwater streams". *Water Research* 33 (1), 239–247.

Lorenz, S., J. J. Rasmussen, A. Süß, T. Kalettka, B. Golla, P. Horney, M. Stähler, B. Hommel, and R. B. Schäfer (2016). "Specifics and challenges of assessing exposure and effects of pesticides in small water bodies". *Hydrobiologia*, 1–12.

Malaj, E., P. C. v. d. Ohe, M. Grote, R. Kühne, C. P. Mondy, P. Usseglio-Polatera, W. Brack, and R. B. Schäfer (2014). "Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale". *Proceedings of the National Academy of Sciences* 111 (26), 9549–9554.

Moschet, C., I. Wittmer, J. Simovic, M. Junghans, A. Piazzoli, H. Singer, C. Stamm, C. Leu, and J. Hollender (2014). "How a Complete Pesticide Screening Changes the Assessment of Surface Water Quality". *Environmental Science & Technology* 48 (10), 5423–5432.

Murrell, D. S., I. Cortes-Ciriano, G. J. P. van Westen, I. P. Stott, A. Bender, T. E. Malliavin, and R. C. Glen (2015). "Chemically Aware Model Builder (camb):

an R package for property and bioactivity modelling of small molecules". *Journal of Cheminformatics* 7 (1).

Newman, M. C. (2008). ""What exactly are you inferring?" - A closer look at hypothesis testing". *Environmental Toxicology and Chemistry* 27 (7). Newman, M. C., 1633–1633.

Newman, M. C. (2012). *Quantitative ecotoxicology*. Boca Raton, FL: Taylor & Francis.

Newman, M. C. (2015). *Fundamentals of ecotoxicology: the science of pollution*. Boca Raton: CRC Press, Taylor & Francis Group.

Nichols, J. and B. Williams (2006). "Monitoring for conservation". *Trends in Ecology & Evolution* 21 (12), 668–673.

Persson, L. M., M. Breitholtz, I. T. Cousins, C. A. de Wit, M. MacLeod, and M. S. McLachlan (2013). "Confronting unknown planetary boundary threats from chemical pollution". *Environmental science & technology* 47 (22), 12619–12622.

Ritz, C. (2010). "Toward a unified approach to dose-response modeling in ecotoxicology". *Environmental Toxicology and Chemistry* 29 (1), 220–229.

Rockström, J., W. Steffen, K. Noone, A. Persson, 3. Chapin F. S., E. F. Lambin, T. M. Lenton, M. Scheffer, C. Folke, H. J. Schellnhuber, B. Nykvist, C. A. de Wit, T. Hughes, S. van der Leeuw, H. Rodhe, S. Sorlin, P. K. Snyder, R. Costanza, U. Svedin, M. Falkenmark, L. Karlberg, R. W. Corell, V. J. Fabry, J. Hansen, B. Walker, D. Liverman, K. Richardson, P. Crutzen, and J. A. Foley (2009). "A safe operating space for humanity". *Nature* 461 (7263), 472–5.

Schäfer, R. B., B. Kühn, E. Malaj, A. König, and R. Gergs (2016). "Contribution of organic toxicants to multiple stress in river ecosystems". *Freshwater Biology*. DOI: 10.1111/fwb.12811.

Schipper, A. M., L. Posthuma, D. de Zwart, and M. A. J. Huijbregts (2014). "Deriving Field-Based Species Sensitivity Distributions (f-SSDs) from Stacked Species Distribution Models (S-SDMs)". *Environmental Science & Technology* 48 (24), 14464–14471.

Schulz, R. (2004). “Field Studies on Exposure, Effects, and Risk Mitigation of Aquatic Nonpoint-Source Insecticide Pollution: A Review”. *Journal of Environmental Quality* 33 (2), 419–448.

Schwarzenbach, R. P., T. Egli, T. B. Hofstetter, U. v. Gunten, and B. Wehrli (2010). “Global Water Pollution and Human Health”. *Annual Review of Environment and Resources* 35 (1), 109–136.

Schwarzman, M. R. and M. P. Wilson (2009). “New Science for Chemicals Policy”. *Science* 326 (5956), 1065–1066.

Steffen, W., A. Persson, L. Deutsch, J. Zalasiewicz, M. Williams, K. Richardson, C. Crumley, P. Crutzen, C. Folke, L. Gordon, et al. (2011). “The Anthropocene: From global change to planetary stewardship”. *Ambio* 40 (7), 739–761.

Stehle, S., A. Knäbel, and R. Schulz (2013). “Probabilistic risk assessment of insecticide concentrations in agricultural surface waters: a critical appraisal”. *Environmental Monitoring and Assessment* 185 (8), 6295–6310.

Stehle, S. and R. Schulz (2015). “Pesticide authorization in the EU—environment unprotected?” *Environmental Science and Pollution Research* 22 (24), 19632–19647.

Stone, W. W., R. J. Gilliom, and K. R. Ryberg (2014). “Pesticides in U.S. Streams and Rivers: Occurrence and Trends during 1992–2011”. *Environmental Science & Technology* 48 (19), 11025–11030.

Strayer, D. L. and D. Dudgeon (2010). “Freshwater biodiversity conservation: recent progress and future challenges”. *Journal of the North American Benthological Society* 29 (1), 344–358.

Suter, G. W., ed. (2007). *Ecological risk assessment*. Boca Raton: CRC Press/Taylor & Francis.

Umweltbundesamt (2016). *ETOX: Information System Ecotoxicology and Environmental Quality Targets*. URL: http://webetox.uba.de/webETOX/index.do.

U.S. EPA (2016). *The ECOTOXicology knowledgebase (ECOTOX)*. URL: http://cfpub.epa.gov/ecotox/.

Van den Brink, P. J., C. B. Choung, W. Landis, M. Mayer-Pinto, V. Pettigrove, P. Scanes, R. Smith, and J. Stauber (2016). "New approaches to the ecological risk assessment of multiple stressors". *Marine and Freshwater Research* 67 (4), 429.

Van Der Hoeven, N. (1998). "Power analysis for the NOEC: What is the probability of detecting small toxic effects on three different species using the appropriate standardized test protocols?" *Ecotoxicology* 7 (6), 355–361.

Vörösmarty, C. J., P. B. McIntyre, M. O. Gessner, D. Dudgeon, A. Prusevich, P. Green, S. Glidden, S. E. Bunn, C. A. Sullivan, C. R. Liermann, and P. M. Davies (2010). "Global threats to human water security and river biodiversity". *Nature* 467 (7315), 555–561.

Warne, M. S. J. and R. van Dam (2008). "NOEC and LOEC data should no longer be generated or used". *Australasian Journal of Ecotoxicology* 14, 1–5.

Waters, C. N., J. Zalasiewicz, C. Summerhayes, A. D. Barnosky, C. Poirier, A. Galuszka, A. Cearreta, M. Edgeworth, E. C. Ellis, M. Ellis, et al. (2016). "The Anthropocene is functionally and stratigraphically distinct from the Holocene". *Science* 351 (6269), aad2622.

WWF (2016). *Living Planet Report 2016 - Risk and resilience in a new era*. URL: http://wwf.panda.org/about_our_earth/all_publications/lpr_2016/.

Xing, Z., L. Chow, H. Rees, F. Meng, S. Li, B. Ernst, G. Benoy, T. Zha, and L. M. Hewitt (2013). "Influences of Sampling Methodologies on Pesticide-Residue Detection in Stream Water". *Archives of Environmental Contamination and Toxicology* 64 (2), 208–218.

2 GENERAL DISCUSSION AND OUTLOOK

TOPICS IN STATISTICAL ECOTOXICOLOGY

The simulation study performed in chapter **??** clearly showed that common experimental designs 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 (D. R. Fox and Landis, 2016). Nevertheless, the NOEC is still one of the standard endpoints for mesocosm experiments in higher tier risk assessment (EFSA, 2013).

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 designs but also regression designs. Indeed, simulations could be used to determine optimal designs 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). Regression designs in mesocosm experiments, assigning the replicates to more tested concentrations, might also provide additional insights. Currently, statistical tools to analyse a community dose-response relations, providing a $EC_{x,mesocosm}$ are not well explored. Separate dose-response models could be fit to each species (Ritz, 2010), leading to a EC_x for each species in a mesocosm study. Subsequently, this EC_x values could be combined and summarised using

Species Sensitivity Distributions (Posthuma et al., 2002), providing 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 well-replicated regression designs with two predictors. We both found that the negative-binomial GLMs surprisingly were prone to Type I errors, although the assumptions of this model closely matched the data. However, as we show in chapter **??**, 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 also provide an alternative. 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 by us. Recently, the currently 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. However, there are still open questions regarding the use of GLMs for count data (see e.g. raised by Prof. John Maindonald, `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 **??** we applied new statistical modelling techniques that explicitly consider the limit of quantification. The currently most commonly used method to deal with such censored data is to omit or substitute non-detects. Censoring is very common when dealing with chemical and ecological datasets, however rarely taken into account (G. A. 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 (D. Helsel, 2010; D. R. 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 **??** 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 know as *hurdle* models (Martin et al., 2005). The log-normal Tobit model has not 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). The same holds for differences between the lognormal and Gamma distribution. Indeed, a tobit-like model could be also fitted assuming a Gamma distribution (Sigrist and Stahel, 2010).

Given the inherent variability of chemical measurements in streams (Wittmer et al., 2010) and the associated uncertainty of the absolute value of concentration, we still can learn from the process generating values above LOQ, even if the measurement miss the peak concentrations. This is also highlighted by the results of chapter **??**, with estimated coefficients for the absolute concentration showing much larger uncertainty than coefficients for the probability of exceeding LOQ (Figure **??**). Currently, models explicitly taking the censored nature of chemical monitoring data are not well explored and seldom applied. Further research on those might provide useful information for analysing monitoring data, assessing chemical status and trends thereof.

LEVERAGING MONITORING DATA FOR ECOLOGICAL RISK ASSESSMENT

CHALLENGES TO UTILISE '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 similary 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 (Heller et al., 2015). Integrating these databases is a current challenge in ERA. 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.

The webchem package, presented in chapter **??**, can foster such an integration. However, to be efficient such data must be accessible. 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 bu used to improve the characterisation of ecotoxicity in life cycle assessment (LCA) (Müller et al., 2016). Although, this database hosts data used risks assessment it is 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).

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 various usages. For the data analyses performed in chapter **??**, we needed to integrate monitoring, chemical and risk assessment data.

These examples show that researchers have been missing such tools in the past. If they can reduce the time consumed for data retrieval and handling, they could focus more on the quintessence of their research. Moreover, is an integration of different datas ources crucial for an integrative ecological risk assessment.

CONCLUSIONS

REFERENCES

Brack, W., V. Dulio, and J. Slobodnik (2012). "The NORMAN Network and its activities on emerging environmental substances with a focus on effect-directed analysis of complex environmental contamination". *Environmental Sciences Europe* 24 (1), 1–5.

Brock, T. C. M., M. Hammers-Wirtz, U. Hommen, T. G. Preuss, H.-T. Ratte, I. Roessink, T. Strauss, and P. J. Van den Brink (2015). "The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems". *Environmental Science and Pollution Research* 22 (2), 1160–1174.

Cottingham, K. L., J. T. Lennon, and B. L. Brown (2005). "Knowing when to draw the line: designing more informative ecological experiments". *Frontiers in Ecology and the Environment* 3 (3), 145–152.

Cuffney, T. F., M. D. Bilger, and A. M. Haigler (2007). "Ambiguous taxa: effects on the characterization and interpretation of invertebrate assemblages". *Journal of the North American Benthological Society* 26 (2), 286–307.

EFSA (2013). "Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters". *EFSA Journal* 11 (7), 3290.

Fox, D. R. and W. G. Landis (2016). "Comment on ET&C perspectives, November 2015-A holistic view". *Environmental Toxicology and Chemistry* 35 (6), 1337–1339.

Fox, G. A., S. Negrete-Yankelevich, and V. J. Sosa, eds. (2015). *Ecological statistics: contemporary theory and application*. Oxford: Oxford University Press.

Gelman, A. and H. Stern (2006). "The difference between "significant" and "not significant" is not itself statistically significant". *The American Statistician* 60 (4), 328–331.

Gelman, A., J. B. Carlin, H. S. Stern, D. B. Dunson, A. Vehtari, and D. B. Rubin (2014). *Bayesian data analysis*. CRC Press.

Goulson, D. (2015). “Neonicotinoids impact bumblebee colony fitness in the field; a reanalysis of the UK’s Food & Environment Research Agency 2012 experiment”. *PeerJ* 3, e854.

Hansen, C. T., C. Ritz, D. Gerhard, J. E. Jensen, and J. C. Streibig (2015). “Re-evaluation of groundwater monitoring data for glyphosate and bentazone by taking detection limits into account”. *Science of the Total Environment* 536, 68–71.

Heller, S. R., A. McNaught, I. Pletnev, S. Stein, and D. Tchekhovskoi (2015). “InChI, the IUPAC International Chemical Identifier”. *Journal of Cheminformatics* 7 (1).

Helsel, D. (2010). “Much ado about next to nothing: incorporating nondetects in science”. *Ann Occup Hyg* 54 (3), 257–62.

Helsel, D. R. (2006). “Fabricating data: how substituting values for nondetects can ruin results, and what can be done about it”. *Chemosphere* 65 (11), 2434–2439.

Hoenig, J. M. and D. M. Heisey (2001). “The abuse of power”. *The American Statistician* 55 (1), 19–24.

Huckele, S. and T. Track (2013). “Risk management of emerging compounds and pathogens in the water cycle (RiSKWa)”. *Environmental Sciences Europe* 25 (1), 1. URL: https://enveurope.springeropen.com/articles/10.1186/2190-4715-25-1.

Ives, A. R. (2015). “For testing the significance of regression coefficients, go ahead and log-transform count data”. *Methods in Ecology and Evolution* 6 (7), 828–835.

Johnson, P. C. D., S. J. E. Barry, H. M. Ferguson, and P. Müller (2015). “Power analysis for generalized linear mixed models in ecology and evolution”. *Methods in Ecology and Evolution* 6 (2), 133–142.

Kim, S., P. A. Thiessen, E. E. Bolton, J. Chen, G. Fu, A. Gindulyte, L. Han, J. He, S. He, B. A. Shoemaker, J. Wang, B. Yu, J. Zhang, and S. H. Bryant (2016). “PubChem Substance and Compound databases”. *Nucleic Acids Research* 44 (D1), D1202–D1213.

Kleiber, C. and A. Zeileis (2016). "Visualizing Count Data Regressions Using Rootograms". *The American Statistician*, 1–25.

Lewis, K. A., J. Tzilivakis, D. J. Warner, and A. Green (2016). "An international database for pesticide risk assessments and management". *Human and Ecological Risk Assessment: An International Journal* 22 (4), 1050–1064.

Maltby, L., N. Blake, T. C. M. Brock, and P. J. Van Den Brink (2005). "Insecticide species sensitivity distributions: Importance of test species selection and relevance to aquatic ecosystems". *Environmental Toxicology and Chemistry* 24 (2), 379–388.

Martin, T. G., B. A. Wintle, J. R. Rhodes, P. M. Kuhnert, S. A. Field, S. J. Low-Choy, A. J. Tyre, and H. P. Possingham (2005). "Zero tolerance ecology: improving ecological inference by modelling the source of zero observations". *Ecology Letters* 8 (11), 1235–1246.

Min, Y. and A. Agresti (2002). "Modeling nonnegative data with clumping at zero: a survey". *Journal of Iranian Statistical Society* 1 (1), 7–33.

Müller, N., D. de Zwart, M. Hauschild, G. Kijko, and P. Fantke (2016). "Exploring REACH as a potential data source for characterizing ecotoxicity in life cycle assessment: Exploring REACH for characterizing ecotoxicity in LCA". *Environmental Toxicology and Chemistry*.

Münch, D. and C. G. Galizia (2016). "DoOR 2.0 - Comprehensive Mapping of Drosophila melanogaster Odorant Responses". *Scientific Reports* 6, 21841.

Nakagawa, S. and T. M. Foster (2004). "The case against retrospective statistical power analyses with an introduction to power analysis". *Acta Ethologica* 7 (2), 103–108.

Pence, H. E. and A. Williams (2010). "ChemSpider: An Online Chemical Information Resource". *Journal of Chemical Education* 87 (11), 1123–1124.

Posthuma, L., G. W. Suter, and T. P. Traas (2002). *Species sensitivity distributions in ecotoxicology*. Environmental and ecological risk assessment. Boca Raton and Fla: Lewis.

Ranke, J. (2016). *jranke/chents*. URL: https://github.com/jranke/chents.

Ritz, C. (2010). “Toward a unified approach to dose-response modeling in ecotoxicology”. *Environmental Toxicology and Chemistry* 29 (1), 220–229.

Schäfer, R. B., M. Bundschuh, A. Focks, and P. C. von der Ohe (2013). “Letter to the Editor”. *Environmental Toxicology And Chemistry* 32 (4), 734–735.

Sigrist, F. and W. A. Stahel (2010). “Using the censored gamma distribution for modeling fractional response variables with an application to loss given default”. *arXiv preprint arXiv:1011.1796*. URL: http://arxiv.org/abs/1011.1796.

Stieger, G., M. Scheringer, C. A. Ng, and K. Hungerbühler (2014). “Assessing the persistence, bioaccumulation potential and toxicity of brominated flame retardants: Data availability and quality for 36 alternative brominated flame retardants”. *Chemosphere* 116, 118–123.

Szöcs, E., P. J. v. d. Brink, L. Lagadic, T. Caquet, M. Roucaute, A. Auber, Y. Bayona, M. Liess, P. Ebke, A. Ippolito, C. J. F. t. Braak, T. C. M. Brock, and R. B. Schäfer (2015). “Analysing chemical-induced changes in macroinvertebrate communities in aquatic mesocosm experiments: a comparison of methods”. *Ecotoxicology* 24 (4), 760–769.

Szöcs, E. and R. B. Schäfer (2016). “Statistical hypothesis testing—To transform or not to transform?” *Integrated Environmental Assessment and Management* 12 (2), 398–400.

Ter Braak, C. J. and P. Šmilauer (2015). “Topics in constrained and unconstrained ordination”. *Plant Ecology* 216 (5), 683–696.

Tobin, J. (1958). “Estimation of Relationships for Limited Dependent Variables”. *Econometrica* 26 (1), 24.

Umweltbundesamt (2016). *ETOX: Information System Ecotoxicology and Environmental Quality Targets*. URL: http://webetox.uba.de/webETOX/index.do.

U.S. EPA (2016). *ECOTOX database*. URL: http://cfpub.epa.gov/ecotox/.

Van den Brink, P. J., C. B. Choung, W. Landis, M. Mayer-Pinto, V. Pettigrove, P. Scanes, R. Smith, and J. Stauber (2016). “New approaches to the ecological risk assessment of multiple stressors". *Marine and Freshwater Research* 67 (4), 429.

Van den Brink, P. J., N. W. van den Brink, and C. J. F. ter Braak (2003). "Multivariate analysis of ecotoxicological data using ordination: demonstrations of utility on the basis of various examples". *Australasian Journal of Ecotoxicology* 9, 141–156.

Van Der Hoeven, N. (1998). "Power analysis for the NOEC: What is the probability of detecting small toxic effects on three different species using the appropriate standardized test protocols?" *Ecotoxicology* 7 (6), 355–361.

Warton, D. I., F. G. Blanchet, R. B. O'Hara, O. Ovaskainen, S. Taskinen, S. C. Walker, and F. K. C. Hui (2015a). "So Many Variables: Joint Modeling in Community Ecology". *Trends in Ecology & Evolution* 30 (12), 766–779.

Warton, D. I., S. D. Foster, G. De'ath, J. Stoklosa, and P. K. Dunstan (2015b). "Model-based thinking for community ecology". *Plant Ecology* 216 (5), 669–682.

Warton, D. I., M. Lyons, J. Stoklosa, and A. R. Ives (2016). "Three points to consider when choosing a LM or GLM test for count data". *Methods in Ecology and Evolution*.

Warton, D. I., S. T. Wright, and Y. Wang (2012). "Distance-based multivariate analyses confound location and dispersion effects". *Methods in Ecology and Evolution* 3 (1), 89–101.

Weininger, D. (1990). "SMILES. 3. DEPICT. Graphical depiction of chemical structures". *Journal of Chemical Information and Computer Sciences* 30 (3), 237–243.

Wittmer, I. K., H. P. Bader, R. Scheidegger, H. Singer, A. Luck, I. Hanke, C. Carlsson, and C. Stamm (2010). "Significance of urban and agricultural land use for biocide and pesticide dynamics in surface waters". *Water Research* 44 (9), 2850–2862.

Yee, T. W. (2015). *Vector Generalized Linear and Additive Models*. Springer Series in Statistics. New York, NY: Springer New York.

Yee, T. W. and T. J. Hastie (2003). "Reduced-rank vector generalized linear models". *Statistical modelling* 3 (1), 15–41.