Spatial variation in the ecological vulnerability of freshwater invertebrate assemblages to chemical stressors

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“With my horse proudly trotting in the spring breeze, I see all Chang'an flowers in one day.”

Meng Jiao

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Abstract

Most current ecological risk assessments of chemicals adopt a single threshold approach. However, the single threshold approach has been criticized for being rather general. Natural assemblages may vary their sensitivity to a chemical, and spatial variation in assemblage recovery is not considered. Therefore, it is necessary to evaluate the impact of spatial variation in sensitivity and recovery on ecological risk assessment.

Ecological vulnerability to chemicals consists of external exposure, intrinsic sensitivity, and recovery potential, and this thesis focused on spatial variation in the latter two elements. The thesis has three principal objectives: to assess spatial variation in the sensitivity of freshwater invertebrate assemblages to chemicals; to relate spatial patterns in assemblage sensitivity to river typology descriptors and land use; to investigate spatial variation in the recovery process of freshwater invertebrate assemblages.

There is spatial variation in the sensitivity of species assemblages to chemicals, with the magnitudes of variation to specifically acting chemicals being greater than general acting chemicals. There is an association between the similarity in species composition and the variation in assemblage sensitivity to chemicals. The river typology descriptors and land use significantly impact spatial variation in assemblage sensitivity to some chemicals. The recovery potential of species assemblages also varies spatially and exhibits certain patterns. Spatial variation in assemblage sensitivity and recovery potential was considered to develop a spatially defined ecological vulnerability framework.

Considering the spatial variation in assemblage sensitivity to chemicals, the current environmental quality standards are protective of biodiversity for most of the chemicals investigated; for many chemicals, the standards are very conservative; potentially unnecessarily restricting the use of chemicals that provide significant societal benefits. Spatially defined ecological vulnerability analysis suggests that spatial variation in assemblage sensitivity and recovery potential can exacerbate or mitigate chemical risks compared to using the single threshold approach.

Contents

Chapter 1 | General introduction

1.1 Introduction

Chemicals, many of which have brought tremendous benefits to modern human society, may be intentionally or unintentionally released into the environment during production, use, and disposal (Holdgate, 1979; Tundo et al., 2000; Anastas and Eghbali, 2010; Maltby, 2013). Chemical pollution has been reported to cause harm to natural assemblages, ecological systems, and human health (Cairns Jr and Niederlehner, 1994; Zala and Penn, 2004; Schwarzenbach et al., 2010; Noyes and Lema, 2015) and has been considered as one of the five major threats to global biodiversity (Hirsch, 2010). Ecosystems provide the essential material basis for human survival, production, and livelihood (e.g. food, water, and recreation in nature) (Corvalan et al., 2005). Chemical pollution has been reported to lead to biodiversity loss and thus affects the benefits humans obtain from ecosystems (Millennium ecosystem assessment, 2005; Cardinale et al., 2012).

There are approximately 140,000 chemicals on the market in Europe, more than half of which are frequently used in agriculture, industry, household products, and pharmaceuticals (Judson et al., 2009; Johnson et al., 2020). Moreover, as global chemical production has a predicted growth rate of 1,000 new chemicals per year (UNEP, 2019), the pressure from chemical pollution on ecosystems and the services they provide is predicted to increase unless the adverse effects of chemicals can be reduced by chemical regulation and management (Maltby et al., 2018; Maltby et al., 2021).

The effective implementation of chemical regulation and management requires the risks of chemicals to ecosystems and the services they provide to be assessed (Maltby et al., 2018; Faber et al., 2021). The ecological risk assessment method has been most widely used to assess the adverse effects of chemicals (Norton et al., 1992; Solomon and Sibley, 2002). The traditional ecological risk assessment characterizes the risk of a chemical by comparing the exposure concentration and effective concentration. In the ecological risk assessment of a chemical, the exposure concentration usually uses the concentration of the pollutant measured in the environment. The effective concentration is usually described using a single threshold approach based on the toxicity data of multiple species. In addition, the single threshold approach to measure effect concentration is also used to derive environmental standard values (e.g. Regulatory Acceptable Concentrations (RACs) (Brock et al., 2006) or Environmental Quality Standards (EQSs) (Rämö et al., 2018) to protect the vast majority of wildlife in the environment in all places. The chemicals industry in Europe is highly regulated compared to other regions of the world (e.g. developing countries in Asia) (Wittcoff et al., 2012). RACs and EQSs provide legally permissible binding concentrations of harmful substances in the environment within a certain time and space frame. However, the RAC and EQSs values in the current legislation are still criticized as being rather general (e.g. Brown, et al., (2017)). This indicates that there is a need to define spatially specific conservation objectives.

The use of a single threshold assumes that either the threshold is conservative enough to protect all natural assemblages that may be exposed or that all assemblages are equally sensitive to a chemical. However, it is known that species vary in their sensitivity to a chemical (i.e. interspecific variation, Maltby et al., 2005) and that the relative sensitivity of species is chemical dependent (i.e. the myth of the most sensitive species, Cairns, 1986). In addition, spatial variation in the taxonomic composition of natural assemblages provides the potential for spatial variation in the sensitivity of assemblages to chemical exposure. Furthermore, the single threshold value is usually derived from toxicity tests performed on a limited number of species, which may not be protective and representative of species that exist in natural assemblages (Del Signore et al., 2016; Posthuma et al., 2019). Therefore, risk management decisions based on a single threshold approach may either be under- or over-protective of natural ecosystems. If the risk management decision is highly overprotective, it may unnecessarily restrict the use of chemicals with societal benefits.

The ecological vulnerability of assemblages to stressors is a function of external exposure, intrinsic sensitivity, and recovery potential (De Lange et al., 2010), and therefore, the ecological vulnerability of assemblages exposed to chemical stressors is a function of both their ability to resist the stressor (i.e., sensitivity) and their ability to recover from any stressor-induced impacts once the stressors have been removed (De Lange et al., 2010; Beroya-Eitner, 2016). Chemicals have different exposure patterns depending on their use pattern and chemical properties. For example, many household chemicals released via wastewater treatment plants may have relative constant exposure patterns (Bai et al., 2022; Chacón et al., 2022), whereas many agricultural pesticides have short-term intermittent exposures patterns (Van Drooge et al., 2001; Reinecke and Reinecke, 2007). Intermittent chemical exposures may provide species with an opportunity to recover to their original state between exposure events and assemblages. The European Food Safety Authority (EFSA) has proposed a conceptual framework to consider the importance of recovery processes in assessing the environmental risk of plant protection products (EFSA Scientific Committee, 2016). Internal recovery refers to the recovery of native species to their pre-disturbance state through their own reproductive capacity, while external resilience refers to the recovery of external species to pre-disturbance levels through immigration across the landscape surface to replenish local assemblages (Fuentes and Arriagada, 2022). Spatial variation in assemblage composition potentially influences internal and external recovery, while spatial variation in land use and landscape features influences external recovery.

The aim of this thesis was to assess spatial variation in the ecological vulnerability of freshwater invertebrate assemblages to chemical stressors and to consider the implications for environmental risk assessment. Freshwater invertebrates were used as the focal taxonomic group as they are distributed widely across different freshwater ecosystems and vary greatly in their chemical sensitivity (Maltby et al., 2005; Maltby et al., 2009; Rico and Van den Brink, 2015). The following sections discuss spatial variation in species composition and ecological vulnerability in more detail before outlining the thesis aims, objectives, and underpinning hypotheses.

1.2 Spatial variation in assemblage composition

1.2.1 Drivers of spatial variation in assemblage composition

Spatial variation in specie compositions is a common feature of natural assemblages (e.g. algae, fish, invertebrate assemblages) (Anderson and Millar, 2004; Sushko, 2021; Wang et al., 2021). Assemblage compositions were mainly shaped by habitat filtering functions and spatial dispersal processes (Beisner et al., 2006). Spatial variation in environmental conditions provides different selection regimes for species. The habitats with different environmental conditions have been considered to have a function of filtering for species, and this “filtering” phenomenon has been boiled down to habitat templet theory (Poff and Ward, 1990; Blanck et al., 2007). Habitat templet theory highlights that species vary in their response to different habitats, resulting in spatial variation in assemblage compositions (Townsend and Hildrew, 1994; Townsend et al., 1997). For example, habitat heterogeneity may be the largest contributor to the beta diversity of freshwater invertebrate assemblages in New Zealand streams (Astorga et al., 2014).

The dispersal and community assembly processes emphasize the relationship between the assemblages and the spatial dispersal processes. The dispersal process is when external species pass over the landscape surface to reach local communities. The dispersal process is influenced not only by the ability of species or vectors to immigrate but also by the travel resistance caused by landscape elements (Pineda et al., 2022). The community assembly describes how trait composition and species richness in communities are affected by a series of local environmental filters and the effects of species interactions (Farooq et al., 2022; Shi et al., 2022).

Dispersal process and habitat templet theory both have important roles in assemblage construction but make different contributions in different situations (He et al., 2022). For example, Li (2020) found that spatial dispersal processes are more important than habitat templet filtering in rare cases in the Ganjiang River, China (Li et al., 2020). In addition, the influence of spatial dispersal processes on taxonomic compositions is also considered to be related to the dispersal ability of taxa. For example, freshwater organisms with poor mobility are more restricted by spatial dispersal processes than habitat templets (Mergeay et al., 2007; Karpowicz, 2014).

1.2.2 Importance of river typology and land use in driving spatial variation in assemblage composition

River typology descriptors aim to classify rivers with similar characteristics (e.g. altitude, catchment size, geology, slope, land use) into different categories, thus simplifying river management in practice (Stefanidis et al., 2022). Rivers within the different typology descriptors potentially provide various habitat types and thus affect the traits composition (González-Paz et al., 2022). Broad river typology descriptors (e.g. System A in the Water Framework Directive (WFD)) have been developed to support environmental management in Europe (European Commission, 2000). River typology descriptors, such as altitude, catchment size, and geology, impact the spatial variation in assemblage compositions. The relationship between altitude and spatial variation in trait compositions of freshwater invertebrate assemblages has been well studied, such as the decrease in species richness with the increase in altitude (Suren, 1994; Pardo et al., 2014). In addition, catchment size has been reported to affect the spatial distribution of species compositions, with the freshwater gatherers frequently found in large streams (LeCRAW and Mackereth, 2010).

Furthermore, catchment geology also affects the spatial distribution of species. For example, crustaceans (Gammaridae) are usually present in calcareous rivers, but it is difficult to find them in siliceous rivers (Pardo et al., 2014). River typology descriptors describe different types of rivers based on the physic-chemical, geologic or hydrologic characteristics of river ecosystems that provide habitat for freshwater organisms. Freshwater invertebrate assemblages in the rivers with the same typologies generally have similar trait compositions based on niche assembly rules.

Spatial variation in assemblage compositions is associated with the surrounding land use. Land use has become a critical stressor affecting river ecosystems. Anthropogenic land use, such as agricultural and urban areas, has been reported to lead to the degradation of river habitat, the deterioration of water quality, and the changes in hydrological rhythms, which directly or indirectly affect the structure of river biological assemblages. For example, more sensitive taxa (e.g. Ephemeroptera, Plecoptera, and Trichoptera) can be found in natural land types than in urban New Zealand streams (Snyder et al., 2003). In addition, land-use types can characterize exposure scenarios based on geographic proximity to pollution sources (Hopkins and Hippe, 1999; Beaulieu et al., 2020).

1.2.3 Importance of chemical stressors in driving spatial variation assemblage composition

Freshwater ecosystems are facing various environmental (e.g. drought, flood) and anthropogenic stressors (e.g. chemical pollution) (Aldous et al., 2011; Carpenter et al., 2011; Malaj et al., 2014; Castello and Macedo, 2016). Many previous studies have shown that single or combined effects of these stressors may affect taxonomic compositions in natural assemblages (Schäfer et al., 2011; Segurado et al., 2018). In Iberian rivers, the proportion of sensitive species decreases with the increased risk of chemical pollution (Kuzmanović et al., 2016). The relative importance of chemical stressors compared to non-chemical stressors requires a case-by-case analysis. For example, in Melbourne streams, urbanization is thought to be the main factor for the degradation of invertebrate community composition, with a few tolerant taxa that can be observed (Walsh et al., 2001).

Many studies have confirmed that chemical pollution can reduce biodiversity and alter taxonomic composition (Diamond et al., 2015; Landrigan et al., 2018; Wilson and Fox, 2021). In heavily polluted areas, species richness has been observed to be lower than in other polluted areas (Xiong et al., 2020; MacLeod et al., 2021). The types of chemicals may have significant impacts on driving spatial variation assemblage composition. For example, some highly toxic insecticides (organophosphates, organochlorines, and pyrethroids) kill species quickly by causing their neurotoxicity, thus altering the taxonomic compositions (Richardson et al., 2019; Tsai and Lein, 2021). For some persistent organic pollutants, long period bioaccumulation and biomagnification would result in species mortality and alter the taxonomic compositions (Kuehr et al., 2021; Borgå et al., 2022). In chemical-contaminated sites, sensitive freshwater invertebrates were eliminated, and only two tolerant invertebrates survived (Datry, 2003).

1.2.4 Predicting species assemblage composition in the absence of environmental stressors

The distribution and occurrence of species could be affected by various external stressors (including chemical pollution). Knowing the species composition before being affected by external stressors can help derive EQSs for chemicals in uncontaminated conditions, thus protecting biodiversity.

Predicting assemblages expected at sites based on their environmental characteristics assumes that reference and impacted sites share ecological features, and impacted sites may be restored to a similar biological status to reference sites after removing external stressors. Reference sites are defined as those under minimally-impacted natural conditions with good biological integrity. Many predictive models have been developed based on environmental characteristics approaches across the world (e.g. River Invertebrate Prediction and Classification System (RIVPACS) in the UK, AusRivAS (Australian River Assessment Scheme) models in Australia, South African Scoring System (SASS) in Africa, Benthic Assessment of Sediment (the BEAST) in Canada) (Reynoldson et al., 1995; Wright et al., 1998; Smith et al., 1999; Wright, 2000; Dickens and Graham, 2002).

Setting environmental standards based on current taxonomic composition can limit the taxonomic compositions from returning to their original state, as current taxonomic composition could be affected by past exposure. Predicting species assemblage composition in the absence of environmental stressors can help eliminate the influence of external stressors (including history stressor exposure) and derive reference regulatory acceptable concentrations of chemical pollutants from protecting natural assemblages.

1.3 Ecological vulnerability

The ecological vulnerability to external stressors is a function both of their ability to resist the stressor (i.e., sensitivity) and their ability to recover from any stressor-induced impacts once the stressors have been removed (De Lange et al., 2010; Beroya-Eitner, 2016). Ecological vulnerability analysis has been widely used to assess the potential risks of external stressors, such as global warming, melting glaciers, drought, floods, and chemical pollution. Many studies have focused on the vulnerability of ecosystems to climate change (Okey et al., 2015; Jiang et al., 2018; Kling et al., 2020), while some other studies research the ecological vulnerability of coal mining (Liao et al., 2013; Xiao et al., 2020). There are also a small number of studies considering the vulnerability of ecosystems to chemical pollutants (Lange et al., 2009; Ippolito et al., 2010; Thomsen et al., 2012).

The definition of the term ecological vulnerability used in this thesis is from De Lange et al (2010), who define ecological vulnerability as “the sensitive response and self-recovery ability under chemical exposure in a certain time and space.” Ecological vulnerability in ecotoxicology consists of three main elements: external exposure, intrinsic sensitivity, and recovery potential (Van Straalen, 1993; De Lange et al., 2010; Ippolito et al., 2010; Rubach et al., 2011). With reference to these previous publications, a conceptual framework is shown in Figure 1.1.

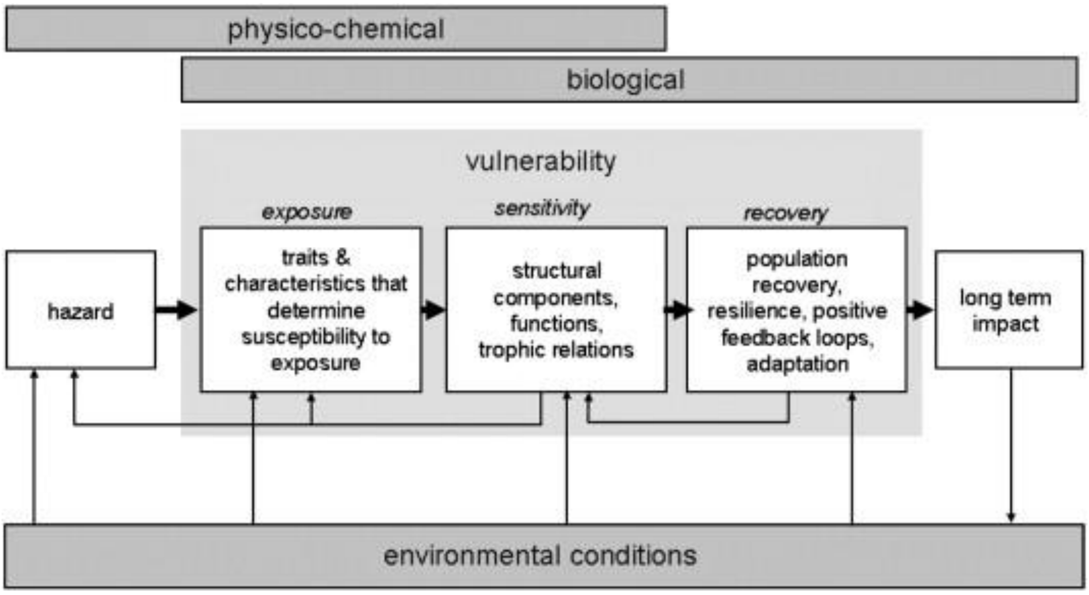

Figure 1.1 A conceptual framework of ecological vulnerability in ecotoxicology proposed by De Lange et al. 2010.

1.3.1 External exposure

External exposure describes the external pressures on ecosystems, which can come from chemical contamination. External exposure is the first component of vulnerability that affects biological organisms. The distribution of chemicals in the environment is not homogeneous. Certain chemicals have fixed spatial usage scenarios. For example, plant protection products are primarily applied to agricultural landscapes, while household and personal care products are most abundant in urban and suburban areas. After chemicals enter the environment, they undergo various fate and transport processes (e.g. dilution, adsorption, and precipitation), which also affects spatial variation in the concentrations of chemicals (Lindim et al., 2015; Chan et al., 2020; Bednarska et al., 2022; Hua et al., 2022).

There is a distinction between chemical presence and chemical exposure. The bioavailability of chemicals plays an important role in species' exposure to chemicals. For example, for heavy metals, most of the bioavailable heavy metals are in the form of cations in rivers (Kalembkiewicz et al., 2018; Miranda et al., 2021). The bioavailability of heavy metals is heavily influenced by various water parameters (e.g. pH, organic matter, alkalinity, hardness) (Zhang et al., 2014; Väänänen et al., 2018). In addition, the bioavailability may vary by chemical class. The bioavailability of pyrethroid insecticides was found to be lower than persistent organic pollutants (Aznar-Alemany and Eljarrat, 2020).

Species traits (e.g. habitat preferences, food choice) also affect the exposure of species to chemicals (Rubach et al., 2011). Habitat preferendum determines whether the scene where species can be exposed to pollutants. For example, freshwater invertebrates (*Gammarus* sp. and *Asellus* sp.) were found to avoid chemical pollution and choose unpolluted habitats (De Lange et al., 2006). Some freshwater invertebrates (*Ceriodaphnia* sp. and *Daphnia* sp.) living in rivers with rich dissolved organic matter, high pH, and high water hardness can effectively avoid the toxic effects of heavy metals (Gensemer et al., 2018). For some soil invertebrates, food is one of the essential pathways for exposure to chemical exposure. Due to the different levels of pollutants in various foods, the variation in food choices may affect external exposure (Peijnenburg et al., 2012).

1.3.2 Intrinsic sensitivity

Sensitivity describes the ability of assemblages to resist stressors (i.e., chemicals). The sensitivity of a species is usually expressed as a concentration or dose value that triggers a specific response in organisms, which may be lethal (i.e., mortality) or sublethal (e.g., reproduction, growth, biochemical response). Species vary in their sensitivity to chemicals, and interspecific variation in sensitivity is a function of interspecific variation in toxicokinetics and toxicodynamics processes (Ippolito et al.; Veltman et al., 2014; Products et al., 2018). Toxicokinetics describes the uptake and internal fate processes of a chemical in organisms (e.g. bioaccumulation, internal distribution, metabolism, and excretion), and toxicodynamics describes the interactions between a chemical and its target sites and the dynamic response of organisms to chemical exposure (e.g. antioxidant stress response, energy allocation) (Dalhoff et al., 2020; Van den Berg et al., 2021). *Lymnaea stagnalis* was found to be more tolerant to diazinon than *Gammarus* sp due to the variation in their biotransformation abilities (Nyman et al., 2014). It has been an argument that there is no single species or specific taxonomic group that can be sensitive to all chemicals (Cairns, 1986). The relative sensitivity of species to toxicants has been considered chemical-specific (Maltby et al., 2005). Therefore, the thresholds based on multi-species testing can provide valuable evidence for biodiversity protection. The spatial variation in taxonomic composition should be taken into account in the ecological risks of chemicals.

The magnitude of interspecific variation in sensitivity exhibited by species to chemicals was influenced by species identities, species traits, and chemical classes (Vaal et al., 1997). The sensitivity of *Daphnia magna* and *Scapholeberis mucronata* to copper varies by 10 fold, while there is no significant difference in the sensitivity of *Ceriodaphnia* sp. and *Simocephalus* sp. (Bossuyt and Janssen, 2005). The magnitude of interspecific variation between *Gammarus pulex* and *Daphnia Magna* was 2-3 orders of magnitude compared to different organic chemicals (Ashauer et al., 2011). The species traits (e.g. body size) can be used to explain the interspecific variation in sensitivity to Triphenyltin hydroxide (Gergs et al., 2015). In addition, large interspecific variation was often found to some specifically acting chemicals, and this variation can be up to 6 orders of magnitude (Vall et al., 2000).

There is a common phenomenon that assemblage compositions vary over space (potential influencing factors have been described in section 1.2). Based on the observations of interspecific variation in sensitivity and species chemical-dependent sensitivity, assemblages with different toxicokinetic and toxicodynamic related traits composition potentially exhibit varying sensitivity to chemicals. Although some tested species may exist in the natural community to be assessed, they only account for a very small proportion, and the sensitivity of most species in assemblages is unknown. For example, close to 90% of the freshwater invertebrate species recorded in the UK (https://environment.data.gov.uk/ecology/explorer/) have not been used in toxicity tests for any of the more than 5000 chemicals included in the USEPA Ecotoxicology knowledgebase (https://cfpub.epa.gov/ecotox/). Therefore, there is a large data gap in assessing assemblage-level sensitivity. Species sensitivity distributions (SSDs) that describe interspecific variation in chemical sensitivity can be used to derive a measure of assemblage sensitivity, such as the HC5 (e.g. the concentration that is hazardous to 5% of species) (Posthuma et al., 2001; Forbes and Calow, 2002; Wheeler et al., 2002). Although the SSD method has been widely used in ecological risk assessment to extrapolate from single-species toxicity tests to community-level sensitivity (Hose and Van den Brink, 2004), SSDs are generally based on toxicity data for a few species that may or may not exist in the natural assemblage to be protected (Kefford et al., 2005). This has resulted in criticism of the approach (Forbes and Calow, 2002; Belanger et al., 2017) and highlights the need to generate more toxicity data to be able to adequately describe interspecific variation in chemical sensitivity. However, conducting toxicology experiments is time-consuming and laborious. In addition, it is an impossible task to test all species to all chemicals due to the even large number of both chemicals and species.

Several prediction approaches have been developed to predict the sensitivity of untested species, which have recently been reviewed by van den Berg et al. 2021. The full review is in Appendix S1.1, and the key points are summarized here. Prediction approaches can be generally divided into two categories: chemical-related (e.g. chemical-property-based methods) and species-related methods (e.g. interspecies correlation, trait-based, genomic-based and taxonomy-based methods) (Gramatica, 2007; Cherkasov et al., 2014; van den Berg et al., 2021). The quantitative structure-activity relationships (QSAR) model, an example of chemical-related methods, uses chemical properties to predict toxicological effects (Golbraikh et al., 2003; Dudek et al., 2006). QSAR models are usually constructed based on the chemicals within the same type of toxic mode of action. As many chemicals have no toxicity data available or are limited to a few standard test species (https://cfpub.epa.gov/ecotox/), QSAR models have limited capability to predict the sensitivity for a great majority of wild species.

Species-related methods consider the correlation between species. Interspecies correlation methods (e.g., interspecies correlation estimation (ICE)) utilize the correlation of toxicological effects across multiple pairs of species (Dyer et al., 2006; Raimondo et al., 2007). The major limitation of interspecies correlation methods is that they are unable to predict the sensitivity of species that have not been tested for any chemicals. Trait-based methods use sensitivity-related traits, such as body size, respiratory modes, and feeding modes, to predict sensitivity (Baird and Van den Brink, 2007; Rubach et al., 2012). By using trait databases (Tachet et al., 2000; Usseglio‐Polatera et al., 2000), trait-based methods can predict the sensitivity of more untested species than interspecies correlation methods. However, most of the traits in currently available trait databases (e.g., reproduction, life cycle duration, and current velocity preferendum) have low relatedness to intrinsic sensitivity. There is a need for more toxicokinetic and toxicodynamic related traits (e.g. uptake and depuration kinetics) in order to develop his approach further (Rubach et al., 2012; Van den Berg et al., 2019). Genomic-based methods consider the similarity in orthologous and the toxicology pathway to predict the sensitivity of untested species. Genomic-based methods face genomics data gaps, although the availability of genomics data is increasing. High-throughput sequence results can be obtained using the Environmental DNA, but a very low number of species (especially for wild freshwater invertebrates) can be identified due to the low coverage of taxa in current genomic databases (Thomsen and Willerslev, 2015; Barnes and Turner, 2016).

The taxonomy-relatedness methods provide a good compromise to predict species sensitivity before the complete genomic databases are available. Taxonomy-relatedness methods consider the evolutionary relationship between species and assume that species with closer taxonomic distance share more similar sensitivity to chemicals (Craig et al., 2012; Craig, 2013). Taxonomy-relatedness can be a good proxy of genetic relatedness and has been proven to make reliable predictions for species sensitivity (Guénard et al., 2011). The hierarchical species sensitivity distribution (hSSD) method was developed by Craig, 2013 to extrapolate the sensitivity of tested species to the sensitivity of untested species. The hSSD method is based on the established hierarchical taxonomy for both tested and untested species. Different taxonomic groups of species may vary in their sensitivity to a chemical, and this variation may be related to the taxonomic structure (e.g. non-arthropods are normally tolerant to insecticides). In addition, species with close taxonomic distance potentially have similar toxicokinetic and toxicodynamic related traits, which may affect their sensitivity to a chemical. Interspecies correlation in sensitivity exists and can be linked to taxonomic similarity. Sinclair (2021) continued to improve the hSSD model and used it to predict the sensitivity of freshwater invertebrates from the RIVPACS reference database.

The hSSD method was chosen compared to other methods, as taxonomic data is easily accessible. The hSSD model was developed using the Bayesian method, which is an optimization of simple regression methods (Craig et al., 2012; Craig, 2013). Extrapolation models provide opportunities to investigate the sensitivity of assemblages with untested species and, therefore, to assess the variation in the sensitivity of species assemblages to chemicals and whether this variation is spatially patterned.

1.3.3 Recovery potential

Recovery potential, the third aspect of ecological vulnerability, is the ability to recover to the original state after removing external stressors. Recovery potential can be divided into internal and external recovery (Rubach et al., 2011). Internal recovery processes mainly depend on species-specific traits (e.g., generation time, number of offspring), and external recovery processes are a function of both species-specific traits (e.g., dispersal, life-cycle) and landscape factors (e.g., source populations, landscape connectivity) (EFSA Scientific Committee, 2016). Short-term intermittent exposure may provide a window period for community recovery. Beketov et al. (2008) found that the population quantity of multivoltine species can cause recovery to the pre-exposure levels after 10 weeks following intermittent exposure to thiacloprid.

Internal recovery describes the process by which the local population is restored to its original level through population growth. Species with high reproductive capacity need a short time to recover to their original state, while species with low reproductive capacity need a long time to recover. Traits related to reproductive capacity include life stage, generation time, voltinism, reproduction mode, and adult life span. Voltinism is an important trait affecting internal recovery. Multivoltine species (e.g. *Asellus*, *Brachionus*, *Paramelita*, *Lumbriculus*) have two or more generations per year, while univoltine species (e.g. *Astacopsis*, *Cambarus*, *Drunella*, *Ephemerella*) only have one generation per year (Rico and Van den Brink, 2015). Different taxonomic groups of species may vary in their reproduction-related traits. Considering the spatial variation in assemblage compositions, the internal recovery potential of freshwater invertebrate assemblages varies spatially.

External recovery refers to the immigration of species to replenish local populations or re-establish a new population. Dispersal capability and mode play important roles in external recovery processes. Different taxonomic groups of species differ in their ability to disperse (Malmqvist, 2002). Insect taxa (e.g. Ephemeroptera, Plecoptera, and Trichoptera) were demonstrated to have stronger dispersal abilities than non-insect taxa (e.g. Oligochaeta, Gastropoda) (Li et al., 2016). A field investigation shows that benthic invertebrates with strong dispersal capability reach the new habitat first, and those with weak dispersal capability need a long time to arrive (Winking et al., 2014). In addition, the dispersal mechanism of different taxonomic groups of species is different. Dispersal modes can be classified as passive and active. Passive dispersal requires an external force such as water currents, wind, or animal vectors. For example, some freshwater invertebrates are moved from upstream to downstream with the river flow (i.e. drift) (Williams and Williams 1993). Some invertebrates (e.g. Naididae, Nematoda) may be attached to animal vectors, such as frogs, snakes, and water birds (Lopez et al., 1999; Frisch et al., 2007). The dispersal modes of freshwater invertebrates may vary between life stages. For example, some freshwater insects (e.g. Ephemeroptera, Plecoptera, and Trichoptera) exhibit passive and active channel dispersal in the larva stage, but in the adult stage, they exhibit active air dispersal (Parkyn and Smith, 2011).

The external recovery process is influenced by landscape factors, such as distance from the source, landscape cost surface, and river connectivity (Trekels et al., 2011; Peterson et al., 2018; Shackelford et al., 2018). Landscape cost surface describes the resistance that species encounter during migration (Zeller et al., 2012; Ma et al., 2022). For active dispersal of insect taxa, the greater the distance between the source and destination, the more efforts species need to pay (Smith et al., 2009; Heino et al., 2017). Distance from the source was thought to be the second most important factor in driving recolonization, and the source within 1000 m is particularly important for external recovery (Tonkin et al., 2014). River connectivity was considered more important for channel dispersal (many taxa) and less important for aerial dispersal (insects only) (Morán‐Ordóñez et al., 2015; Tonkin et al., 2018). Some poor dispersers (non-insects and insects with poor flying strength) were limited to reaches and ponds with poor connectivity. In contrast, river connectivity did not limit the insects with strong flying strength (Sarremejane et al., 2017). Spatial heterogeneity in landscape elements makes a spatial variation in resistance during dispersal. Usually, for aquatic dispersal, there is less resistance from upstream to downstream than from downstream to upstream (Tonkin et al., 2018). For some freshwater invertebrates (e.g. crayfish and crabs) with strong dispersal capabilities, they can go upstream from downstream (Bubb et al., 2004; Torres et al., 2006). The concept of metacommunity theory can well explain the correlation between the various assemblages, emphasizing the importance of the landscape factors in the region (Vanschoenwinkel et al., 2008). The immigration of species from one community to another community can be affected by landscape elements.

1.4 Aims and Objectives

This thesis aims to investigate spatial variation in the ecological vulnerability of freshwater invertebrate assemblages to chemical exposure. Freshwater invertebrates have been regarded as ideal bioindicators to reflect river health. Many programs have been performed by monitoring freshwater invertebrates to assess water quality

(Marchant et al., 1997; Resh, 2008; Clews and Ormerod, 2009). Spatial variation in the ecological vulnerability can be disassembled into spatial variation in external exposure, intrinsic sensitivity, and recovery potential. Six groups of 20 chemicals (organophosphates (diazinon, fenitrothion, malathion, and parathion-methyl (PM)), organochlorines (dichlorodiphenyltrichloroethane (DDT), endosulfan and endrin), pyrethroids (cypermethrin, deltamethrin, and permethrin), heavy metals (cadmium, copper, nickel, and zinc), narcotics (phenol, benzenamine and glyphosate isopropylamine salt (GIS) and surfactants(linear alkylbenzene sulfonates (LAS), nonylphenol, sodium dodecyl sulfate (SDS)) were selected for this thesis, with considering the representation of chemical classes, different exposure patterns, rich toxicity databases, and high taxonomic diversity. England was chosen for this study, as freshwater invertebrate assemblages are monitored site-specifically in the long term. The thesis is mainly focused on two aspects of ecological vulnerability (intrinsic sensitivity and recovery potential) and is structured around four specific objectives below:

Objective 1: To investigate whether the sensitivity of freshwater invertebrate assemblages to chemicals varies spatially and presents spatial patterns (Chapter 2)

The taxonomic composition of natural assemblages varies spatially (Section 1.2.1), and the relative sensitivity of species to chemical exposure is chemical-specific (Section 1.3.2). The different taxonomic composition potentially provides the different possibility of assembling sensitive and tolerant species, thereby affecting the assemblage sensitivity to chemicals. The taxonomic composition in assemblages could show spatial patterns, and environmental "filtering" functions drive these. It can be expected that the assemblages with similar taxonomic compositions exhibit identical sensitivity to a chemical. Therefore, it is hypothesized that spatial variation in the sensitivity of freshwater invertebrate assemblages exhibits spatial patterns to chemicals. Species vary greatly in their sensitivity to specifically acting chemicals (e.g., insects are particularly sensitive to insecticides, while non-insects are relatively tolerant). The interspecific variation in sensitivity to specifically acting chemicals can be six orders of magnitude for aquatic species (Vall et al., 2000). The taxonomic composition of natural assemblages varies spatially (Section 1.2.1), potentially providing a combination of mechanisms. If the natural assemblages are composed of extremely sensitive or tolerant species, there will be a large variation in assemblage sensitivity to chemicals. Therefore, it is hypothesized that the magnitude of spatial variation in assemblage sensitivity will be greater for specifically acting than generally acting chemicals (Section 1.3.2). Prior stressor exposure may alter taxonomic compositions in natural assemblages. Species richness could be reduced by historical exposure, and sensitive species will be filtered out by stressors. The protection thresholds may not be protective enough if only historically exposed assemblages are focused on. The historically exposed assemblages may be less sensitive to chemical exposure than the assemblage of species expected if the site was minimally impacted (Section 1.2.4). Therefore, it is hypothesized that the assemblage sensitivity under minimally impacted conditions is different from those which were observed in the field. To address Objective 1, a multivariate approach will be used to predict the assemblage composition of over 2318 river sites in England under minimally impacted conditions, and a taxonomy-based method will be used to predict the sensitivity of untested species in actual and expected assemblages to 20 chemicals. This will assess the magnitude and pattern of spatial variation in the sensitivity of observed and expected freshwater invertebrate assemblages to chemical exposure.

Objective 2: To address whether the sensitivity of freshwater invertebrate assemblage sensitivity is related to river typology descriptors and land use where the assemblages are in the field (Chapter 3)

The rivers with different typology descriptors may provide different habitats and thus lead to spatial variation in taxonomic composition (Section 1.2.2). Different taxonomic groups of species vary their sensitivity to a chemical (Section 1.3.2). The land-use patterns can also affect species distribution and composition (Section 1.2.2). Therefore, it is hypothesized that the assemblage sensitivity varies across different typology descriptors and land use types. In addition, some types of chemicals with specific land use application patterns. For example, pesticides are applied in agricultural lands (Goessens et al., 2022), while personal care products are used in urban and suburban areas (Meng et al., 2022). Altitude, catchment size, and catchments are important drivers for species compositions (Section 1.2.2). River typology descriptors influence the spatial composition of species, with the expectation that assemblage sensitivity to chemicals can be related to the existing systems of river classification (e.g. WDF system A). Relate spatial variation in assemblage sensitivity to land use can outline likely exposure scenarios in the natural environment. To address Objective 2, the river typology descriptors and land use were related to spatial variation in the sensitivity of 2318 observed assemblages.

Objective 3: To explore whether the recovery potential, including internal and external recovery, of freshwater invertebrate assemblages, shows spatial variation and patterns (Chapter 4)

Recovery potential is divided into internal and external recovery (Section 1.3.3). Internal recovery depends on the reproductive capacity of the species. External recovery relies on the ability of the species to disperse and on landscape elements such as distance to the source, landscape resistance, and the connectivity of the river network (Section 1.3.3). Certain types of chemicals (e.g. pesticides) exhibit intermittent exposure patterns, which may provide a window period for recovery (Kanu et al., 2021). Freshwater invertebrate assemblages differ in taxonomic composition spatially (Section 1.2.1), and different taxonomic groups of species vary in their reproduction and immigration abilities (Section 1.3.3). In different combinations of species with varying reproduction and immigration abilities, freshwater invertebrate assemblages are expected to exhibit spatial variability in their recovery potential. To address Objective 3, 2318 observed assemblages in chapter 2 and 3 were used as a case study. Recovery-related traits and landscape factors were used to describe internal recovery, external recovery, and recovery potential to test the hypothesis of whether the recovery potential of freshwater invertebrate assemblages varies spatially.

Objective 4: To assess the ecological vulnerability of freshwater invertebrate assemblages to chemicals by synthesizing the results of chapters 2 to 3 (Chapter 5)

The single threshold approach to describe the assemblage sensitivity has been considered rather generic, and recovery potential has been seen as an important process after chemical exposure (Section 1.1). Ecological vulnerability analysis comprehensively considers external exposure, intrinsic sensitivity, and recovery potential (Section 1.3). External exposure, the first aspect of ecological vulnerability, exhibits spatial heterogeneity (Section 1.3.1). On the assumption that the sensitivity and recovery potential of natural assemblages vary spatially, the ecological vulnerability of freshwater invertebrate assemblages to chemicals may potentially vary over space. Objective 4 aims to synthesize results from Objective 1, 2, and 3 to assess spatial variation in the ecological vulnerability of freshwater invertebrate assemblages to chemicals. Firstly, the variation in assemblage sensitivity to chemicals was compared to the EQSs (e.g. HC5 values generated from global toxicity datasets to investigate whether the EQSs are protective enough or overprotected to natural assemblages. Secondly, spatial variation in assemblage sensitivity to chemicals was applied to ecological risk assessment to assess whether chemical risks are over- or under-estimated for natural assemblages. Finally, a comprehensive assessment framework of spatially defined ecological vulnerability to chemicals was constructed to explore whether recovery potential may exacerbate or mitigate the chemical risks to natural assemblages.

Chapter 2 | Assessing spatial variation in the sensitivity of observed and expected freshwater invertebrate assemblages to chemicals

2.1 Introduction

Natural assemblages vary in their compositions spatially. Environmental variables and external stressors are important factors affecting spatial variation in species compositions of natural assemblages (Mooraki et al., 2009; Ding et al., 2017; Baranov et al., 2020). Different species have different reactions to the same chemical (Maltby et al., 2005), and there is no one species that is equally sensitive to all chemicals (Cairns, 1986). Based on the above observations, natural assemblages with different combinations of species may potentially vary over space. Therefore, it is necessary to investigate whether there is a large variation in the sensitivity of species assemblages to chemicals over space. This chapter aims to investigate spatial variation in the sensitivity of freshwater invertebrate assemblages to chemicals by assessing the magnitude and spatial patterning of variation.

Chemical toxicity is closely related to its physical and chemical properties (Blum and Speece 1990). Chemicals with different physical and chemical properties may cause varying degrees of toxic effects on organisms. Based on the relationship between chemical structure and toxic effects, several classification methods have been proposed (e.g. Verhaar scheme, EPA MOAtox database) (Verhaar et al., 1992; Kienzler et al., 2017). Species sensitivity has great variability for specifically acting chemicals and small variability for narcotics (Escher and Hermens, 2002, 2004). For example, the sensitivity of fishes to nonpolar narcotics presented the smallest variation compared to specifically acting chemicals and heavy metals (Roex et al., 2000). Based on the large difference in sensitivity variation between specifically acting chemicals and nonpolar narcotics, a binary classification was proposed to classify over 3,000 organic chemicals (Kienzler et al., 2019). It has been well known that species vary in their sensitivity to different groups of chemicals (Vaal et al., 2000; Sorgog and Kamo, 2019). Whether the patterns in sensitivity variation between specifically acting chemicals and general acting chemicals at the species level can be extrapolated to the assemblage level needs to be investigated and it is expected that the assemblage may also show great variability in sensitivity to specifically acting chemicals. In addition, chemicals in the same class have the same toxic mode of action on organisms based on the similarity of physicochemical properties (Blum and Speece, 1990). Therefore, the sensitivity of freshwater invertebrate assemblages to chemicals in the same class potentially shares similar spatial patterns.

The species sensitivity distribution (SSD) is a cumulative-probability distribution of single-species sensitivity for multiple species to describe the assemblage-level sensitivity to chemicals (Newman et al., 2000; Posthuma et al., 2001). The hazardous concentration 5% (HC5) derived from the SSD curves is the maximum concentration of acceptable chemical contamination when 95% of species in ecosystems can be protected (Belanger et al., 2017). A suitable taxonomic coverage is required when using the SSD method to assess the ecological risks of chemicals (Capdevielle et al., 2008). Some studies suggest a minimum of 10 species that can cover algae, invertebrates and fish, to construct the SSD curves (DeForest et al., 2012; Rämö et al., 2018). The tested species used to construct the SSDs curves are often not in the assemblages to be assessed and may introduce a high degree of uncertainty in determining the HC5 values to protect natural assemblages. It has been suggested to use species that can represent the local natural community (Forbes and Calow, 2002; Fox et al., 2021). However, there is a challenge that toxicity data are lacking for most species in natural assemblages (Xu et al., 2015; Berger et al., 2016). For example, only 10.6% of the 1381 aquatic invertebrate species (https://environment.data.gov.uk/ecology/explorer/) recorded in the UK have been used in toxicity tests for any of the 5131 chemicals included in the USEPA Ecotoxicology knowledgebase (https://cfpub.epa.gov/ecotox/). For individual chemicals with extensive toxicity datasets, such as cadmium and chlorpyrifos, 4.9% and 3.15% of aquatic invertebrate species in the UK have been used in toxicity tests, respectively. There are hundreds or even thousands of species in natural ecosystems (Giller et al., 1998; Collen et al., 2014). It is impossible to perform toxicity tests on most of these species for any single chemical and let alone for the vast number of chemicals in use today. Given that it is not possible to test all species that may be exposed to chemicals in the environment, other approaches are needed to predict the sensitivity of untested species in natural communities.

Taxonomy relatedness methods considered the evolutionary relationship between species, with species with a more recent common ancestor being more highly related. Compared to molecular sequence similarity methods, taxonomy relatedness methods are traditionally based on morphological characters and have a great advantage: taxonomy data are easily accessible from taxonomy databases (e.g. National Center for Biotechnology Information (NCBI), Integrated Taxonomic Information System (ITIS), and national biodiversity network (NBN)). Craig et al. (2012) established the hierarchical species sensitivity distribution (hSSD) model for the sensitivity extrapolation with the considerations of the hierarchical taxonomic relatedness between species with measured toxicity data and untested species whose sensitivity is to be predicted. Due to the easy availability of taxonomic data, taxonomy-relatedness methods have the potential to cover most species in natural assemblages.

Freshwater ecosystems are one of the natural ecosystems severely affected by human activities potentially and could be exposed to a range of environmental stressors, including chemical pollutants. Consequently, stress-tolerant taxa are found at highly impacted sites, and more sensitive taxa are restricted to minimally-impacted sites (Clarke et al., 2003). The River Invertebrate Classification Tool (RICT), originally named River Invertebrate Prediction and Assessment Scheme (RIVPACS), assess river health by comparing observed species richness at monitoring sites with expected species richness in the absence of significant anthropogenic disturbance (Clarke and Davy-Bowker, 2014). RIVPACS focused on the most common invertebrates in the UK and established 43 End-groups covering 685 reference sites in Great Britain (Davy-Bowker et al., 2008). These 685 reference sites have high species compositional integrity and are rarely affected by human activities (Wright, 2000; Clarke et al., 2003).

RICT has been developed to predict which invertebrate species should be present at minimally-impacted river sites in the UK (Clarke et al., 2003; Kral et al., 2017). Expected freshwater invertebrate assemblages refers to predicted natural river invertebrate community without the impacts of human activity, which may provide the species compositions of natural communities under minimally impacted conditions and minimize the impacts of history exposure from external stressors. Expected assemblages also vary in species compositions over space, as they are predicted from the similarity in environmental information compared to reference sites. Observed freshwater invertebrate assemblages refer to the observation of samples collected in the field. The collected samples may be in areas with intensive human activities, where the species have undergone external stressors filtering. Species compositions in natural communities may be altered and fail to reflect the original state before being affected.

2.2 Objectives:

The overall objective of this Chapter was to investigate the magnitude and spatial patterns of the sensitivity of species assemblages to chemicals. This was addressed through three sub-objectives:

(1) Linking variation in species composition to variation in assemblage sensitivity to chemicals.

The taxonomic composition of natural assemblages varies over space in response to environmental heterogeneity, as species vary in their functional traits and spatial distribution. Chemicals are divided into different categories according to their toxic effects (e.g. baseline chemicals, specifically acting chemicals). The chemical sensitivity of species can also vary considerably (especially for specifically acting chemicals), while the variation in chemical sensitivity to baseline chemicals is very small. Therefore, the magnitude of variation in assemblage sensitivity is expected to be greater for specific acting than generally acting chemicals. The assemblages with similar taxonomic composition will exhibit similar sensitivities to a chemical. However, the situation is unknown for assemblages with different species compositions. Different taxonomic groups of species may show similar chemical sensitivity because they share similar TD-TK-related traits. The association between taxonomic composition and the variation in assemblage sensitivity to chemicals was investigated to test whether assemblages with relatively different species compositions could vary slightly in their sensitivity to a chemical.

(2) Exploring spatial variation and patterns of assemblage sensitivity to chemicals.

Species composition shows certain patterns in spatial distribution, which can be explained by ecological niche and habitat templet theories. The selection of species by different environmental variables and ecological niches leads to different spatial distributions of species. Similar environmental conditions in regional scales could shape similar species composition, potentially influencing the assemblage sensitivity to chemicals. The hypothesis was investigated: assemblages vary in their sensitivity to chemicals spatially, and this variation is spatially patterned. The spatial variation in assemblage sensitivity to chemicals was captured using the river basins. If there was spatial variation, the patterns would be further investigated.

(3) Comparing the sensitivity of observed and expected assemblages to chemicals

Observed assemblages were sampled in the field. Some of them may be impacted by external stressors, and the taxonomic composition has been altered. Therefore, the RICT was used to predict expected assemblages under minimally impacted conditions at the same locations where observed assemblages were sampled. Looking into the sensitivity of expected assemblages can help to understand protection thresholds for chemicals under minimally impacted conditions. Therefore, it is hypothesized that observed assemblages present different sensitivity to chemicals compared to expected assemblages.

2.3 Methodology

The framework used to assess spatial variation in the sensitivity of species assemblages to chemicals is illustrated in Figure 2.1. The framework adopted a stepwise approach and consisted of four steps: (1) data collection and pre-processing, (2) predicting the sensitivity of untested species in the species pool, (3) describing the assemblage-specific sensitivity, and (4) mapping and assessing spatial variation in assemblage sensitivity to chemicals. This framework was applied to invertebrate assemblages recorded in riverine sites (i.e., observed assemblages) and to invertebrate assemblages expected to occur at the same sites if they were minimally impacted by environmental stressors (i.e., expected assemblages). Assemblage sensitivity was assessed for chemicals with different uses and toxic modes of action (i.e., insecticides, narcotics, heavy metals, and surfactants). The framework was applied to river sites in England.

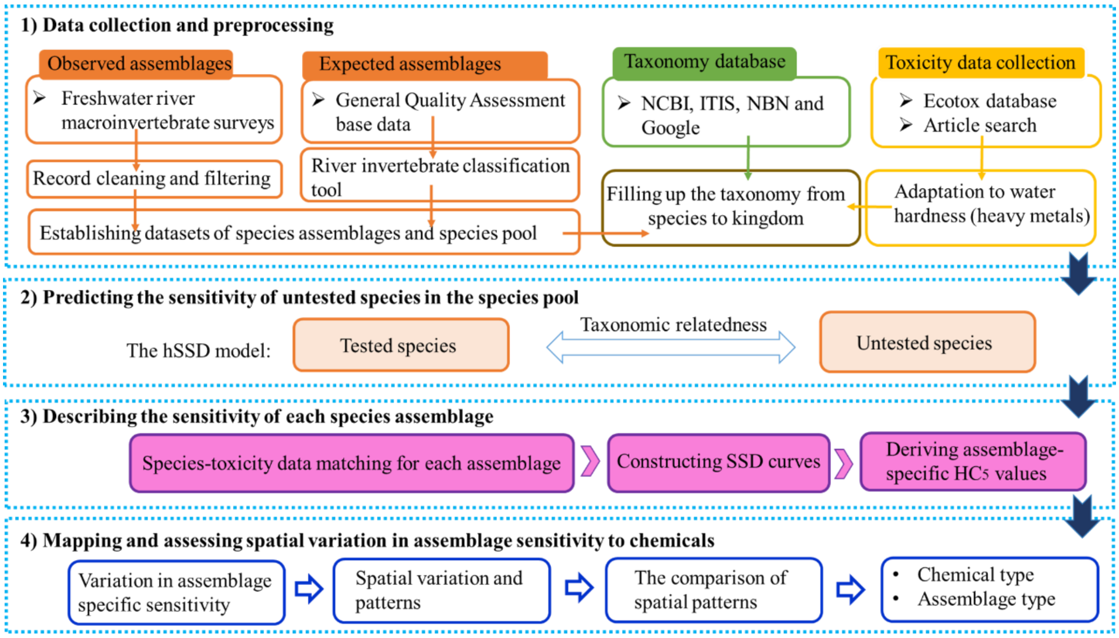

Figure 2.1 A framework for assessing spatial variation in the sensitivity of species assemblages to chemicals

2.3.1 Data collection and preprocessing

Four types of data had to be obtained, collated and pre-processed: freshwater invertebrate assemblages observed at study sites; freshwater invertebrate assemblages expected to occur at study sites if they were minimally impacted; toxicity data for study chemicals; taxonomic information for study species.

Information on invertebrate assemblages was obtained from the Biosys database, which contains taxa information from river surveys carried out across England from

1965 onwards (https://environment.data.gov.uk/ecology/explorer/). Invertebrate records were extracted and collated for the period 2015 to 2020. The most recent year was selected for sites sampled in multiple years and the most recent sampling data was selected for sites sampled on more than one occasion within a year. Each record comprised the sample date, sample location (Site ID) and taxon name. Obvious errors in taxonomy were corrected and any records for marine invertebrates and non-invertebrates were excluded from the extracted database. Some taxa were only recorded at the taxonomic levels of Family (e.g. Limnephilidae), Order (e.g. Acarina) or Class (e.g. Oligochaeta), but toxicity data are usually recorded at the level of Species or Genus. Therefore, percentage analysis was conducted to exclude the sites where a large proportion of taxa (i.e. >30%) were recorded at Family and higher taxonomic levels. Sites where the total number of taxa recorded was less than ten, were also excluded.

The dataset of expected assemblages was derived from the River Invertebrate Classification Tool (RICT) predictions. RICT is a web tool that implements the RIVPACS IV predictive model and uses site-specific environmental data to predict the invertebrate taxa expected to occur at a site under minimally impacted conditions. The tool is hosted by the Freshwater Biological Association and is available at https://fba.org.uk/FBA/Public/Discover-andLearn/Projects/RICT%20Application.aspx. The input data required by RICT (i.e., site, year, National Grid Reference (NGR), easting, northing, altitude, slope, discharge category, distance from the source, stream width and stream depth) were extracted from General Quality Assessment (GQA) base data compiled by the Environmental Agency for river sites in England. The GQA base data are benchmark values for long-term monitoring of environmental variables and can be downloaded from Environmental Agency, UK. The RICT estimates the probability of occurrence for each taxon at a site, which ranges from 100% to less than 1%. A cut-off level for the probability of occurrence for species in expected invertebrate assemblages was determined by comparing the similarity of expected and observed assemblages at reference sites. TL4 predictions were selected to obtain the taxonomic information of RIVPACS species across different sites. Taxonomic information for 421 reference sites in England was obtained from the RIVPACS database (https://www.ceh.ac.uk/services/rivpacs-reference-database). Observed and expected assemblages at each reference site were compared using the Jaccard similarity index (Ivchenko and Honov, 1998). A probability of occurrence ≥ 30% resulted in the highest similarity between observed and expected assemblages (Appendix Figure S1) and this was therefore used as the cut-off value for compiling the dataset of expected assemblages.

Expected assemblages were generated for all Environment Agency river survey sites in England that met the selection criteria for observed assemblages and had corresponding GQA base data for use in RICT. Taxonomic lists were generated for observed and expected invertebrate assemblages for 2318 river sites across England (Figure 2.2). A total species pool was generated by merging the datasets of observed and expected assemblages across all sites for further analysis.

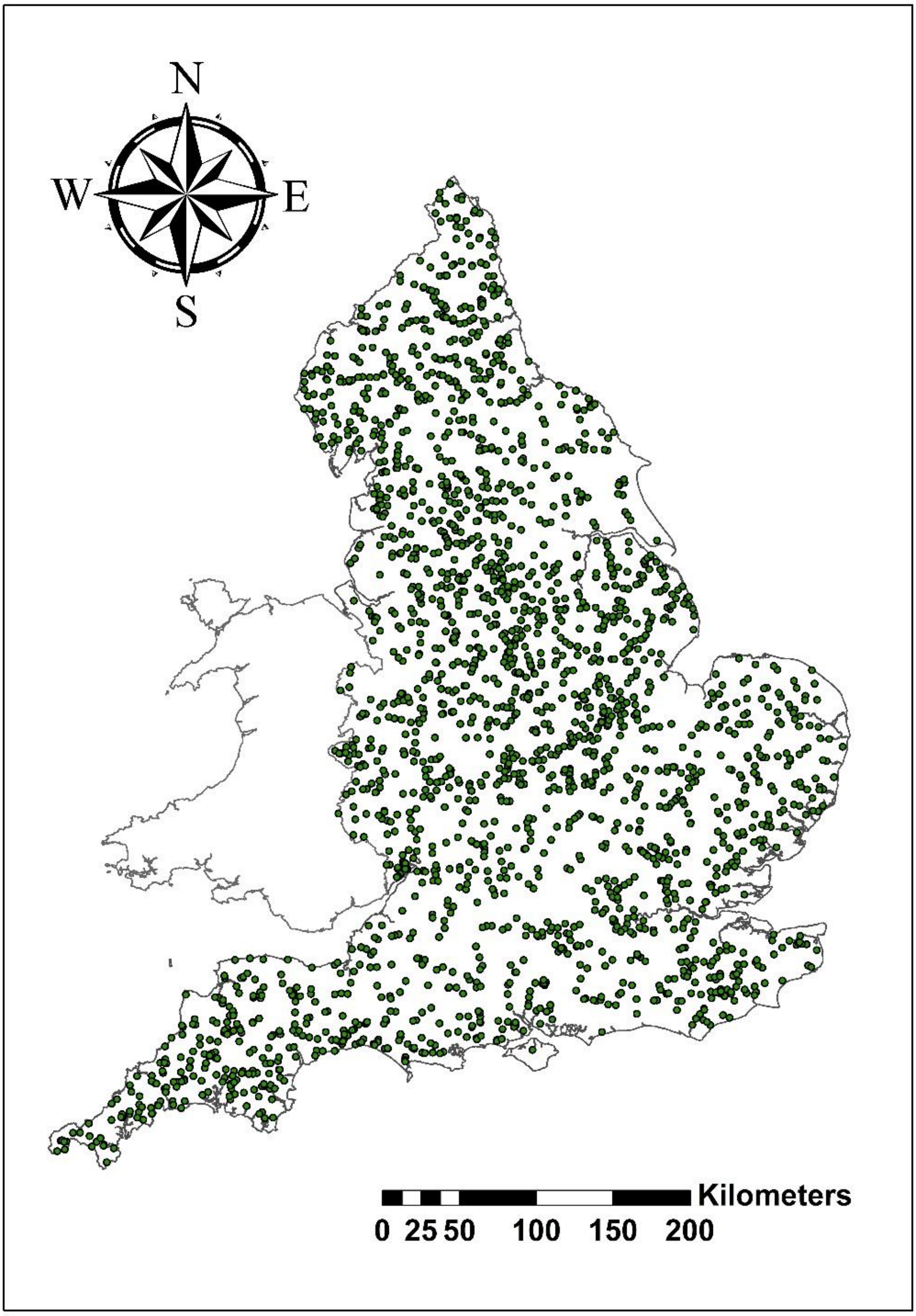

Figure 2.2 Distribution of 2318 study sites across England. For each site, information was collated for invertebrates recorded at the site (observed assemblages) and predicted to occur at the site if it was minimally impacted by environmental stressors (expected assemblage).

Toxicity data for freshwater invertebrates were extracted from USEPA Ecotoxicology Knowledgebase (ECOTOX) (https://cfpub.epa.gov/ecotox/). Data were collated for chemicals that have rich toxicity datasets with relatively high taxonomic diversity and that could be classified as having narrow spectrum toxicity (e.g. selective pesticides, specifically acting chemicals) or broad spectrum toxicity (e.g. biocides, general acting chemicals). Toxic mode of action information was obtained from the MOA-aquatic toxicity database (Barron et al., 2015). A total of 20 chemicals were selected: ten specifically acting pesticides, which included organophosphates (diazinon, fenitrothion, malathion, and parathion-methyl (PM)), organochlorines (dichlorodiphenyltrichloroethane (DDT), endosulfan and endrin), and pyrethroids (cypermethrin, deltamethrin, and permethrin) and 10 generally acting chemicals, which included heavy metals (cadmium, copper, nickel and zinc), narcotics (phenol, benzenamine and glyphosate isopropylamine salt (GIS) and surfactants (linear alkylbenzene sulfonates (LAS), nonylphenol, sodium dodecyl sulfate (SDS).

The criteria for selecting toxicity data followed those used in previous studies (i.e., Maltby et al., 2005; Maltby et al., 2009) and were: LC50 (mortality) and EC50 (immobility) as endpoints and exposure time ranged from 1 to 7 days. Toxicity data were reported as equal and approximate values were collated. The geometric mean of toxicity data values was calculated when multiple values were reported for the same species, chemical and endpoint. Toxicity data values were collated for genera if no species-specific information was reported. Metal toxicity is strongly dependent on water hardness (Di et al., 2001). Water hardness data were extracted from the source references and used to adjust toxicity values for heavy metals to a common water hardness level (i.e. 50 mg/L as CaCO3) using the USEPA Aquatic Life Criteria Calculator (https://www.epa.gov/wqc/aquatic-life-criteria-and-methods-toxics). All toxicity data were converted to µg/L and any apparent outliers were checked by reviewing the source reference.

The taxonomy of species in the toxicity and assemblage datasets was completed from Species up to Kingdom using the R package “taxize” (https://cran.r-project.org/web/packages/taxize/index.html) and the following taxonomy databases:

National Center for Biotechnology Information (NCBI), Integrated Taxonomic Information System (ITIS) and the National Biodiversity Network (NBN). If taxonomic information for a species was not available in these taxonomy databases, a google search was performed.

The compiled datasets of assemblages, toxicity data and taxonomic information were used to provide an overview of the composition and similarity of assemblages as well as the taxonomic overlap between the toxicity datasets and the assemblages to be assessed. The Jaccard similarity index (Ivchenko and Honov, 1998) was used to assess inter-assemblage variation by comparing species compositions for pairs of observed or expected assemblages. Paired T-tests (or paired Wilcoxon Test) were used to assess differences in taxa richness (paired by sites) and similarity (paired by pairwise comparison) between observed and expected assemblages. The total species pools for observed and expected assemblages were used to assess the proportion of assemblage taxa represented in the toxicity data set of each chemical.

2.3.2 Predicting the sensitivity of untested species in the assemblages

The hSSD model was used to predict chemical-specific toxicity values for untested species. This model utilizes the hierarchical taxonomic relatedness between species with known toxicity data and species to be predicted (Craig et al., 2012; Craig, 2013). The hSSD model was structured as equation (1) – (4) (Craig et al., 2012 and 2013). For chemical x and species y, the observed toxicity value is equal to the sum of true toxicity values and measurement error (Equation 1). True value equals the sum of the central value for chemical x, the tendency of species y and the interaction between chemical x and species y (Equation 2). The taxonomically related structure was introduced into the model using the Equation 3 and 4. Then, the model parameters were estimated based on the known toxicity data and the taxonomy of known toxicity data.

$\text{Observed value}_{xyz} = \text{True value}_{xy} + \text{Measurement error}_{xyz}$

(Equation 1)

Ture value$_{xy}$ = Central value$_{x}$ + Tendency$_{y}$ +Interaction$_{xy}$ (Equation 2)

Tendency$_{y}$ = Tendency1 * taxonomic level (1)$_{y}$ + · · · + Tendency$_{L}$ * taxonomic level (L)$_{y}$ (Equation 3)

Interaction$_{xy}$ = k$_{x}$ * (Interaction$_{x1}$ * taxonomic level (1)$_{y}$ + · · · + Interaction$_{xL}$ * taxonomic level (L)$_{y}$) (Equation 4)

where x is x-th chemical, y is y-th species, z is z-th measurement, kx is the variation for chemical x and "taxonomic level (L) y" is the taxonomic rank of species y at the level "L".

The current hSSD model was written in R scripts (Sinclair, T, 2021). The updated hSSD model has strict requirements for data input formats (Sinclair, T, 2021). It requires three inputs: toxicity data, the taxonomy of species for which there is toxicity data and the taxonomy of species in the dataset to be predicted. Toxicity data are inputted as log10 transformed data. Blank values were filled with NA where the taxonomy is unknown. Once the required files were imported and the hSSD model run, the predicted toxicity data values for untested species in the assemblage were saved. As some species in species assemblages had been tested, any species with available toxicity data in natural assemblages would be removed to avoid processing duplicate data. The hSSD model was validated using the leave-one-out cross-validation of 20 study chemicals (Figure S2.2, Sinclair, T., 2021)

2.3.3 Describing the assemblage-specific sensitivity

The predicted and known toxicity data were pooled together to establish datasets of species-specific toxicity values for each study chemical. Then, the species-specific sensitivity was matched to the species in the study assemblages based on their taxon names using the merge function in R. The function of merge in R is to splice two data tables based on the same intersection and returns the matching rows in output (https://www.rdocumentation.org/packages/base/versions/3.6.2/topics/merge). HC5 values were derived from the SSD curves. A separate SSD curve was generated for each assemblage by establishing a loop structure in R. The SSD curves were plotted using the lognormal distribution using the "fitdistr()" function from the MASS package in R. The "fitdistr()" from the MASS package was used to fit a lognormal distribution (https://www.rdocumentation.org/packages/MASS/versions/7.3-58.1/topics/fitdistr). Assemblage-specific HC5 values were calculated based on constructed SSD curves to describe the sensitivity of observed and expected assemblages.

2.3.4 Linking variation in species composition to variation in assemblage sensitivity to chemicals.

The descriptive statistics for the HC5 values for the 20 study chemicals were calculated for both observed and expected invertebrate assemblages. The magnitudes of variation (ratio of maximum to minimum HC5 values) were calculated to reflect inter-assemblage variation for each chemical and compared between specifically acting and general acting chemicals. The assumptions (e.g. normality, variance homogeneity) were tested first to decide to use parametric or non-parametric analysis. For comparing the magnitudes of variation in sensitivity to specifically acting and general acting chemicals, the normality was checked using the Shapiro-Wilk test, and variance homogeneity was checked using F-test. If both the Shapiro-Wilk test and F-test are passed, an unpaired two-sample t-test was used; otherwise, an unpaired two-sample Wilcoxon test was adopted.

The relationship between the similarity in species composition and the variation in assemblage sensitivity to chemicals was investigated using the subset dataset of the similarity analysis of species composition in assemblages. Firstly, 100 assemblages were randomly selected and matched to their sensitivity to 20 study chemicals. The similarity in species compositions between each pair of these 100 assemblages was derived from the similarity analysis of species composition in assemblages (in Section 2.3.1). The assemblage sensitivity to 20 study chemicals was matched to each pair of these 100 assemblages. The ratio of the greater HC5 value to the smaller HC5 values between each pair of 100 assemblages was used to describe the inter-assemblage variation in sensitivity. The association between similarity in assemblage composition and similarity in assemblage sensitivity was analyzed to investigate the impacts of assemblage composition on assemblage sensitivity.

2.3.5 Mapping and assessing spatial variation in assemblage sensitivity to chemicals

The sensitivity of both observed and expected assemblages in England was mapped using ArcGIS software. Assemblage sensitivity was first related to the 9 groups of River Basin Districts in England (Figure 2.3) to assess whether there was spatial variation in assemblage sensitivity. The tag numbers of River Basin Districts increase from north to south and from west to east (Figure 2.3). Dee and Severn were grouped together and tagged with 5, as only two sites were located in the Dee basin. The assemblage sensitivity across different river basin districts was compared using one-way ANOVA followed by the Tukey test when pre assumptions were met. Otherwise, Kruskal–Wallis test followed by Dunn's test was used. The normality was checked using the Shapiro-Wilk test, and variance homogeneity was checked using Bartlett's test.

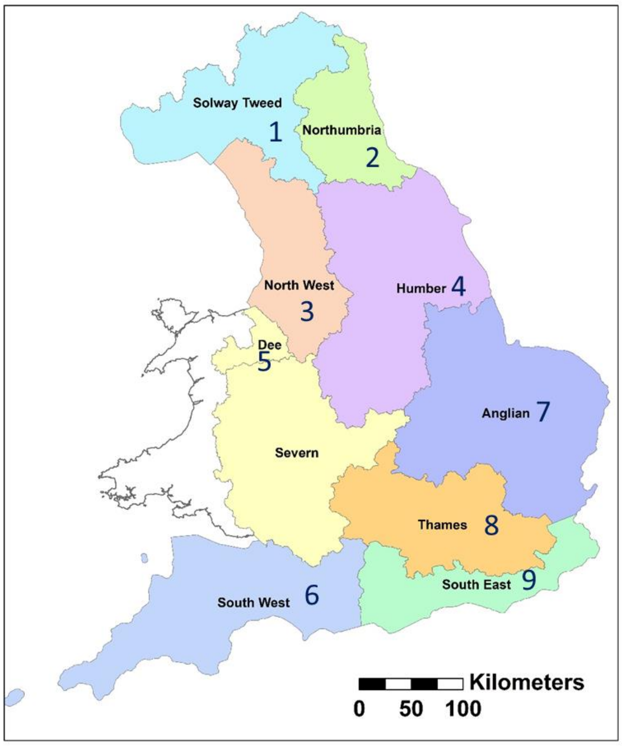

Figure 2.3 River basin districts in England (The tag numbers of river basin districts increase from north to south and from west to east; Dee and Severn were grouped together and tagged with 5)

Spatial patterns of assemblage sensitivity to chemicals were analyzed using Anselin Local Moran's I method in ArcGIS. HC5 values were log-transformed prior to spatial cluster analysis. Anselin Local Moran's I was used to judge whether the attribute value has clustering characteristics over space (Zhang et al., 2008). This method identifies high-value density, low-value density and spatial outliers. The Anselin Local Moran's I method in ArcGIS calculates the local Moran's I value, z-score, pseudo-p-value and the code that represents the clustering type of each statistically significant feature. The z-score and pseudo-p-value indicate the statistical significance of the calculated index value. False discovery rate correction was applied when performing cluster analysis. Five cluster types were obtained: High-High clusters with large HC5 values (i.e. tolerant assemblages); Low-Low clusters with small HC5 values (i.e. sensitive assemblages); High-Low Outliers with large HC5 values surrounded by small HC5 values; Low-High Outliers with small HC5 values surrounded by large HC5 values; Not significant clusters.

2.3.6 Comparing the sensitivity of observed and expected assemblages to chemicals

The assemblage-specific sensitivity and spatial pattern were compared for observed and expected assemblages to 20 study chemicals. Observed and expected assemblages were paired according to the unique ID for 2318 sites. A pairwise comparison was performed for observed and expected assemblages using the Wilcoxon test for each chemical. Spatial patterns in the sensitivity of observed and expected assemblages to 20 study chemicals were compared using the similarity index. The similarity in spatial patterns of sensitivity between observed and expected assemblages was described using the formula (Similarity index (%) =100* (The number of sites sharing the same cluster types / the total number of sites)). Spatial patterns between observed and expected assemblages were compared using one-way ANOVA with posthoc Tukey HSD Test across the chemical class.

2.4 Results

2.4.1 Overview of species assemblages and toxicity data

The dataset of macroinvertebrates recorded across 2318 English river sites between 2015 and 2020 comprised a total of 876 taxa, which represented 6 phyla, 11 classes, 35 orders, 130 families and 373 genera. Taxonomic compositions for observed assemblages are presented in Figure 2.4. The taxonomic richness of assemblages ranged from 10 - 86 (note that assemblages with less than 10 species were excluded from the analysis) and Jaccard similarity between pairs of assemblages ranged from 0 - 0.71 (Figure 2.5).

The dataset of macroinvertebrates expected to occur at the 2318 study sites if they were minimally impacted comprised a total of 267 taxa, which represented 4 phyla, 7 classes, 26 orders, 84 families and 190 genera. Taxonomic compositions for expected assemblages are presented in Figure 2.4. The number of taxa expected to occur in assemblages at the study sites if they are minimally impacted ranged from 40 to 104 and the pairwise Jaccard similarity index ranged from 0.04 - 1 (Figure 2.5). Although the database for expected assemblages included fewer taxa than the database for actual assemblages, taxonomic richness was considerably greater for expected assemblages (Wilcoxon rank sum test with continuity correction, W = 5220220, p-value < 0.001, n = 4636), as was Jaccard similarity (Wilcoxon signed rank test with continuity correction, V = 1124874301, p-value < 0.001, n = 5370806).

Toxicity data were collected mainly from the USEPA Ecotox Database. For observed assemblages, the average coverage proportions at phylum are 71% for heavy metals, 72% for narcotics, 72% for organochlorines, 67% for organophosphates, 50% for pyrethroids and 67% for surfactants. For expected assemblages, the average coverage proportions at phylum are 75% for heavy metals, 87% for narcotics, 80% for organochlorines, 85% for organophosphates, 60% for pyrethroids and 80% for surfactants. The species pools of observed and expected assemblages have relatively good coverage at a high taxonomic rank (Figure 2.6). With the decrease in taxonomic levels, an increased proportion of taxa in species pools have lower coverage by toxicity data (Figure 2.6). The proportion of species that have been tested for study chemicals is extremely low. Only around 6% of taxa at the genus level have been tested for study chemicals (Figure 2.6). Therefore, the sensitivity of a large proportion of untested taxa (around 94% at genus level) was extrapolated from around 6% of species at genus or species level with available toxicity data.

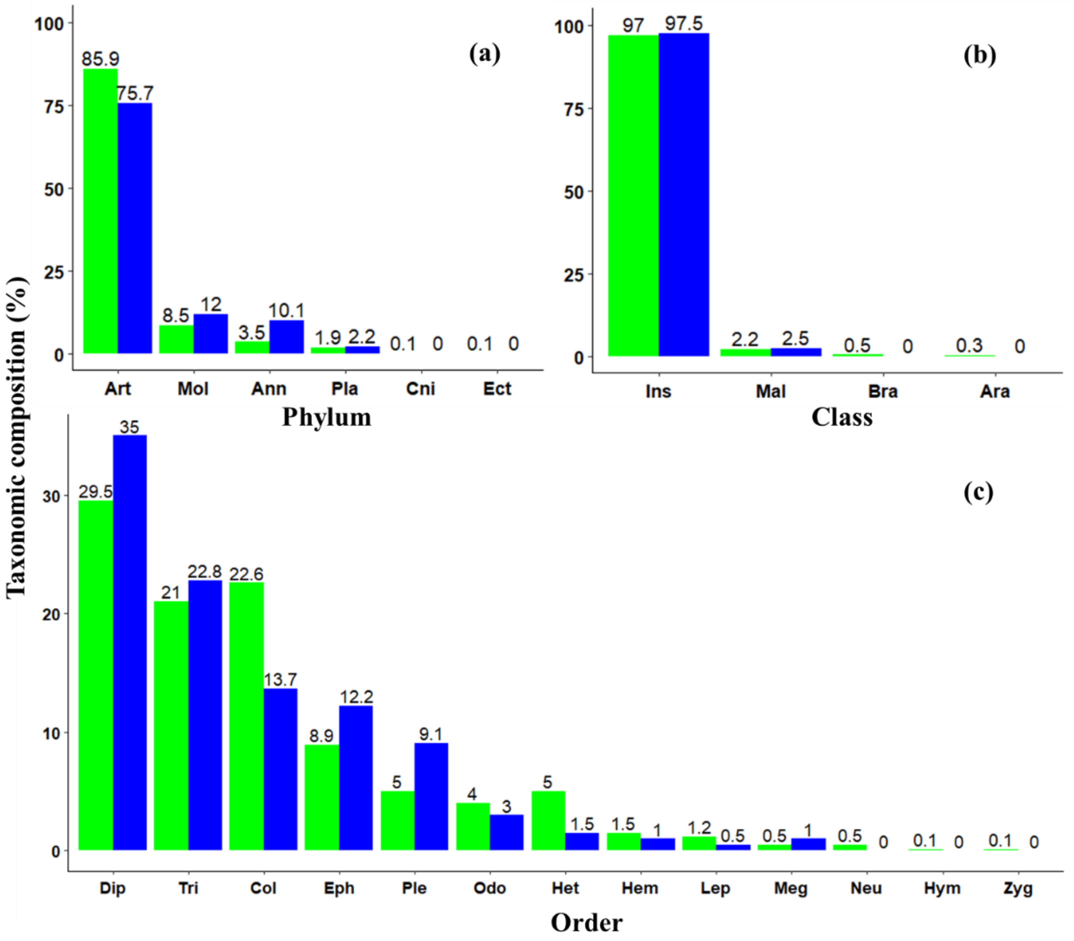

Figure 2.4 Taxonomic compositions of observed (green bars) and expected (blue bars) assemblages are compared across phyla (a), across the classes within the Arthropoda (b) and across orders within the Insecta (c). Phylum (Art – Arthropoda; Mol – Mollusca; Ann – Annelida; Pla – Platyhelminthes; Ect – Ectoprocta; Cni – Cnidaria); Class (Ins – Insecta; Ara – Arachnida; Mal – Malacostraca; Bra – Branchiopoda; Ent – Entognatha; Col – Collembola); Order (Dip – Diptera; Tri – Trichoptera; Col – Coleoptera; Eph – Ephemeroptera; Ple – Plecoptera; Odo – Odonata; Het – Heteroptera; Hem – Hemiptera; Lep – Lepidoptera; Meg – Megaloptera; Neu – Neuroptera; Hym – Hymenoptera; Zyg - Zygoptera)

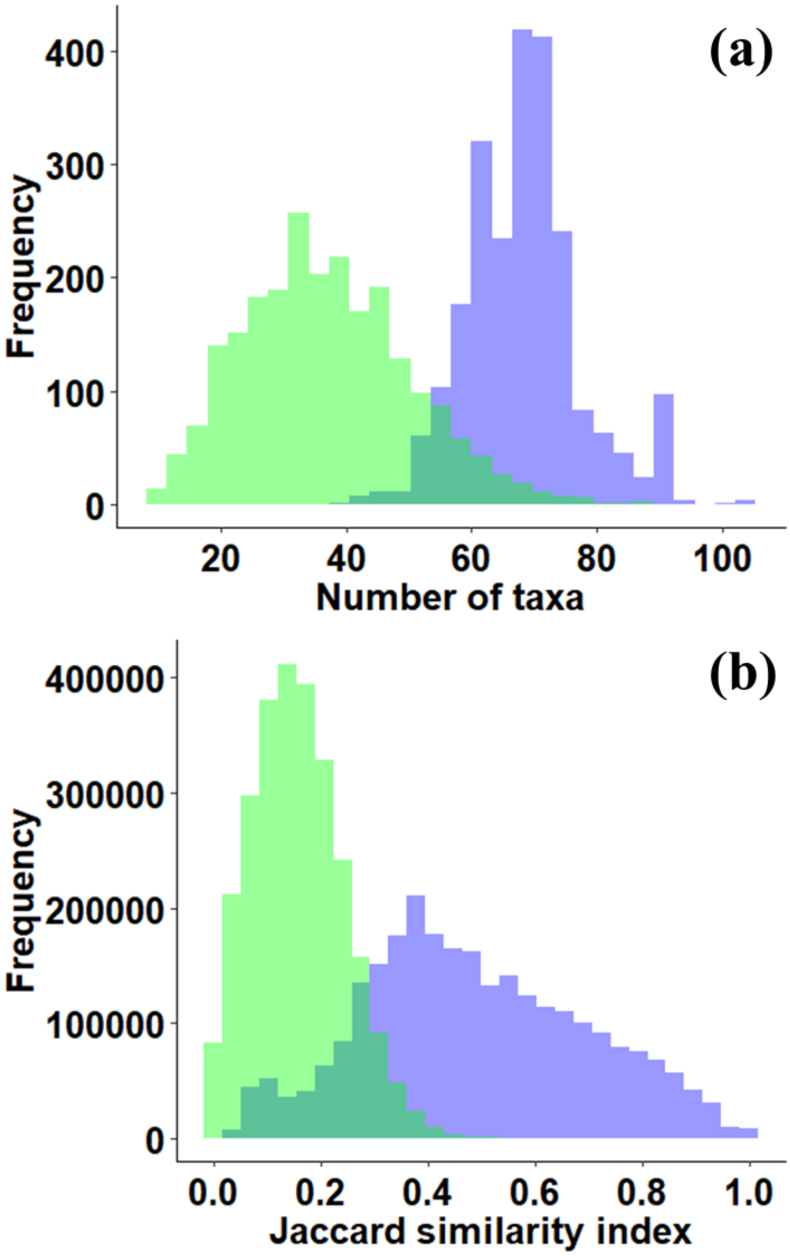

Figure 2.5 Frequency distribution of (a) the number of taxa in observed (green bars) and expected (blue bars) assemblages and (b) Jaccard similarity index based on pairwise comparison of species compositions in observed (green bars) or expected (blue bars) assemblages.

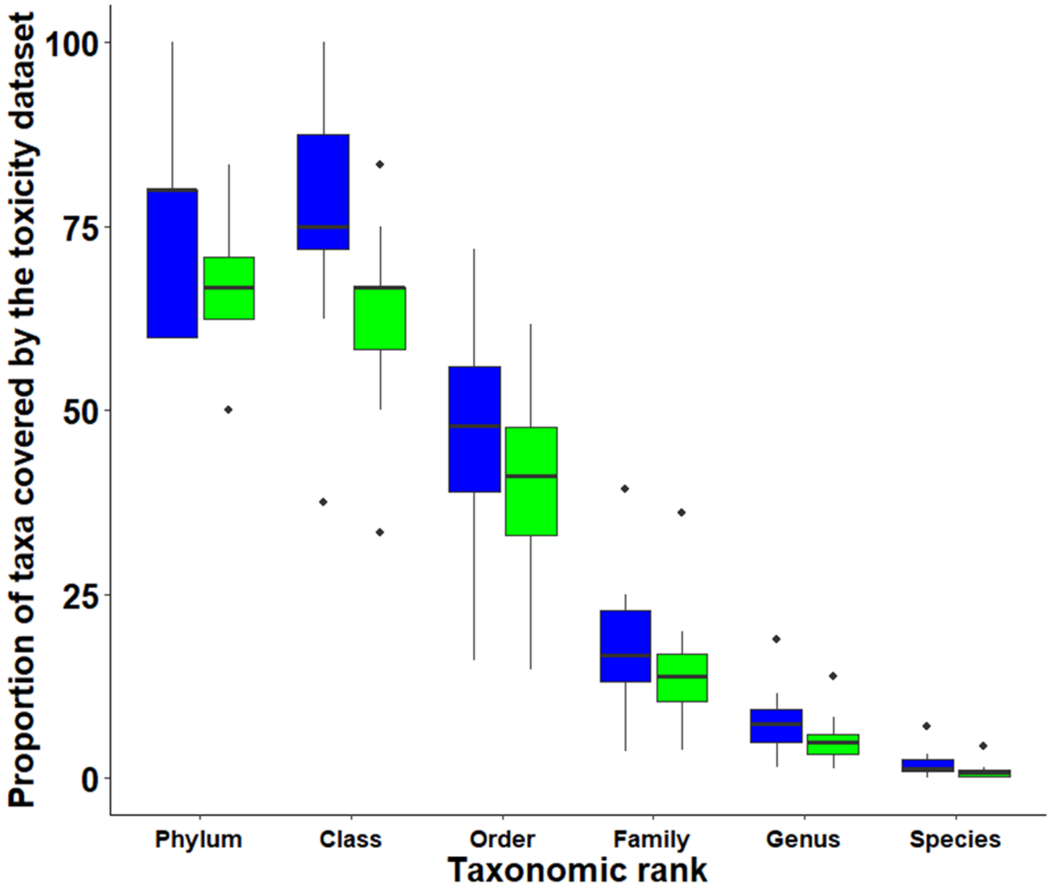

Figure 2.6 Median proportion of taxa in observed (in green) and expected (in blue) species pools represented by the toxicity dataset for the 20 study chemicals. Data are presented for different taxonomic ranks. Boxplots represent the lower quartile (25th percentile), median (50th percentile), and upper quartile (75th percentile) with whiskers and outliers.

2.4.2 Linking variation in species composition to variation in assemblage sensitivity to chemicals

For each study chemical, LC50 values predicted for untested species were combined with experimentally derived LC50 values of tested species to produce SSDs for the actual and expected assemblages at each of the 2318 sites.

Comparing across all assemblages, the ratio of the maximum HC5 to minimum HC5 was used as a measure of magnitudes of variation in sensitivity of assemblages to a chemical. For observed assemblages, the magnitude of variation in assemblage sensitivity ranged from less than one order of magnitude (e.g. glyphosate isopropylamine salt, 5) to greater than 5 orders of magnitude (e.g. deltamethrin, 12556) (Table S2.1) and was significantly greater for specifically acting chemicals than for general acting toxicants (Wilcoxon rank sum exact test, W = 0, $p < 0.001$, n = 20, Figure 2.7 a). For most specifically acting chemicals, the maximum HC5 values are hundreds or even thousands of times greater than the minimum ones.

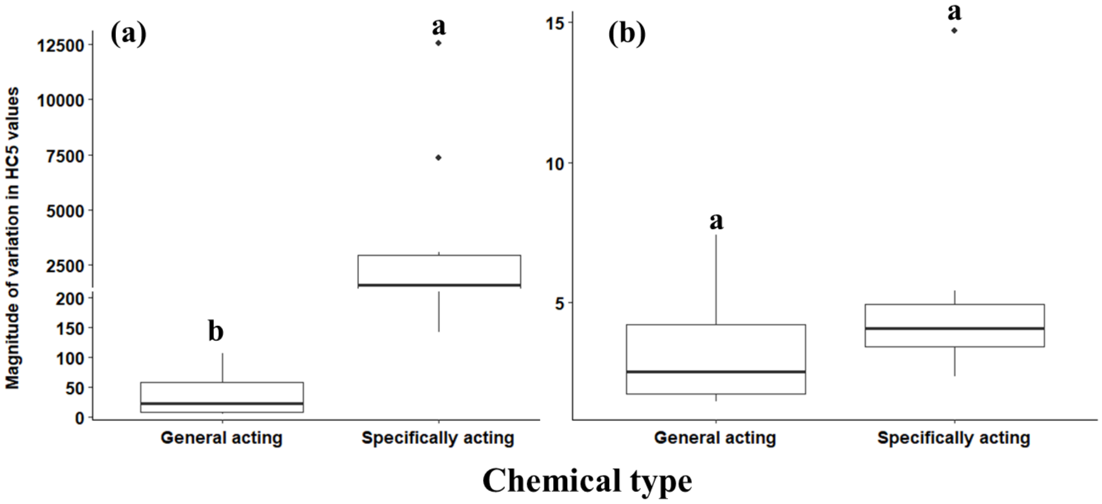

Figure 2.7 Median ratio of maximum to minimum HC5 values for observed (a) and expected (b) assemblages exposed to specifically acting and general acting toxicants. (Boxplots represent the lower quartile (25th percentile), median (50th percentile), and upper quartile (75th percentile) with whiskers and outliers; Different letters mean significant difference across groups, $p < 0.05$)

The magnitude of variation in HC5 values was significantly less for expected assemblages than for observed assemblages, and this was consistent across all study chemicals (Wilcoxon signed rank exact test, V = 0, $p < 0.001$, n = 40). For expected assemblages, the magnitude of variation was less than one order of magnitude for all study chemicals except endrin (Table S2.1), and although, on average, the magnitude of variation was greater for specifically acting than general acting chemicals, the difference was not statistically significant (Wilcoxon rank sum exact test, W = 29, $p > 0.05$, n = 20, Fig. 2.7 b).

The correlation between the compositional similarity (Jaccard index) and sensitivity (HC5) of assemblages are presented in Figure 2.8 (observed assemblages) and Figure 2.9 (expected assemblages) for each study chemical. For observed assemblages, the association between similarity in species composition and similarity in assemblage sensitivity presents Pareto front profile (Figure 2.8). The assemblages with similar species compositions are prone to show similar sensitivity to chemicals. The assemblages with different species compositions could exhibit similar or vary greatly in sensitivity to chemicals. This association differs across chemicals and seems to be stronger to some specifically acting chemicals (e.g. diazinon, endrin, malathion, cypermethrin, permethrin) than other chemicals. Expected assemblages exhibit similar patterns as observed assemblages exhibit, although they show small variation in their sensitivity to chemicals (Figure 2.9).

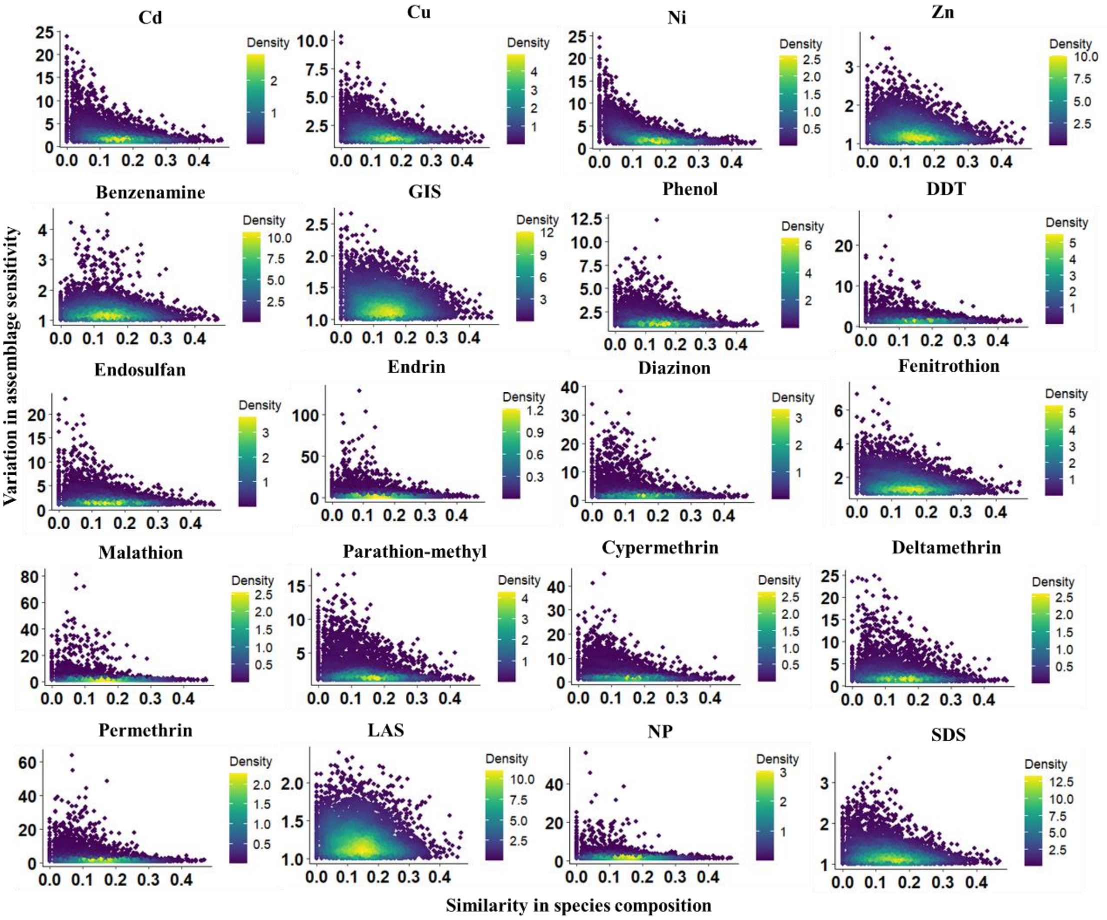

Figure 2.8 The association between similarity in observed assemblage composition and similarity in observed assemblage sensitivity to chemicals. Chemical abbreviations: glyphosate isopropylamine salt (GIS), linear alkylbenzene sulfonates (LAS), nonylphenol (NP), sodium dodecyl sulfate (SDS).

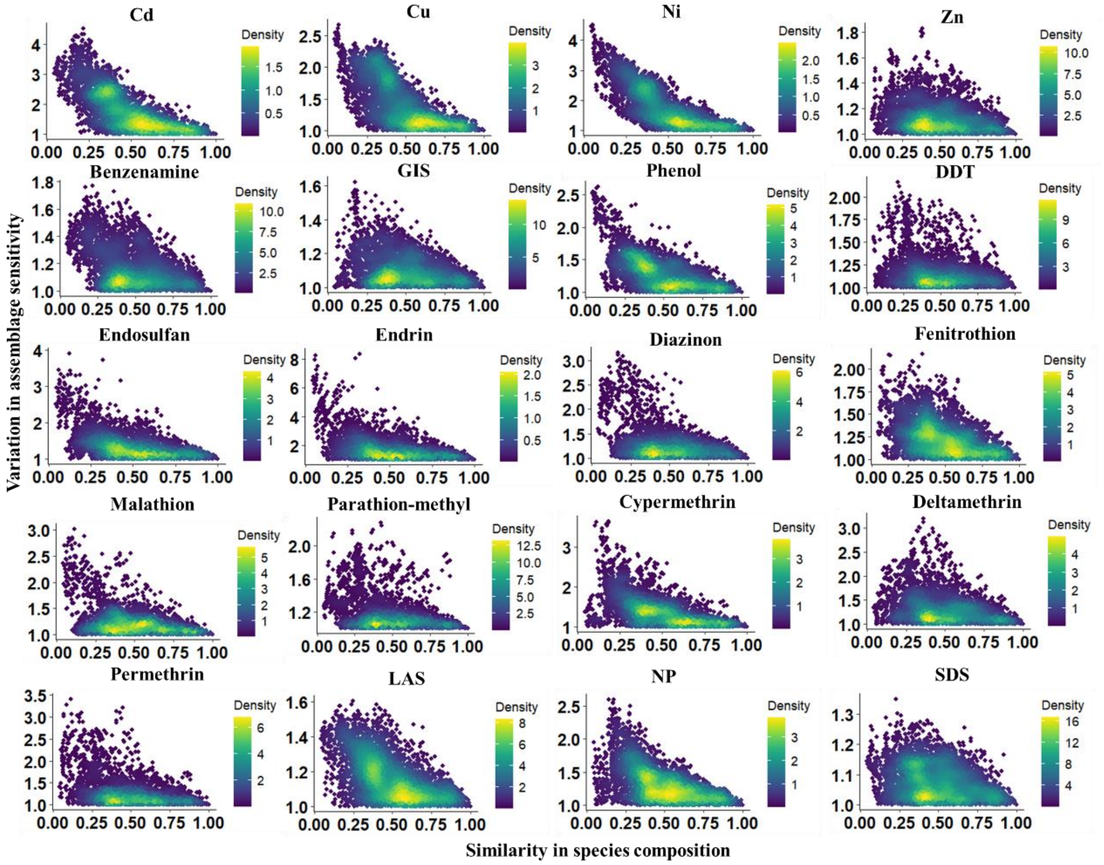

Figure 2.9 The association between similarity in expected assemblage composition and similarity in expected assemblage sensitivity to chemicals. Chemical abbreviations: glyphosate isopropylamine salt (GIS), linear alkylbenzene sulfonates (LAS), nonylphenol (NP), sodium dodecyl sulfate (SDS).

2.4.3 Exploring spatial variation and patterns of assemblage sensitivity to chemicals

The sensitivity of observed invertebrate assemblages to all study chemicals varies significantly varies across River Basin Districts (Kruskal–Wallis test, chi-squared = 43.521 – 737.66, df = 8, $p < 0.001$, n = 2318), suggesting that there is spatial variation in the sensitivity of observed invertebrate assemblages to all study chemicals. Assemblage sensitivity was spatially clustered, although the strength of this clustering was greater for some chemicals than others (Figure 2.10). High-High clusters indicate assemblages with large HC5 values that are tolerant to chemicals, while Low-Low clusters indicate assemblages with small HC5 values that are sensitive to chemicals. Invertebrate assemblages that are sensitive to organophosphates (diazinon, fenitrothion, and parathion-methyl) are mainly clustered in the west and central England and tolerant assemblages are clustered in the east. In contrast, assemblages that are sensitive to heavy metals (cadmium, copper, nickel) are clustered in the east and central of England, while tolerant assemblages are clustered in the north and southwest. Assemblages that are sensitive to endosulfan, endrin, cypermethrin and permethrin are clustered in central and southern areas and assemblages sensitive to nonylphenol are mainly clustered in central and southeastern areas. High-High clusters for endosulfan, phenol, LAS and SDS are mainly scattered in the eastern areas. Assemblage sensitivity to deltamethrin and glyphosate isopropylamine salt exhibits weak spatial clustering patterns.

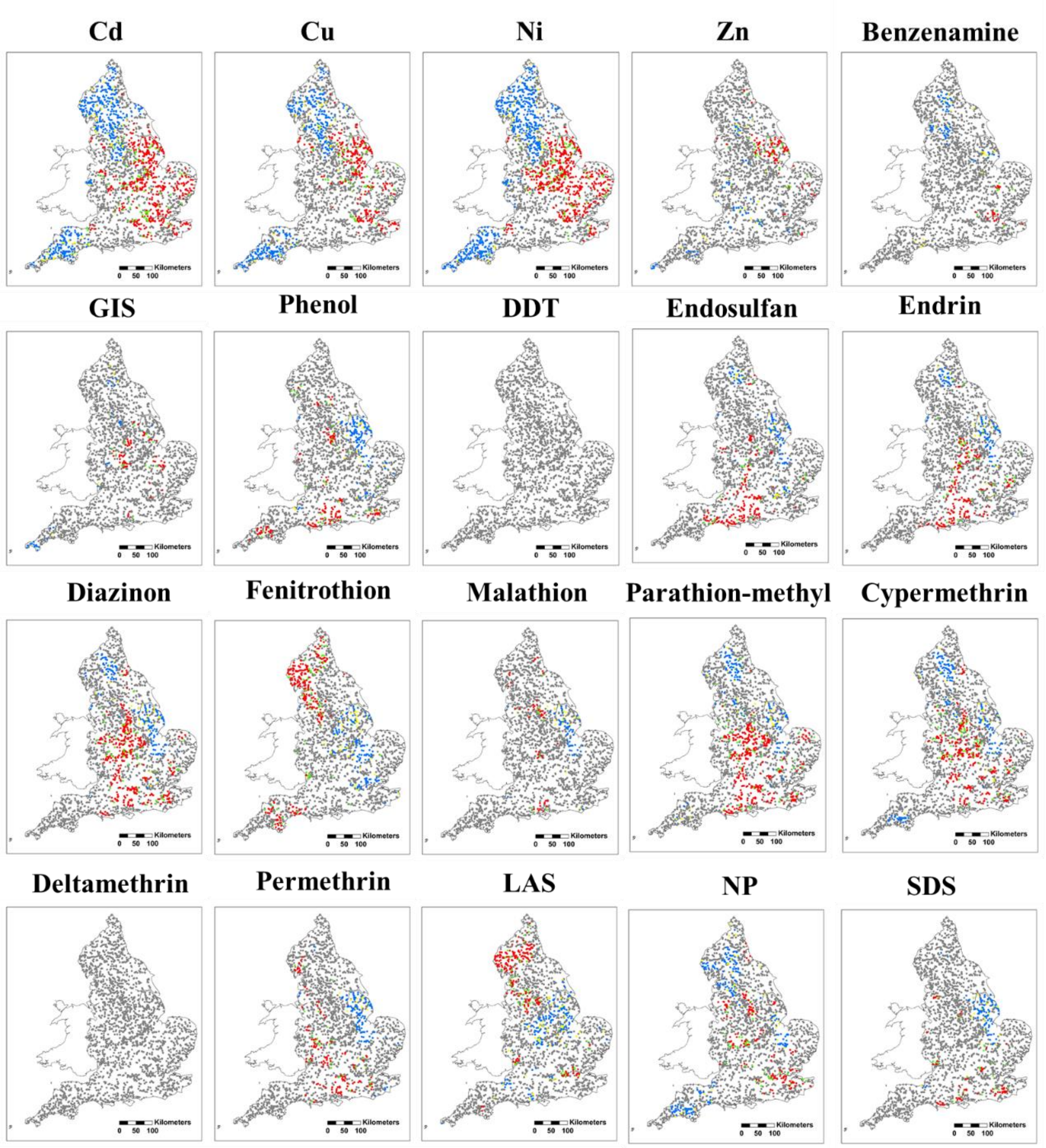

Figure 2.10 Spatial patterns of the sensitivity of observed assemblages to 20 chemicals. High-High clusters with large HC5 values indicate tolerant assemblages (blue dot); Low-Low clusters with small HC5 values indicate sensitive assemblages (red dot); High-Low Outliers with large HC5 values surrounded by small HC5 values (green dot); Low-High Outliers with small HC5 values surrounded by large HC5 values (yellow dot); No strong spatial patterns (grey dot). Chemical abbreviations: glyphosate isopropylamine salt (GIS), linear alkylbenzene sulfonates (LAS), nonylphenol (NP), sodium dodecyl sulfate (SDS).

The sensitivity of expected assemblages to 20 chemicals also varies significantly across river basin districts (Kruskal–Wallis test, chi-squared = 99.478 - 925.66, df = 8, $p < 0.001$, n = 2318), indicating that expected assemblages vary their sensitivity to a chemical spatially.

The sensitivity of expected assemblages to study chemicals also presents spatial clustering patterns (Figure 2.11). Sensitive assemblages to Cd, Cu, and Ni, are distributed in the central and east of England, while tolerant assemblages to all heavy metals are in the north and southwest. To cypermethrin, LAS and nonylphenol, expected assemblages exhibit similar spatial patterns to heavy metals, with sensitive assemblages being in the east and central of England. Sensitive assemblages of phenol, endosulfan, endrin, diazinon, and fenitrothion are mainly scattered in the north and southwest of England. Low-Low clusters for parathion-methyl are scattered in the middle and south of England. High-High clusters for DDT and parathion-methyl are distributed in northern areas. The sensitivity of expected assemblages to these 20 study chemicals also varies spatially and but glyphosate isopropylamine salt and permethrin exhibit weak spatial clustering patterns.

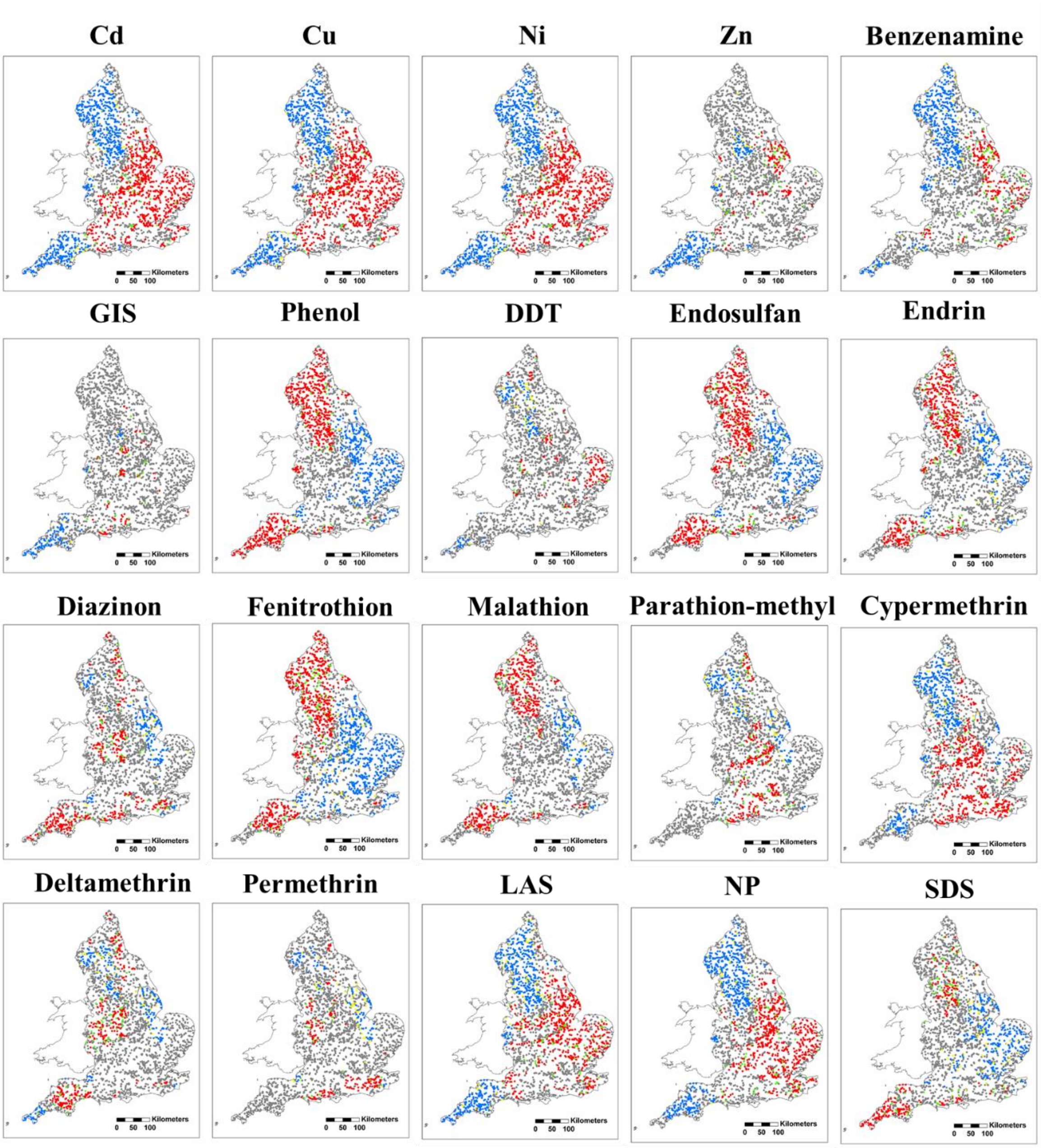

Figure 2.11 Spatial patterns of the sensitivity of expected assemblages to 20 chemicals. High-High clusters with large HC5 values indicate tolerant assemblages (blue dot); Low-Low clusters with small HC5 values indicate sensitive assemblages (red dot); High-Low Outliers with large HC5 values surrounded by small HC5 values (green dot); Low-High Outliers with small HC5 values surrounded by large HC5 values (yellow dot); No strong spatial patterns (grey dot). Chemical abbreviations: glyphosate isopropylamine salt (GIS), linear alkylbenzene sulfonates (LAS), nonylphenol (NP), sodium dodecyl sulfate (SDS).

2.4.4. Comparing the sensitivity between observed and expected assemblages to chemicals

The sensitivity of observed and expected assemblages paired by site was significantly different for all study chemicals except cypermethrin (Paired-Wilcoxon signed rank test with continuity correction, V = 16420 - 2482559, $p < 0.001$, n = 4636). The observed assemblages were more sensitive than expected assemblages to cadmium, zinc, benzenamine, GIS, phenol, endosulfan, endrin, diazinon, parathion-methyl, cypermethrin and SDS, but they were less sensitive to other chemicals (Fig. 2.12). In addition, the expected assemblages exhibit smaller variation in sensitivity than observed assemblages to all study chemicals.

Spatial patterns in the sensitivity of observed and expected assemblages to some study chemicals show a certain similarity (Figure 2.13), although there is a large variation in sensitivity to the same chemical. Proportions of sites where observed and expected assemblages share the same cluster types were calculated to reflect the similarity in spatial patterns (Figure 2.13). For each chemical class, the similarity in spatial cluster types is more than 50%. No significant difference was detected in the similarity of spatial cluster types across chemical types (One-way ANOVA test, df = 5, F= 0.521, $p > 0.05$, n = 20).

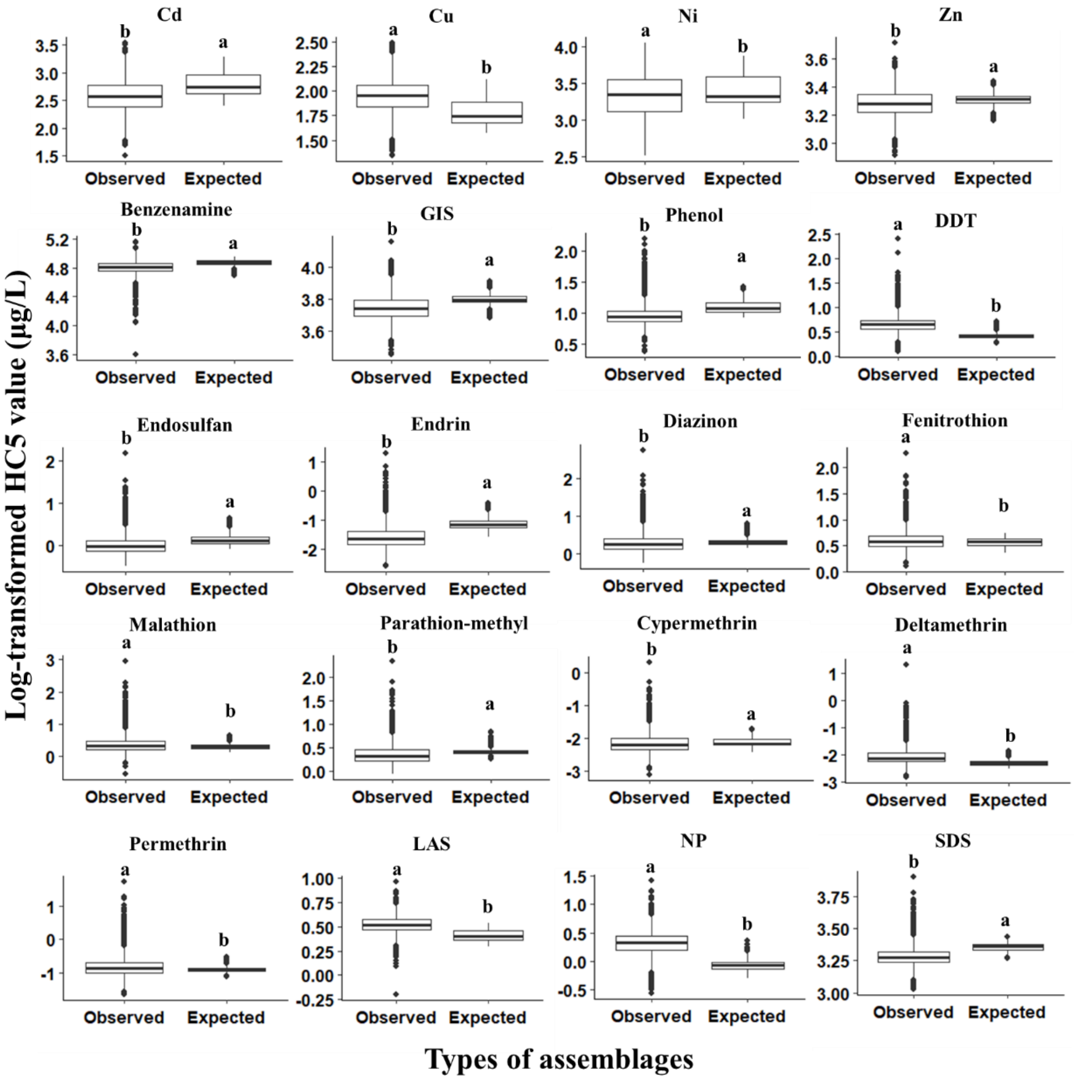

Figure 2.12 Log-transformed HC5 values (µg/L) for observed and expected assemblages to 20 chemicals (Different letters indicate significant differences ($p < 0.05$)). Chemical abbreviations: glyphosate isopropylamine salt (GIS), linear alkylbenzene sulfonates (LAS), nonylphenol (NP), sodium dodecyl sulfate (SDS).

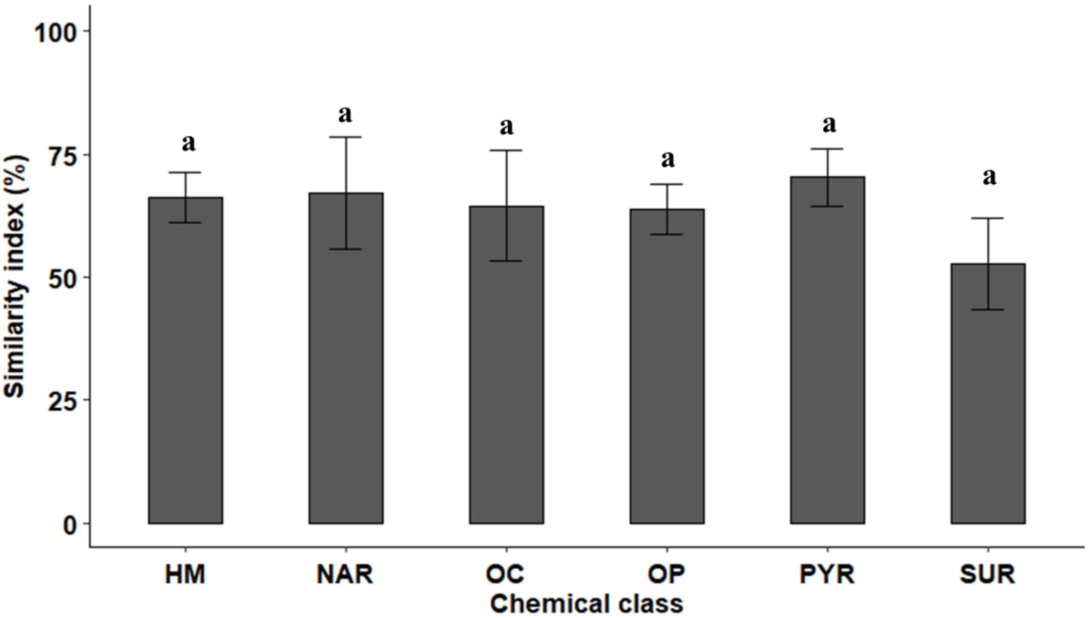

Figure 2.13 Proportions of sites where observed and expected assemblages share the same cluster types (High-High clusters, Low-Low clusters, High-Low Outliers, Low-High Outliers, and Not Significant); Chemical class: heavy metals (HM), narcotics (NAR), organochlorines (OC), organophosphates (OP), pyrethroids (PYR) and surfactants (SUR); The same letters indicate no significant differences ($p > 0.05$).

2.5 Discussion

This study aimed to investigate the magnitude and spatial patterning of variation in the sensitivity of freshwater invertebrate assemblages to chemical exposure. It evaluated the link between variation in species composition and variation in assemblage sensitivity to chemicals, assessed spatial variation and patterns of assemblage sensitivity to chemicals, and compared the sensitivity of observed and expected assemblages to chemicals.

The results from this study support the hypotheses that assemblages vary in their sensitivity to chemicals, and the magnitude of variation in the sensitivity of observed assemblages was greater for specific acting than generally acting chemicals. Furthermore, assemblages with similar species compositions exhibit minor chemical sensitivity variations. In contrast, the assemblages with different species compositions can show relatively similar or completely different sensitivity to chemicals. There is a significant difference in the magnitude of variation in assemblage sensitivity across chemical types and between observed and expected assemblages.

The magnitude of variation in the sensitivity of species assemblages depended on the chemical type and species composition. Different taxonomic groups of species may have different traits that affect toxicokinetics (e.g. uptake, bioaccumulation, and distribution) or toxicodynamics (e.g. depuration kinetics), resulting in interspecies variation in sensitivity to chemicals (Ashauer, Agatz et al. 2011, Van den Brink, Baird et al. 2013, Groh, Carvalho et al. 2015, Fahd, Khan et al. 2017). This study found that the magnitude of variation in observed assemblage sensitivity was significantly higher for specifically acting than general acting toxicants at the assemblage level. This may be the result of extrapolation from the species level to the assemblage level. For example, if species have large variation to chemicals, the sensitivity of assemblages with the sensitive taxa will vary greatly compared to that for assemblages with the tolerant taxa. But if the variation in species sensitivity to chemicals is small, the sensitivity of assemblages with the sensitive and tolerant taxa will vary slightly. Empirical evidence shows that when compared across a wide taxonomic range (e.g. crustaceans, insects, annelids, and fish) species exhibit greater interspecific variation in sensitivity to specifically acting chemicals than to generally acting chemicals such as narcotics (Hoekstra et al., 1994; Vaal et al., 1997; Robinson et al., 2021). Interspecies variation in sensitivity to specifically acting chemicals has been demonstrated to vary by 6 orders of magnitude, while that to generally acting narcotic chemicals has very little variation (i.e. 2 orders of magnitude (Vaal et al., 2000). Based on laboratory toxicity tests, sensitivity among arthropods to a specifically acting chemical (λ-cyhalothrin) varied by 3 orders of magnitude (Wiberg-Larsen, Graeber et al. 2016). Variation in the species composition also plays an important role in inter-assemblage variation in sensitivity to chemicals. The results of this study demonstrate that if the assemblages have similar species compositions, they will exhibit similar assemblage sensitivity to a chemical. However, if assemblages have different species compositions, their sensitivity to a chemical can be similar or completely different. Differences in species compositions could result in significant variation in assemblage sensitivity to chemicals (Awkerman, Raimondo et al. 2008). For example, mesocosm experiments have demonstrated that species composition was the primary factor that influenced assemblage-level response to copper and carbaryl (Havens 1994). However, variation in species composition may not necessarily result in differences in assemblage sensitivity. Assemblages may have different species compositions but similar trait profiles and hence similar sensitivity to a chemical. For example, species traits (i.e., surface contact area) were considered more important than species identity in determining interspecific variation in sensitivity to λ-cyhalothrin. Invertebrates with large surface contact areas were more sensitive to the chemical (Wiberg-Larsen, Graeber et al. 2016).

The results from this study support the hypothesis that the sensitivity of assemblages to chemicals varies spatially, resulting in clusters of sensitive and tolerant assemblages. Observed and expected assemblages both show a significant difference in their sensitivity to a chemical across different river basin districts. Spatial variation in species compositions is probably the main determinant of spatial variation in assemblage sensitivity to chemicals. Spatial heterogeneity of environment factors results in spatial variation in the distribution of species and the composition of assemblages (Gilinsky 1984, Cooper, Barmuta et al. 1997, Heino 2000). Previous studies have used species sensitivity distribution (SSDs) to describe interspecific variation in sensitivity and to determine spatially defined sensitivity thresholds (Feng, Wu et al. 2013, Wu, Liu et al. 2015, Zhang, Wang et al. 2017). However, these studies only predicted the sensitivity of a very small number of untested species at the national scale with no consideration of spatial differences in assemblage sensitivity. Most other studies considering the spatial variation in chemical impact have focused on spatial variation in pollutant concentrations without considering the spatial variation of species composition (Shi, Xu et al. 2018, Posthuma, van Gils et al. 2019, Lu, Wang et al. 2022). Van den Berg et al (2020) used a trait-based method to extrapolate species sensitivity and considered the spatial variation in species compositions. They also demonstrated the spatial variation in the sensitivity of invertebrate assemblages to narcotic and AChE-inhibiting chemicals at the UK national scale and the European scale. The work reported here, builds on these previous studies by using more detailed spatial datasets, increased taxonomic coverage of invertebrate assemblages and more study chemicals and chemical types. It also uses a different approach (i.e. hSSD) to predict the chemical sensitivity of untested species in assemblages.

The sensitivity of observed assemblages seems to exhibit relatively high similarity in spatial patterns within the same chemical groups (e.g. heavy metals, endosulfan and endrin), while expected assemblages exhibit relatively high similarity in sensitivity for heavy metals, pyrethroids and organophosphates. Apart from assemblages sharing similar species compositions at similar environmental conditions (Chase, 2007; Astorga et al., 2012), the toxic mode of action may be one of the important drivers for interpreting the high similarity in spatial patterns of assemblage sensitivity to chemicals within the same chemical groups. The metals studied (cadmium, copper, and nickel) cause toxic effects on aquatic organisms by impairing osmoregulatory processes (Solomon 2008, de Paiva Magalhães, da Costa Marques et al. 2015). Endosulfan and endrin are highly toxic to insects and cause neurotoxicity by inhibiting neurotransmitters (Kaushik and Kaushik 2007). Diazinon, fenitrothion, malathion and parathion-methyl cause toxic effects on aquatic organisms through inhibiting the enzyme activities of acetylcholinesterase (Sapbamrer and Hongsibsong 2014). Cypermethrin, deltamethrin, and permethrin affect sodium regulation resulting in neurotoxicity (Werner and Moran 2008).

The sensitivity of observed is significantly different from that of expected assemblages to all study chemicals. Observed assemblages were more sensitive to 11/20 study chemicals than expected assemblages. The difference in the sensitivity between observed and expected assemblages is mainly due to the difference in variation in species compositions. The species pool for observed assemblages was drawn from 2318 river sites across England and is much larger than expected species pools. The expected assemblages were based on 267 RIVPACS species (Kim et al., 2016), which include the most common species within the dominant phyla (e.g. Arthropoda, Mollusca, Annelida and Plathylminthes). In contrast, information on observed assemblages was obtained from monitoring of actual assemblages and recording of all species sampled, resulting in a taxonomically diverse dataset containing 907 taxa. The difference in species compositions between observed and expected assemblages may be affected by several factors. RICT predicts assemblages expected at a site if it was minimally impacted. However, many of the study sites will have been affected by external stressors (Murphy and Davy 2005; Sarriquet et al., 2006; Armitage et al., 2007) and therefore the observed and predicted invertebrate fauna will be different (Wright et al., 1998; Wright 2000; Wright et al., 2000). RICT provides estimates of the probability of occurrence for each of the RIVPACS species at each site. To define expected assemblages, a decision had to be made regarding the probability of occurrence at which taxa would be deemed present in the assemblage. The cut-off used in this study was a 30% probability of occurrence, with taxa with a lower probability of occurrence being filtered out (Clarke et al., 2003). This will potentially exclude species that could actually be present at a site and therefore included in the observed assemblages. Furthermore, RICT predicts expected assemblages by comparing physical and chemical environmental variables with reference sites and establishing relevant taxa end groups (Clarke et al., 2003). Consequently, RICT provides the same compositions for predicted assemblages at sites in the same region with similar environmental conditions (Clarke et al., 2011; Clarke and Davy 2014). The spatial resolution of RICT is relatively low and the 2318 expected assemblages were predicted based on less than 40 end-groups in England. Except for the currently used probability of occurrence 30% cut-off level, all possible occurring species at study sites were tested in the initial analysis. The general pattern is similar to the current results for expected assemblages. The impacts of the inclusion or exclusion of the species predicated to be infrequent on assemblage-specific sensitivity may depend on their positions in the hierarchical taxonomy structure. If infrequent species have a very close taxonomic distance to extremely sensitive or tolerant species with known sensitivity, assemblage-specific sensitivity will be affected. Although inclusion or exclusion of the species predicated to be infrequent in RICT predictions potentially change the assemblage-specific sensitivity to chemicals, these changes are very limited compared to the large variation in sensitivity of observed assemblages.

Although observed and expected assemblages differ in the magnitude of variation in assemblage sensitivity, they share the same spatial patterns of assemblage sensitivity to most chemicals. Spatial patterning was particularly strong for heavy metals. This may be because heavy metals are widespread in the natural environment and are closely related to geological characteristics (Okumur et al., 2007; Jaishankar et al., 2014; Masindi and Muedi 2018). The release of other study chemicals (narcotics, organochlorines, organophosphates, pyrethroids and surfactants) was mainly influenced by anthropogenic activities (Hanif, Adnan et al. 2012, López-Pacheco, Silva-Núñez et al. 2019, Xu, Zhang et al. 2021). As the species composition observed at some sites may have been altered by exposure to external stressors (e.g. decreasing sensitive species and increasing tolerant species), assessing the sensitivity of expected assemblages could provide minimally impacted scenarios or the unaffected conditions that could be used to inform risk assessment and environmental management. This work is the first attempt to assess and compare spatial variation in the sensitivity of both observed and expected assemblages.

Overall, species compositions have effects on assemblage sensitivity to chemical exposure. For observed assemblages, the magnitude of variation in assemblage sensitivity was greater for specific acting than generally acting chemicals. Assemblages that had similar species composition tended to exhibit similar sensitivity to a chemical, while assemblages with different species compositions had similar or largely different sensitivities. There was variation in the sensitivity of observed and expected assemblages to chemicals and this variation was spatially patterned for most of the chemicals studied. The sensitivity of observed and expected assemblages to chemicals was significantly different. This study indicates that the sensitivity of species assemblages to chemicals can vary greatly and spatially depending on chemical and assemblage types. Possible environmental drivers of this variation are considered in Chapter 3 and potential implications for ecological risk assessment are discussed in Chapter 5.

Chapter 3 | Relating assemblage sensitivity to chemicals to river catchment typologies and land use

3.1 Introduction

Chapter 2 explored the importance of spatial variation in assemblage composition and its consequence for spatial variation in community sensitivity. This chapter aims to broaden the analysis to consider the environmental drivers of spatial variation in community composition and how these drivers relate to spatial variation in assemblage sensitivity to chemicals. Relating these drivers to spatial variation in assemblage sensitivity to chemicals can help in understanding possible exposure scenarios and enhancing the environmental management of chemicals.

The assemblage of species present at a specific location is a function of environmental filtering and habitat template. How species assemble a community has been known to be affected by both environmental and biological factors. The theories “environmental filtering” and “habitat template” were proposed to be commonly used to explain the mechanisms of species assembly in natural communities. The environment has been considered to have a function of filtering species at local. Species adapted to “environmental filtering” can find their suitable niche locally. Predatory invertebrates were observed to be highly related to the biochemical and chemical oxygen demand in Malaysian streams, while benthic invertebrate shredders were highly associated with the river velocity and pH (Md Rawi, et al. 2014). In addition, environmental heterogeneity provides diverse habitats for species and also affects species composition. The distribution of species is controlled by their niche differentiation and local environmental conditions (i.e. environmental screening). Therefore habitats with similar ecological conditions would have similar species compositions in assemblages. The assemblage composition varies over space, as different freshwater invertebrates prefer different environmental habitats (Collier and Smith, 2006; de Haas and Kraak, 2008).

Rivers are nested within a hieratical structure in the catchment and exhibit diverse morphological and hydrological characteristics (Chainho et al., 2006; Heling et al., 2018). The physicochemical characteristics of rivers could be controlled and driven by the properties of their catchments (e.g. land use, catchment altitude, size and geology).

In Scotland, high levels of nutrients were detected in rivers within the catchments that were covered by high proportions of farmland (Benzie et al. 1991). In River Ganga, India, lowland rivers were heavily influenced by anthropological activities with high concentrations of pollutants (Sood et al. 2008). The catchment size was found to have a significant effect on the electrical conductivity of the river, as high flow volumes in large river catchments may dilute electrons (Ouyang et al. 2006). The physicochemical characteristics of Amazon rivers were found to be highly associated with the catchment geology and there were high levels of silica in siliceous rivers (Ríos-Villamizar et al. 2020). The properties of rivers and their catchments (e.g. land use, catchment altitude, size, and geology) are important drivers of the variation in physicochemical characteristics of rivers.

The physicochemical characteristics of rivers are strongly influenced by their catchment land use, and different land use heavily affects the physicochemical characteristics of rivers, thereby influencing species compositions of freshwater invertebrate assemblages (Carlisle and Hawkins, 2008; Pavlin et al., 2011; Johnson and Angeler, 2014; Fierro et al., 2017). Land use can reflect the disturbance process of anthropogenic activities on freshwater ecosystems to a certain extent and is considered as one of the good descriptors to measure the comprehensive impacts of anthropogenic activities (Fianko et al., 2009; Williams et al., 2016; Wu et al., 2017). In some human-intensive activity land use, anthropogenic activities affect river health, alter river physicochemical conditions and thus affect species composition (Nerbonne and Vondracek, 2001; Bu et al., 2014; Wang et al., 2014). For example, species richness and taxonomic diversity of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) show a positive correlation with agricultural land and a negative correlation with the urban area (Herringshaw et al., 2011). More shredder and predator invertebrates were observed in forests than in other land use in the Dongjiang River, China (Fu et al. 2016). There is the difference in species compositions of freshwater invertebrate assemblages near agricultural land and forests (Bu et al., 2014). In addition, land use has been used to describe exposure scenarios based on the geographic proximity of species to the source of pollutants (Maund et al., 2001; Bonzini et al., 2006; Maxwell et al., 2010). For chemicals with certain spatial exposure characteristics, such as pesticides, surfactants, pharmaceutical and personal care products, land-use can be used as an alternative method to predict environmental exposure when determining environmental concentrations at a large spatial scale is uneconomic (Bonzini et al., 2006). Spatial variation in species composition may result in natural assemblages and potentially differ in their sensitivity to chemicals (the findings of Chapter 2). The impacts of land use on spatial variation in assemblage sensitivity to chemicals need to be investigated to understand the levels of assemblage sensitivity under specific exposure scenarios where specific chemicals are frequently used.

The river catchment typologies (e.g. catchment altitude, size and geology) are highly associated with the physicochemical characteristics of rivers, thereby shaping species compositions in assemblages (Dodkins et al., 2005). Jacobsen et al. (1997) found that the number of insect orders and families decreases with the increase in altitude. In Southern England, altitude was one of the important factors in explaining the variation in the specie composition of assemblages (Williams et al. 2004). In New Zealand streams, there was distinct variation in species compositions of freshwater invertebrate communities across the different size catchments (Death and Joy 2004). In Belizean streams, more non-insect invertebrates (e.g. snails) were found in calcareous rivers than those in siliceous rivers (Carrie et al. 2015).

A variety of classification systems to group the rivers and streams systematically based on their topologies have been proposed for environmental management (EU Water Framework Directive system A and B; U.S. Stream Classification System (USSCS)). The classification of rivers mainly considered geospatial factors, environmental variables, and hydraulic indicators based on the multivariate analysis (Olden et al. 2009). The classification of rivers followed the principles of maximizing between-group differences, minimizing within-group differences, and balancing the trade-off between broad representations and limited management units (Tag 2012). Different regions considered different variables in developing classification systems (e.g. altitude, catchment area and geology in EU Water Framework Directive system A;

slope, flow velocity, width, catchment area and geology in national river typology descriptors of the Netherlands; types of water bodies and land use in Romania) (European Commission, 2000). The EU Water Framework Directive system A has been one of the most widely used classification systems in Europe for river management (European Commission, 2000)). Altitude, catchment size, and geology are the fixed typologies of Systems A for river management in the EU Water Framework Directive. These three typologies are also considered as important factors affecting spatial variation in species compositions of freshwater invertebrate assemblages (Jacobsen et al., 1997; Richards et al., 1997; Brosse et al., 2003; Olson, 2012; Milner et al., 2015). WFD typologies have been regarded as useful evaluation systems which can explain most of the spatial variation in species compositions of freshwater invertebrate assemblages at a large spatial scale (Verdonschot and Nijboer, 2004). The Water Framework Directive (WFD) is an important legal document for river health assessment management in Europe (European Commission, 2000).

Spatial variation in the sensitivity of freshwater invertebrate assemblages to chemicals has been investigated in Chapter 2. WFD typologies (catchment altitude, size, geology) and land use have been regarded as important drivers for spatial variation in species compositions and may potentially affect spatial variation in assemblage sensitivity to chemicals. Therefore, this study aims to relate assemblage sensitivity to river WFD river typology descriptors and land use, thereby integrating spatial variation in assemblage sensitivity into the existing legal system to support river safety assessment in Europe. WFD typology was used to capture 3 river characteristics – altitude, catchment size, and UKCEH land use was used to outline likely exposure scenarios. The objectives were addressed using the chemical sensitivity data of observed assemblages across 2318 sites in England (Chapter 2). The specific objectives of this chapter were to investigate:

(1) how WFD river typology descriptors (river catchment altitude, catchment size, and geology) are related to variation in the sensitivity of species assemblages to different chemical classes;

It has been reported that taxonomic composition in invertebrate assemblages are associated with river catchment typologies (Extence et al., 1999; Morecroft et al., 2009), e.g., altitude, catchment area, and geology (Bis et al., 2000; Skoulikidis et al., 2009; Olson, 2012; Buendia et al., 2013). For example, Dean Jacobsen (2004) investigated how altitude affected species compositions in stream invertebrate assemblages in Ecuador. Therefore, river typology descriptors may influence taxonomic compositions and thus affect assemblage sensitivity to chemicals. The sensitivity of species assemblages to chemicals was related to WFD river typology descriptors (river catchment altitude, catchment size, and geology) to test the hypothesis: of whether the sensitivity of species assemblages to chemicals varies across different river typology descriptors.

(2) how land-use types are related to variation in the sensitivity of species assemblages to different chemical classes;

Land use type not only affects nutrient input but also outlines potential exposure scenarios. Different input nutrients can affect the species composition, and exposure to external stressors also has impacts on the species composition, thus influencing the variation in assemblage sensitivity to chemicals. Therefore, the sensitivity of species assemblages to chemicals was examined in relation to land use (improved grassland, urban and suburban, arable, woodland, semi-natural grassland, mountain, health, and bog) to test the hypothesis that land use has an impact on the variation in assemblage sensitivity to chemicals.

(3) to assess the impacts of grouping chemicals by their type with considering whether sensitive or tolerant assemblages to chemicals within the same chemical type have similar species compositions.

The assemblage sensitivity to chemicals was grouped within the same class and compared across WFD river typology descriptors or land use. Therefore, the impacts of grouping chemicals by their type were also investigated. Organophosphates, pyrethroids, and organochlorine all belong to neurotoxic insecticides which target the nervous system of invertebrates (Yamamoto, 1970; Coats, 1990; O'Brien, 2014; Antwi and Reddy, 2015; Rathnayake and Northrup, 2016). Cadmium, copper, nickel, and zinc disorder invertebrate osmoregulatory and impair their circulatory in similar toxic pathways (Flick et al., 1971; Spicer et al., 1998; Lignot et al., 2000; Brooks and Mills, 2003). Chemicals in the same class share similar chemical structures and thus exhibit the same toxic mode of action on species. The taxonomic compositions between all sensitive and tolerant taxa lists were compared for each chemical within a chemical class and investigated using the Jaccard similarity measure to test the hypothesis that sensitive assemblages to the chemicals within a chemical class share similar taxonomic compositions.

3.2 Method

These objectives were addressed using chemical-specific HC5 values calculated for 2318 observed freshwater invertebrate assemblages in Chapter 2, which was expanded to include information on land use and WFD typologies. The 20 chemicals used in this study are the same as those described in Section 2.3.1.

3.2.1 River catchment typologies

Following the WFD river typology, the 2318 river sites were categorized using three descriptors: catchment altitude, catchment size and geology (European Commission, 2000). Catchment altitude was defined as the average elevation of the river catchment area and catchment size was defined as the area of the river catchment.

Mean catchment altitude and catchment area data were downloaded from the Catchment Characterisation Model River and Catchment Database (CCM2), available from the European Commission Joint Research Centre (De Jager, Alfred; Vogt, Jürgen (2007). The CCM2 database covers the main continent of Europe, Great Britain and Ireland, providing characteristic data of more than 2*10^6 primary catchments (e.g. area; minimum, average, and maximum elevation; minimum, average, and maximum slope; minimum, average, and maximum rain; average, and maximum temperature).

The spatial resolution of shapefile layers in the CCM2 database is 100 m. Catchment geology data were obtained from Solheim et al. (2019). The collected shapefiles of mean altitude, catchment area and geology were imported into ArcGIS 10.7.1 and classified according to the WFD typology. The specific typology descriptors, categories and range of values, used to define river catchment typologies in this study, are given in Table 3.1. The field calculator in ArcGIS was used to categorize river catchment typologies according to Table 3.1 and spatial classification maps were created for each descriptor (Figure 3.2). Freshwater invertebrate assemblages were related to each descriptor using the spatial join function in ArcGIS.

Table 3.1 River catchment typology descriptors, categories and range of values

Descriptor	Category	Range(Value I)
Altitude	Lowland	$I < 200$ m
	Midland	$200 \leq I \leq 800$ m
	Highland	$I > 800$ m
Size	Very Small	$I < 10$ km2
	Small	$10 \leq I \leq 100$ km2
	Medium	$100 < I \leq 1000$ km2
	Large	$1000 < I \leq 10000$ km2
	Very large	$I > 10000$ km2
Geology	Calcareous	
	Siliceous	
	Mixed	
	Organic	

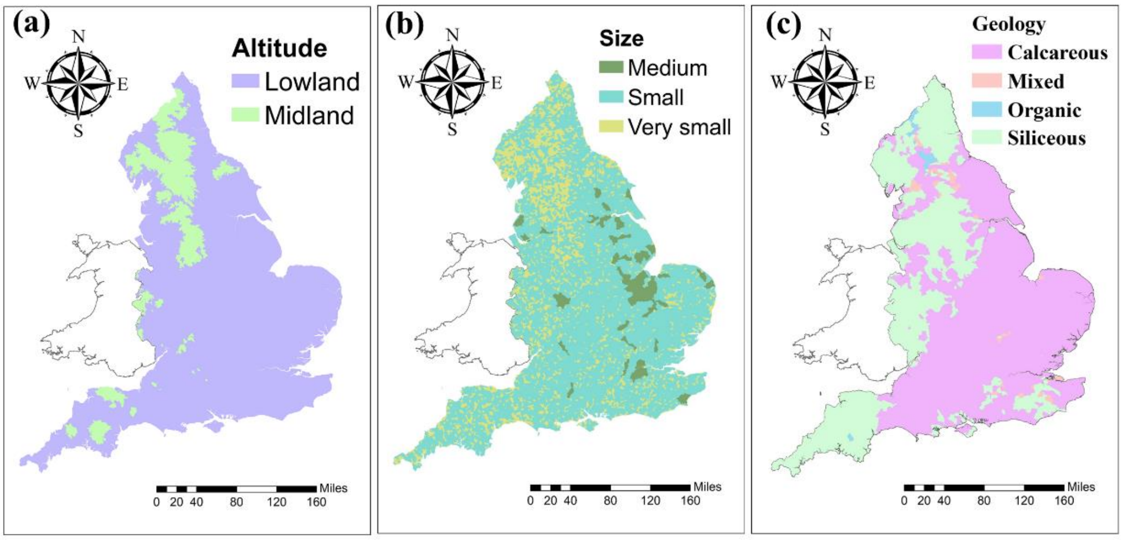

Figure 3.2 Spatial distribution of WFD river typology descriptors: altitude (a), catchment size (b) and geology (c) in England

Chemical-specific HC5 values for all assemblages and each chemical were related to river catchment altitude, catchment size and geology using the merge function in R based on unique Site-ID. Species assemblages may vary greatly in their sensitivity to chemicals, even within the same types. Chemical-specific HC5 values were nondimensionalized using min-max normalization to eliminate dimensional effects across chemicals when the HC5 values were grouped by chemical type. The standardized HC5 values were grouped by chemical type: organophosphates (diazinon, fenitrothion, malathion, and parathion-methyl (PM)); organochlorines (endosulfan, endrin and dichlorodiphenyltrichloroethane (DDT)); pyrethroids (cypermethrin, deltamethrin, and permethrin)); heavy metals (cadmium, copper, nickel and zinc); narcotics (phenol, benzenamine and glyphosate isopropylamine salt (GIS)) and surfactants (linear alkylbenzene sulfonates (LAS), nonylphenol (NP), sodium dodecyl sulfate (SDS)). The effect of altitude, catchment size and geology on the sensitivity of assemblages (standardized HC5 values) was assessed for each chemical type.

All the data failed the Anderson-Darling test for normality and therefore, an unpaired two-sample Wilcoxon test was used to check if there was a significant difference in assemblage sensitivity (i.e. standardized HC5 values) between lowland and midland sites for each chemical type. Kruskal–Wallis one-way analysis of variance

(ANOVA) test was used to examine significant differences in assemblage sensitivity (i.e. standardized HC5 values) across different river catchment size and geology for each chemical type. If the Kruskal–Wallis ANOVA test was significant ($p < 0.05$), multiple comparisons were performed using Dunn's test with the Benjamini-Hochberg adjustment.

3.2.2 Land use

The UK Centre for Ecology & Hydrology land cover map for 2020 (LCM2020) with a 25m spatial resolution was obtained via the Digimap Service (https://digimap.edina.ac.uk/). The land cover map provides 21 specific land cover classes and 10 aggregate classes for the UK. The land cover map for 2020 with 21 specific land cover classes was imported into ArcGIS 10.7.1. The 21 specific land cover classes were aggregated into 9 categories in England, as shown in Figure 3.3. Freshwater invertebrate assemblages were related to land use based on geographical proximity using spatial join in the ArcGIS software. This thesis used the freshwater invertebrate assemblages as the case study, therefore the categories of coastal and saltwater were excluded for further investigations. This study focused on six categories of the land cover map: woodland arable, improved grassland, urban & suburban, semi-natural grassland, mountain, heath and bog. As a very small proportion of freshwater invertebrate assemblages were related to the categories of freshwater and no data, the land use type for the assemblages in the category of freshwater or no data was replaced by one of other six study categories through the google satellite map identification. The sensitivity of assemblage (i.e., standardized HC5) was compared across different land use for each chemical type. The normality was checked using the Anderson-Darling test, and variance homogeneity was checked using Bartlett's test. The data failed to pass the premise hypothesis of one-way analysis of variance test. Kruskal–Wallis ANOVA test was used to examine significant difference in assemblage sensitivity across different land use ($p < 0.05$). Dunn's test with the Benjamini-Hochberg adjustment was used to compare assemblage sensitivity across different land use.

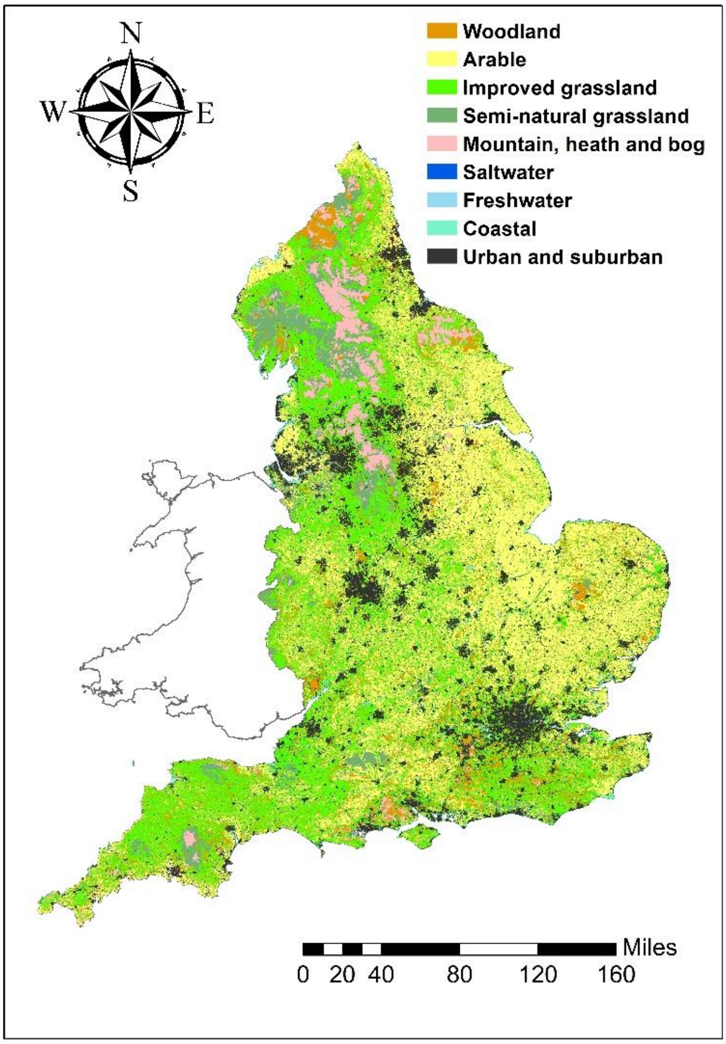

Figure 3.3 Land use map in England (based on the LCM2020 with a 25m resolution)

3.2.3 Relating taxonomic compositions to WFD river typology descriptors and land use

The freshwater invertebrate assemblages recorded at the 2318 study sites were linked to descriptors and categories of WFD river typology descriptors and land use. For each descriptor or category, the sites were extracted and used to subset the taxa dataset. The taxa dataset for each category of WFD river typology descriptors and land use were matched to the taxonomy database to obtain the taxonomic rank from phylum to order. The percentage composition of datasets for each category of WFD river typology descriptors and land use were compared at the phylum level, at the class level within the dominant phylum (Arthropoda), and at the order level within the dominant class (Insecta).

3.2.4 Assessing impacts of grouping chemicals by their type

The distribution profile for the sensitivity (HC5) of invertebrate assemblages recorded at the 2318 study sites was calculated to identify ‘sensitive’ and ‘tolerant’ assemblages for each chemical. Sensitive assemblages were defined as those in the lowest 5% of HC5 values, whereas the tolerant assemblages were defined as those in the highest 5% of HC5 values. The median values of the top 5% of sensitivity and top 5% of tolerant assemblages and the ratio of median values were calculated for each chemical. There are 116 assemblages (5%) pooled to produce combined taxa lists for sensitive and tolerant groups, separately. For each chemical, a single taxa list for all sensitive assemblages and a single taxa list for all tolerant assemblages were generated using the R, separately. The number of taxa in the sensitive and tolerant group for each chemical was counted and corresponding Jaccard similarity indexes between the sensitive and tolerant groups for each chemical were calculated. Then, the sensitive and tolerant taxa lists for each chemical within a chemical class were compared using the package “forestmangr” in R (https://rdrr.io/cran/forestmangr/). The package “forestmangr” was used to compare the similarity in taxonomic compositions across different groups. The Jaccard similarity matrices were obtained for each chemical class. Hierarchical dendrograms were made to compare differences in species composition between sensitive and tolerant groups within each chemical class. The height of hierarchical dendrograms was calculated using “dist” (https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/dist) and “hclust” (https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/hclust) in R based on Jaccard similarity matrices for each chemical class.

3.3 Results

3.3.1 Relating assemblage sensitivity to river typology descriptors

Assemblage sensitivity to chemicals was related to the three WFD river catchment typology descriptors (i.e., catchment altitude, catchment size, and geology) to explore the impacts of river typology descriptors on variation in the sensitivity of assemblages to chemical stress.

Eighty-seven percent of the assemblages (i.e., 2008 out of 2318) were classified as being in 'Lowland' river sites and the remainder were classified as being in 'Midland' river sites. None of the study assemblages were classified as being in 'Highland' river sites (i.e., > 800 m). As shown in Figure 3.4, the altitude had a significant effect on the sensitivity of assemblages to all chemical types (Wilcoxon rank sum test with continuity correction, W = 2145080 - 4241453, $p < 0.001$, n = 6954 (narcotics; organochlorines; pyrethroids; surfactants) and 9272 (heavy metals; organophosphates)). In all cases, the standardized HC5 values for assemblages in Lowland rivers were significantly smaller than those in Midland rivers, indicating that assemblages in Lowland rivers are more sensitive to chemical stress (Table 3.2 and Fig. 3.4). The largest difference between median values is for heavy metals, with the median value for Midland rivers being 1.7 times greater than that for Lowland rivers (Table 3.2 and Fig. 3.4). There is a small variation in median values for organochlorines, organophosphates pyrethroids, and surfactants, although the results of the unpaired two-samples Wilcoxon test achieved a significant level. (Wilcoxon rank sum test with continuity correction, W = 2145080 - 4241453, $p < 0.001$, n = 6954 (organochlorines; pyrethroids; surfactants) and 9272 (organophosphates)). In Fig. 3.4 c-e, there are a small proportion of outliers for Lowland rivers, indicating that a small number of assemblages in low-altitude rivers present strong resistance to organochlorines, organophosphates and pyrethroids.

Taxonomic comparisons of freshwater invertebrate assemblages recorded in lowland (<200 m) or midland (200 – 800 m) river sites in England are different, as shown in Figure 3.5. Freshwater invertebrate assemblages in Lowland rivers have higher taxonomic diversity at phylum, and order under Insecta than those in Midland rivers. At phylum, the taxa in Lowland rivers are Arthropoda, Mollusca, Annelida, Platyhelminthes, Cnidaria and Ectoprocta, while the taxa in Midland rivers are Arthropoda, Mollusca, Annelida and Platyhelminthes. Within the class Insecta, Diptera accounts for the largest proportion of the taxa in Lowland rivers, while the caddisflies (Trichoptera) make up the largest proportion of the taxa in Midland rivers.

Table 3.2 Number of sites/assemblages in each category of river typology descriptors and median values for each chemical type (heavy metals (HM), narcotics (NAR), organochlorines (OC), organophosphates (OP), pyrethroids (PYR) and surfactants (SUR))

Descriptor	Category (Number of sites)	Medians of standardized HC5 values (µg/L)					
		HM	NAR	OC	OP	PYR	SUR
Altitude	Lowland (2008)	0.182	0.228	0.004	0.004	0.002	0.13
	Midland (310)	0.312	0.284	0.006	0.006	0.003	0.151
Size	Very Small (684)	0.24	0.246	0.005	0.004	0.002	0.135
	Small (1553)	0.187	0.231	0.004	0.004	0.002	0.131
	Medium (81)	0.086	0.219	0.009	0.01	0.003	0.202
Geology	Calcareous (1251)	0.156	0.224	0.005	0.004	0.002	0.135
	Siliceous (1012)	0.248	0.249	0.005	0.004	0.002	0.13
	Mixed (32)	0.23	0.241	0.005	0.004	0.002	0.141
	Organic (23)	0.294	0.315	0.009	0.007	0.003	0.163

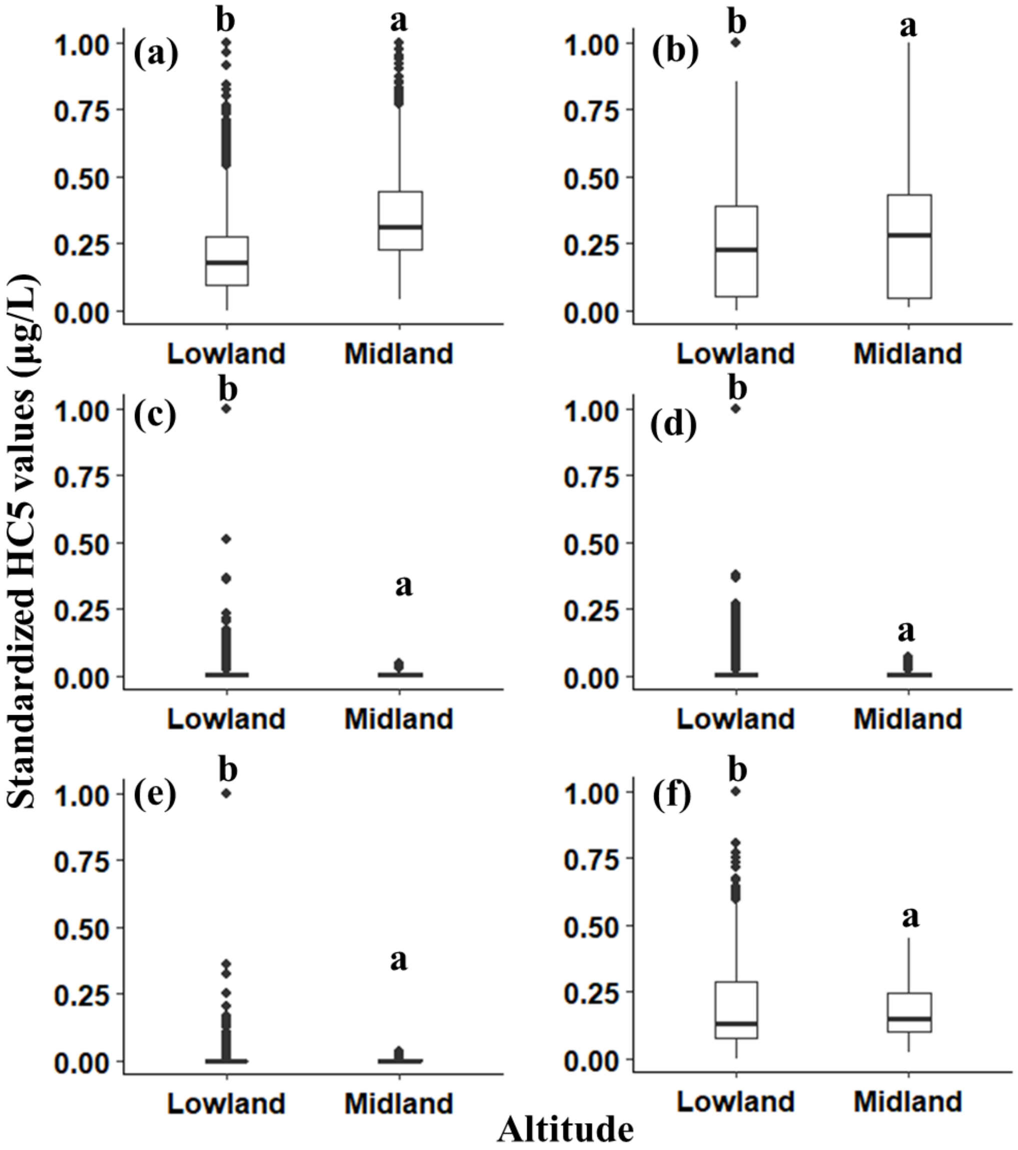

Figure 3.4 Comparisons of the median chemical sensitivity (standardized HC_5 values) of invertebrate assemblages recorded in lowland (< 200 m) or midland (200 – 800 m) river sites in England. Assemblages are compared for six chemical types: heavy metals (a), narcotics (b), organochlorines (c), organophosphates (d), pyrethroids (e) and surfactants (f). The boxes indicated the interquartile range, the horizontal lines are the median values, the vertical whiskers are quartiles, and the dots are outliers. The different letters above the box plots indicate significant differences in median chemical sensitivity ($p < 0.05$).

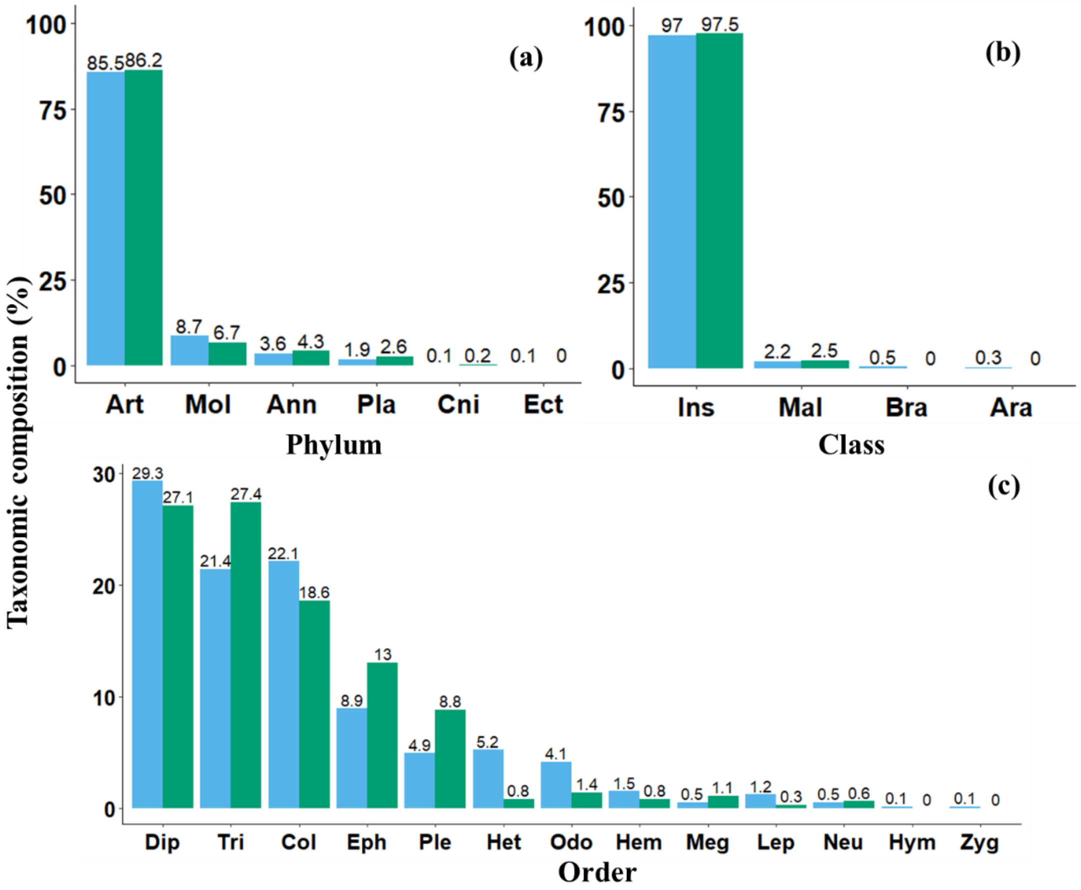

Figure 3.5 Taxonomic comparisons of freshwater invertebrate assemblages recorded in lowland (< 200 m, sky blue bars) or midland (200 – 800 m, green bars) river sites in England. Taxonomic compositions are compared across phyla (a), across the classes within the Arthropoda (b), and across orders within the Insecta (c). Phylum (Art – Arthropoda; Mol – Mollusca; Ann – Annelida; Pla – Platyhelminthes; Cni – Cnidaria; Ect – Ectoprocta); Class (Ins – Insecta; Mal – Malacostraca; Bra – Branchiopoda; Ara – Arachnida); Order (Dip – Diptera; Tri – Trichoptera; Col – Coleoptera; Eph – Ephemeroptera; Ple – Plecoptera; Het – Heteroptera; Odo – Odonata; Hem – Hemiptera; Meg – Megaloptera; Lep – Lepidoptera; Neu – Neuroptera; Hym – Hymenoptera; Zyg - Zygoptera)

Thirty percent of the assemblages (i.e., 684) were classified as belonging to 'Very small' catchments, 67% (i.e., 1553) to 'Small' catchments, and 3% (i.e., 81) to 'Medium' catchments. None of the study assemblages were classified as belonging to 'Large' (i.e., 1000-10000 km2) or 'Very large' (i.e., > 10000 km2) catchments. In all cases except narcotics, variation in the sensitivity of natural assemblages was significantly affected by river catchment size (Kruskal-Wallis test, chi-squared = 6.75 - 488.25, df = 2, p < 0.001, Figure 3.6). The sample size is 9272 for heavy metals and organophosphates, and 6954 for narcotics, organochlorines, pyrethroids and surfactants, respectively. The standardized HC5 values for assemblages in very small catchments are 2.79 times greater than that for assemblages in medium catchments for heavy metals (Table 3.2 and Figure 3.6 a). The standardized HC5 values for assemblages in small catchments were significantly lower than that for assemblages in very small catchments for heavy metals (Table 3.2 and Figure 3.6 a). Whereas the sensitivity of assemblages to heavy metals increased with increasing catchment size, for organophosphates, organochlorines, pyrethroids and surfactants, assemblage sensitivity decreased with increasing catchment size (Figure 3.6 a and c-f). For organophosphates, organochlorines, and surfactants, the standardized HC5 values for medium catchments are 1.5 -2.5 times greater than that for small catchments. As freshwater invertebrate assemblages in small and very small catchments account for a great majority (97%) in the dataset, the comparisons deserve more attention. The comparisons of assemblage sensitivity to narcotics, organophosphates, organochlorines, pyrethroids and surfactants show no significant difference between small and very small catchments.

Taxonomic comparisons of freshwater invertebrate assemblages recorded in very small (< 10 km2), small (10 - 100 km2), or medium (100 - 1000 km2) river catchments in England are presented in Figure 3.7. Freshwater invertebrate assemblages in small river catchments exhibit higher taxonomic diversity than those in other river catchments. The proportion of species ranked at Mollusca in medium-size river catchments is greater than those in other river catchments. For arthropods, assemblages in very small small and medium river catchments cover the same taxonomy: Insecta, Malacostraca, Branchiopoda and Arachnida. The proportion of insects in medium-size river catchments is lower than in other size catchments. For insects, the taxonomy of Diptera accounts for the largest proportion in the small and very small catchments but drops to third place in medium-size catchments. Coleoptera accounts for the largest proportion in medium catchments, followed by Trichoptera.

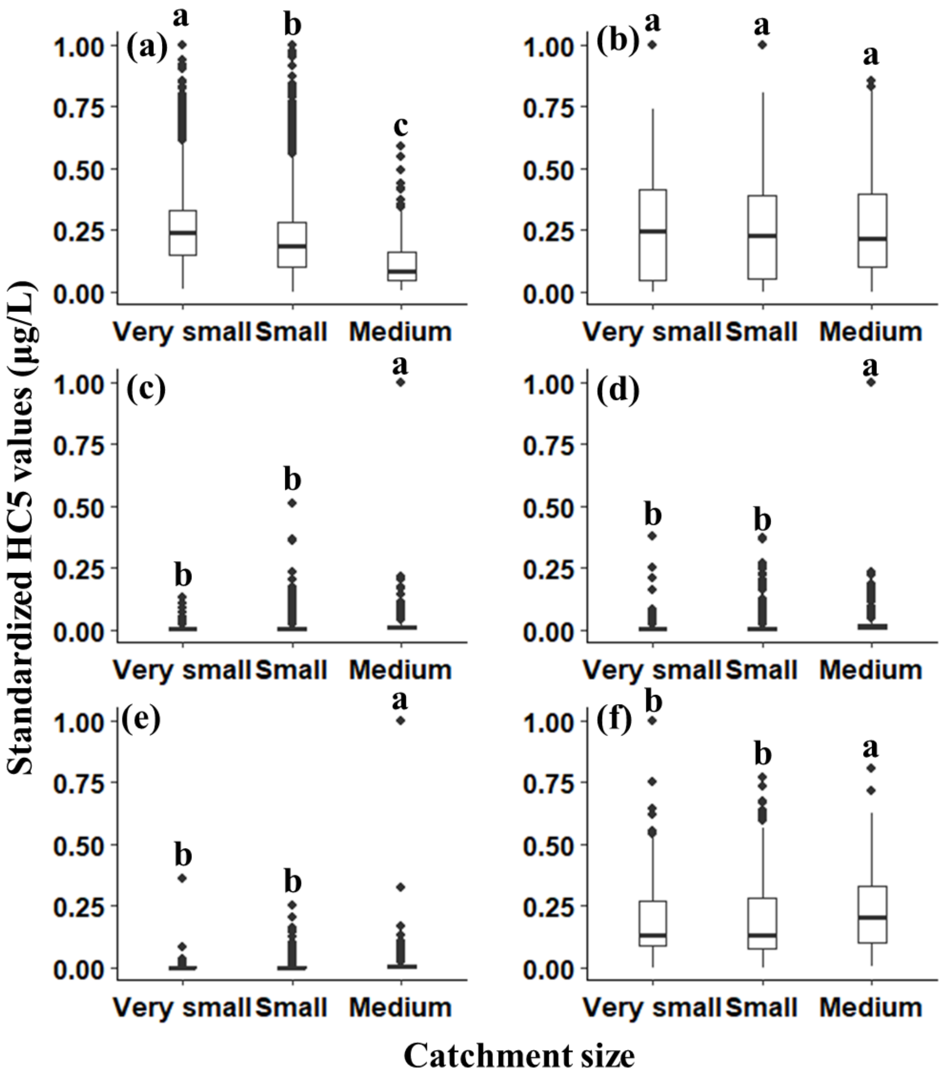

Figure 3.6 Comparisons of the median chemical sensitivity (standardized HC_5 values) of invertebrate assemblages recorded in very small (< 10 km^2), small (10 - 100 km^2), or medium (100 - 1000 km^2) river catchments in England. Assemblages are compared for six chemical types: heavy metals (a), narcotics (b), organochlorines (c), organophosphates (d), pyrethroids (e), and surfactants (f). The boxes indicated the interquartile range, the horizontal lines are the median values, the vertical whiskers are quartiles, and the dots are outliers. The different letters above the box plots indicate significant differences in median chemical sensitivity ($p < 0.05$).

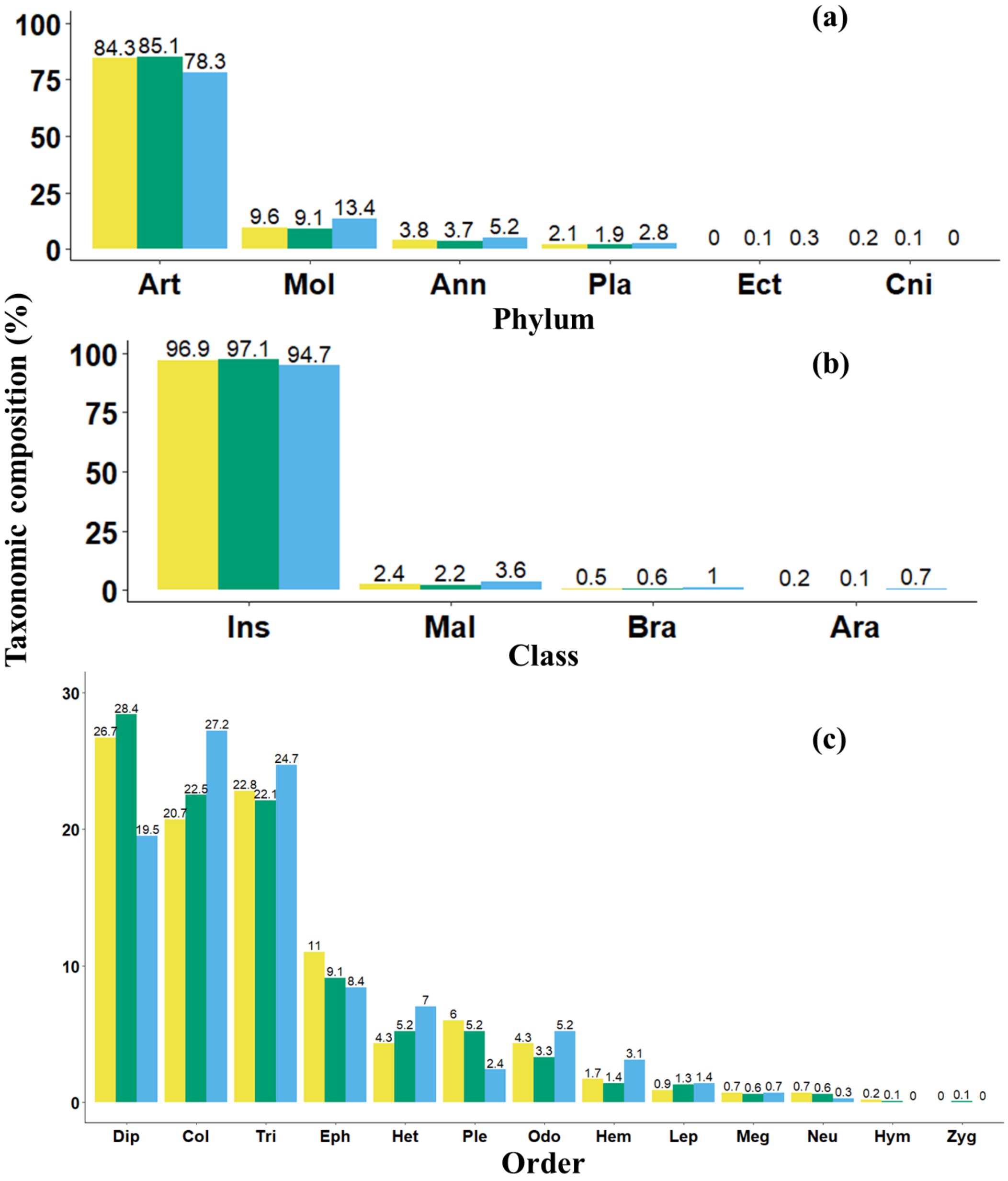

Figure 3.7 Taxonomic comparisons of freshwater invertebrate assemblages recorded in very small (< 10 km2, yellow bars), small (10 - 100 km2, green bars), or medium (100 - 1000 km2, sky blue bars) river catchments in England. Taxonomic comparisons are analysed at phylum (a, d & g), class under Arthropoda (b, e & h), and order under insect (c, f & i). Phylum (Art – Arthropoda; Mol – Mollusca; Ann – Annelida; Pla – Platyhelminthes; Ect – Ectoprocta; Cni – Cnidaria); Class (Ins – Insecta; Mal – Malacostraca; Bra – Branchiopoda; Ara – Arachnida); Order (Dip – Diptera; Col – Coleoptera; Tri – Trichoptera; Eph – Ephemeroptera; Het – Heteroptera; Ple – Plecoptera; Odo – Odonata; Hem – Hemiptera; Lep – Lepidoptera; Meg – Megaloptera; Neu – Neuroptera; Hym – Hymenoptera; Zyg - Zygoptera)

Fifty-four percent of assemblages (i.e., 1251) were classified as ‘Calcareous’, 44% (i.e., 1012) were classified as ‘Siliceous’, 1 % (i.e., 32) were classified as ‘Mixed’ and 1 % (i.e., 23) were classified as ‘Organic’ (Table 3.2). The sample size is 9272 for heavy metals and organophosphates, and 6954 for narcotics, organochlorines, pyrethroids and surfactants, respectively. River catchment geology significantly influenced assemblage sensitivity to heavy metals, organochlorines, organophosphates, and pyrethroids (Kruskal-Wallis test, chi-squared = 12.61 - 976.07, df = 3, $p < 0.05$). Considering that 98% of assemblages were either classified as ‘Calcareous’ or ‘Siliceous’, there is a significant difference in the median sensitivity of assemblages only to heavy metals between calcareous and siliceous rivers. To heavy metals, the assemblages in siliceous rivers have greater HC5 values than others in calcareous rivers (Table 3.2). To pyrethroids, there is a slight variation in the median sensitivity of assemblages between calcareous and siliceous rivers (Table 3.2). For organochlorines and organophosphates, there is no significant difference in the median sensitivity of assemblages in calcareous or siliceous sites. To heavy metals, organochlorines, organophosphates and pyrethroids, assemblages exhibit stronger tolerance in organic rivers than those in other types of rivers (Kruskal-Wallis test, chi-squared = 12.61 - 976.07, df = 3, $p < 0.05$, Figure 3.8 a, c, d and e).

Taxonomic comparisons of freshwater invertebrate assemblages recorded in calcareous, siliceous, mixed, or organic rivers in England are presented in Figure 3.8. The freshwater invertebrate assemblages in calcareous sites present greater taxonomic diversity at the phylum, the class within Arthropoda and the order within Insecta than the assemblages in the catchments with other geologies. For Arthropods, assemblages in siliceous sites cover Insecta, Malacostraca and Branchiopoda, while assemblages in calcareous sites cover another three: Insecta, Malacostraca, Branchiopoda and Arachnida. For insects, the taxonomy of Diptera accounts for the top proportion in calcareous and siliceous sites, while the taxonomy of Trichoptera has the largest proportion in the catchments with organic and mixed geologies.

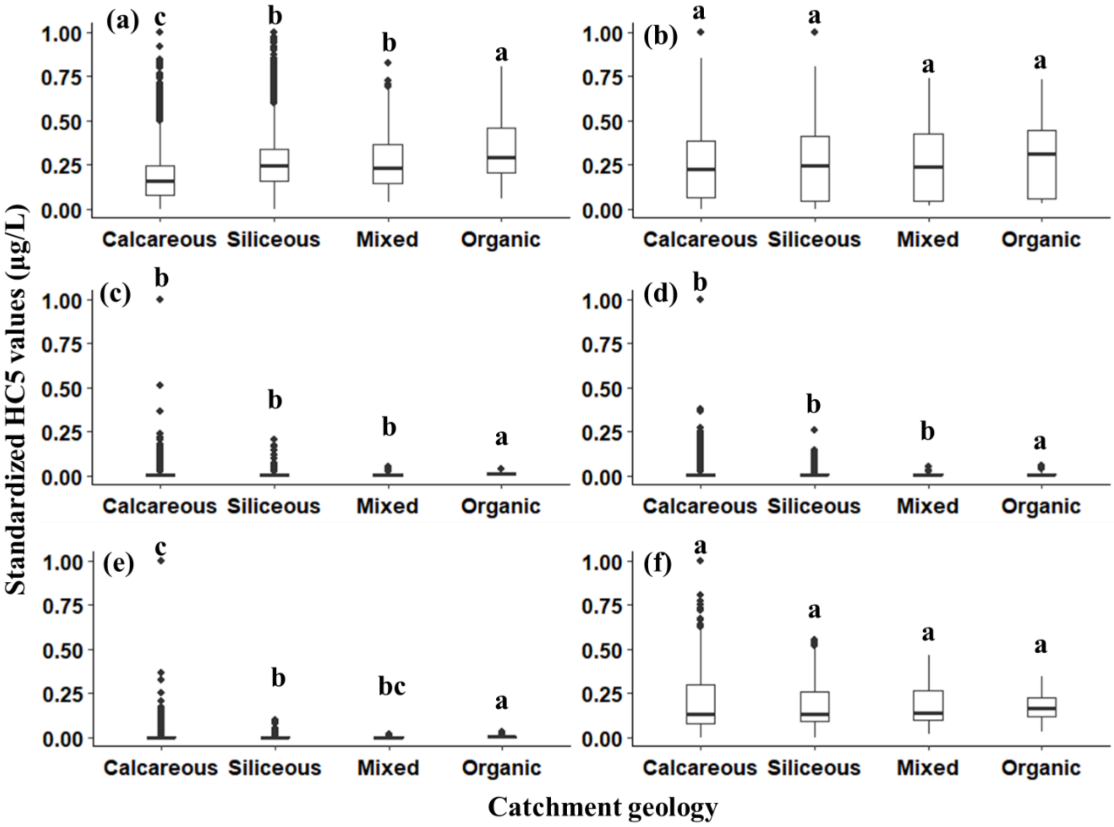

Figure 3.8 Comparisons of the median chemical sensitivity (standardized HC_5 values) of invertebrate assemblages recorded in calcareous, siliceous, mixed, or organic rivers in England. Assemblages are compared for six chemical types: heavy metals (a), narcotics (b), organochlorines (c), organophosphates (d), pyrethroids (e), and surfactants (f). The boxes indicated the interquartile range, the horizontal lines are the median values, the vertical whiskers are quartiles, and the dots are outliers. The different letters above the box plots indicate significant differences in median chemical sensitivity ($p < 0.05$).

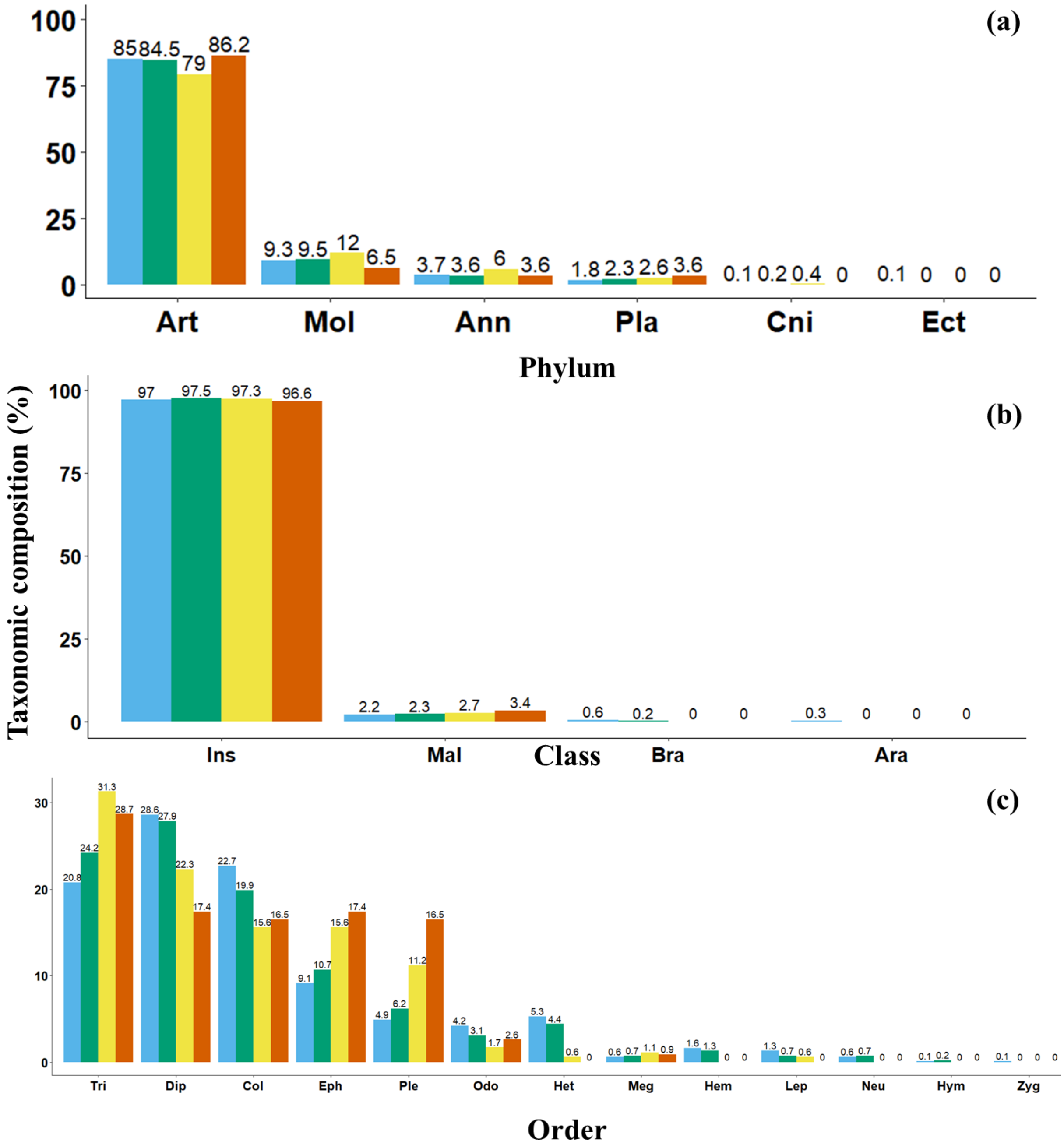

Figure 3.9 Taxonomic comparisons of freshwater invertebrate assemblages recorded in calcareous (sky blue bars), siliceous (green bars), mixed (blue bars), or organic (vermilion bars) rivers in England. Taxonomic comparisons are analysed at phylum (a), class under Arthropoda (b), and order under insect (c). Phylum (Art – Arthropoda; Mol – Mollusca; Ann – Annelida; Pla – Platyhelminthes; Ect – Ectoprocta; Cni – Cnidaria); Class (Ins – Insecta; Ara – Arachnida; Mal – Malacostraca; Bra – Branchiopoda; Ent – Entognatha; Col – Collembola); Order (Dip – Diptera; Col – Coleoptera; Tri – Trichoptera; Eph – Ephemeroptera; Ple – Plecoptera; Odo – Odonata; Hem – Hemiptera; Lep – Lepidoptera; Meg – Megaloptera; Neu – Neuroptera; Hym – Hymenoptera; Zyg - Zygoptera)

3.3.2 Relating assemblage sensitivity to land use

Forty-three percent (i.e., 995) of the study assemblages were surrounded by improved grassland, 20% (i.e., 473) were in urban or suburban areas, 18 % (i.e., 410) were in arable catchments, 15 % (i.e., 359) were in woodland, 3% (i.e., 67) were in semi-natural grassland, and 1 % (i.e., 14) were in mountain, heath or bog (Table 3.3). 81% of assemblages are in modified and managed landscapes (arable, improved grassland, urban & suburban) and the other 19% of assemblages are in woodland, semi-natural grassland, mountain, heath, or bog.

Table 3.3 Number of sites/assemblages in each category of land use and median values for each chemical type (heavy metals (HM), narcotics (NAR), organochlorines (OC), organophosphates (OP), pyrethroids (PYR) and surfactants (SUR))

Land use (Number of sites)	Medians of standardized HC5 values (µg/L)					
	HM	NAR	OC	OP	PYR	SUR
Improved grassland (995)	0.210	0.235	0.004	0.004	0.0018	0.130
Urban and suburban (473)	0.179	0.231	0.004	0.004	0.0018	0.132
Arable (410)	0.155	0.227	0.005	0.005	0.0018	0.142
Woodland (359)	0.245	0.253	0.005	0.004	0.0019	0.132
Semi-natural grassland (67)	0.201	0.246	0.007	0.005	0.0021	0.154
Mountain, heath and bog (14)	0.320	0.252	0.007	0.006	0.0025	0.177

Land use has a significant impact on the sensitivity of assemblages to heavy metals, organochlorines, organophosphates, and pyrethroids (Kruskal-Wallis test, chi-squared = 22 - 389.28, df = 5, $p < 0.001$, n = 6954 (narcotics; organochlorines; pyrethroids; surfactants) and 9272 (heavy metals; organophosphates), Figure 3.10). Assemblages sensitive to heavy metals have the smallest HC5 values in arable land, and the second smallest HC5 values in urban and suburban areas (Figure 3.10 a). For heavy metals, the HC5 values for assemblages in improved and semi-natural grassland are significantly greater than those in arable land, urban and suburban areas (Figure 3.10 a). Assemblages have the largest HC5 values in mountain, heath and bog, and the second-largest HC5 values in woodland (Figure 3.10 a). Although there is a significant difference for organochlorines, organophosphates, and pyrethroids, the medians of standardized HC5 values vary very slightly. For heavy metals, organochlorines, organophosphates and surfactants, assemblages in less modified land use are more tolerant than those in modified land use, although the comparisons of modified and less modified landscapes were very uneven (81% modified v 19 % less modified).

The freshwater invertebrate assemblages have coverage on the common phyla: Arthropoda, Mollusca, Annelida, and Platyhelminthes across different land use (improved grassland, urban and suburban, arable, woodland, semi-natural grassland, mountain, health and bog) (Figure 3.11 a). For Arthropoda, the assemblages only cover insects and Malacostraca in mountain, health and bog, while those assemblages in other land use can cover 3-4 classes (Figure 3.11 b). For insects, the taxonomy of Diptera makes up the largest proportion in improved grassland, urban and suburban and arable, and dropped to second place for the assemblages in woodland, semi-natural grassland, mountain, heath, or bog (Figure 3.12). The taxonomy of Trichoptera has the largest proportion in woodland, semi-natural grassland, mountain, health or bog.

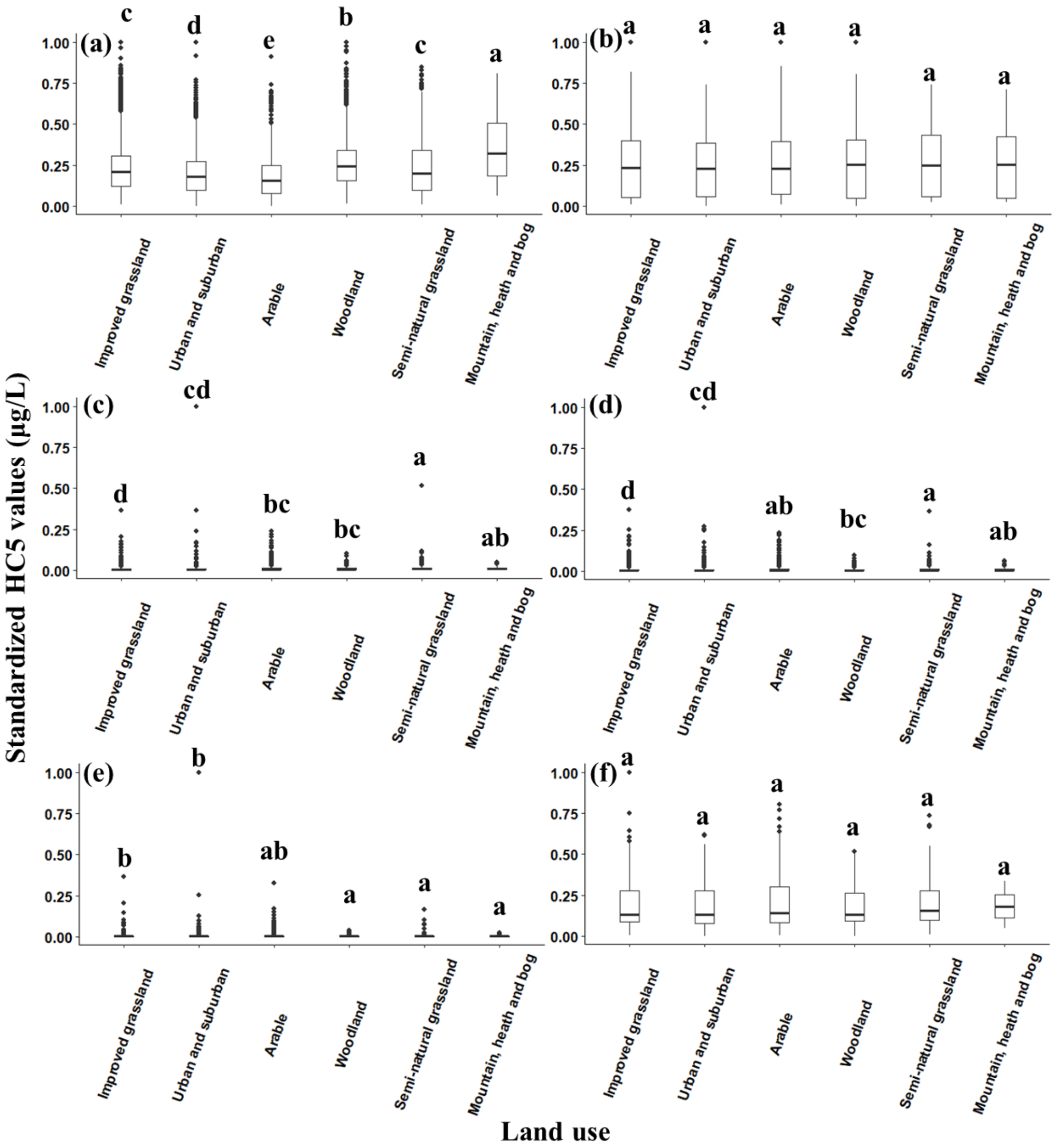

Figure 3.10 Comparisons of the median chemical sensitivity (standardized HC5 values) of invertebrate assemblages across different land use in England. Assemblages are compared for six chemical types: heavy metals (a), narcotics (b), organochlorines (c), organophosphates (d), pyrethroids (e), and surfactants (f). The boxes indicated the interquartile range, the horizontal lines are the median values, the vertical whiskers are quartiles, and the dots are outliers. The different letters above the box plots indicate significant differences in median chemical sensitivity ($p < 0.05$).

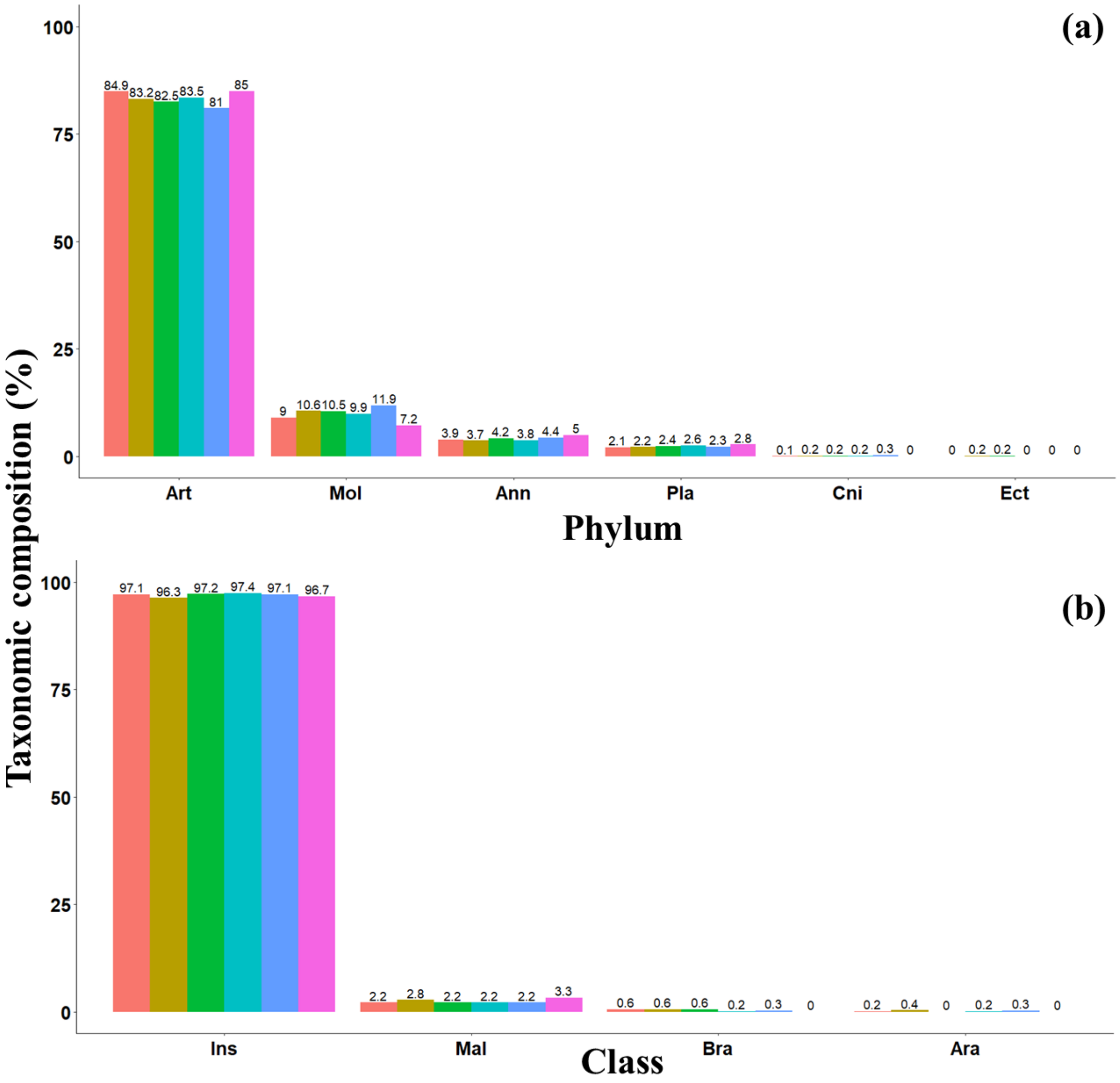

Figure 3.11 Taxonomic comparisons of freshwater invertebrate assemblages across different land use (improved grassland (red bars), urban and suburban (brown bars), arable (green bars), woodland (light blue bars), semi-natural grassland (dark blue bars), mountain, health and bog (purple bars)) in England. Taxonomic comparisons are analyzed at phylum (a) and class under Arthropoda (b). Phylum (Art – Arthropoda; Mol – Mollusca; Ann – Annelida; Pla – Platyhelminthes; Cni – Cnidaria; Ect – Ectoprocta); Class (Ins – Insecta; Mal – Malacostraca; Bra – Branchiopoda; Ara – Arachnida).

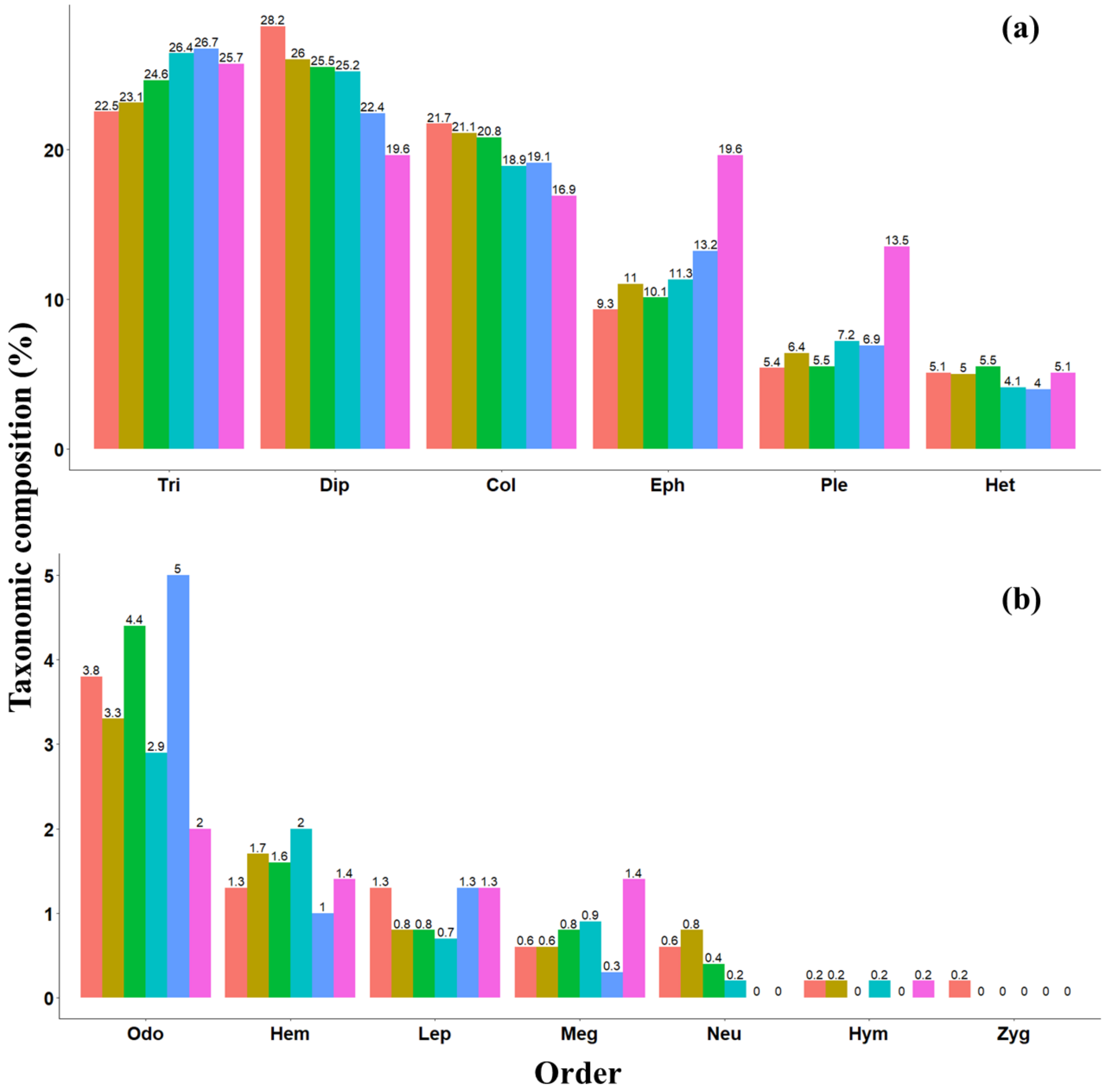

Figure 3.12 Taxonomic comparisons of freshwater invertebrate assemblages in order under insect across different land use (improved grassland (red bars), urban and suburban (brown bars), arable (green bars), woodland (light blue bars), semi-natural grassland (dark blue bars), mountain, health and bog (purple bars)) in England. Order: Tri – Trichoptera (a); Dip – Diptera (a); Col – Coleoptera (a); Eph – Ephemeroptera (a); Ple – Plecoptera (a); Het – Heteroptera (a); Odo – Odonata (b); Hem – Hemiptera (b); Lep – Lepidoptera (b); Meg – Megaloptera (b); Neu – Neuroptera (b); Hym – Hymenoptera (b); Zyg – Zygoptera (b).

3.3.3 Assessing the impacts of grouping chemicals by their type

The median values of the top 5% and bottom 5 % of assemblage sensitivity for each chemical are presented in Table 3.4. The smallest median HC5 value for the top 5% sensitive assemblages is 0.002 μg/L for cypermethrin, while the greatest median HC5 value for the top 5% tolerant assemblages is 93893 μg/L for benzenamine (Table 3.4). The ratio of the median HC5 value of the top 5% sensitive assemblages to that of the top 5% tolerant assemblages ranges from 2 to 50 (Table 3.4). The top 5% of sensitive and tolerant assemblages have the largest variation in median HC5 values to endrin (Table 3.4). Five percent of assemblages are 116 for generating sensitive and tolerant taxa groups, separately. The number of taxa for sensitive ranges from 319 to 463, while that of tolerant groups ranges from 247 to 446 (Table 3.4). Jaccard similarity indexes sensitive and tolerant taxa groups range from 0.17 to 0.54 (Table 3.4).

Species compositions were related to sensitive and tolerant assemblages to chemicals. Species compositions exhibit low similarity between sensitive and tolerant assemblages to all chemicals (Figure 3.13). For all study chemical classes (heavy metals, narcotics, organochlorines, organophosphates, pyrethroids, and surfactants), there is a higher similarity in the composition of either sensitive or tolerant assemblages across chemicals than between sensitive and tolerant assemblages for the same chemical class (Figure 3.13). For instance, assemblages that are sensitive to zinc are more similar in composition to assemblages that are sensitive to other heavy metals than to assemblages that are tolerant to zinc (Fig. 3.13 a). The grouping of sensitive and tolerant assemblages across chemicals is less pronounced for DDT (Fig. 3.13 c).

Table 3.4 The median values of top 5% and bottom 5 % assemblage sensitivity to each chemical, the number of taxa in the sensitive or tolerant group and their corresponding Jaccard similarity index.

Chemical	Median of HC5 values (µg/L)			Number of taxa		Jaccard similarity index
	Sen[a]	Tol[b]	Ratio	Sen[a]	Tol[b]	Range (0 - 1)
Cd	112	1805	16	348	270	0.32
Cu	37	194	5	328	321	0.35
Ni	549	7650	14	319	247	0.17
Zn	1182	3056	3	357	401	0.47
Ben	39070	93893	2	401	418	0.54
GIS	3913	7966	2	368	369	0.49
Phenol	5.238	28.134	5	409	337	0.38
DDT	2.212	11.341	5	403	397	0.48
Endrin	0.006	0.315	50	345	407	0.44
Endo	0.454	4.091	9	356	369	0.46
Dia	0.851	10.206	12	341	379	0.44
Feni	2.113	9.853	5	343	335	0.39
Mal	1.021	12.346	12	367	365	0.35
PM	1.23	11.162	9	407	334	0.48
Cyp	0.002	0.043	18	349	361	0.47
Del	0.003	0.057	16	463	342	0.5
Per	0.053	1.168	22	370	394	0.4
LAS	2.245	4.851	2	354	401	0.44
NP	0.71	5.764	8	340	446	0.39
SDS	1449	3166	2	385	381	0.43

Note: Sen[a] (sensitive group) and Tol[b] (tolerant group); Chemical abbreviations: cadmium (Cd), copper (Cu), nickel (Ni), and zinc (Zn); benzenamine (Ben), glyphosate isopropylamine salt (GIS), and phenol; dichlorodiphenyltrichloroethane (DDT), endosulfan (Endo) and endrin; diazinon (Dia), fenitrothion (Feni), malathion (Mal), and parathion-methyl (PM)); cypermethrin (Cyp), deltamethrin (Del), and permethrin (Per); linear alkylbenzene sulfonates (LAS), nonylphenol (NP), sodium dodecyl sulfate (SDS).

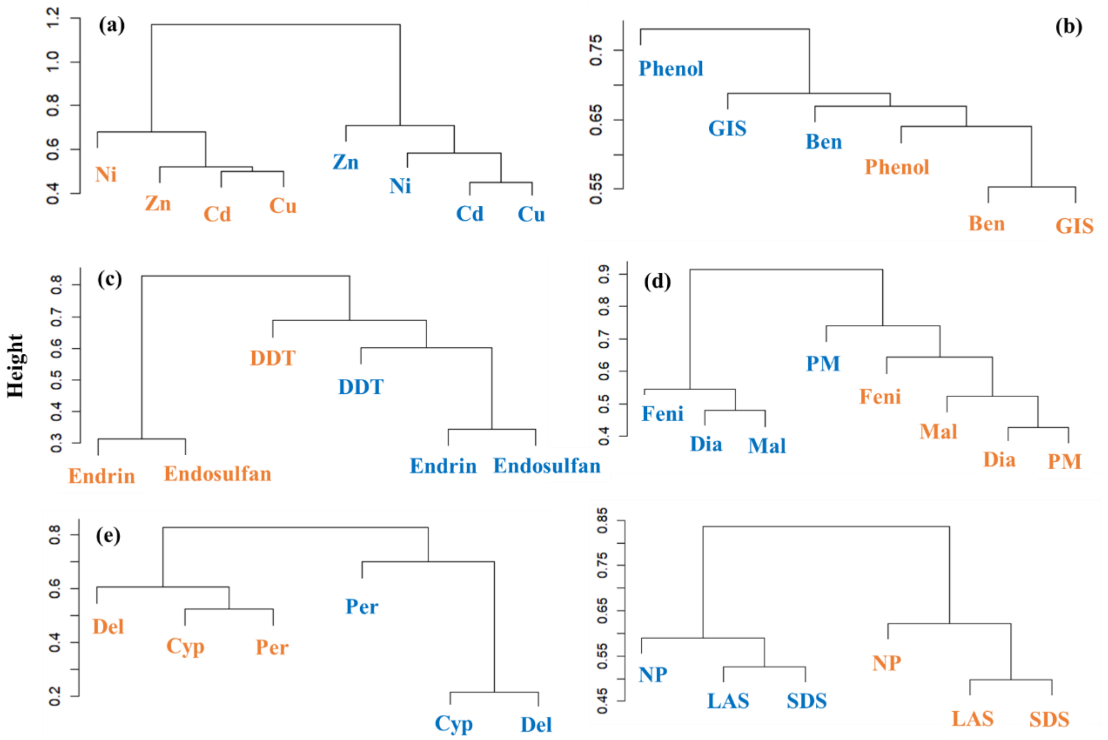

Figure 3.13 Hierarchical dendrograms for comparing differences in species composition between sensitive (orange) and tolerant (blue) assemblages to (a) heavy metals, (b) narcotics, (c) organochlorines, (d) organophosphates, (e) pyrethroids, and (f) surfactants. Chemical abbreviations: cadmium (Cd), copper (Cu), nickel (Ni), and zinc (Zn); benzenamine (Ben), glyphosate isopropylamine salt (GIS), and phenol; dichlorodiphenyltrichloroethane (DDT), endosulfan and endrin; diazinon (Dia), fenitrothion (Feni), malathion (Mal), and parathion-methyl (PM)); cypermethrin (Cyp), deltamethrin (Del), and permethrin (Per); linear alkylbenzene sulfonates (LAS), nonylphenol (NP), sodium dodecyl sulfate (SDS).

3.4 Discussion

This chapter aimed to investigate potential drivers of the spatial variation in assemblage sensitivity to chemical stressors reported in Chapter 2. It addressed three specific objectives: to investigate how WFD river typology descriptors (river catchment altitude, catchment size, and geology) are related to variation in the sensitivity of species assemblages to different chemical classes; to investigate how land-use types are related to variation in the sensitivity of species assemblages to different chemical classes; to assess the impacts of grouping chemicals by their type with considering whether sensitive or tolerant assemblages to chemicals within the same chemical type have similar species compositions. To address these objectives, the sensitivity of 2318 assemblages to 20 study chemicals has been related to WFD typology descriptors and land use in England. The similarity in taxonomic compositions of all sensitive and tolerant taxa lists was compared for each chemical within each of the six chemical classes.

Altitude had significant effects on the variation in assemblage sensitivity to all study chemical classes. In general, results support the hypothesis that species assemblages in lowland rivers were more sensitive to chemical stress (i.e. smaller HC5 value) than those in midland rivers. Variation in assemblage sensitivity between lowland and midland rivers is greater for heavy metals than for other chemical classes. Some taxa were only observed in lowland rivers (e.g. Branchiopoda, Arachnida). Previous studies have found that Branchiopoda was very sensitive to organochlorines and organophosphates and pyrethroids (Hyder et al. 2004, Friberg-Jensen et al. 2010, Demetrio et al. 2014, Varó, et al. 2015). In addition, the variation in the richness of taxa that are common between lowland, and midland rivers could also be a driver for variation in assemblage sensitivity to chemicals. Diptera was sensitive to heavy metals (Martinez et al. 2002, Dornfeld et al. 2019), and more Diptera was observed in lowland than in midland rivers, possibly contributing to the variation in assemblage sensitivity to heavy metals.

Catchment size affects variation in assemblage sensitivity to all chemical types except narcotics. For heavy metals, species assemblages were tolerant in very small catchments and sensitive in medium-size catchments, probably because the proportion of Mollusca in medium-size catchments is higher than that in very small catchments. Some freshwater Mollusca (e.g. snails, clams, mussels) have been tested and considered as sensitive to heavy metals (Gupta and Singh 2011, Gawad 2018). Assemblages in small and very small catchments did not differ significantly in their sensitivity to organochlorines, organophosphates, and pyrethroids, probably because assemblages in small and very small catchments share similar taxonomic compositions.

River catchment geology significantly influenced variation in the sensitivity of assemblages to heavy metals, organophosphates, organochlorines, and pyrethroids. Most river sites (i.e. 97%) were classified as calcareous or siliceous. Species assemblages in calcareous sites were sensitive to heavy metals but more tolerant to surfactants than those in siliceous sites. These observations may be because there are some relatively sensitive taxa (*Anodonta sp. Asellus sp. Chironomus sp.*) to heavy metals in calcareous sites (Migliore and de Nicola Giudici, 1990; Ochieng et al., 2008; Kurnia et al., 2010), but these species are tolerant to insecticides (Bressan et al., 1989; Mäenpää and Kukkonen, 2006; Sobrino-Figueroa, 2018). Invertebrate assemblages in organic river catchments are tolerant to most of the study chemicals, especially heavy metals. This may be due to the interaction between organic matter and pollutants (Karickhoff et al., 1979). In organic rivers, high organic matter content has a sorption effect on chemicals (especially for heavy metals).

The impacts of land use on taxonomic compositions in freshwater invertebrate assemblages have been recognized and investigated (Weijters et al., 2009; Mesa, 2010; Herringshaw et al., 2011; Hanna et al., 2020). Land use may directly affect species composition and thus influence assemblage sensitivity to chemicals. Land use has significant impacts on the variation in assemblage sensitivity to heavy metals, organochlorines, organophosphates and pyrethroids. For heavy metals, invertebrate assemblages in arable sites are most sensitive to heavy metals, whereas assemblages in mountain, heath and bog sites are most tolerant to heavy metals. The invertebrate assemblages in arable show strong tolerance to organochlorines and organophosphates.

Evidence from compositional analysis and ecotox studies suggest that sensitive species in arable may be eradicated by past pesticide use (Relyea 2005, Duflot, Georges et al. 2014).

As expected, assemblages that are sensitive to chemicals have different species compositions compared to tolerant assemblages. Sensitive or tolerant assemblages to the chemicals within a chemical class share similar taxonomic compositions for heavy metals, narcotics, organochlorines, organophosphates, pyrethroids and surfactants. The study chemicals were grouped by toxic mode of action. The toxic mode of action for the metals, cadmium, copper, nickel and zinc, is to disrupt osmotic pressure regulation (Kahlon, Sharma et al. 2018, Kravchik, Novikov et al. 2020). The pesticides studied all caused neurotoxicity: organochlorines inhibit acetylcholinesterase activity; organophosphates inhibit acetylcholinesterase, and pyrethroids inhibit the transmission of sodium in the neural pathway (Davies et al. 2007, Van Dyk and Pletschke 2011, Androutsopoulos et al. 2013, Williamson et al. 2019, Zhong et al. 2021). Phenol and benzenamine are polar narcotics (Aptula et al. 2002, Westbury et al. 2004, Furuhama et al. 2015, Gergs et al. 2016).

Overall, WFD typology descriptors are associated with variation in assemblage sensitivity to some chemical classes. Freshwater invertebrate assemblages in lowland rivers are sensitive to heavy metals, narcotics, organochlorines, organophosphates, pyrethroids and surfactants. Invertebrate assemblages in very small catchments are most tolerant to heavy metals, while those in medium catchments are most sensitive to heavy metals. Assemblages in calcareous sites are sensitive to heavy metals. Assemblages in arable sites are sensitive to heavy metals and tolerant to organochlorines, organophosphates, and surfactants. The impacts of WFD typology descriptors and land use on variation in assemblage sensitivity to chemicals may be explained in terms of differences in the taxonomic composition of assemblages. Sensitive assemblages to chemicals with the same class share a similar taxonomic composition and this also applies to tolerant assemblages.

Chapter 4 | Spatial variation in the recovery potential of freshwater invertebrate assemblages: the importance of species traits and landscape elements

4.1 Introduction

Different groups of chemicals exhibit different exposure patterns based on different usage scenarios and frequencies (Chapter 1 1.3.3 section). For example, pesticide use results in intermittent chemical exposure. At the same time, heavy metals are discharged from abandoned mines, and pharmaceuticals and personal care products are discharged via domestic sewage systems, resulting in more continuous exposure patterns. Ecosystems exposed to variable chemical exposure have the potential to recover from adverse effects once the chemical concentration has decreased below the threshold for those effects. Understanding the processes by which communities and populations recover after chemical exposure is important for risk assessment, as highlighted by the recent publications on this topic by the European Food Safety Authority (EFSA) (EFSA PPR Panel, 2013; EFSA Scientific Committee, 2016). Ecological recovery is the process by which impacted communities or populations return to their pre-impacted state or range of control systems (i.e. normal operating range) (Gergs et al., 2016). Recovery potential considers the potential processes by which impacted communities or populations may recover to their original state after the removal of external stressors (e.g. chemical pollution) (Underwood, 1989; Norton et al., 1992; Barnthouse, 2004). Empirical studies and model simulations have demonstrated that freshwater invertebrate populations and communities have the potential to recover after chemical exposure is removed (e.g. Van den Brink et al., 1996; Sherratt et al., 1999; Barnthouse, 2004; Hayashi et al., 2008; Baveco et al., 2014; Gabsi and Preuss, 2014) and the implications of recovery for environmental risk assessment were reviewed by Gergs et al. (2016).

The recovery potential of freshwater invertebrate assemblages consists of two processes: internal and external recovery (Chapter 1 1.3.3 section). Internal population recovery describes the potential capability of individuals surviving a chemical impact to reproduce and increase population abundances to their original level (EFSA Scientific Committee, 2016). Internal recovery is closely associated with the demographic characteristics and traits of the species impacted. Traits that are important for internal recovery include the adult life span, the number of cycles per year, the number of reproductive events per year, and the number of offspring

(Rubach et al., 2011; Hamilton et al., 2020). Many population-level experiments (e.g. life table demography experiments) have demonstrated the important impacts of these demographic characteristics and traits on population growth (Levin et al., 1996; Muro-Cruz et al., 2002; Gama-Flores et al., 2007; Zhao et al., 2009). Freshwater invertebrates exhibit diverse demographic characteristics and traits (Usseglio-Polatera et al., 2000; Culp et al., 2011). For example, it only takes a few days for some rotifers and cladocerans to reproduce, while some larger insects may take 1-2 years to reach maturity (Barnthouse, 2004). Spatial variation in the species composition of freshwater invertebrate assemblages (Chapter 2, 3) will potentially influence assemblage trait profiles, suggesting that assemblage-level internal recovery may also vary spatially.

External recovery depends on the immigration of species from an external source to the local area. External recovery can be divided into four categories based on the dispersal modes of freshwater invertebrates: aquatic active, aquatic passive, aerial active, and passive (e.g. transport by animal vectors or wind) (Bilton et al., 2001; Bohonak and Jenkins, 2003). Aquatic active dispersal is influenced by flow velocity and an organism’s mobility (i.e., swimming and crawling capability), while aquatic passive is primarily controlled by flow velocity, although an organism’s habitats and behaviors are also important. Freshwater invertebrates in rivers with active aquatic dispersal may disperse both upstream and downstream, although dispersal downstream should be easier than dispersal to upstream. Aquatic passive dispersal is dependent on flow direction and is unidirectional in rivers (i.e. downstream). There is substantial inter-specific variation in the dispersal ability of riverine invertebrates. For example, in experiments with stream invertebrates, *Perlodes*, *Rhyacophila* and *Isoperla* dispersed 9.5-13.5 m in 24 h, whereas *Ecdyonurus*, *Hydropsyche*, *Gammarus* and *Baetis* moved 5.5-7 m in 24 h. Interesting, for nine of the 10 species investigated, movement over the 24-h study period was predominately upstream (Elliott, 2003).

Landscape elements (e.g. distance from the source, altitude， and land use) are considered to be important factors affecting both aerial active and passive dispersal (Thomas, 1996; Heino and Muotka, 2006). Altitude and land use have been reported to have strong impacts on the dispersal of adult insects (Didham et al., 2012; Carlson et al., 2016). These two elements can act on freshwater invertebrates for aerial active and passive dispersal (Jansson and Malmqvist

2007). Aerial active dispersal refers to immigration by flying species (e.g. adult insects), while aerial passive dispersal means the movement of freshwater invertebrates or their eggs or resting stages, by mobile animal vectors (e.g. frogs, water birds, dogs) or by the wind (Figuerola and Green, 2002; Vanschoenwinkel et al., 2008; Vanschoenwinkel et al., 2011). Aerial passive dispersal has higher uncertainty than active aerial dispersal. For aerial dispersal, all aquatic environments (e.g. rivers, lakes, ponds) in the landscape can be potential sources of colonizing individuals, but sources for aquatic dispersal are limited to the connected network of upstream and downstream water bodies. Considering both the spatial variation in the composition of freshwater invertebrate assemblages and the impacts of landscape elements on aerial dispersal process, it is expected that assemblage-level external recovery will potentially vary spatially.

Different approaches have been applied to investigate internal and external recovery. For example, Barnthouse (2004) used life history information (age-specific reproductive rate, survival probability, longevity, generation time, development time) and the logistic population growth model, to quantify and compare recovery rates for freshwater vertebrates, invertebrates and plants. Other studies have combined population growth models with dynamic energy budget (DEB) theory to describe internal recovery processes (van der Meer, 2006; Nisbet et al., 2010; Martin et al., 2012). The use of population growth models is limited by the lack of appropriate input data for many species in freshwater invertebrate assemblages. Jepson (1989) described the recovery potential of terrestrial invertebrates (e.g. Carabidae, Staphylinidae and Linyphiidae) using a scoring method that considered life-history parameters and dispersal capability.

The investigation of external recovery needs to incorporate a spatial analysis as it is determined by both the capability of species to disperse and landscape elements. Van den Brink (2007), for example, incorporated drift processes into a spatially explicit model to describe the internal and external recovery of a freshwater crustacean (*Asellus aquaticus*). This type of spatially explicit model requires a lot of data to support, and this makes it difficult to cover freshwater invertebrates in natural assemblages. Rico and Van den Brink (2015) used the weight index method to describe the internal and external recovery with biological traits, but this work did not consider landscape resistance.

The main aim of this chapter is to investigate the recovery potential of freshwater invertebrate assemblages after removing chemical impacts based on species traits and landscape elements. Spatial variation in the species composition of freshwater invertebrate assemblages drives spatial variation in the sensitivity of those assemblages to chemicals (Chapter 2 and 3). Variation in species composition may also drive spatial variation in recovery potential by affecting the trait profiles of assemblages. This chapter has three specific objectives:

Objective 1: comparing recovery-related trait profiles between sensitive and tolerant freshwater invertebrate assemblages to chemicals.

The recovery potential of assemblages could be determined by their trait profile, which may be driven by the trait profiles of their constituent species. Recovery-related trait profiles for chemical-sensitive and chemical-tolerant assemblages were compared using the redundancy analysis. From a risk management perspective, if sensitive and tolerant taxa groups are associated with the recovery-related traits, the protection thresholds could be used as representation indexes for describing the recovery potential. This will thus simplify the process of risk evaluation on chemicals. Therefore, it was hypothesized that sensitive taxa groups to chemicals show different recovery-related trait profiles from tolerant taxa groups.

Objective 2: investigating whether the recovery potential of freshwater invertebrate assemblages exhibits spatial variation and spatial patterns.

Recovery-related traits and landscape elements were used to evaluate the recovery potential of freshwater invertebrate assemblages and to investigate spatial variation and spatial patterns in recovery potential. Differences in species composition drive variation in the trait profile of assemblages. Whereas internal recovery potential is determined by the trait profile of an assemblage, external recovery potential is a function of trait profile and landscape factors that influence connectivity and the distribution of source populations. As assemblage composition and landscape factors vary spatially, it is expected that both the internal and external recovery potential of freshwater invertebrate assemblages exhibit spatial variation and that this variation exhibits spatial patterns.

Objective 3: exploring the relationship between assemblage sensitivity to chemicals and assemblage-specific recovery potential.

The resilience of assemblages to chemical stressors is a function of their chemical sensitivity (i.e. magnitude of effect) and recovery potential. For example, assemblages with high sensitivity and low recovery will be more susceptible to chemical exposure than assemblages with high sensitivity and high recovery. If sensitive and tolerant assemblages share the same recovery-related trait profiles, the importance of recovery processes in influencing the outcome of ecological risk assessments is limited. The relationship between chemical sensitivity and recovery potential was investigated to assess the extent to which risk is enhanced by the co-occurrence of assemblages with high sensitivity but low recovery potential. If the assemblage-specific sensitivity and recovery potential are highly correlated, then only one of them will need to be focused on in ecological risk assessment. If the two are not correlated, then both indicators will need attention, especially in the situation when assemblages with high sensitivity but have low recovery potential. Therefore, it is hypothesized that the assemblage sensitivity is correlated to its recovery potential.

4.2 Method

4.2.1 Data collection and preliminary processing

The dataset of 2318 freshwater invertebrate assemblages described in Section 2.3.1 and Section 3.2.1 was used as a case study in this Chapter. The 20 chemicals and chemical-specific HC5 values used in this study are the same as those described in Section 2.3.1 and Section 3.2.1.

Trait data were collected from the following three studies. The source databases used by these three studies are the Tachet Database, Pond-FX database, Poff database, and DISPERSE database (Heneghan et al., 1999; Usseglio-Polatera et al., 2000; Poff et al., 2006; Sarremejane et al., 2020), which were supplemented by information from expert consultation and literature searches (Rico and Van den Brink 2015; Van den Berg et al., 2019). Recovery-related traits selected for this analysis are presented in Table 4.1 and are either classified as continuous and factor types (Table 4.1). The Tachet and DISPERSE databases were used as the primary data sources. Trait data for the 'number of cycles per year', 'aquatic stage' and 'dispersal mode' were obtained from the Tachet database, which contains information on 22 biological and ecological traits for 472 European invertebrate species. Trait data for the 'number of eggs per female', 'adult life span', 'female wing length', 'wing pair type' and 'propensity to drift' were obtained from the DISPERSE database, which covers 480 European invertebrate species. Trait data for 'crawling capacity' and 'swimming ability' were derived from Pond-FX database, Poff database and supplementary trait data by provided by Rico and Van den Brink (2015). Mostthe of the trait data in the Tachet and DISPERSE databases were recorded at Genus, whereas trait data from the other sources used was mainly recorded at Family or Order. The final trait dataset covered 94% of species in 2318 freshwater invertebrate assemblages.

Elevation data, land use ,and river maps for England were obtained from EDINA digimap (https://digimap.edina.ac.uk/). The original maps were cropped to England (as shown in Figure 4.1).

Table 4.1 Recovery-related traits and their categories, codes and types

Recovery-related process	Trait	Category	Code	Type
Internal recovery	Number of eggs per female	< 100 100 to 1000 1000 to 3000 > 3000	Eggs	Continuous
	Number of cycles per year	< 1 1 > 1	Ncpy	Continuous
	Adult life span	<1wk 1wk - 1m 1m - 1yr > 1yr	Als	Continuous
	Aquatic stage	Adult Nonadult	As	Factor
External recovery	Female wing length (mm)	<5 5 to 10 10 to 15 15 to 20 20 to 30 30 to 40 40 to 50 50 to 60	Fwl	Continuous
	Wing pair type	No wings 1 pair + halters 1 pair + 1 pair of small hind wings 1 pair + 1 pair of elytra or hemielytra 2 similar-sized pairs	Wnp	Continuous
	Propensity to drift	Rare/Only during catastrophic events Occasional Frequent	Drift	Continuous
	Crawling capacity	<10cm per hour 10-100 cm per hour >100 cm per hour	Crac	Continuous
	Swimming ability	Not typically swimmers Weak (slow/clumsy) Strong	Swia	Continuous
	Number of cycles per year	< 1 1 > 1	Ncpy	Continuous
	Dispersal mode	Aquatic passive Aquatic active Aerial passive Aerial active	Disp	Factor

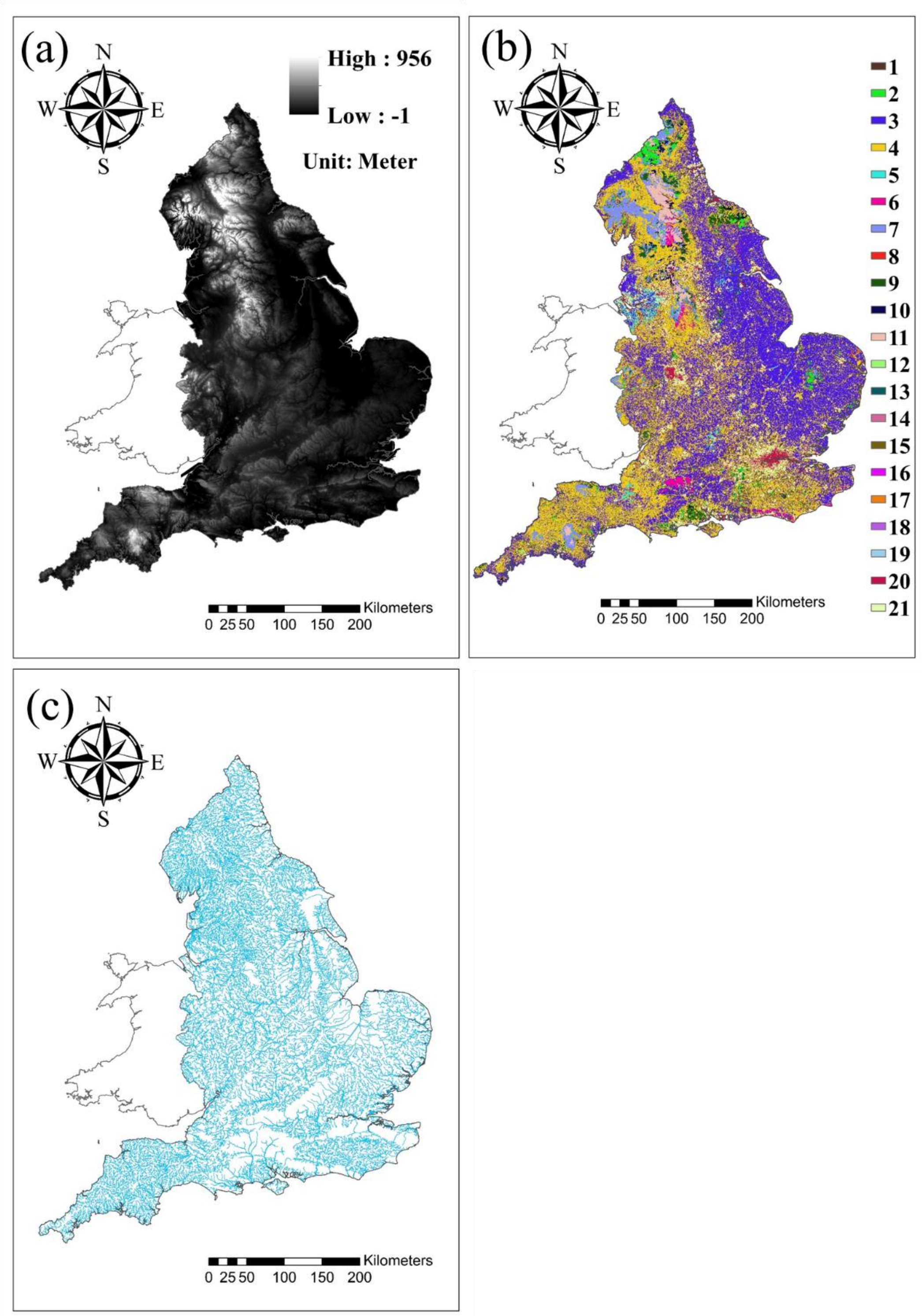

Figure 4.1 Elevation map (a), land use map (b), and river network map (c). The land use map shows 21 categories: 1 - Deciduous woodland, 2 - Coniferous woodland; 3 - Arable; 4 - Improved grassland; 5 - Neutral grassland; 6 - Calcareous grassland; 7 - Acid grassland; 8 – Fen; 9 –Heather; 10 - Heather grassland; 11 - Bog, 12 - Inland rock; 13 – Saltwater; 14 – Freshwater; 15 - Supralittoral rock; 16 - Supralittoral sediment; 17 - Littoral rock; 18 –Littoral sediment; 19 – Saltmarsh; 20 - Urban. 21 - Suburban.

4.2.2 Comparing trait profiles between sensitive and tolerant taxa groups

The recovery-related trait profiles between sensitive and tolerant taxa groups were compared using redundancy analysis (RDA). For each chemical, the assemblages with the lowest 5% of HC5 values (116 sensitive assemblages) and the assemblages with the highest 5% of HC5 values (116 tolerant assemblages) were extracted to generate separate sensitive and tolerant species pools. The unique taxa from the sensitive and tolerant species pools were identified and filtered out to constitute a sensitive taxa list and a tolerant taxa list. The species matrix was made up of sensitive taxa lists for all study chemicals. For each chemical, sensitive taxa are assigned to a sensitive group, while tolerant taxa are assigned to a tolerant group. If the species that exist in the 90% common assemblages to a chemical A and also in the 5% most sensitive and tolerant assemblages to other chemicals, this species will be assigned to the indifferent group.

To perform the multivariate analysis, the recovery-related traits need to be recoded based on their type. The weighted sum method (Rubach et al., 2010) was adopted to recode continuous traits, whereas factor traits were recoded based on the most important categories. If the categories of traits are equally important, a new code category is generated. The recoding of factor traits is provided in Appendix S4.1. The species in sensitive, tolerant and indifferent groups were related to the taxonomy datasets from Section 3.2.1 to obtain the taxonomy information from genus to order levels. Species-trait matching was performed at the lowest taxonomic level possible. Firstly, species-trait matching was performed at species or genus levels. Median values were calculated when multiple data were available for the same trait and species. Median values were also used when species-trait matching was performed at family or order levels. Species without trait data were removed from the dataset.

The redundancy analysis was performed using the species by trait matrix as response variables and the species by being sensitive, tolerant, or indifferent as explanatory variables. For the species matrix, the sensitive, indifferent, and tolerant groups were recoded as -1, 0, and 1, respectively. The recoded species and trait matrices were standardized using z score standardization with a mean = 0 and a standard deviation (SD) = 1 for each variable (Baird and Van den Brink, 2007). Then, the redundancy analysis was performed for species-chemical combinations, and the significance of redundancy analysis was tested using the function ANOVA.cca.The function ANOVA.cca test also provides a separate explanation for each chemical which indicates whether the taxon is sensitive or tolerant of that particular chemical. Furthermore, the model for redundancy analysis was simplified using forward selection to identify the chemicals with a relatively strong explanation. The redundancy analysis was performed using the package 'vegan' (https://cran.r-project.org/web/packages/vegan/vegan.pdf). The package 'vegan,' also named the community ecology package, provides analysis tools for the community ecology (e.g. the response of species communities to multiple environmental variables).

4.2.3 Spatial variation and patterns in recovery potential of freshwater invertebrate assemblages

A framework for describing the internal and external recovery potential for species was constructed that considered species traits, river density, river order, and landscape elements (altitude and land use) (Figure 4.2). Internal recovery assessments begin with identifying species that have an aquatic adult stage and therefore have the possibility of producing offspring in the local area. For species with an aquatic adult stage, the key traits are the number of eggs per female, the number of cycles per year, and the adult life span. External recovery assessments consider the dispersal mode (aerial active, aquatic active, aquatic passive), dispersal capability (swimming ability and crawling capability Figure 4.2), distance from the source, and landscape resistance. Aerial passive dispersal was not taken into account, as it is highly uncertain and mainly depends on the dispersal animal vectors (e.g. water birds).

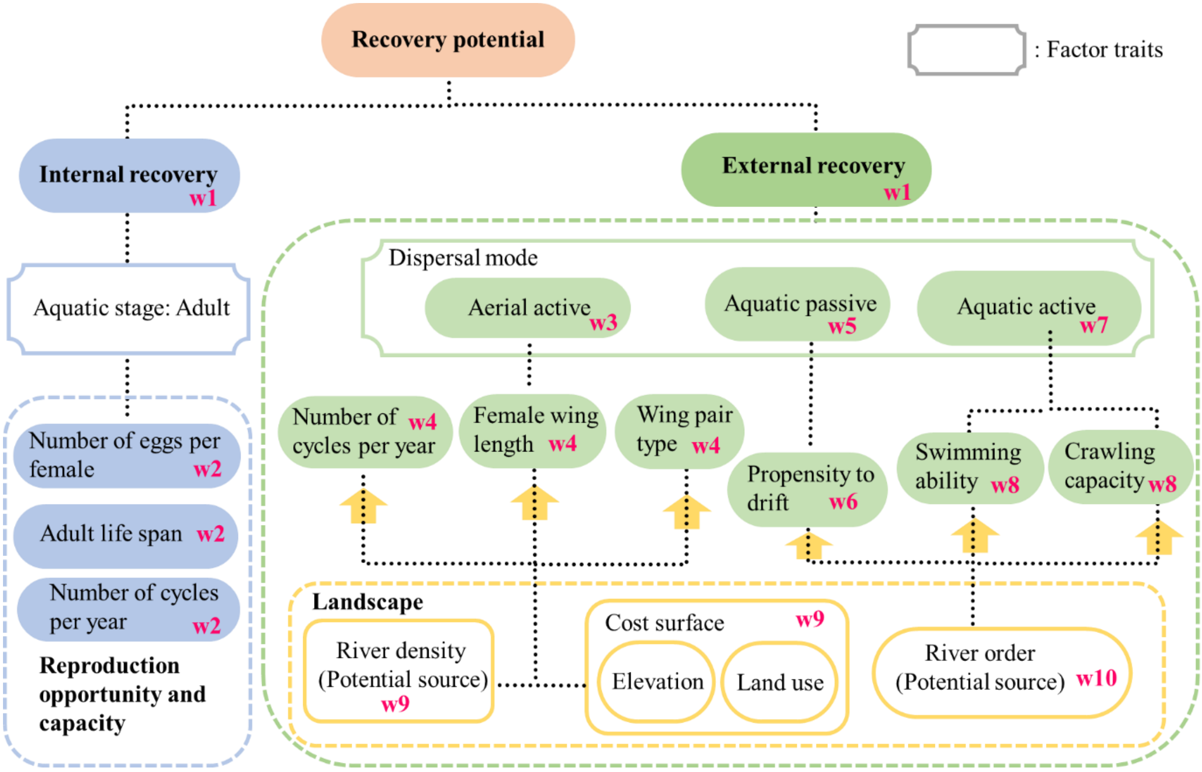

Figure 4.2 A framework for describing the internal and external recovery potential for freshwater invertebrate species (w1-w12 represent the weights for variables; yellow arrows represent the impacts of landscape on external recovery (potential source for aerial and aquatic dispersal; travel surface cost for active aerial dispersal)).

Internal and external recovery of species in assemblages to be assessed was described using the weighted sum of scaled traits. The weighted sum method (Rubach et al., 2010) was adopted to recode continuous traits using the same approach as section 4.2.2. In the framework illustrated in Figure 4.2, factor traits play a role in assigning the weights to dispersal modes (aerial active, aquatic active, and aquatic passive) (e.g. w3, w15, and w7 in Figure 4.2). Dispersal mode trait data were used to calculate the weights of dispersal processes using the formula (e.g. Trait value$_{\text{Aquatic-active}}$ / (Trait value $_{\text{Aerial-Active}}$ + Trait value $_{\text{Aquatic-Passive}}$+ Trait value$_{\text{Aquatic-active}}$). The trait data for aquatic stages were recoded as a binomial distribution (0 or 1) (S4.1). In the stream scenarios (Rico and Van den Brink, 2015), eight experts generally scored equal weights for different variables (e.g. 0.52 for internal recovery and 0.48 for external recovery). Therefore, referring to the expert scores under stream scenarios in Rico and Van den Brink (2015), equal weights were given to other variables (e.g. w1 for internal and external recovery; w2 for the number of eggs per female, adult life span and the number of cycles per year; w4 for the number of cycles per year, female wing length and wing pair type; w8 for swimming ability and crawling capability and w11 for elevation and land use) at the corresponding hierarchical levels. All variables except factor traits were rescaled from 0 to 1 using the min-max normalization. Landscape cost is a negative variable for external recovery. Therefore they were transformed by subtracting the actual value from the maximum value. Internal recovery potential for each species that has an adult aquatic stage was calculated using the equation (Internal recovery = w2 * (Number of eggs per female + Adult life span + Number of cycles per year). Species-specific external recovery potential was calculated using the equation (External $\text{recovery}_{\text{species}}$ = w4 * (Female wing length + Wing pair type + Number of cycles per year) + w6* Propensity to drift + w8* (Swimming ability + Crawling capacity)). Detailed calculations of weights were provided in appendix S4.2.

Species-trait matching was performed at the lowest taxonomic possible, as described in section 4.2.2, and species without the trait data were removed from the dataset. Species-trait matching and weighting calculations were performed in R to obtain the species-specific internal recovery and external recovery. The mean values of internal recovery and external $\text{recovery}_{\text{species}}$ indices were calculated at order level based on individual species to reflect the variation in recovery-related traits. Assemblage-specific internal recovery and external recovery were described using the mean values of species-specific internal recovery and external $\text{recovery}_{\text{species}}$ (external recovery did not consider the impacts of landscape processes at this stage).

The effects of landscape processes (potential sources and potential travel cost) were incorporated to describe assemblage-specific external recovery. The river density was used to characterize the potential source for active aerial dispersal, while elevation and land use were selected as resistance factors for active aerial dispersal (Figure 4.2). River order was used to characterize the potential source for aquatic dispersal (Figure 4.2).

The river density used the linear density method that calculated the total length of the river segments in a unit area. The 1*1 km grids were created along with England and clipped using the layer of England in ArcGIS 10.7.1. The river network and the clipped grids were intersected to obtain a new map layer with information on the length of the river and the area of clipped grids. The total length of the river segments and the area of each unit were calculated using the function of the calculating geometry tool in ArcGIS 10.7.1. The river density for each unit was calculated using the field calculator based on the equation (River density = the total length of the river segments / the area of each unit). The raster data of river density was converted to point data using the feature to point. The points that contain river density data for their corresponding grids were interpolated using the inverse distance method to obtain a smooth surface of river network density in England.

The cost surface for active aerial dispersal was generated based on elevation and land use. The raster layers of elevation and land use were reclassified in ArcGIS 10.7.1. The elevation was reclassified into 200 categories from -1 to 956 meters. The land use was aggregated to 10 classes from 21 specific classes (Rowland et al., 2017) and then reclassified based on assigned weights. The elevation and land use were given equal weights (Figure 4.2). Elevation data and land use were used to create a cost surface using the raster calculator tool in ArcGIS 10.7.1.

River order was derived using the elevation data in ArcGIS 10.7.1. The fill tool in ArcGIS 10.7.1 was used on elevation data to fill the sinks or remove peaks. This aimed to derive a continuous river network. Flow direction was identified based on a filled elevation raster, and continuous river networks were outlined using the flow accumulation in ArcGIS 10.7.1. The stream order tool was used to generate the river order based on the flow direction and river network.

Locations of freshwater invertebrate assemblages were imported into ArcGIS 10.7.1. Then, river density, surface cost, and river order were related to freshwater invertebrate assemblages based on geospatial proximity using the Spatial Join tool or Extract Values to Points tool. The assemblage-specific data (river density, surface cost, and river order) was exported in txt format. Due to missing raster data in elevation and land use (12 sites (0.5% of study assemblages) were excluded for further analysis), the remaining 2306 freshwater invertebrate assemblages were used as the dataset to investigate spatial variation and pattern in assemblage-specific internal recovery, external recovery and recovery potential. The surface cost was seen as landscape resistance for aerial active dispersal, therefore, was transformed by subtracting the actual value from the maximum value. Assemblage-specific river density, surface cost, and river order were incorporated into describing assemblage-specific external recovery by weight sum method. The indices of assemblage-specific internal recovery, external recovery, and recovery potential were then imported into ArcGIS 10.7.1 to analyze spatial variation and spatial patterns. Internal recovery, external recovery, and recovery potential were classified into five quintiles to display spatial variation. The Anselin Local Moran's I method in ArcGIS was used to analyze the spatial patterns.

4.2.4 The relationship between the sensitivity and recovery potential of freshwater invertebrate assemblages to chemicals

Data on the sensitivity of assemblages to the study chemicals were derived from Section 3.2.1. The relationships between assemblage sensitivity and recovery potential from chemical exposure were analyzed using Spearman’s rank correlation. Before performing the correlation analysis, the normality of the data was checked using the Shapiro-Wilk Test. For each of the 20 study chemicals, freshwater invertebrate assemblages were allocated to one of nine groups depending on their sensitivity (e.g. HC5 values μg/L) and recovery potential (Figure 4.3). Assemblages that were identified as either being of ‘extremely high concern’ (i.e. high sensitivity/low recovery) were extracted to investigate their spatial distributions.

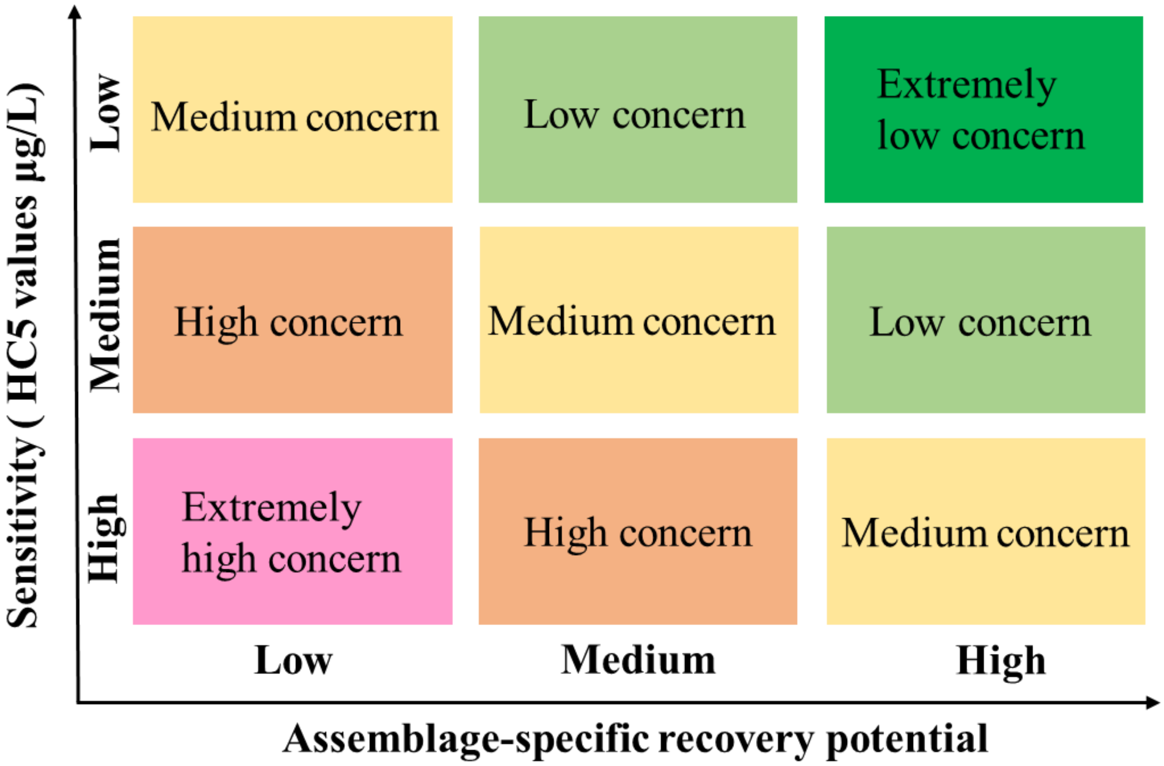

Figure 4.3 Classification of freshwater invertebrate assemblages based on their chemical sensitivity and recovery potential.

4.3 Result

4.3.1 Comparing recovery-related trait profiles between sensitive and tolerant taxa groups

Redundancy analysis was performed to compare recovery-related trait profiles between sensitive and tolerant taxa groups for each chemical. The redundancy analysis shows that the sensitive and tolerant groups can explain 16.56% of the variation in the recovery-related trait matrix, indicating that sensitivity has low relevance to recovery-related traits. The study chemicals, except for endrin, glyphosate isopropylamine salt, phenol, and SDS, present significantly explanatory effects for the RDA ($df = 1$, $F = 2.694 - 19.677$, $p < 0.05$). The forward selection shows that the chemicals (e.g. malathion, NP, Ni, permethrin, endosulfan, diazinon, LAS, Zn, fenitrothion, endrin, deltamethrin, phenol, and GIS) show stronger explanatory power than other chemicals.

4.3.2 Internal and external recovery of taxa

The order-level mean internal and external recovery indices (excluding the effects of landscape processes) are presented in Figure 4.4. The clams 'Venerodia' have the largest mean internal recovery index, followed by snails 'Basommatophora' and hydroids 'Hydroida'. The order 'Diptera', 'Ephemeroptera', 'Megalopteran', 'Odonata', 'Plecoptera', 'Trichoptera' and 'Zygoptera' have the smallest mean internal recovery index. Invertebrates with the largest mean external recovery indices are damselflies 'Zygoptera', followed by water bugs 'Heteroptera', whereas freshwater mussels 'Unionoida' have the lowest mean external recovery indices, followed by clams 'Veneroida' and earthworms 'Lumbriculida'. Flatworms 'Tricladida' and clams 'Rhynchonellida' are general low ranking on both measures.

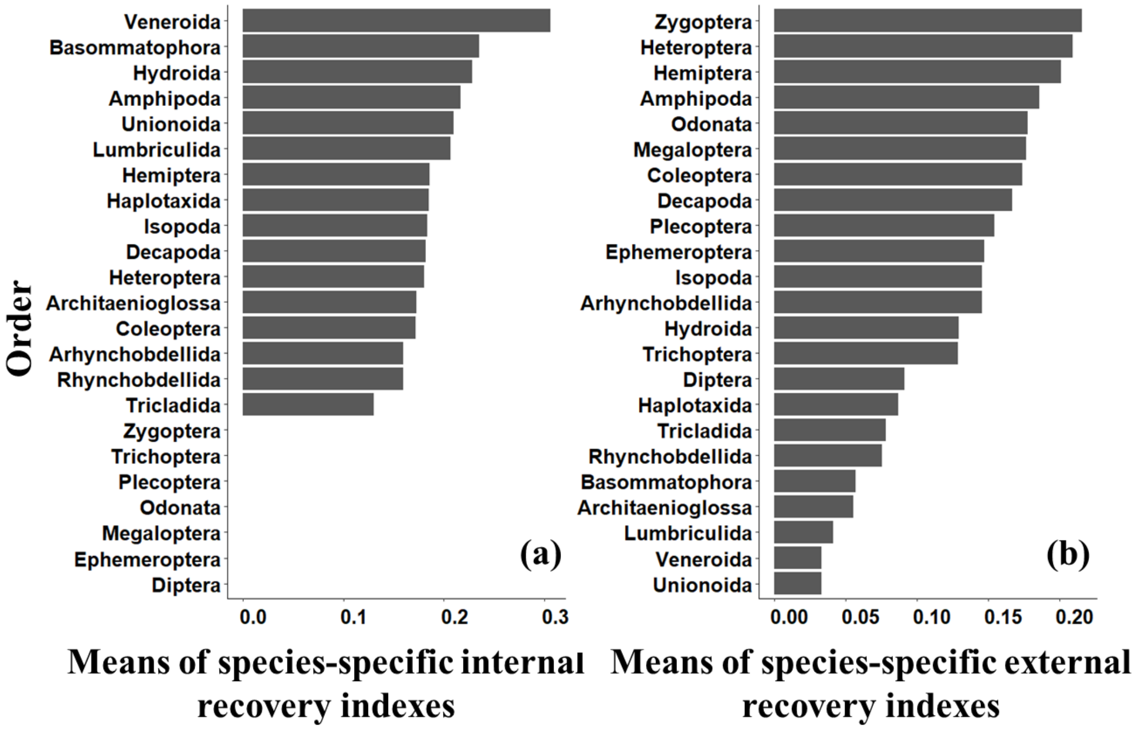

Figure 4.4 Mean internal (a) and external (b) recovery indices for freshwater invertebrate orders recorded in 2318 sites in rivers in England. External recovery does not consider the impacts of landscape processes.

4.3.3 Spatial variation and patterns in recovery potential of freshwater invertebrate assemblages

Assemblage-specific indices were calculated for internal recovery, external recovery and recovery potential. Assemblage-specific external recovery and recovery potential were investigated by considering the potential source (characterized by river density) and cost (characterized by cost surface based on elevation and land use for aerial active dispersal and the potential source (characterized by river order) for aquatic dispersal.

The River network in England has high densities in the northwest and southwest, potentially providing sources for invertebrates with active aerial dispersal. The River network exhibits relatively low densities in the east and southeast (Figure 4.5 a). Invertebrates with active aerial dispersal face a high cost to dispersal in the north of England and Greater London areas and a low cost to dispersal in the east, midlands, and south west of England (Figure 4.5 b). Rivers with many tributaries are mainly across central England, while other rivers with few tributaries are across England (Figure 4.5 c). Rivers with high order be the sink of the rivers with low order, indicating that the invertebrates with either aquatic active or passive dispersal have higher chances of entering the high order rivers. The assemblage-specific external recovery index with and without considering landscapes was compared for 2306 invertebrate assemblages in English rivers (Figure 4.6). It can be seen that the different slopes are layered among the points (Figure 4.6), meaning that the external recovery of assemblages was affected by varying degrees of landscape factors (e.g. potential source and travel cost).

There is variation in internal recovery, external recovery, and recovery potential of freshwater invertebrate assemblages (Figure 4.7). The internal recovery index ranges from 0.007 to 0.244 (theoretical range: 0-0.5), with the maximum 35 times greater than the minimum. The external recovery index ranges from 0.096 to 0.303 (theoretical range: 0-0.5), with the maximum 3 times greater than the minimum. The maximum recovery potential index is 3 times greater than the minimum one, with a range from 0.168 to 0.466 (theoretical range: 0-1). The indices of internal recovery, external recovery, and recovery potential follow the unimodal distribution.

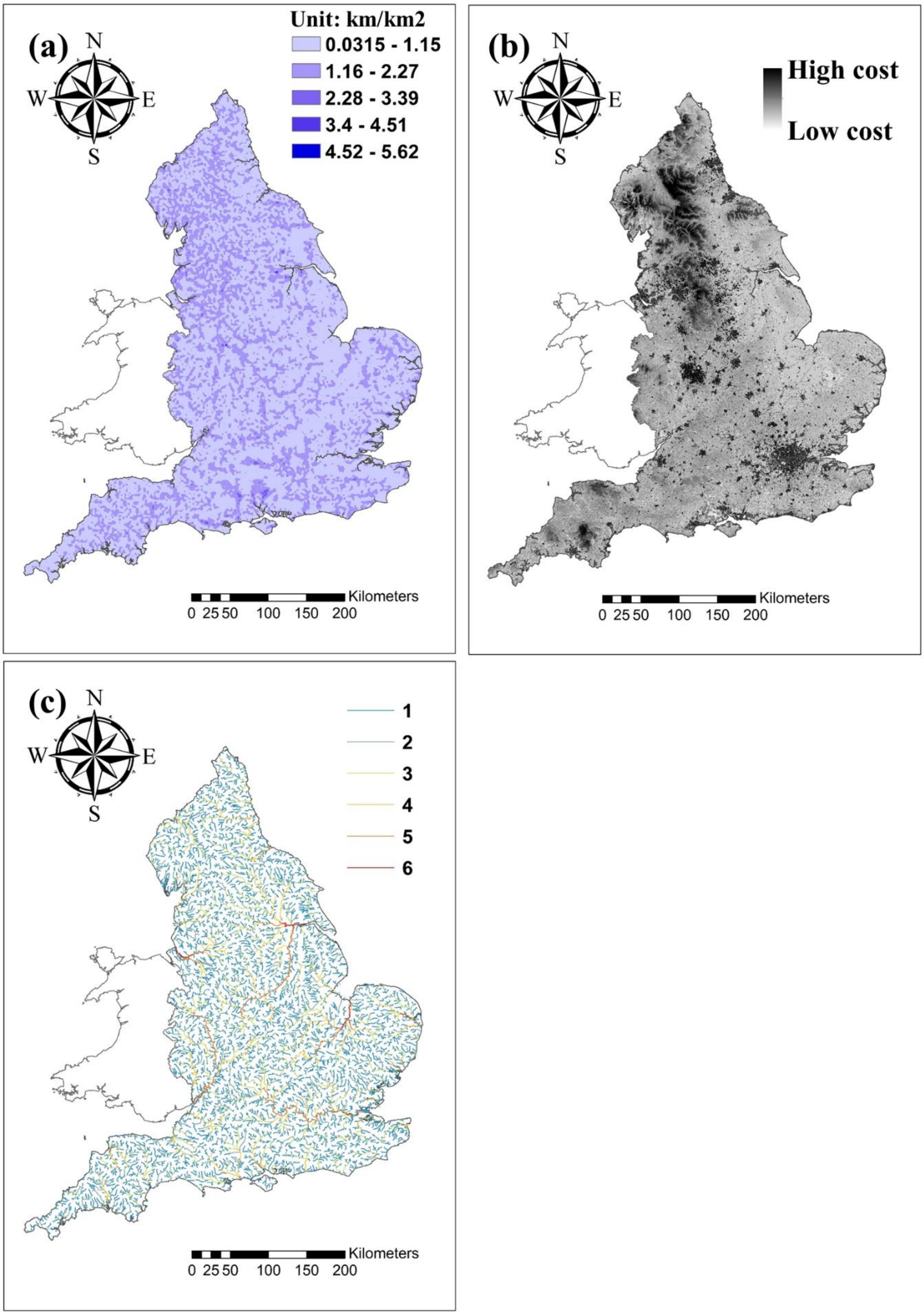

Figure 4.5 River density (a), and the cost surface based on altitude, land use (b) and steam order (c) in England

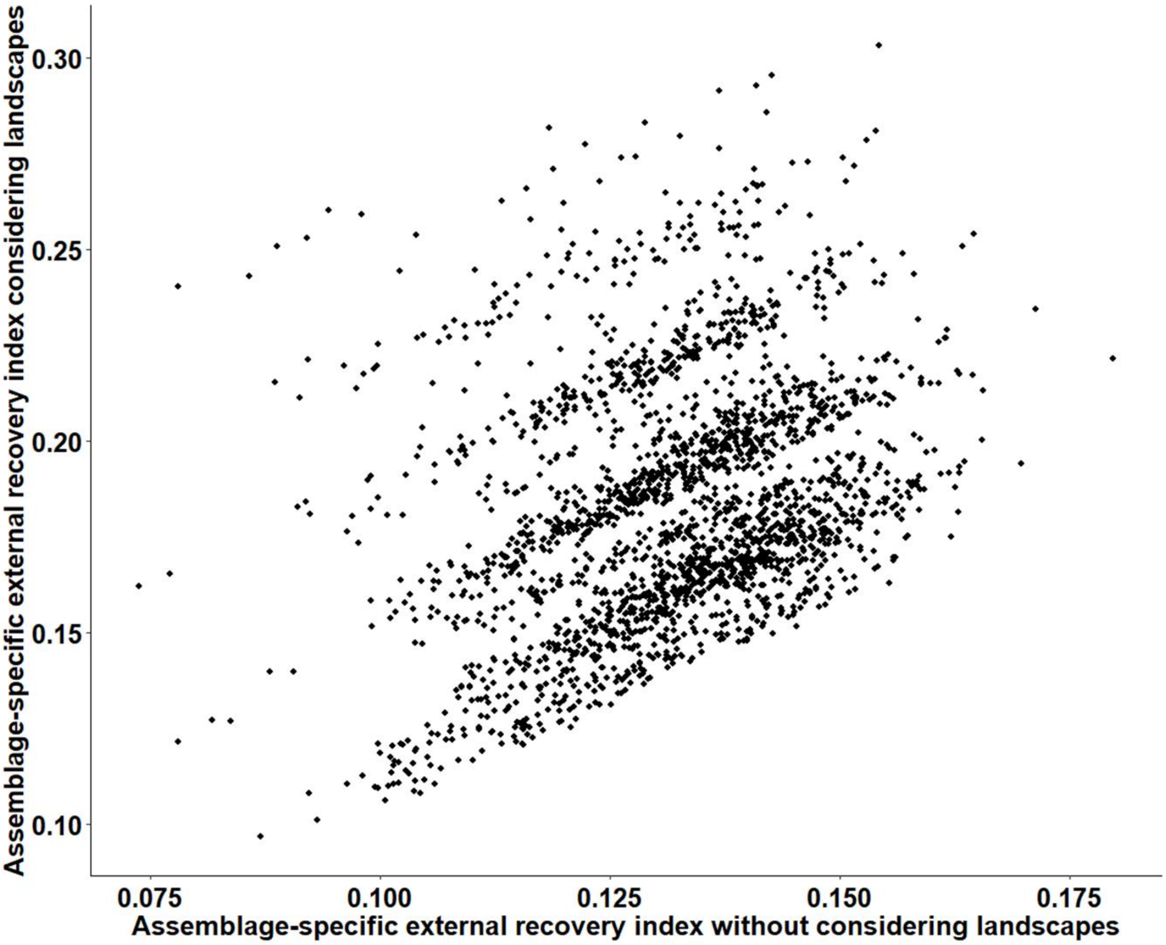

Figure 4.6 Comparisons of assemblage-specific external recovery index between with and without considering landscapes for 2306 invertebrate assemblages in English rivers.

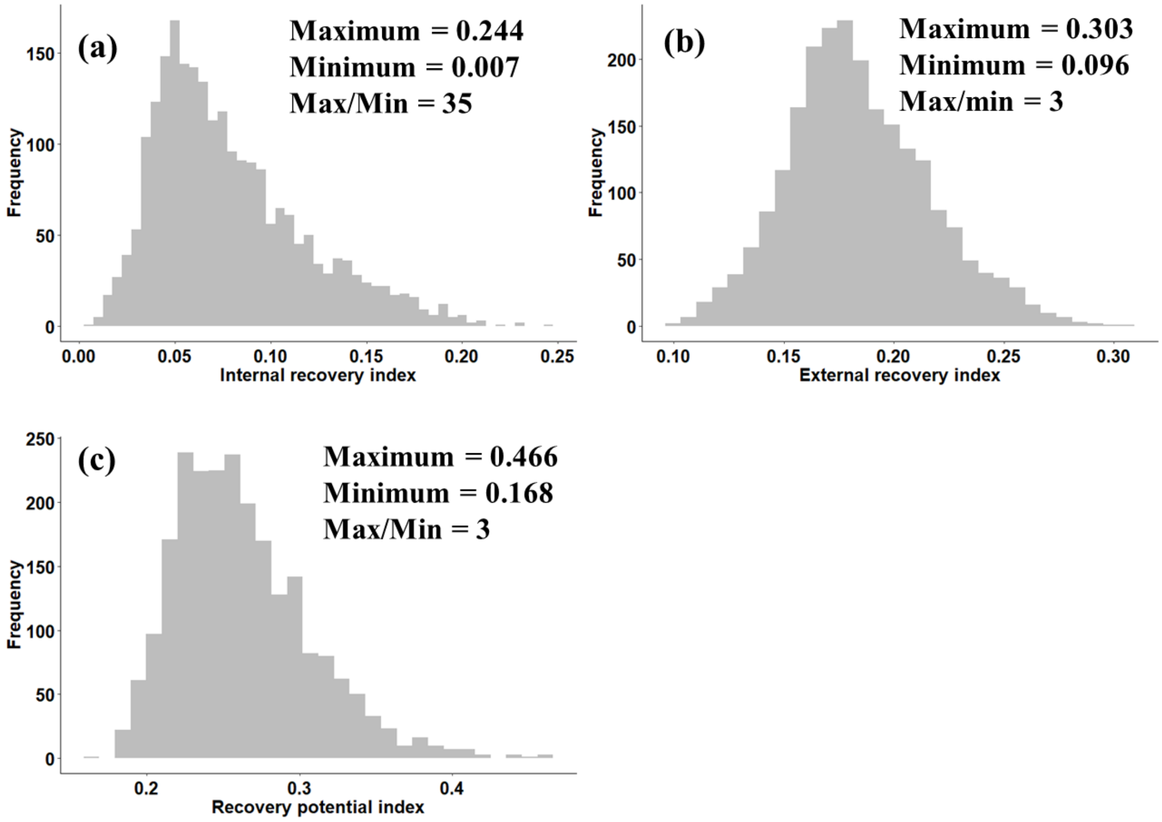

Figure 4.7 Distributions of assemblage-specific internal recovery, external recovery and recovery potential indices for 2306 freshwater invertebrate assemblages in English rivers.

Spatial distributions of internal recovery, external recovery, and recovery potential are presented in Figure 4.8. Most invertebrate assemblages with low internal recovery (Fig. 4.8 a, red and orange symbols) are in the north and southwest of England, whereas assemblages with high internal recovery (Fig. 4.8 a, blue symbols) are mainly distributed in eastern England. In contrast, assemblages that have low external recovery (Fig. 4.8 b, red and orange symbols) are distributed in central and eastern areas. Although a small number of assemblages with high external recovery (Fig. 4.8 b, blue symbols) are populated in the north and southwest corners of England, most are distributed across England. Recovery potential shows a similar spatial distribution as internal recovery, with assemblages with low recovery potential distributed in the north and southwest and high recovery potential distributed in eastern England (Fig. 4.8 c).

Spatial patterns of internal recovery, external recovery, and recovery potential indices were analyzed for 2306 invertebrate assemblages in English rivers using the Anselin Local Moran's I method (Figure 4.9). The internal recovery of freshwater invertebrate assemblages shows obvious spatial distribution patterns with assemblages with low internal recovery assemblages (Fig. 4.9 a, red symbols) in the north and southwest, and assemblages with high internal recovery assemblages (Fig. 4.9 a, blue symbols) in the east. Spatial patterns for external recovery are less distinct, but assemblages with low external recovery (Fig. 4.9 b, red symbols) are identified in the east of England, whilst assemblages with high external recovery (Fig. 4.9 b, blue symbols) are primarily in the north of England. The spatial patterns for recovery potential (Fig. 4.9 c) are relatively similar to the spatial pattern for internal recovery. Assemblages with low recovery potential are mainly disturbed in the north and southwest, while assemblages with high recovery potential are mainly disturbed in the east (Fig. 4.9 c).

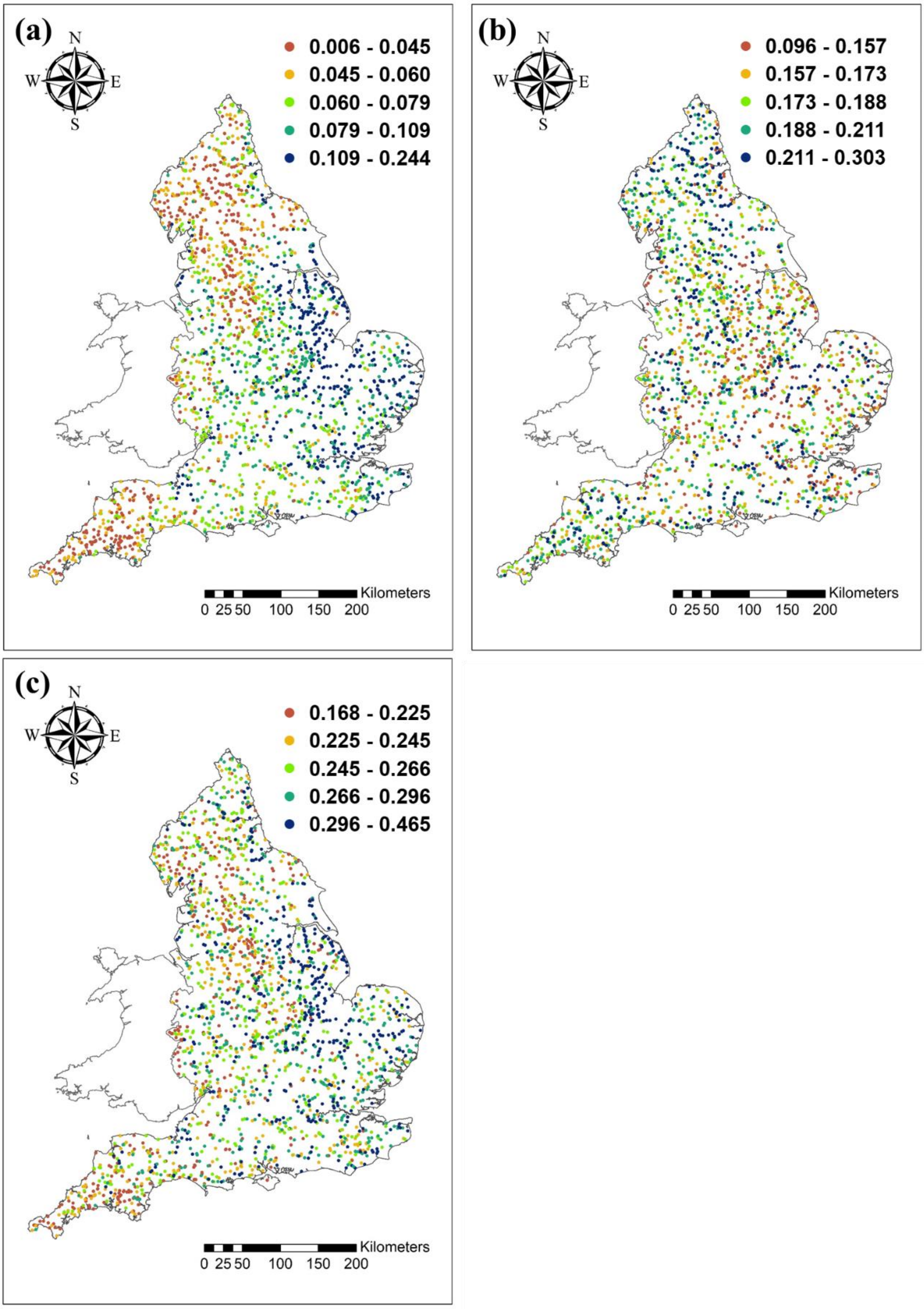

Figure 4.8 Spatial distributions of internal recovery (a), external recovery (b), and recovery potential (c) indices for 2306 invertebrate assemblages in English rivers. Each index is divided into quintiles. Red symbols denote assemblages with indices in the lowest quintile, and dark blue symbols denote assemblages with indices in the highest quintile.

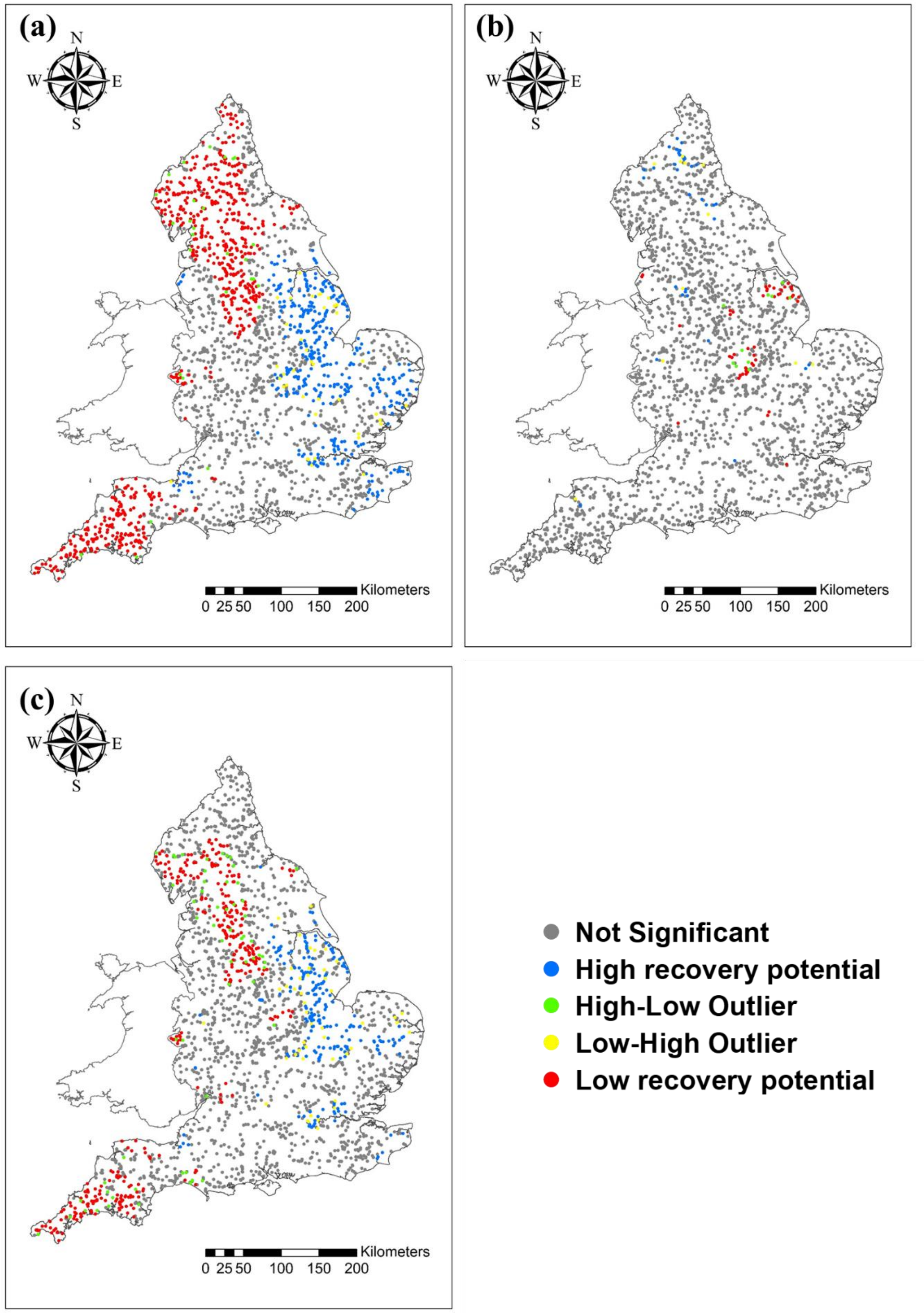

Figure 4.9 Spatial patterns of internal recovery, external recovery, and recovery potential indices for 2306 invertebrate assemblages in English rivers.

4.3.4 The relationship between the sensitivity and recovery potential of freshwater invertebrate assemblages to chemicals

Recovery potential has low relevance for most study chemicals, although spearman correlation analysis achieves significant levels ($p < 0.05$). To heavy metals, recovery potential was negatively correlated to the sensitivity of assemblages with correlation coefficients ranging from -0.621 to -0.223. To phenol, recovery potential presents a moderately positive correlation to the sensitivity of assemblages (correlation coefficients: 0.385). For most study chemicals, correlation coefficients ranged from -0.282 to 0.262, indicating a very weak association between sensitivity and recovery potential. There was also no consistency in the direction of the relationship, with the correlation being positive for 45% of chemicals and negative for the other 55% (Table 4.2).

Assemblages with a combination of high sensitivity and low recovery potential that was classified as ‘extremely high concern’ were identified and mapped for 20 study chemicals (Figure 4.10). To cadmium, copper, nickel, and zinc, ‘extremely high concern’ assemblages were distributed in central and south, to linear alkylbenzene sulfonates and fenitrothion in north and southeast. For other chemicals, extremely high concern sites are distributed across England.

Table 4.2 Spearman correlation analysis of assemblage-specific sensitivity and recovery potential to different chemicals (“*” indicates $p < 0.05$)

Type	Chemical	Spearman correlation coefficient	The p-value
Organophosphate	Diazinon	0.069	< 0.001*
	Fenitrothion	0.205	< 0.001*
	Malathion	0.198	< 0.001*
	Parathion-Methyl	-0.063	0.002*
Organochlorine	DDT	-0.082	< 0.001*
	Endosulfan	0.062	0.003*
	Endrin	0.068	0.001*
Pyrethroid	Cypermethrin	-0.071	< 0.001*
	Deltamethrin	-0.102	< 0.001*
	Permethrin	0.181	< 0.001*
Narcotic	Benzenamine	-0.058	0.005*
	GIS	-0.282	< 0.001*
	Phenol	0.385	< 0.001*
Heavy metal	Cd	-0.538	< 0.001*
	Cu	-0.460	< 0.001*
	Ni	-0.621	< 0.001*
	Zn	-0.223	< 0.001*
Surfactant	LAS	0.257	< 0.001*
	NP	-0.070	< 0.001*
	SDS	0.262	< 0.001*

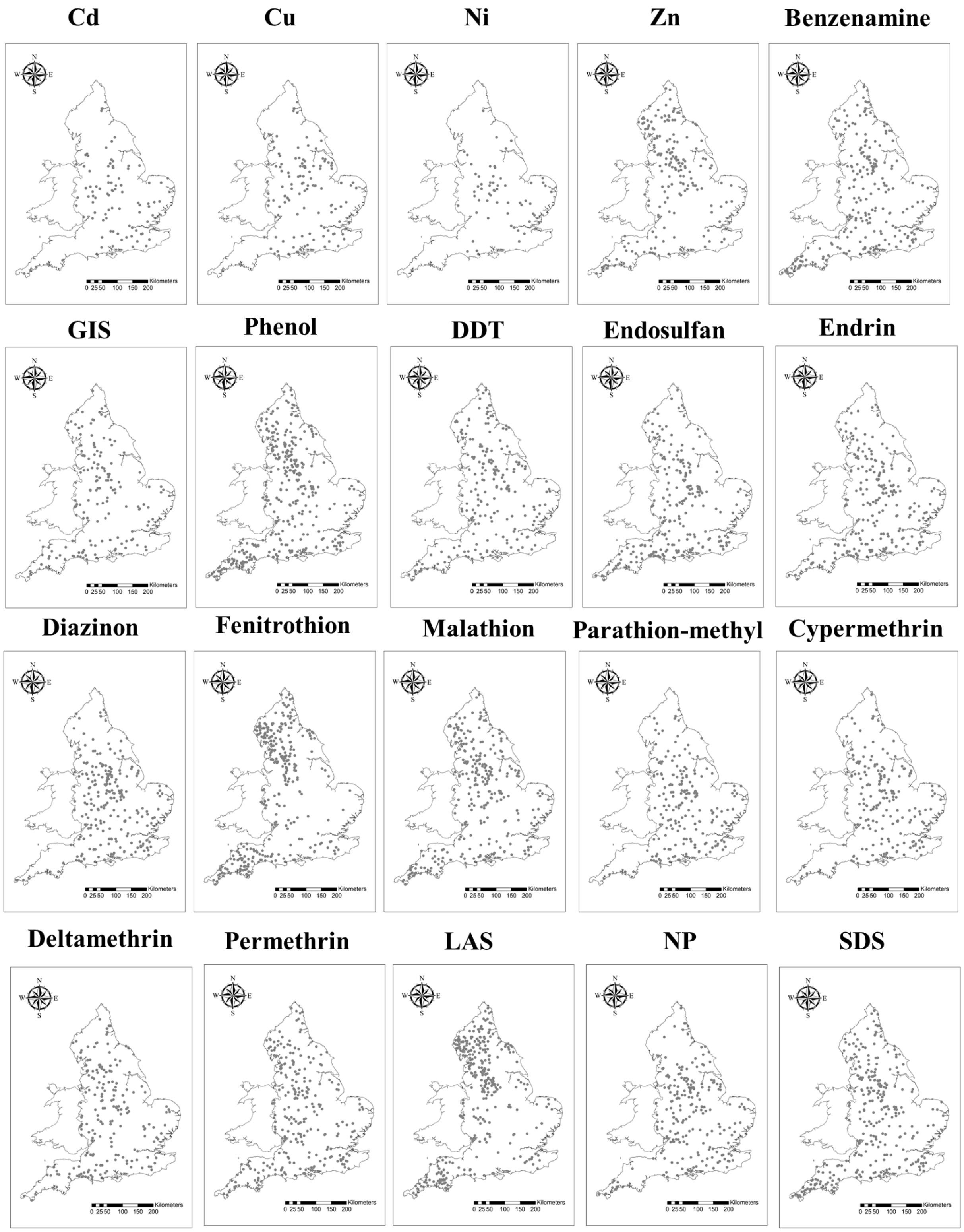

Figure 4.10 Spatial distributions of 'extremely high concern' sites for 20 study chemicals

4.4 Discussion

This chapter aimed to investigate spatial variation in the recovery potential of freshwater invertebrate assemblages. Three specific objectives were addressed: (1) whether sensitive freshwater invertebrate assemblages to chemicals show the same recovery-related trait profiles as tolerant freshwater invertebrate assemblages; (2) whether the recovery potential of freshwater invertebrate assemblages exhibit spatial variation and spatial patterns; (3) whether the sensitivity of freshwater invertebrate assemblages is associated with their recovery potential.

Sensitive and tolerant groups have low explanatory power to recovery-related trait profiles, although sensitive and tolerant groups show significant difference to most study chemicals except endrin, GIS (glyphosate isopropylamine salt), phenol and (SDS) sodium sodecyl sulfate. The sensitivity of species to chemicals has usually been considered to be related to TK-TD related traits (e.g. size, uptake rate), while recovery potential is relevant to reproductive migration capacity. Malathion, NP, Ni, permethrin, endosulfan, diazinon, LAS, Zn, fenitrothion, endrin, deltamethrin, phenol, and GIS show stronger explanatory power than other chemicals, probably because sensitive and tolerant taxa groups are associated with the taxa with major contributor traits. Some snails (e.g. *Physella acuta, Indoplanorbis exustus, Biomphalaria alexandrina*) have been known to be tolerant to fenitrothion, and have relatively strong crawling capacity (Dalesman and Rundle, 2010, Adetunji and Salawu, 2010, Hofkin et al., 1992, Covich et al., 1994, Barata et al., 2015). Some crayfishes (e.g. *Procambarus sp.*), crabs (e.g. *Oziotelphusa sp.*), and fairy shrimps (e.g. *Streptocephalus sp.*) have been reported to be very tolerant to malathion and also observed to be good swimmers (Kane et al., 2005, Rebelo and Cruz, 2005, Moore and Burn, 1968). To deltamethrin, the highly tolerant taxa are mussels (e.g. Unionoida) and snails (e.g. Basommatophora) (Caquet et al., 1996, Falfushynska et al., 2013, Srisawat et al., 2010). The dispersal mode for mussels (e.g. Unionoida) and snails (e.g. Basommatophora) are mainly aquatic (Doucet-Beaupré et al., 2012, Cordellier and Pfenninger, 2009), while sensitive mosquito larvae (e.g. Diptera) will emerge to enter their adult stage and may leave the aquatic environment (Kay and Farrow, 2000).

The second hypothesis is supported by the findings of this study. Recovery potential, including internal and external recovery, all exhibit spatial variation and spatial patterns. The assemblage-specific internal recovery, external recovery, and recovery potential present spatial variation, and these spatial variations exhibit certain patterns. It could be that the assemblages with similar species compositions and therefore exhibit similar spatial patterns of recovery potential. Or assemblages with different species compositions, but these species have similar recovery-related traits (Poff et al., 2006, Brown et al., 2018).

The trait-based method was used to capture the internal and external recovery potential of taxa in the assemblages to be assessed. The taxa with the largest internal recovery potential are clams (Venerodia), followed by snails (Basommatophora), as these taxa have been observed to have limited dispersal abilities, relatively strong reproductive capacities, and adult stage in rivers (Sarremejane et al., 2020). For example, freshwater snails (Radix balthica) were found to have very low chances of being able to migrate to other habitats (Pfenninger et al., 2011). The taxa with the largest external recovery potential are beetles (Coleoptera), followed by damselflies (Zygoptera) and dragonflies (Odonata). They were all considered as excellent flies with fast flight speeds (e.g. beetles: 2m/s; damselflies: 0.71 m/s; dragonflies: 5.8 m/s) (Byers, 1999, Sato and Azuma, 1997). Flatworms (Tricladida) and clams (Rhynchonellida) are low rankings on both internal and external recovery, as they have poor dispersal and reproduction abilities (Sarremejane et al., 2020). Poor dispersal may limit dispersal processes, resulting in high spatial autocorrelation in species compositions (Shurin et al., 2009).

External recovery was affected by the landscape elements: river density, elevation, land use, and river order. River density was used to characterize the potential source for aerial dispersers, while the most important limiting factors (elevation and land use) for aerial dispersal were used to characterize the travel cost (Didham et al., 2012, Malmqvist, 2002). River order was used to describe flow direction and connectivity, thereby characterizing the potential sources for aquatic dispersers. In contrast to the point-to-point calculation of the least-cost path (Verbrugghe et al., 2017), the aim of this analysis was to incorporate as many species as possible across a large number of sites. The landscape elements used in this study present the effects of landscape elements on external recovery processes. Previous studies have also found that landscape elements (e.g., elevation and river network properties) were the important factors for aquatic insect dispersal (Altermatt et al., 2013, Razeng et al., 2016, Polato et al., 2017).

The sensitivity of freshwater invertebrate assemblages is not strongly associated with their recovery potential, although there is some evidence that recovery potential was moderately correlated to the sensitivity of assemblages to metals (the third hypothesis). To cadmium, copper, nickel, and zinc or linear alkylbenzene sulfonates and fenitrothion, 'extremely high concern' assemblages present similar spatial patterns. For other chemicals, extremely high concern sites are distributed across England. Assemblages in the northwest are tolerant to heavy metals (Chapter 2) but have low recovery potential. Assemblages in the east are tolerant to linear alkylbenzene sulfonates, and nonylphenol (Chapter 2) and also have high recovery potential. Sensitivity is the response to chemicals, while recovery potential is a combination of internal and external recovery based on taxa-specific reproduction capabilities, dispersal abilities, and landscape elements (De Lange et al., 2010). Sensitive assemblages could vary in their recovery potential (Van den Brink et al., 2011). Therefore, assemblages with high sensitivity and low recovery potential should deserve additional attention.

Using traits and landscape elements to describe the recovery potential of freshwater invertebrate assemblages has a number of advantages. A high proportion of species in freshwater invertebrate assemblages can be covered by current trait databases (e.g., 94% of taxa were covered in this study). The approach can capture the main drivers of potential internal and external recovery processes and also provide spatially-specific information on recovery potential. This study developed the work of Rico and Van den Brink (2015) by: (1) the selection of species-specific weights for the dispersal mode; (2) considering more recovery-related traits and hence incorporating more possible recovery processes; (3) incorporating landscape elements into the description of external recovery potential; (4) extrapolating recovery potential from species level to assemblage level; (5) investigating spatial distribution and patterns in recovery potential.

This study shows the importance of recovery-related traits and landscape elements in describing the recovery potential of freshwater invertebrate assemblages from chemical stress. Sensitive and tolerant assemblages exhibited significantly different recovery-related trait profiles for four of the 20 study chemicals (fenitrothion, malathion, deltamethrin, and sodium dodecyl sulfate). The internal recovery, external recovery, and recovery potential of invertebrate assemblages varied spatially. The assemblages with co-occurrence of high sensitivity with low recovery potential were identified as an extremely high concern. The assemblages with low recovery potential and high sensitivity to chemicals are the situation that requires attention, as these sensitive assemblages may be difficult to restore to their original state after strong chemical exposure.

Chapter 5 | General discussion: spatially defined ecological vulnerability to chemicals

5.1 Introduction

This thesis mainly aims to assess the spatially-defined ecological vulnerability of freshwater ecosystems to chemicals by taking freshwater invertebrate assemblages in England as a case study. This thesis investigated spatial variation in the vulnerability of freshwater invertebrate assemblages to chemicals, with a specific focus on (i) the associations between assemblage sensitivity and river catchment typologies, land use, and species compositions and (ii) spatial variation in the recovery potential of freshwater invertebrate assemblages. The objectives of this chapter are: (i) to synthesize the results from chapters 2-4 and construct a framework for assessing the spatially-defined ecological vulnerability of freshwater invertebrate assemblages to toxic chemicals (Figure 5.1); and (ii) to use this framework to investigate the implications of a spatial variation in ecological vulnerability for the derivation of environmental quality standards and chemical risk assessments.

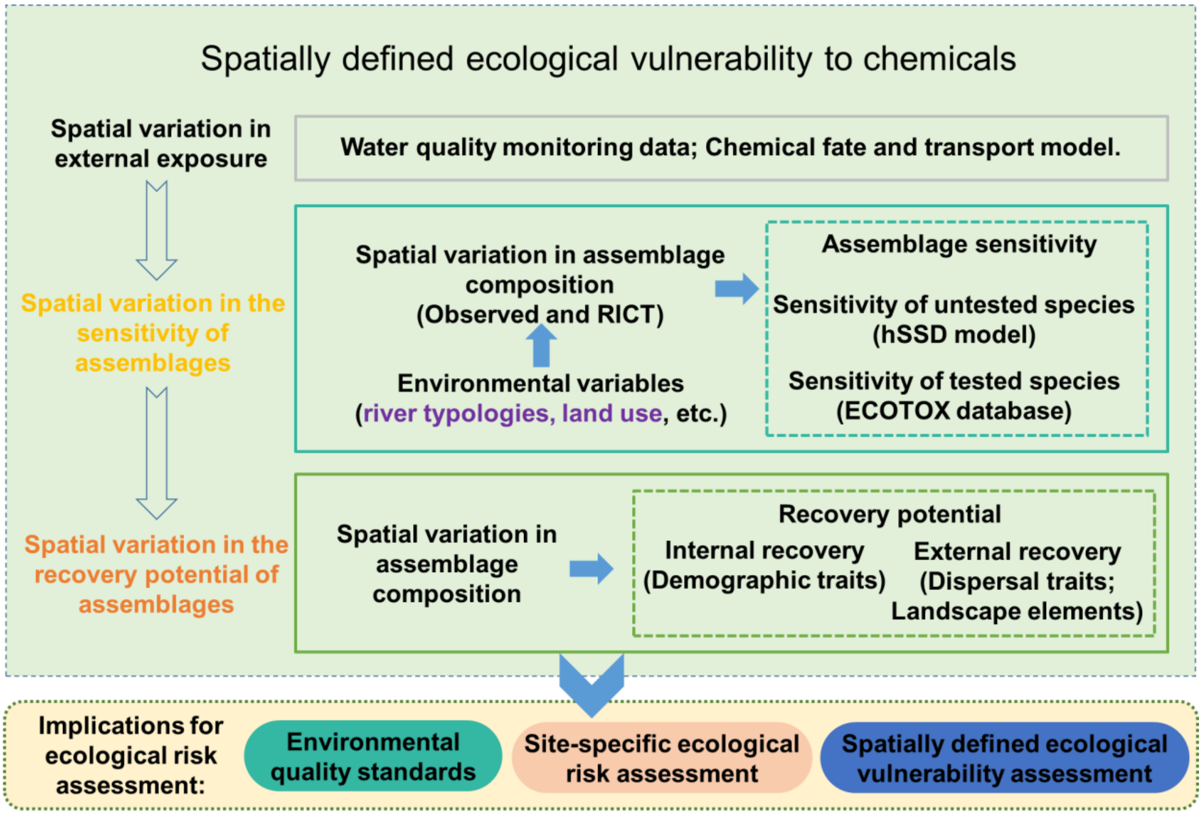

Figure 5.1 An assessment framework for spatially-defined ecological vulnerability, which synthesizes results from chapters 2, 3, and 4.

The principal research findings are discussed (Section 5.2) before applying the framework to explore the implications of spatial variation in assemblage sensitivity and ecological vulnerability on the derivation of environmental quality standards and chemical risk assessment (Section 5.3). The uncertainties and limitations are then discussed for each chapter (Section 5.4). The wider implications of this research are finally discussed (Section 5.5).

5.2 Principal findings

5.2.1 Spatial variation in assemblage-specific sensitivity

Natural assemblages vary spatially in their species compositions, as different species occupy different ecological niches (Jackson et al., 2001; Wiens et al., 2009; Niedrist and Füreder, 2016). Species also vary in their sensitivity to chemical stressors (Blanck, 1984; Van den Berg et al., 2021), and the relative sensitivity of species is chemical-specific (Teather and Parrott, 2006). Species composition influences the sensitivity of assemblages to chemical stressors (Forbes and Calow, 2002; Wheeler et al., 2002). Assemblages with high similarity in species composition would be expected to exhibit a small difference in chemical sensitivity. However, assemblages with low similarity may exhibit a small or large difference in chemical sensitivity depending on how the sensitivity profile is influenced by differences in species composition. Chapter 2 investigates the hypotheses: that the magnitude of variation in assemblage sensitivity is greater for specific acting than generally acting chemicals; assemblages with similar species composition exhibit similar sensitivities to a chemical, while assemblages with relatively different species composition vary greatly in their sensitivity to a chemical; assemblages vary in their sensitivity to chemicals spatially, and this variation in assemblage sensitivity to chemicals is spatially patterned, potentially influenced by chemical type.

The species composition and chemical sensitivity of assemblages will be a function of prior exposure to environmental stressors (Malmqvist, 2002; Stubbington et al., 2021). Therefore, the hypotheses were investigated both for assemblages recorded at river sites in England (i.e., observed) and for assemblages predicted to be present at each site if it was minimally impacted (i.e. RICT model predictions; Wright et al., 1996; Wright et al., 1998; Wright et al., 2000; Beane et al., 2016; Kral et al., 2017). Inter-assemblage variation in sensitivity was assessed for 20 chemicals, selected based on the availability of aquatic toxicity data with high taxonomic coverage. Toxicity data were available for a low proportion of species present in invertebrate assemblages, so the sensitivity of untested species was predicted by using the cross-species predictive sensitivity model hSSD (Craig, 2013).

The relationship between community similarity and variation in chemical sensitivity was investigated. The pattern is more obvious for observed assemblages than expected assemblages, as observed assemblages have greater intra-assemblage variation among species compositions. This thesis demonstrated that the assemblages with similar species compositions show small variation in their sensitivity to chemicals; when assemblages have different species compositions, the variation in assemblage sensitivity to chemicals could be particularly small or may vary considerably. Different species may share similar TKTD-related traits and thus exhibit similar sensitivity to a chemical (Baird and Van den Brink, 2007; Rubach et al., 2012), potentially driving the small variation in the sensitivity of assemblages with different species compositions. The large variation in assemblage sensitivity to chemicals, especially for specifically acting chemicals, could be explained by the fact that these chemicals are very toxic and have targeted taxonomic groups. Previous studies have reported that aquatic species exhibit limited variation in their sensitivity to narcotics, while the magnitude of variation to specifically acting chemicals can be >100-fold (Vaal et al., 1997).

Both observed and minimally-impacted assemblages varied in their sensitivity to chemicals, and the magnitudes of variation ranged from one to several orders of magnitude. As predicted, the magnitudes of variation in HC5 values for observed assemblages exposed to specifically-acting chemicals were significantly greater than that of generally-acting chemicals. A similar pattern was observed for minimally-impacted assemblages, although the difference was not statistically significant. This ma be because there is less variation in the species composition of predicted assemblages. Observed assemblages have higher species diversity and higher variability in species compositions than minimally-impacted assemblages, as the setting of the RICT model focuses on common benthic organisms, and the source database was derived from the 421 uncontaminated sites in England (Clarke et al., 2011; Clarke and Davy

2014).

This chapter shows that there is spatial variation in the sensitivity of species assemblages to chemicals, and this spatial variation can show certain patterns depending on species composition and chemical types. This chapter also reveals the relationship between community similarity and chemical sensitivity and how this varies by assemblage and chemical type. Furthermore, this chapter assesses the variation in sensitivity of the expected assemblages focusing on common taxa and comparing them to observed assemblages, providing some thoughts on describing the assemblage sensitivity to chemicals under minimally-impacted conditions.

5.2.2 The important of river typology descriptors and land use to assemblage-specific sensitivity

The composition of freshwater invertebrate assemblages is influenced by river catchment typology and land use (Brosse et al., 2003; Death and Joy, 2004; Webb and Lott, 2006; Mendes et al., 2017). The objective of Chapter 3 was to relate river typology descriptors and land use to assemblage-specific sensitivity to chemicals with expecting to describe different habitat types where natural assemblages are located or scenarios where they may be exposed to chemicals. The impacts of river typology descriptors and land use on assemblage-specific sensitivity to 6 chemical classes were explored in Chapter 3. The taxonomic compositions in different river typology descriptors and land use were also investigated. WFD typologies (catchment altitude, size, geology) and UK CEH land use classes were related to 2318 assemblages in England to test the hypotheses: the sensitivity of freshwater invertebrate assemblages varies across river typology descriptors and land use; freshwater invertebrate assemblages in their types that can be linked to one or more river typology descriptors and land use are particularly sensitive to certain types of chemicals.

The hypothesis regarding whether the sensitivity of freshwater invertebrate assemblages varies across river typology descriptors is supported by river catchment altitude to all study chemical classes. But for catchment size and geology, they support the hypothesis to some study chemical classes. For all study chemical classes, the assemblages in lowland rivers (i.e., < 200 m above sea level) were more sensitive to chemical stressors and exhibited higher inter-assemblage variation in sensitivity than those in midland rivers (i.e. 200 - 800 m above sea level). The invertebrate assemblages found in lowland rivers are more sensitive to chemicals than those in midland rivers, probably due to the higher proportion of sensitive species (e.g. Branchiopoda, Arachnida) living in lowland rivers (Van Helsdingen et al., 1996; Neretina et al., 2017).

Variation in the sensitivity of natural assemblages to 5 study chemical classes (except narcotics) was significantly affected by river catchment size, whereas the sensitivity of assemblages to heavy metals increased with increasing catchment size. For other chemical classes, assemblage sensitivity decreased with increasing catchment size. The relatively higher proportions of Mollusca in medium size river catchments than in small and very small catchments. Some Mollusca species (e.g. freshwater snails *Viviparus sp.* and *Lymnaea stagnalis*) have been found to be sensitive to heavy metals (Gawad, 2018; Pinkina et al., 2019), while some other Mollusca species were observed to organic chemicals (e.g. fenitrothion which was also assessed in this thesis) (Sabater and Carrasco, 2001). River catchment geology has significant influences on the variation in assemblage sensitivity to 4 study classes, especially for heavy metals. Invertebrate assemblages in calcareous sites are more sensitive on average than those in siliceous sites. This is probably because some soft-body species (e.g. snails, worms, leeches) exist in calcareous sites and are sensitive to heavy metals (Singh et al., 2007; Gupta and Singh, 2011), while some insects, ‘Clitellata’ and ‘Rhabditophora’ in siliceous sites are tolerant to heavy metals (Clements et al., 1988).

Land use has significant impacts on the sensitivity of natural assemblages to heavy metals, organochlorines, organophosphates, and pyrethroids to support the hypothesis about whether the sensitivity of freshwater invertebrate assemblages varies across land use. The most sensitive assemblages to heavy metals are in arable land, followed by urban and suburban areas. The most sensitive assemblages to organochlorines are in improved grassland, while those assemblages most sensitive to organophosphates are in improved grassland, woodland, urban and suburban areas. To surfactants, the most sensitive assemblages are in woodland land, followed by urban and suburban areas. The general rule is the proportion of sensitive taxa in certain land use is greater than those in other land use. Compared to mountain, heath, and bog, assemblages in other land use have higher propositions of sensitive taxa (e.g., Branchiopoda, Arachnida) and taxonomic diversity (Van Helsdingen et al., 1996; Neretina et al., 2017). In addition, the potential for exposure to study chemicals in different land use should also be considered. For example, some fertilizers contain high levels of heavy metals (e.g., Cadmium, Lead) (Atafar et al., 2010), while the most sensitive assemblages to heavy metals are in arable land.

Chapter 3 attempted to relate the river typology descriptors and land use can be to assemblage-specific sensitivity based on environmental filtering and habitat template theories. It is demonstrated that invertebrate assemblages could vary their sensitivity to chemicals across different river typology descriptors and land use depending on chemical classes. The variation in assemblage-specific sensitivity across different river typology descriptors and land use is largely attributable to the variation in species composition. Chapter 3 found that the assemblage-specific sensitivity to chemicals is associated with river typology descriptors and can be integrated into the present river management system. The assemblage-specific sensitivity to chemicals can also be linked to possible exposure scenarios based on land use, thus helping to manage the risks of chemicals.

5.2.3 Spatial variation in site-specific recovery potential

The recovery potential of freshwater invertebrate assemblages is a function of two processes: internal and external recovery. Internal recovery describes the self-reproduction ability of adult aquatic species, while external recovery depends on species dispersal ability and landscape elements. Species composition is spatially diverse, and species vary in their reproductive and dispersal abilities. In addition, external recovery is also affected by landscape factors, which also vary spatially. The objective of Chapter 4 was to assess spatial variation in the recovery potential of invertebrate assemblages to test the specific hypotheses: whether sensitive and tolerant assemblages present different recovery-related traits profiles; whether invertebrate assemblages vary their recovery potential spatially, and if so, what is the pattern in space. In addition, Chapter 4 considers the correlation analysis between sensitivity and resilience and screens out the assemblages with high sensitivity to chemical pollution and low recovery potential.

The sensitive and tolerant assemblages have low explanatory power to recovery-related traits profiles to chemicals, although statistical tests reached a significant level for most study chemicals. The significant difference in recovery-related trait profiles of sensitive and tolerant species assemblages to some chemicals (e.g. malathion, NP, Ni, permethrin, endosulfan). Some sensitive taxa show a positive correlation with reproductive capacity (e.g. the trait “eggs”). The trait “eggs” describes the number of offspring produced by adult female species and reflects the reproductive capacity of the species. Some tolerant taxa present a positive correlation with some traits related to dispersal (e.g. wing pair type, crawling capacity, swimming abilities). These observations may be related to the survival strategies of species selection (Montalto and Marchese, 2005).

The internal recovery, external recovery, and recovery potential of natural assemblages vary spatially but present certain spatial patterns. The taxa that contribute largely to internal recovery are clams ‘Venerodia’, worms ‘Lumbriculida’ and snails, ‘Architaenioglossa,’ and those taxa have poor mobility and strong reproductive ability. Beetles ‘Coleoptera’, stoneflies ‘Plecoptera’, and the hog-louse ‘Isopoda’ make large contributions to the external recovery process, and these taxa have strong immigration capability. Field observation experiments found that stoneflies present more active mobility than other taxonomic groups (Winterbottom et al., 1997).

Overall, Chapter 4 found that assemblage-specific sensitivity to chemicals has low explanatory power to recovery-related trait profiles. With limited explanatory power, sensitive and tolerant invertebrates potentially present different recovery-related profiles to some chemicals (e.g. fenitrothion, malathion, and deltamethrin). For sensitive invertebrates, reproductive capacity plays a major role in the recovery process, while dispersal abilities (e.g. flying strength, clawing, and swimming abilities) are important for tolerant invertebrates. Chapter 4 demonstrates the potential importance of spatial variation in internal recovery, external recovery, and recovery potential, and this spatial variation present cluster patterns. Chapter 4 extrapolated recovery potential from individual species to the assemblage level by considering the impacts of landscapes and assessing spatial variation in site-specific recovery potential at the national scale.

5.3 Application of findings to chemical risk assessments

5.3.1 A framework of spatially defined ecological vulnerability

The results reported in chapters 2 to 4 demonstrate that the sensitivity and recovery potential of species assemblages varies spatially, which may have important consequences for the derivation of environmental quality standards and the assessment of chemical risk. Most regulatory ecological risk assessments mainly focus on spatial variation in external exposure with limited consideration of spatial variation in the sensitivity and recovery potential of species assemblages to chemical stressors. A spatially specific framework of ecological vulnerability assessment was proposed in Figure 5.1. A consequence of spatial variation in the chemical sensitivity of species assemblages is that adopting a single threshold, as used in environmental quality standards, may overprotect or underprotect the assemblages exposed. Similarly, ecological risk assessments that use a single quotient (i.e., the ratio of chemical exposure and effect) may also overestimate or underestimate risks to natural assemblages. In addition, not all regulatory risk assessments consider the recovery potential of natural assemblages. If sensitive assemblages have very high recovery potential, the actual risk posed by chemicals may be less than originally estimated. Therefore, the ecological vulnerability assessment, which considers spatial variation in sensitivity and recovery, was applied to assess chemical risk, especially for pesticides with episodic exposure patterns.

Three case studies were used to illustrate the practical application of the framework. The first considers the derivation of environmental quality standards. The second case is a site-specific ecological risk assessment that considers both spatial variation in external exposure and spatial variation in assemblage-specific sensitivity to chemical stressors. The third is a site-specific assessment of ecological vulnerability that considers spatial variation in external exposure, assemblage-specific sensitivity, and recovery potential.

5.3.2 Deriving environmental quality standards (EQSs)

Species sensitivity distributions (SSDs) have been one of the commonly used methods to derive the EQSs for rivers. Recommended minimum number of species ranges from 5 to 10 based on different guidelines (Wheeler et al., 2002). HC5 values derived from the SSD curves based on a small number of tested species were usually combined with an assessment factor to make up for low taxonomic diversity coverage due to limited toxicity data. This method has two problems: selected species may not be in the assemblages to be assessed, and this method follows a single threshold principle. Some studies have tried extrapolating species sensitivity using the ICE model, and increased the number of species in their SSD curves, but EQSs they derived are still general (Shen et al., 2022, Feng et al., 2013, Qi et al., 2011).

In this case study, EQSs were derived from acute toxicity data used in Section 2.3.1. The acute HC5 for both the global SSD and the site-specific hSSDs was compared to investigate whether: (1) EQSs are protective enough for all natural assemblages; (2) there is overprotection using EQSs; and if there is, what the implications for assessment factors used in EQSs derivation. This case study used the minimum and maximum HC5 values for observed assemblages calculated in Chapter 2 and compared them to the EQSs derived from the global SSD curves.

Environmental quality standards based on global HC5 values are underprotective for at least some invertebrate assemblages in English rivers potentially exposed to endrin, malathion, phenol, sodium dodecyl sulfate, deltamethrin and permethrin (Table 5.1). The EQSs from the global SSD curve failed to protect 21.2 % of invertebrate assemblages exposed to endrin, 3.6 % of assemblages exposed to permethrin, 1% of assemblages exposed to deltamethrin and <1% of assemblages exposed to the other chemicals (Figure 5.2). The 5th HC5 values for the endrin where the EQS is underprotected are 1.76 times less than EQS, indicating that an assessment factor of 1.76 on HC5 derived from the global SSD would protect the majority invertebrate assemblages from the case study chemicals. However, the general application of an assessment factor of 1.76 would be very precautionary and result in a high level of overprotection for most assemblages to some chemicals. The maximum HC5 values for observed assemblages are 6.8 to 5902 times greater than EQSs, while the 95th HC5 values for observed assemblages are 2.3

to 197.5 times greater than EQSs. This indicates that EQSs could be overprotective for some assemblages exposed to some chemicals. For example, more than 95% of assemblages exposed to metals, DDT, fenitrothion, parathion-methyl, benzenamine, glyphosate isopropylamine salt and nonylphenol are overprotected by the 1.76*EQSs (Figure 5.3). This case study indicates that a single threshold approach may underprotect or overprotect the assemblages to be assessed to some chemicals and that EQSs based on spatial region refinement could be developed to protect natural communities.

Table 5.1 The environmental quality standards (EQSs, HC5 values derived from global SSDs using acute data), minimum, 5th, 95th and maximum HC5 values for observed assemblages (The numbers highlighted in red indicate that EQSs fail to protect the assemblages)

Chemical	Environmental quality standards (μg/L)	Observed assemblages			
		Minimum (μg/L)	5^{th} (μg/L)	95^{th} (μg/L)	Maximum (μg/L)
Cadmium	8.05	31.68	133.9	1290.73	3372.58
Copper	6.49	21.84	44.17	173.36	305.39
Nickel	169.4	326.82	661.37	6482.62	10933.33
Zinc	131.81	817.88	1299.03	2826.36	5109.58
DDT	0.49	1.205	2.511	9.247	252.269
Endrin	0.014	0.003	0.008	0.168	19.497
Endosulfan	0.282	0.314	0.514	2.764	143.163
Diazinon	0.389	0.553	0.944	7.009	563.564
Fenitrothion	0.27	1.274	2.329	7.811	180.918
Malathion	0.53	0.283	1.179	7.611	868.385
Parathion-methyl	0.294	0.866	1.301	8.153	218.551
Ben	453	3898	44054	89393	143123
GIS	283	2834	4156	7554	14239
Phenol	2.546	2.341	5.769	21.548	154.365
LAS	0.321	0.629	2.394	4.535	9.006
NP	0.049	0.273	0.893	5.001	25.464
SDS	1157	1062	1520	2668	7859
Cypermethrin	0.001	0.001	0.003	0.031	1.938
Deltamethrin	0.003	0.001	0.004	0.041	18.833
Permethrin	0.057	0.023	0.062	0.661	50.248

Note: The HC5 values derived from global SSDs using acute data from the toxicity datasets used in Section 2.3.1.

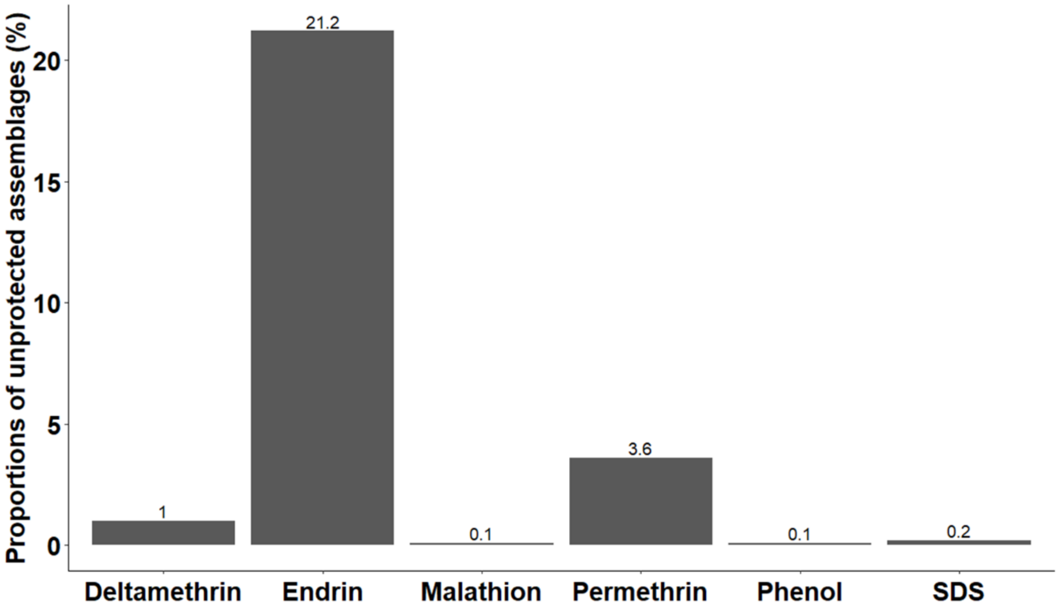

Figure 5.2 Proportions of unprotected observed assemblages (%) to cypermethrin, deltamethrin, diazinon, endrin, malathion, permethrin, phenol, and sodium dodecyl sulfate (SDS)

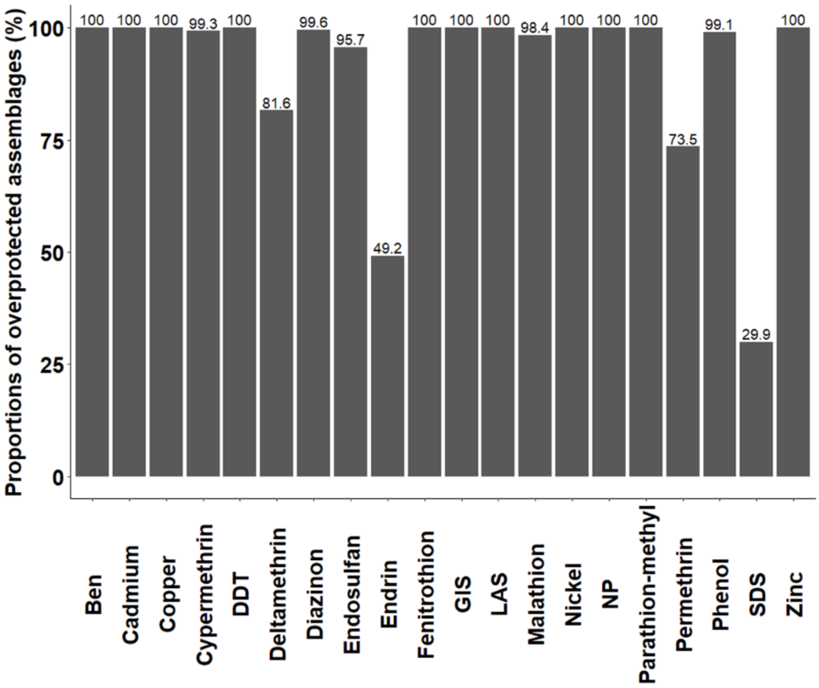

Figure 5.3 Proportions of overprotected observed assemblages (%) with the assessment factor of 1.76 * EQSs to heavy metals (a), narcotics (b), organochlorines (c), organophosphates (d), pyrethroids (e) and surfactants (f). (Ben – benzenamine; GIS - glyphosate isopropylamine salt; PM - parathion-methyl; LAS - linear alkylbenzene sulfonates; NP - nonylphenol, SDS - sodium dodecyl sulfate (SDS))

5.3.3 Site-specific ecological risk assessment

This case study focussed on copper, cypermethrin, and diazinon as they are routinely monitored in rivers in England by the Environment Agency (Watterson, 1999). Copper detected in the rivers can come from various sources, including use in fertilizers and pesticides, industrial and domestic wastes, mining, and vehicle exhausts (Rader et al., 2019). Copper has been reported to present ecological risks to freshwater organisms in England (Johnson et al., 2017). Cypermethrin and diazinon are pesticides that are highly toxic to insects (Saadati and Mirzaei, 2016). The concentrations of copper, cypermethrin, and diazinon were obtained from the Environment Agency. The ecological risks of selected chemicals were determined for assemblages using a risk quotient method (Risk = Environmental concentrations/ assemblage sensitivity). The distribution of environmental concentrations (Figure 5.4) and site-specific ecological risk assessments (Figure 5.5) were mapped using ArcGIS10.7.1. The proportions of sites where considering spatial variation in assemblage sensitivity may exacerbate or mitigate the ecological risks were calculated based on changes in risk levels.

High concentrations of copper are found mainly in central, southwest, and southeast England (Figure 5.4 a). High concentrations of cypermethrin are in central England (Figure 5.4 b), whereas high concentrations of diazinon are found across the north, central, and east England (Figure 5.4 c). Assemblages in central and southern areas are at relatively high risk of copper exposure (Figure 5.5 a). High ecological risks of cypermethrin are in west England (Figure 5.5 b), whereas a high risk of diazinon is found in the north, central and east England (Figure 5.5 c). Spatial patterns for ecological risks generally follow exposure patterns for copper and diazinon, with little mitigation by variation in assemblage sensitivity (Figures 5.4 and 5.5). For cypermethrin, there are differences in spatial patterns of the environmental concentration and ecological risk in western areas, indicating that spatial variation in assemblage sensitivity is important.

Spatial variation in assemblage sensitivity is important in determining ecological risk. As shown in Figure 5.6, the chemical risk level of around 24 to 50% sites for these copper, cypermethrin, and diazinon altered. Considering the spatial variation in assemblage sensitivity may exacerbate or mitigate the ecological risks.

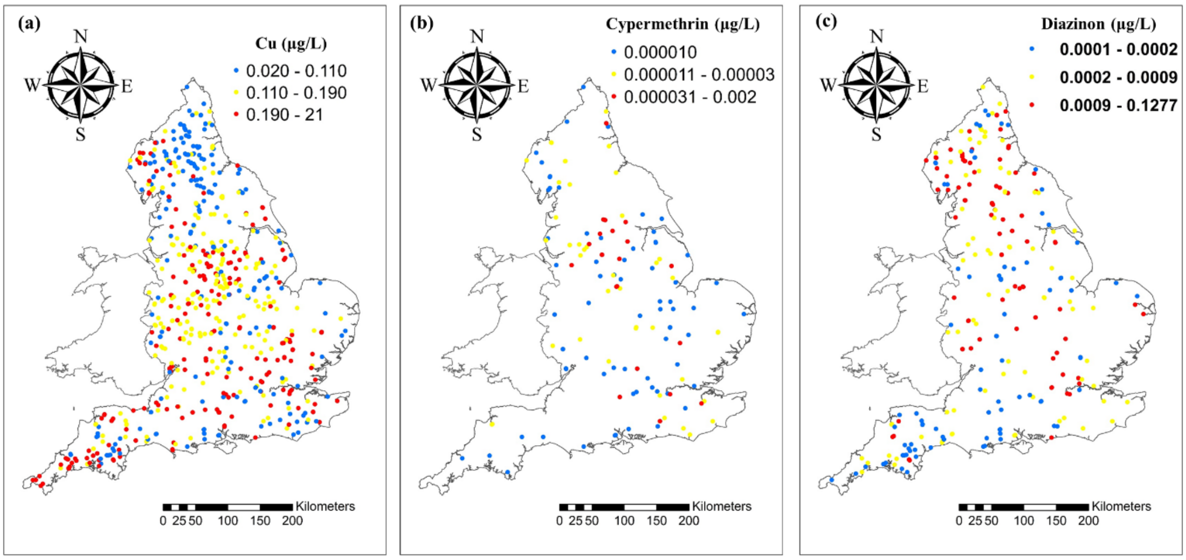

Figure 5.4 Environmental concentrations of copper (a), cypermethrin (b), and diazinon (c)

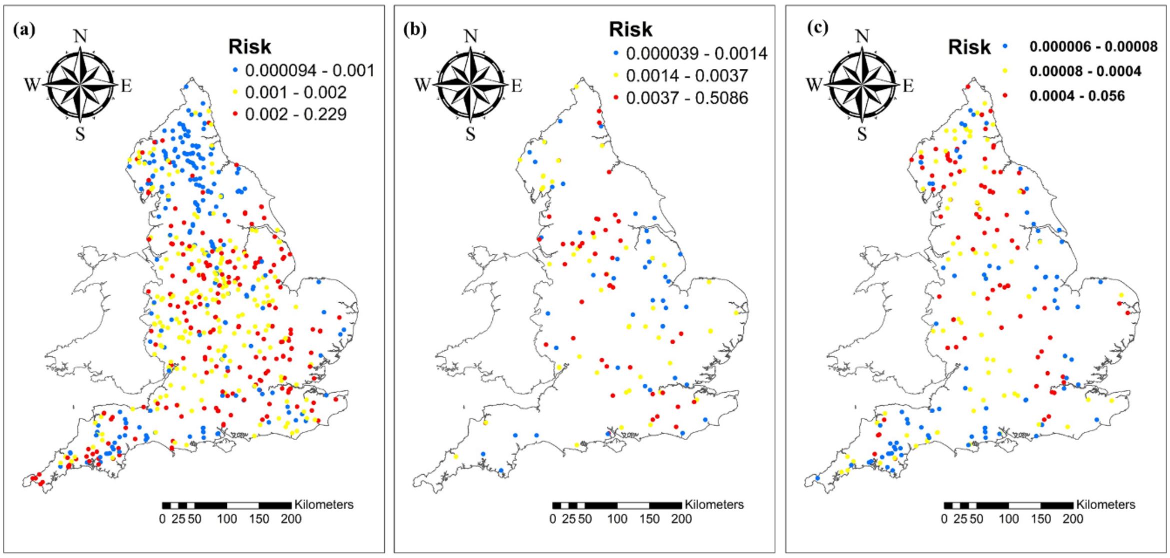

Figure 5.5 The site-specific ecological risk of copper (a), cypermethrin (b), and diazinon (c)

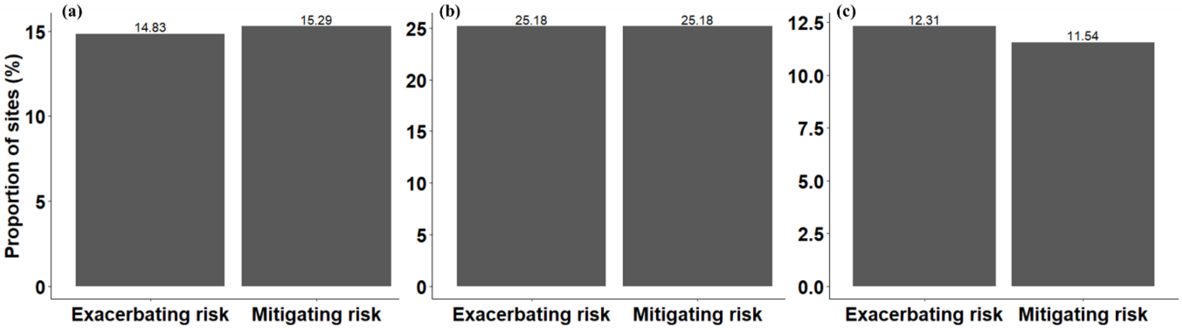

Figure 5.6 Proportion of sites where considering spatial variation in assemblage sensitivity may exacerbate or mitigate the ecological risks to copper (a), cypermethrin (b), and diazinon (c)

5.3.4 Spatially define ecological vulnerability assessment

The recovery potential of assemblages was described using the findings from Chapter 4. The data for external exposure, assemblage sensitivity, and recovery potential were standardized to the scale (0-1) using the min-max normalization. The scaled data for assemblage sensitivity and recovery potential were reversed by calculating the difference from 1. An ecological vulnerability index (EVI) was calculated using the formula:

$$\text{EVI} = \text{external exposure} + \text{assemblage sensitivity}_{\text{reversed}} + \text{recovery potential}_{\text{reversed}}$$

The higher the EVI value, the more vulnerable the assemblage is to a chemical. The spatial distribution of EVI values for assemblages exposed to cypermethrin and diazinon were mapped using ArcGIS10.7.1. The proportions of sites where considering recovery potential may exacerbate or mitigate the risks to cypermethrin and diazinon were calculated based on changes in risk levels.

The site-specific ecological vulnerability assessment was performed on cypermethrin and diazinon (Figure 5.6). Highly vulnerable assemblages to cypermethrin are mainly in the west (Figure 5.6 a), while highly vulnerable assemblages to diazinon are mainly in the north and southwest (Figure 5.6 b). It can be seen that different spatial patterns in site-specific ecological risk and vulnerability assessments (Figures 5.5, 5.6, and 5.7). Some previous studies have criticized the single threshold approach used in ecological risk assessments and called for the development of spatially explicit methods (Hope, 2006; Rutgers and Jensen, 2011). The case study fills this gap, and ecological vulnerability assessment further considers spatially explicit recovery potential.

Adding recovery to the ecological risk assessment may exacerbate or mitigate the chemical risks. In figure 5.8, for cypermethrin, recovery potential exacerbates the risks for 30.22% of sites and mitigates the risks for 30.22%. For diazinon, recovery potential exacerbates the risks for 33.08% of sites and mitigates the risks for 33.46%. Recovery potential has been considered in the ecological risk assessment process in Europe (EFSA PPR Panel, 2013; EFSA Scientific Committee, 2016), but the application of resilience in ecological risk assessment needs to be analyzed on a case-by-case basis.

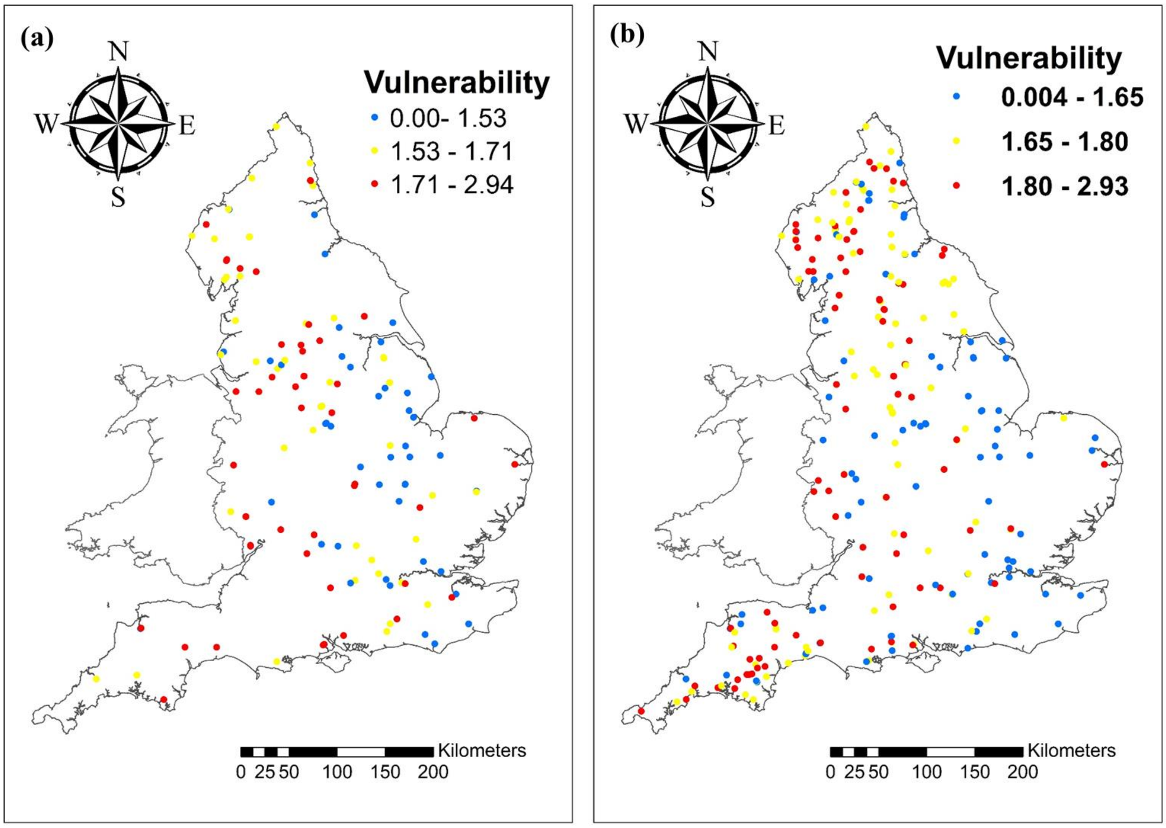

Figure 5.7 Spatially defined ecological vulnerability assessment to cypermethrin (a), and diazinon (b)

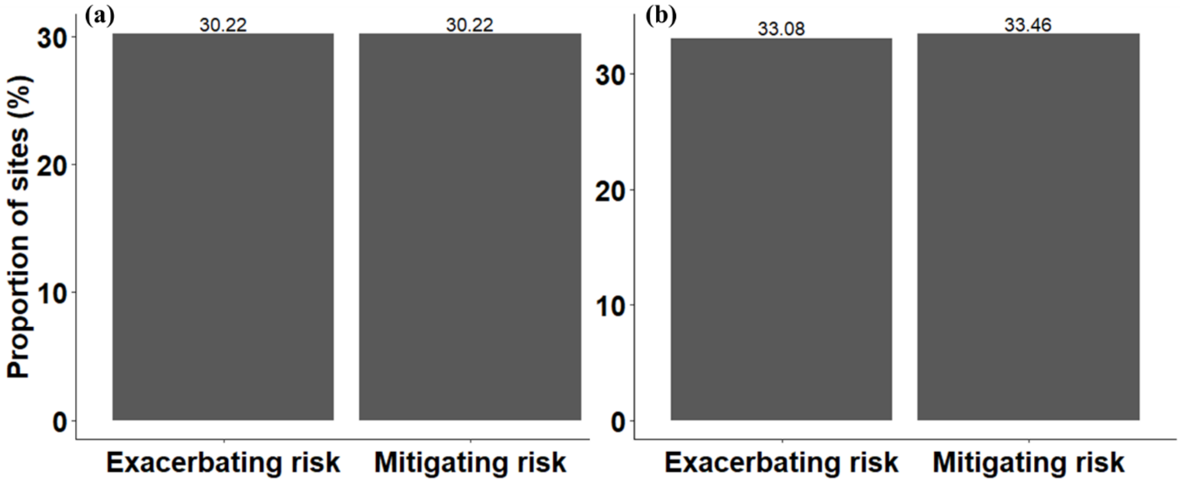

Figure 5.8 Proportion of sites where considering recovery potential may exacerbate or mitigate the risks to cypermethrin (a), and diazinon (b)

5.4 Uncertainties and limitations

This thesis assesses spatial variation in the sensitivity of freshwater invertebrate assemblages to chemicals; river catchment typologies and land use are important to assemblage sensitivity; the recovery potential of freshwater invertebrate assemblages also varies spatially. There are still certain uncertainties and limitations in each chapter that can be improved in future research.

For Chapter 2, data acquisition may limit the results of this thesis to a broader context. The twenty chemicals selected in this study have rich toxicity data and high taxonomic coverage. When the findings are applied to other chemicals with limited toxicity data, toxicity tests will need to be set up to generate new toxicity data. In addition, biological monitoring data (e.g., taxa recorded at genus or species level) is not easy to obtain. Many environmental monitoring projects in Europe mainly focused on general biological indexes rather than species identifications (Van den Berg et al., 2020). This problem may be overcome by other technical means (e.g., artificial intelligence invertebrate identification; environmental DNA) (Lytle et al., 2010; Bohmann et al., 2014; Klymus et al., 2017), although the primary databases of these techniques still need to be established and enriched. The expected assemblages predicted using the RICT can be further developed to improve the spatial resolutions, as current community types are based on 43 end groups. Although it provides good taxonomic coverage, the hSSD model used in Chapter 2 has several uncertainties and limitations. The taxonomic distance is expected to measure the phylogenetic relatedness, but there may be some misrepresentations between taxonomic and phylogenetic relatedness (Ruhí et al., 2013; Hamilton et al., 2020). Furthermore, the results predicted using the hSSD model may have higher uncertainties with the increasing taxonomic distance between tested and untested species.

For Chapter 3, sample sizes across different WDF river catchment typologies and land use are biased. In addition, there is a trade-off between selecting a detailed classification system of river catchment typologies and land use and improving comparability in a broad context. Using the WDF river catchment typologies can make results linked to the current European legal frameworks, but there have been many criticisms that think WDF river catchment typologies are rather general and more detailed frameworks need to be developed (Hering et al., 2010;

Solheim et al., 2019). It is hoped that the classification system is simple to facilitate communication and management but at the same time can interpret and cover many scenarios. In addition, freshwater invertebrate assemblages were related to land use based on geographic proximity and did not consider the potential synthetic effects of multiple land use and the impacts land use upstream has on downstream.

The trait databases used in Chapter 4 remain incomplete and can be further supplemented, although they have provided good coverage for freshwater invertebrates in England. Some specific traits (e.g., crawling capacity and swimming ability) derived from supplementary trait data provided by Rico and Van den Brink (2015) for most species were recorded at Family or Order and can be further refined at Genus or Species as Tachet and DISPERSE databases (Usseglio‐Polatera et al., 2000; Rico and Van den Brink, 2015; Sarremejane et al., 2020). Chapter 4 focused on active aerial dispersal and active and passive aquatic dispersal and did not consider passive aerial dispersal. Incorporating this high uncertainty process (passive aerial dispersal) into the current framework still requires further investigation. The study in Chapter 4 used river density and orders as potential sources of dispersers, and it is an idealized simulation. In natural communities, the distribution of species may be habitat-specific, indicating that more detailed analysis requires more data to support it. In addition, some important indicators (e.g., number of adult survivors after removing exposure; maximum travel distance) need to be further supplemented. Chapter 4 assigned equal weights to variables used to describe the internal and external recovery. In nature, the situations are more complicated than the simulation in Chapter 4. Although different combinations of weights can be used to simulate different scenarios, which specific scenario is close to reality may require field experiments or observations. In addition, Chapter 4 did not consider the contributions of tributaries using the stream order and the changes in the microenvironment of specific river segments.

The case studies in this chapter demonstrate that the findings of this thesis have important impacts on current ecological risk assessments of chemicals but have not been investigated in further research in this thesis. The case studies used chemical monitoring data to describe spatial variation in external exposure, which can also be simulated using transport and fate models of chemicals in the environment. The final case study adopted a commonly used formula for calculating the vulnerability index (Xu et al., 2020) and did not consider interactions among external exposure, sensitivity, and recovery potential. The simple way to deal with this problem is conducting the expert scoring case by case. However, this mainly depends on the subjectivity of the experts and may deviate from the actual situations. Many indicators need to be measured, and how to make convenient and economic applications needs consideration. For the UK, chemical monitoring and biological monitoring data can be derived from a public source. For the countries without a complete monitoring network, rapid non-target screening of chemicals and environmental DNA was recommended to obtain the data on spatial variation in external exposure and species compositions. If there is no local trait database available to describe the recovery potential, the databases from other regions of the world can be used temporarily.

5.5 Contributions to knowledge

This thesis has investigated the spatial variation in the ecological vulnerability of freshwater invertebrate assemblages to chemical exposure with the key considerations of spatial variation in assemblage sensitivity and recovery processes. The following contributions to knowledge can be summarised in this thesis:

(1) Demonstrating that there is a spatial variation in assemblage sensitivity to a chemical

A single threshold approach was widely used to describe assemblage sensitivity to a chemical in deriving EQSs or ecological risk assessment (Brock et al., 2006). This approach is expected to be conservative enough to protect the vast majority of species. However, the one-size-fits-all approach using single values may lead to underestimating the ecological risk of chemicals in different areas or overestimating ecological risk limiting the use of reasonable chemicals (Li et al., 2021). Spatially specific sensitivity assessment methods, therefore, need to be developed. This study provides a technical route for describing the sensitivity of freshwater invertebrate assemblages and finds that the sensitivity of freshwater invertebrate assemblages varies spatially. Especially for specifically acting chemicals, this variation can be 5 orders of magnitude. Based on this great spatial variability in freshwater invertebrate assemblages to chemicals, spatially detailed environmental criteria need to be developed to which this thesis can contribute.

(2) Revealing the relationship between the similarity in species compositions and the variation in assemblage sensitivity to a chemical.

Interspecies variation in chemicals has focused on comparing sensitivities of a small number of tested species due to limited toxicity data (Del Signore et al., 2016; Heaton et al., 2020), and inter-assemblage sensitivity was seldom investigated. This study focused on the inter-assemblage sensitivity by extrapolating the sensitivity of tested species to untested species. This thesis investigates spatial variation in assemblage sensitivity to chemicals by considering two types of assemblages (observed and expected assemblages) and six chemical classes. The results of this thesis reveal the relationship between the similarity in species compositions and the variation in assemblage sensitivity to chemicals. The assemblages with similar species compositions exhibit similar sensitivity to a chemical. The assemblages with large variations in species composition vary their sensitivity slightly or largely. The relationship between the similarity in species compositions and the variation in assemblage sensitivity to a chemical show Pareto restriction curves for assemblages with large variation in species composition.

(3) Relating the assemblage-specific sensitivity to WFD river catchment typology descriptors and land use

Species have relatively specific habitats in ecosystems. This study considers WFD river catchment typology descriptors and land use to capture the features of species habitats and explore the associations between assemblage-specific sensitivity and river catchment typology descriptors or land use. This thesis found that the river catchment typology descriptors and land use are essential to assemblage sensitivity to some chemical classes, especially for heavy metals. Relating the assemblage-specific sensitivity to WFD river catchment typology descriptors and land use can help understand the potential habitats and exposure scenarios of species assemblages to manage the environmental risks of chemicals.

(4) Incorporating landscape elements into describing the spatial variation in recovery

potential of freshwater invertebrate assemblages to chemicals

Past studies of recovery potential have mostly been field observations or focused on a few species (Guisan and Thuiller, 2005; Lusher et al., 2020). The recovery-related traits are used in this study to describe internal and external recovery processes. External recovery also takes into account landscape factors (e.g., to characterize the potential source using the river density and order; to characterize landscape resistance to dispersal processes using the altitude and land use). This study found that internal recovery, external recovery, and recovery potential of freshwater invertebrate assemblages vary spatially and show specific patterns. In addition, taxa-specific weights were used to determine dispersal mode, increasing the ecological reality in describing external recovery.

(5) Integrating spatial variation in the sensitivity and recovery potential into ecological vulnerability to chemicals.

This thesis integrates the three spatially specific external exposure, assemblage sensitivity, and recovery potential to evaluate the risks of chemicals. It assesses the implications for current environmental criteria and evaluation methods. This thesis finds that the current environmental quality standards are protective of biodiversity for most of the chemicals investigated. For many chemicals, the standards are very conservative to potentially unnecessarily restrict the use of chemicals that provide significant societal benefits. The findings of this thesis also contribute to developing site-specific ecological risk assessment and ecological vulnerability to chemicals. When applying the refined ecological risk and ecological vulnerability assessment to chemicals, site-specific sensitivity and recovery potential can be considered to increase ecological realism, which most current ecological risk assessments rarely consider. Ecological vulnerability (external exposure, assemblage sensitivity, and recovery potential) to chemicals varies spatially, suggesting that refined environmental protection values can be developed by region to not only protect natural communities from chemical pollutants but also use chemicals to benefit human life.

5.6 Conclusion

The thesis has shown that there is spatial variation in the sensitivity and recovery of freshwater invertebrate communities to different types of chemicals. Moreover, the magnitude of variation in community sensitivity can be more than five orders of magnitude and is greatest for chemicals that target specific taxonomic groups (i.e., insecticides). This thesis revealed the relationship between the similarity in species compositions and the variation in assemblage sensitivity to a chemical. The assemblages with similar species compositions vary slightly in their sensitivity to a chemical. The assemblages with different species compositions have similar or completely different sensitivity to a chemical. The assemblage-specific sensitivity to some chemical classes varies significantly across river catchment typology descriptors and land use. Spatial variation in the sensitivity and recovery of freshwater invertebrate communities can exacerbate or mitigate chemical risks; therefore, spatially defined ecological vulnerability to chemicals needs to be considered to make chemicals benefit the development of human society while controlling the potential ecological risks of chemicals to natural communities.

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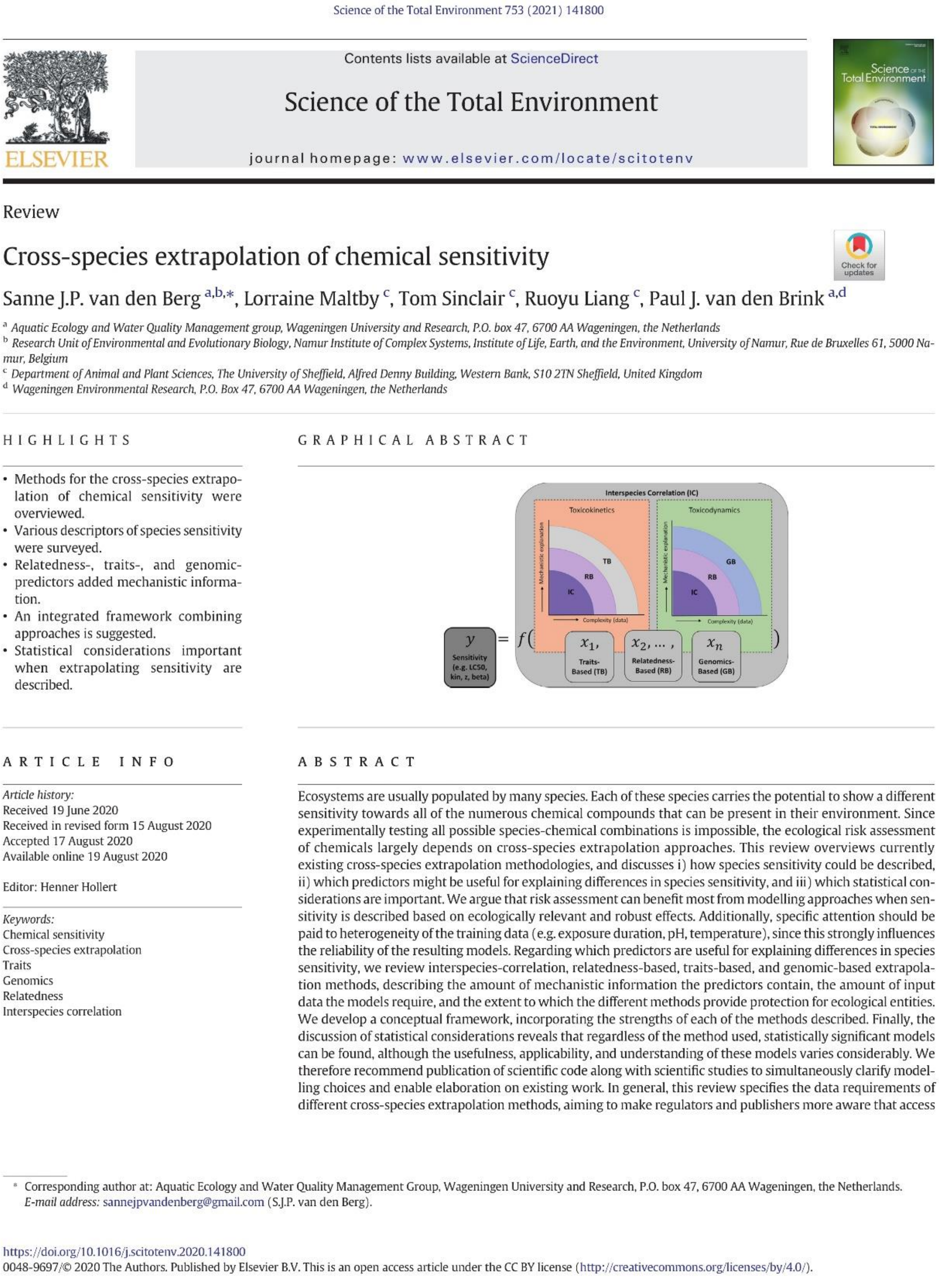
Appendix

S1.1 The full review of cross-species sensitivity prediction **(**Van den Berg, S.J., Maltby, L., Sinclair, T., Liang, R. and van den Brink, P.J., 2021. Cross-species extrapolation of chemical sensitivity. Science of the Total environment, 753, p.141800.**)**



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Review

Cross-species extrapolation of chemical sensitivity

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HIGHLIGHTS

- Methods for the cross-species extrapolation of chemical sensitivity were overviewed.
- Various descriptors of species sensitivity were surveyed.
- Relatedness-, traits-, and genomic-predictors added mechanistic information.
- An integrated framework combining approaches is suggested.
- Statistical considerations important when extrapolating sensitivity are described.

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ABSTRACT

Ecosystems are usually populated by many species. Each of these species carries the potential to show a different sensitivity towards all of the numerous chemical compounds that can be present in their environment. Since experimentally testing all possible species-chemical combinations is impossible, the ecological risk assessment of chemicals largely depends on cross-species extrapolation approaches. This review overviews currently existing cross-species extrapolation methodologies, and discusses i) how species sensitivity could be described, ii) which predictors might be useful for explaining differences in species sensitivity, and iii) which statistical considerations are important. We argue that risk assessment can benefit most from modelling approaches when sensitivity is described based on ecologically relevant and robust effects. Additionally, specific attention should be paid to heterogeneity of the training data (e.g. exposure duration, pH, temperature), since this strongly influences the reliability of the resulting models. Regarding which predictors are useful for explaining differences in species sensitivity, we review interspecies-correlation, relatedness-based, traits-based, and genomic-based extrapolation methods, describing the amount of mechanistic information the predictors contain, the amount of input data the models require, and the extent to which the different methods provide protection for ecological entities. We develop a conceptual framework, incorporating the strengths of each of the methods described. Finally, the discussion of statistical considerations reveals that regardless of the method used, statistically significant models can be found, although the usefulness, applicability, and understanding of these models varies considerably. We therefore recommend publication of scientific code along with scientific studies to simultaneously clarify modelling choices and enable elaboration on existing work. In general, this review specifies the data requirements of different cross-species extrapolation methods, aiming to make regulators and publishers more aware that access

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to raw- and meta-data needs to be improved to make future cross-species extrapolation efforts successful, enabling their integration into the regulatory environment.

Contents

1. Introduction

An ecosystem generally consists of a diverse species assemblage. Each of the species present in such an assemblage has the potential to show a different sensitivity towards each of the many different chemical compounds that can be present in their environment (e.g. Biggs et al., 2007; Clements and Rohr, 2009; Hickey and Clements, 1998). Ecological risk assessment (ERA) is the process used to evaluate the impact of chemicals on species assemblages by seeking the threshold concentration below which ecosystem structure and functioning experience no adverse impacts (e.g. Suter, 2016). At the first tier of this assessment, this threshold is often defined by combining results of single species toxicity tests with assessment factors (e.g. Brock et al., 2006). These assessment factors should reflect the uncertainty and variability related to the extrapolation from a laboratory system (short-term, high exposure, controlled environment, one species) to the natural environment (long-term, low exposure, variable environment, multiple species, and species interactions) (Brown et al., 2017). However, the assessment-factor approach remains generalized, since one threshold value is applicable to all assemblages within an ecosystem, irrespective of the variation in their species composition over space and time. This limits the specificity of the ERA. In contrast, existing higher tier approaches, such as mesocosm studies, do consider species assemblages rather than single species. However, performing multiple mesocosm experiments to account for seasonal and spatial variation would be too time and capital intensive (Van den Brink, 2008). Predictive methodologies extrapolate existing toxicity data to untested organisms. By predicting sensitivity values for a wide range of species, these methods can account for the part of the spatial-temporal variation in species sensitivity that is due to differences in species assemblages within and between sites (e.g. Malaj et al., 2016; Raimondo and Barron, 2019; Van den Berg et al., 2019). However, although several predictive methods have been developed over the last decades, a clear overview of which extrapolation methodologies are currently available, along with a description of their considerations, assumptions, merits, and pitfalls, is still lacking.

Since the need to address spatial-temporal variation requires the sensitivity of a species assemblage to be calculated rather than the sensitivity of a single species, we focus this review on methods extrapolating the sensitivity of multiple species towards one chemical or mode of action (MOA), thereby excluding methodologies extrapolating sensitivity of one species to multiple chemicals (e.g. Quantitative-Structure-Activity Relationships (QSARs), Donkin, 2009). Interspecies Correlation Estimation (ICE) is one of the earliest methods used to extrapolate toxicity data to untested species (Janardan et al., 1984; Mayer and Ellersieck, 1986). A software program to predict acute effects on aquatic and terrestrial species using ICE was developed in the 2000s (Asfaw et al., 2003) and a web-based model is available as Web-ICE (Raimondo et al., 2015). The method has gained popularity for the derivation of water quality criteria (e.g. Dyer et al., 2008; Feng et al., 2013), for example within the WFD (Water Framework Directive, European Commission, 2000).

To understand interspecific differences in species sensitivity towards chemical exposure, it is useful to divide sensitivity into two processes: toxicokinetics (TK) and toxicodynamics (TD) (EFSA PPR Panel (Panel on Plant Protection Products and their Residues) et al., 2018). TK processes describe the uptake, biotransformation and elimination of a chemical by a given organism, whilst TD processes are related to the damage, internal recovery and toxicity thresholds inside the organism after uptake of the chemical. The mechanistic basis of cross-species extrapolation is related to interspecific differences in TKTD processes. Interspecific differences in TKTD processes can be investigated by describing the combined effect of TK and TD processes simultaneously, or by using more specific predictors that split TK and TD into separate processes. In this review, we illustrate these processes in more detail, explain how they can be used as a more accurate description of species sensitivity, and clarify how different predictors can be used to describe different components of interspecific variation in sensitivity to chemical exposure.

The main research question of this review is '*How can we extrapolate species sensitivity?*'. However, a direct answer to this question does not exist, and in order to understand and compare cross-species extrapolation methods, it is necessary to study the three elements that make up predictive models separately, namely: i) the dependent variable (y), ii) the independent variable(s) (x), and iii) the function used to determine the relationship between the independent variable(s) and the dependent variable (f, Fig. 1). Concerning the cross-species extrapolation

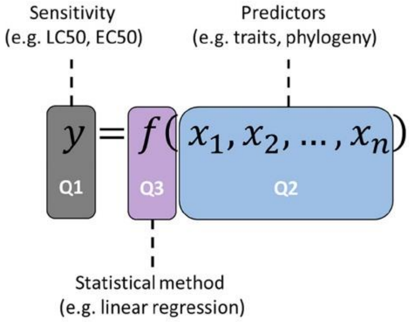

Fig. 1. Schematic overview of the elements making up predictive models. The Qs indicate the elements covered by sub-question 1, 2, and 3 posed in this review.

methods reviewed here, the dependent variable is the sensitivity of an untested species to a chemical. Therefore, the first sub-question this review tries to answer is *'How can we describe species sensitivity?'* (Q1). Although there is a proven distinction between true sensitivity and sensitivity as measured by short-term, laboratory experiments (Craig, 2013), it remains unambiguous that true sensitivity can only be inferred from measured sensitivity. Therefore, we will continue to use the term sensitivity to refer to measured sensitivity, of which we are aware that it is a measure relative to the protocol under which it was determined. The second element making up predictive models is the independent variable(s), or in other words, the predictors required to explain species sensitivity. The second sub-question this review tries to answer is therefore *'Which independent variables are useful for explaining differences in species sensitivity?'* (Q2). Ultimately, the last element concerns the statistical considerations that are of importance when connecting the independent and dependent variables together, or in other words, an answer to the question *'Which statistical considerations are important when extrapolating species sensitivity?'* (Q3). Overall, we aim to identify the range of approaches available for each of the three elements mentioned, along with a description of the considerations and assumptions they make, and to provide guidance on how the optimal combination of these elements can be combined in a conceptual framework. Since our background and expertise lies primarily in the field of aquatic ecotoxicology, most examples mentioned in this review will refer to the aquatic ecosystem. However, the general concepts and theories described and discussed can be applied to any cross-species extrapolation effort.

2. How can we describe species sensitivity?

The first element concerns how sensitivity is described. This description is primarily dependent on choices made in the selection of the input data, since this limits the boundaries of the model. For example, if the input data exclusively contain data on mortality effects, the resulting model will only be capable of predicting effects on mortality. We will discuss important selection criteria in Sections 2.1–2.4. Additionally, when comparing the performance of different models to determine which model is most suitable for answering a specific research question, it is important to consider whether data have been grouped or not (e.g. over chemicals or taxa). This will be discussed in more detail in Section 2.5.

2.1. Effects

Effects on mortality are most frequently incorporated into predictive models (Table 1). This is primarily determined by data availability. More than 40% of all aquatic toxicity tests in the ECOTOX database (U.S. Environmental Protection Agency, 2019) report effects on mortality, making it the most frequently studied effect on aquatic organisms in this database. However, mortality is sometimes not the most important effect to consider, depending on the mode of action of the chemical under study. Additionally, the data used to derive standard endpoints (e.g. LC50 values) can be exploited further to obtain a more mechanistic understanding of sensitivity, for instance, by means of TKTD models.

Effects other than mortality might be ecologically more relevant, or more relevant due to the mode of action of the chemical. Reproduction, for instance, is an indisputable element of population sustainability (see Gleason and Nacci, 2001; for an example with fathead minnow, and see Segner, 2011 for extensive background material). Thus, processes influencing reproductive success might be a better indicator of effects at higher levels of biological organization (e.g. offspring fitness, Hammers-Wirtz and Ratte, 2000). Energy allocation has been suggested as a means to link various levels of biological organization together (Calow and Sibly, 1990), since the energy available for reproduction and other functions depends on the availability of food sources and on the ability of an organism to exploit those (Amiard-Triquet, 2009). Thus, effects on feeding behaviour and reproduction can directly be connected to effects at population level by means of energy allocation modelling (Calow and Sibly, 1990), and might provide a closer approximation of sensitivity compared to when effects on mortality are used. More recently, energy allocation modelling has obtained renewed research interest under the acronym DEBtox (dynamic energy budget for toxicants), promoting simple generic models of animal life history (Baas et al., 2018; Jager et al., 2013; Kooijman, 2020).

Besides incorporating more ecologically relevant measurement endpoints, it is also possible to extract more information from existing data by means of TKTD models. For instance, the General Unified Threshold model of Survival (GUTS) is a TKTD framework that has been developed to obtain more mechanistic understanding from mortality or immobilization data by dynamically describing the process of uptake, elimination, recovery, and survival (Jager et al., 2011). Since GUTS parameters provide a more accurate description of processes determining species sensitivity, additional mechanistic understanding of differences in species sensitivity can be obtained by comparing calibrated GUTS parameter values across species, instead of standard sensitivity endpoints (Rubach et al., 2011; Rubach et al., 2012). To be able to fit GUTS models, however, data on effects at multiple time points are required. Collection of these data is already obligatory under most standard test protocols (e.g. OECD, 2019). However, public access to these data remains difficult, either due to the requirements of journals where these studies are published, or, in case of regulatory studies, the rules of the regulatory frameworks. These difficulties can easily be overcome by a commitment to publish the raw data of experiments along with summary statistics like LC_{50} values, preferably open access.

2.2. Exposure duration

Typically, acute toxicity tests with an exposure duration between 24 and 96 h are used for predictive modelling (Table 1). Again, this is primarily determined by data availability, since >50% of all aquatic toxicity test data available in the ECOTOX database concern tests with an exposure duration of up to 96 h (U.S. Environmental Protection Agency, 2019). Although expanding the exposure duration range may be beneficial for obtaining an adequately-sized dataset, it potentially compromises the integrity of the model and should be avoided if possible. For instance, we are likely to find less (fewer or smaller) effects after a 24 h continuous exposure than after a 96 h continuous exposure, because it takes time for a chemical to reach equilibrium between the exposure concentration and the concentration inside the organism. This difference is likely to become larger when the comparison concerns tests performed with different species, i.e. due to intraspecific differences in size and other traits influencing the uptake and elimination of

Table 1
Overview of modelling decisions made in the construction of interspecies correlation (IC), relatedness-based (RB), taxonomy-based (TB), and genomic-based (GB) models.[a]

	Effects (endpoint)	Exposure duration	Taxa	Transformation or normalization factor	Unit of exposure conc.	Chemicals included per model	Grouping across taxa	Statistical method	Reference(s)
IC	Mortality (LC50), Immobilization (EC50)	48 to 96 h	Fish, algae, birds, mammals, and aquatic invertebrates	$\log LC50$	μg/L	>1 chemical or MOA	Species	Linear regression	(e.g. Bejarano and Barron, 2014; Brill et al., 2016; Dyer et al., 2006; Feng et al., 2013)
RB	Mortality (LC50), Immobilization (EC50)	48 to 96 h	Fish, and aquatic invertebrates	$\log LC50$	μg/L	1 chemical	Species	Bayesian regression	(Craig, 2013)
	Mortality (LC50)	96 h	Amphibians, fish, and aquatic invertebrates	$\log LC50$	μmol/L	> 1 MOA	Species	Bilinear regression	(Guénard et al., 2014)
	Mortality (LC50), Immobilization (EC50)	24 to 96 h	Aquatic invertebrates	$\log LC50$	μg/L	> 1 heavy metal	Species	Bilinear regression	(Malaj et al., 2016)
	Population growth (EC50)	96 h	Algae	$\sqrt{LC50}$	μg/L	1 chemical	Species	Multivariate analysis	(Larras et al., 2014)
TB	Mortality (LC50), Immobilization (EC50)	24 to 96 h	Aquatic invertebrates	$\frac{\log(LC50)-\mu}{\sigma}$	μg/L	1 MOA	Family	Linear regression	(Rubach et al., 2010)
	Mortality (LC50), Immobilization (EC50)	24 to 96 h	Aquatic invertebrates	$\log(\frac{1}{LC50})*F^{b}$	mol/L	1 chemical	Genus, species	Genetic algorithm	(Ippolito et al., 2012)
	Mortality (LC50), Immobilization (EC50), Uptake (k_{in}), Elimination (k_{out})	48 h	Aquatic invertebrates	$\log LC50$	μg/L	1 chemical	Species	Linear regression	(Rubach et al., 2012)
	Mortality (LC50), Immobilization (EC50)	24 to 96 h	Aquatic invertebrates	$\frac{\log(LC50)-\mu}{\sigma}$	μg/L	1 MOA	Order, family, genus	Linear regression	(Rico and Van den Brink, 2015)
	Mortality (LC50)	24 to 96 h	Aquatic invertebrates	$\frac{\log(LC50)-\mu}{\sigma}$	mol/L	1 MOA	Genus	Linear regression	(Van den Berg et al., 2019)
GB	Mortality (LC50)	48 and 96 h	Amphibians, fish, and aquatic invertebrates	$\log LC50$	μg/L	1 chemical	Species	Linear regression	(LaLone et al., 2013)
	Mortality (LD50)	–	Birds	$\log LD50$	nmoles/kg	3 chemicals	Species	Linear regression	(Farmahin et al., 2012)

[a] This table is intended to be illustrative, not exhaustive, due to space constraints.
[b] Normalization factor was used to normalize the data according to exposure duration (Ippolito et al., 2012).

the chemical (e.g. Wiberg-Larsen et al., 2016). The exposure duration required to reach equilibrium is not only species dependent, but also depends on the physical-chemical properties of the compound, as is well-known from QSAR modelling (Cherkasov et al., 2014).

Besides running experiments long enough to ascertain that internal and external concentrations are in equilibrium, internal tissue concentrations could be measured and reported together with external exposure concentration. Several studies have demonstrated that the internal chemical concentration describes toxic effects more closely than the external chemical concentration (Friant and Henry, 1985; McCarty et al., 2011). Focussing on internal chemical concentration would by-pass TK processes, since uptake and elimination processes are redundant when internal concentrations are known, and would enable us to compare differences in species sensitivity originating from internal processes only (TD). Alternatively, a TKTD model like GUTS could be employed, which results in toxicity measures that are independent of exposure time (Jager et al., 2006).

2.3. Additional selection criteria

Imposing additional selection criteria on experimental conditions (e.g. pH, temperature, conductivity) can be useful for improving data homogeneity and hence data quality. Heavy metal toxicity, for example, has been reported to vary greatly according to the physicochemical characteristics of the exposed water (Gerhardt, 1993; Pascoe et al., 1986). The biotic ligand model has been developed to examine the bioavailability of heavy metals under different exposure circumstances, and additionally explains how abiotic conditions influence the affinity of metals to accumulate on the surface of aquatic organisms (Erickson, 2013). Similar models, normalization factors, or additional selection criteria, can be employed for other compound groups when necessary. Whether and which physicochemical properties should be taken into consideration when determining toxicity depends on the specific characteristics of the chemical group under study.

There are many other variables that may be sources of variation in species sensitivity. Consider, for instance, the size (Poteat and Buchwalter, 2014), sex (McClellan-Green et al., 2007), and life stage (van der Lee et al., 2020) of the individuals used in the toxicity test. Although these sources of variation are well-known, setting additional selection criteria on them is nearly impossible, since reporting on these factors is not always, or has not always been, common practise under standard guidelines. Additionally, standard guidelines take a lot of time and effort to develop, and are therefore only available for a limited range of species, making the use of selection criteria on a wide range of species difficult. Similar as before, whether and which of these variables should be taken into consideration when determining toxicity depends on the compound and taxonomic group under study, since the importance of these variables depends on the combination of both. For instance, sex dependent responses towards endocrine disrupting compounds may be common among fish (Orlando and

Guillette, 2007), whilst they may be absent for certain groups of invertebrates due to the large complexity and variation in endocrine systems among species (Janer and Porte, 2007).

2.4. Units

A final, but equally important choice in the description of sensitivity data is the unit in which sensitivity is expressed. This is specifically important when comparing species sensitivity across chemicals, which is sometimes necessary when data availability is restricted (discussed in Section 2.5). Although $\mu g\ l^{-1}$ is still the most frequently used unit in aquatic toxicity tests (almost 50% of all aquatic tests available in the ECOTOX database, U.S. Environmental Protection Agency, 2019, and see Table 1), it is not the most suitable one. It is frequently overlooked that chemical sensitivity is primarily related to molecular activity, and that the use of molar units makes molecule-to-molecule activity comparisons possible. For baseline toxicants exhibiting a non-polar narcosis MOA, the concentration at which mortality occurs will be close to equivalent for all species when internal molar concentrations are used (Escher and Hermens, 2002; Wezel and Opperhuizen, 1995), reducing differences in species sensitivity to TK processes only. To overcome the problems of tests expressed in weight units, attaching an accurate molar mass database (e.g. EPIsuite, U.S. Environmental Protection Agency, 2018) can help with converting mass units to molar units.

2.5. Grouping data, and its effects on explained variance

When data are limited, which is often the case, there is the possibility of grouping data (e.g. across chemicals or taxa) to obtain an adequately sized dataset suitable for modelling purposes.

Classifying chemicals according to their MOA is considered useful, because it provides an organizing scheme using an intermediate level of complexity between molecular mechanisms and physiological or organismal outcomes (Carriger et al., 2016). The rationale for using MOA classification for cross-species extrapolation is that these molecular mechanisms are conserved among biological entities (Escher and Hermens, 2002). However, as in any grouping, using MOA as a grouping variable also introduces variation and errors. The assigned MOA may vary, for instance, between species or life stage depending on the availability of target sites (e.g. in the case of photosynthetic inhibitors, Nendza and Muller, 2000), or between classification scheme used (see Kienzler et al., 2017 for differences in MOA classification according to the approach used). Therefore, MOA grouping only represents a suitable option when it is used with caution, for instance, by restricting the taxonomic range of the model to avoid interspecific variation in MOA, or when there is strong evidence that the MOA is applicable across the species in question (e.g. for baseline narcosis, for which there is strong evidence that the critical body residue for acute lethality in aquatic organisms has a very small range, van Wezel et al., 1995).

Similar to using MOA to group across chemicals, higher taxonomic ranks (e.g. family, order) can be used to group across taxa, and may also be useful for reducing data gaps. Grouping at higher taxonomic ranks has the advantage of reducing bias due to extreme values and spurious data. However, potentially important differences in species sensitivity might be lost by summarising the sensitivity of several species at, for example, family level (Buchwalter et al., 2008; Ippolito et al., 2012), and this trade-off should be carefully considered for the chemical-taxa combination under study.

Whether and how input data are grouped needs to be considered when comparing the performance (e.g. the adjusted R^2, or the cross-validation error) of different models. It is crucial to keep in mind that the variation associated with the grouping that goes into the model, is directly related to the variation related to the predictions that come out of the model (Schultz and Cronin, 2003). Disregarding the variation in input values can result in an overly optimistic view on model performance. Similarly, when comparing the performance of different models, it is important to consider how much variation the model explains, since this largely depends on the number of chemicals considered in the model. For instance, the most complex model of Guénard et al. (2014) explained 80% of the variation in the sensitivity of 25 species towards five compounds, whilst a related model of Van den Berg et al. (2019, both models include AChE inhibition as MOA) explained only 41% of the variation in the sensitivity of 32 genera towards 33 compounds. This large difference in model performance can partially be explained by the fact that the five compounds of Guénard et al. included three MOAs, whilst the 33 compounds of Van den Berg et al. included only one MOA, thereby resulting in a large difference in the absolute amount of variation that each model explains.

3. Which independent variables are useful for explaining differences in species sensitivity?

We divide possible sensitivity predictors into four groups based on the type of mechanistic information that they contain: interspecies-correlation (IC), relatedness-based (RB), trait-based (TB), and genomic-based (GB). Here, we first give an overview of the general concept behind each sub-group (Section 3.1), followed by a discussion of the merits and pitfalls associated with each of them (Section 3.2, Table 2), and close with a description on how the different predictor groups can be combined in a conceptual framework (Section 3.3).

3.1. Overview of methods

Interspecies-correlation (IC) models are log-linear least-squares regressions of the acute toxicity (E/LC_{50}) of chemicals measured in two species (e.g. Awkerman et al., 2008; Awkerman et al., 2014; Dyer et al., 2006; Dyer et al., 2008; Raimondo et al., 2007). IC models aim at predicting the acute toxicity of a chemical to untested species (predicted species) using the known acute toxicity of this chemical to tested species (surrogate species). IC models have been used to predict chemical toxicity for algae (e.g. Brill et al., 2016), aquatic invertebrates and vertebrates (e.g. Awkerman et al., 2014), terrestrial birds (e.g. Raimondo et al., 2007) and mammals (e.g. Awkerman et al., 2009), and have proven to be protective for rare and endangered species (Willming et al., 2016). However, not all predictions made by this kind of model are reliable. Reliable prediction results are those that are derived from models that have a low mean square error, narrow confidence intervals, a high cross-validation success rate, a high R^2 value, and are predicting the sensitivity of closely related taxa (e.g. belonging to the same order, Raimondo and Barron, 2019; Raimondo et al., 2007; Raimondo et al., 2010b).

Relatedness-based (RB) models use the extent of evolutionary relatedness between organisms as a proxy for the similarity in their response to chemical stressors (e.g. Craig, 2013; Guénard et al., 2014; Malaj et al., 2016). The underlying principle of these models is that closely related species exhibit high correlation of sensitivity to chemicals, such that closely related species tend to have similar sensitivity, divergence of sensitivity, and uncertainty. These three aspects subsequently increase for more distantly related species. The correlation of the sensitivity of species with a known relatedness can be used to make extrapolations from species whose sensitivity is known, to closely related untested species. The strength of this correlation decreases as the two species are more distantly related to the point where species that belong to the same higher taxonomic rank exhibit no correlation of sensitivity. Most RB models use taxonomy to predict the sensitivity of untested species (e.g. Craig, 2013), although other relatedness metrics, such as phylogenetics, have also been used (Guénard et al., 2014; Malaj et al., 2016, Table 1).

Trait-based (TB) models use physiological, morphological and ecological characteristics of a species to describe its sensitivity towards chemical stressors (e.g. Rubach et al., 2010). Several traits of organisms are known to directly relate to organism sensitivity (e.g. larger organisms

Table 2
Brief description of the four groups of cross-species extrapolation approaches discussed in this review, along with information on their mechanistic explanation, data demand, and level of protection for ecological entities.

	Main principle	Mechanistic explanation	Data demand	Protection of ecological entities
IC	Correlation between the responses of two species (surrogate and predicted species) to a range of chemicals	Absent	Toxicity data on multiple chemicals (both on the surrogate and predicted species)	Only the sensitivity of well-studied species can be predicted, and so far no examples of extrapolations to higher levels of biological organization exist.
RB	Evolutionary relatedness	Evolutionary related species exhibit similar sensitivity due to overlap in sensitivity-influencing traits and closely-related genetic patterns	Toxicity data, and data on taxonomic relatedness (i.e. a taxonomic or phylogenetic classification)	The sensitivity of real species assemblages can be predicted. Indirect effects of chemicals can only be predicted when chemical effects are restricted within taxonomic or phylogenetic groups carrying specific functions.
TB	Morphological, physiological and ecological relatedness	Differences in sensitivity-influencing morphological, physiological, or ecological characteristics of a species	Toxicity data, traits data, taxonomy data (to match toxicity and traits)	The sensitivity of real species assemblages can be predicted. Indirect effects of chemicals can be predicted based on what might happen to specific functional groups
GB	Similarity in biogeochemical pathways	Differences in sensitivity-influencing biogeochemical pathways	Toxicity data, adverse outcome pathway, data on one or more aspects of the biogeochemical pathway	Only the sensitivity of well-studied species can be predicted, and no examples exist yet on the extrapolation to higher levels of biological organization.

tend to be more tolerant of toxicants) and therefore the relationships between these traits and sensitivity can be used to predict the sensitivity of untested species with known traits. Currently existing trait databases (e.g. Usseglio-Polatera et al., 2000), primarily describe visible, external traits (e.g. size, shape). Therefore, TB models are most appropriate for describing TK related processes, e.g. by considering feeding mode or mode of respiration (Rubach et al., 2012; Van den Berg et al., 2019). Other traits that could help describe internal TD processes (e.g. presence of target receptors) are available, but have so far only been described for a small number of species (see Table 2 in Rubach et al., 2011 for an overview of the availability and linkage of potential toxicodynamic traits).

Genomic-based (GB) models use the relationship between gene expression and biological function as a way to determine the sensitivity of an organism towards specific chemical stressors (Fedorenkova et al., 2010; Snape et al., 2004). Essentially, GB models directly link the genetic code underlying the molecules and pathways of chemical sensitivity to the sensitivity of the organism itself. Therefore, GB methods directly compare the differences between how organisms respond to chemicals internally, rather than the extent of relatedness in RB methods or the traits (which may have multiple genetic or phenotypic origins) of TB models that both partially relate to organism sensitivity. GB models focus on gene and protein expression, integrating transcriptomics (identification of mRNA from actively transcribed genes), proteomics (identification of proteins in a biological sample), and metabolomics (identification of metabolites in a biological sample) into ecotoxicology (Pennie et al., 2001). It is widely recognized that changes in gene expression have the potential to serve as early warning indicators for environmental effects and as useful biomarkers for chemical exposure (Pennie et al., 2001; Poynton et al., 2014), because they can be detected at low concentrations of chemicals and occur well before any morphological or reproductive effects become visible (e.g. Klaper and Thomas, 2004). However, how effects found at a molecular level should be extrapolated to a higher biological level relevant to risk assessment is an area of active research, for which adverse outcome pathways (AOPs) have been suggested as a suitable framework (Ankley et al., 2010). An AOP is a conceptual construct of a sequence of events that starts with a molecular initiating event, spans multiple levels of biological organization, and ends with an adverse outcome on endpoints meaningful to risk assessment (e.g. survival, reproduction). We realize that the boundary between a phylogenetic RB approach and a GB approach can be vague. To avoid ambiguity, we consider an analysis of the sequence similarity in a molecular target a GB approach (because this confirms a deeper understanding of the toxicity process), whilst an analysis of the sequence similarity in the whole genome or in genetic markers frequently used in phylogenetic analysis (e.g. COI, 18S) is considered an RB approach (Table 1).

3.2. Comparison of methods

3.2.1. Mechanistic explanation

Raimondo et al. (2010a) state that taxonomic relatedness is the underlying mechanistic explanation for IC models. However, IC models do not incorporate any phylogenetic or taxonomic predictors, and only take taxonomic distance into account when screening for reliable prediction results (Raimondo and Barron, 2019). Similarly, relatedness between chemicals can be considered the mechanistic explanation of IC models, since these models always include the response of species to multiple chemicals. Indeed, the fact that IC models work well when enough data are available, is likely due to the simultaneous explanation of the variation in sensitivity related to different chemicals and different species. Nevertheless, the lack of either taxonomic or physicochemical predictors raises the possibility of over-fitting the correlation model to the training data, resulting in inaccurate predictions when models are applied beyond the limits of the training data (Johnson and Omland, 2004). In the case of IC models, any chemical untested on the target species lies outside the limits of the training data.

RB models use relatedness as the mechanistic explanation of sensitivity. Relatedness itself does not explain differences in sensitivity, but is used as a proxy for similarity in species response to chemicals (Craig, 2013; Guénard et al., 2014; Malaj et al., 2016), since closely related taxa tend to exhibit similar sensitivity due to shared sensitivity-influencing traits (e.g. size and target receptor, Blomberg et al., 2003). The shared distance from a common ancestor results in closely-related genetic patterns, which leads to a similar biochemistry and phenotype, and therefore, to a shared susceptibility to certain MOAs.

TB models incorporate mechanistic explanations of sensitivity arising from differences in phenotypic or ecological characteristics of species. One TB approach focusing on aquatic invertebrates has, for instance, demonstrated that the uptake rate of chemicals can to a large extent be explained by the lipid content of an organism, whilst elimination rates are negatively correlated with the degree of sclerotization (Rubach et al., 2012). Depending on the taxonomic group under study, mechanistic hypotheses between traits and chemical susceptibility have been established to a greater or lesser extent. See Table 2 in Rubach et al. (2011) for an overview of the availability of a wide range of traits for algae, fish, aquatic plants, birds, mammals, and aquatic invertebrates, and the strength of the trait-process relationship (i.e. plausible but not proven, some evidence for some taxa, relationship available for several taxa).

GB models have the potential to contain a comprehensive mechanistic explanation of sensitivity to chemical exposure. However, in contrast to TB models, GB models often describe complex biochemical pathways that are difficult to understand and to test experimentally (see Forbes et al., 2006 for an overview of the limitations of biomarkers for assessing population level effects). Even if a complete AOP is available, capturing all possible molecular initiating events and/or key events that could be generated by the compound under study, uncertainties in the quantification of one of the intermediate steps required to infer organism level effects from molecular target sequence similarity might prevent a model from performing well, i.e. have a large predictive power. This is largely because these intermediate steps (e.g. related to transcriptomics, proteomics) heavily influence the eventual outcome of the molecular effect. LaLone et al. (2013) found, for example, that the correlation between empirical acute toxicity data and the percent similarity in the molecular target analysis is not very strong ($R^2 = 0.49$, p-value $= 0.121$). They argue that to fully understand chemical susceptibility it is necessary to further assess sequence and even structural information beyond the level of the primary or secondary protein structure (LaLone et al., 2013).

3.2.2. Data demand

IC models only require data on toxicity (e.g. EC_{50}, LC_{50}), which can be obtained from public databases such as the ECOTOX Knowledgebase (U.S. Environmental Protection Agency, 2019). However, the requirement that paired toxicity data (i.e. surrogate and predicted species) must be available for at least three chemicals in order to produce the correlation, restricts data availability (Raimondo et al., 2010a). Nevertheless, the latest IC models for aquatic animals contain >8500 toxicity values covering 316 species and 1499 chemicals (Raimondo et al., 2015). However, the taxonomic coverage of these models is restricted, with >60% of all the models available in WebICE extrapolating from one fish species to another (Raimondo et al., 2015), and of another 26%, either the surrogate or the predicted species is a fish.

As the predictive methods of RB models are based on relatedness, rather than on correlations of sensitivity to chemicals, data on toxicity must be complemented with data on relatedness. Taxonomic classifications for use in taxonomic RB models are readily available for any described species in publicly available databases (e.g. the taxonomy database from the National Center for Biotechnology Information, Federhen, 2011; or the Integrated Taxonomic Information System, ITIS, 2019). A phylogenetic RB model requires the genetic sequencing of a species, and coverage of phylogenies is currently still clade dependent. For instance, sequencing efforts in eukaryotic genomics are strongly biased towards multicellular organisms and their parasites (del Campo et al., 2014), and large projects are available to sequence vertebrate genomes (e.g. the Genome 10 K project, Koepfli et al., 2015). Genomic projects on algae and invertebrates remain limited, however, restricting the use of phylogeny-based RB models to data-rich clades such as fish. To ensure a good performance of RB models, a taxonomically or phylogenetically diverse toxicity dataset is required, because the correlation of sensitivity decreases with decreasing relatedness (Craig, 2013).

The data demand of TB models depends on the traits to be included in the model, as well as the taxonomic group for which the model is constructed. For invertebrates, traits like size and mode of respiration (e.g. having gills or not) are readily available in literature, or can otherwise easily be recorded. Data on more specific traits, like lipid content or target site distribution, require more effort to measure, and are therefore less available in literature (see Table 2 in Rubach et al., 2011). The study of Van den Berg et al. (2019) showed that when a wide range of traits were included in the construction of invertebrate TB models, the modelling effort was primarily limited by a shortage of traits data (loss of 56% of the species for which toxicity data are available). However, only one trait database was used in their study (Usseglio-Polatera et al., 2000), whilst more trait databases are available for invertebrates (Hébert et al., 2016; Poff et al., 2006; Schäfer et al., 2011). For fish, a wide range of traits are available, distributed over several trait databases (Frimpong and Angermeier, 2009; Froese and Pauly, 2000; Lamouroux et al., 2002) and covering a large part of the taxonomic diversity of fish. For algae we are aware of two traits databases currently available (Lange et al., 2016; Reynolds et al., 2002), but have to acknowledge that they are likely to have the lowest taxonomic coverage out of the three standard organism groups discussed here (invertebrates, fish, algae), due to the large biodiversity of this group. Besides data on traits, TB models require data on taxonomy to match the traits with the toxicity data. The taxonomic nomenclature used in the traits database has to exactly match the one used in the toxicity database. If this is not the case, the taxonomy of both the traits and the toxicity database has to be standardized by means of an external taxonomy database. Access to taxonomic data has already been described under RB models.

GB models are the most data demanding, because they require peer-reviewed AOPs, based on validated biomarkers. Currently, 274 AOPs have been described in the AOP wiki in total covering 521 stressors (including chemicals, environmental factors), although the OECD status of the majority of them remains 'under development' (https://aopwiki.org/, accessed on the 25th of January 2020), and taxonomic coverage of these models remains limited. However, powerful advances in genome sequencing technology, informatics, automation, and artificial intelligence are assisting researchers in understanding species differences to a more detailed level (Lewin et al., 2018), and can be expected to lead to a significant increase in the development of AOPs. Promising new techniques, e.g. in vitro cell-lines (Eisner et al., 2019) or enzymatic markers (Arini et al., 2017), are being developed and carry the potential to replace currently used in-vivo concentration-response curves with in-vitro concentration-response curves (see, for instance, Fig. 3 in Zhang et al., 2018). However, these methods are time-, and cost-intensive, and are frequently incomparable due to inconsistent bioinformatic methods for data filtering, concentration-response modelling and quantitative characterization of genes and pathways (Zhang et al., 2018).

3.2.3. Protection of ecological entities

The main objective of all cross-species extrapolation methods is to get an accurate view on the variation in species sensitivity that exists in the real world. Indeed, all methods presented in this review attempt to add realism to ERA by filling in data gaps. However, the methods studied in this review vary in two important ways: i) in the way they are able to consider real species assemblages, and ii) in the way that they can be used to extrapolate effects to higher levels of biological organization (e.g. population, community or ecosystem level). Therefore, the four methods differ in the way they provide protection for ecological entities.

Researchers have known for a long time that real species assemblages vary through time (Murphy, 1978) and space (Vannote et al., 1980). Although we will likely never be able to understand this variation in its entirety, we can reduce uncertainty in ERA by predicting the sensitivity of representative species assemblages. RB and TB methods have this potential, since both methods can predict the sensitivity of species that have never undergone toxicity testing before, provided that data requirements of the species whose sensitivity you want to predict are available or can be collected. This contrasts with IC models, which require sufficient toxicity data to be available for the taxon whose sensitivity we want to predict (Section 3.2.2), and then still might be overfitted to the training data due to the absence of mechanistic relationships. GB models require, at least, to have the part of the genome sequenced that is associated with the key molecular initiating event(s) (LaLone et al., 2013). This is to ensure that divergence of genomic sequences linked to the molecular targets of a chemical can be associated with differences in the sensitivity between species. Consequently, extensive collection of genomic data and understanding of the chemical's toxicity pathway is required to produce a robust GB model. Therefore, IC and GB models are only able to predict the sensitivity of well-studied species.

All four methods have the potential to be used for the construction of species sensitivity distributions (SSDs), a statistical tool considered more protective of ecological entities than single measurements of sensitivity, since they allow only a defined fraction of species present in a species assemblage to be affected (Kooijman, 1987). Again, due to the restrictions in the underlying data, IC and GB models assume standard species assemblages in their SSDs, whilst RB and TB models can also be applied to representative species assemblages. RB approaches have as advantage over TB approaches that data on relatedness is usually more abundant than data on traits, allowing sensitivity to be predicted for a wider range of species. For this reason, RB models can be used to develop spatially-defined protection criteria, whereas TB models can extrapolate found relationships towards assemblages with the same trait profile, but with a different taxonomic composition (Van den Brink et al., 2011). GB approaches have recently been used for the retrospective risk assessment of community-level effects towards ammonia and nitrogen using field-based SSDs (Yang et al., 2017). However, there are many uncertainties in using retrospective risk assessment approaches, for instance, due to the inability to disentangle effects caused by the stressor of interest from other stressors (either natural or anthropogenic) that might be present at the site under study. For this reason, we do not consider retrospective risk assessment studies in our review.

Although SSDs are considered more representative of real species assemblages than when only an algae, an invertebrate, and a fish are evaluated, they still do not consider indirect effects of chemical exposure, i.e. effects on food availability, predation, competitive interactions or feedback mechanisms. Indeed, all studies described in this review only consider direct effects of chemical exposure on organism sensitivity. However, certain methods are better able than others to be used for the extrapolation of effects to higher levels of organization. For instance, TB models permit the derivation of hypotheses on what might happen to specific functional groups, whilst RB can only do this if functions are clearly restricted to taxonomic or phylogenetic groups. Imagine, for example, that predators are more sensitive to a certain chemical than herbivores due to a difference in assimilation efficiency (a relationship found in Hendriks et al., 2001). It is well known from literature that functional traits like feeding guild are not strongly conserved across taxonomy (e.g. see Table 1 in Poteat et al., 2015 for the distribution of feeding guilds over the orders Ephemeroptera, Plecoptera, and Trichoptera). Therefore RB approaches will fail to extrapolate the effect of this relationship to the community level, whilst TB approaches will be able to do so. Additionally, hypotheses derived from TB models can directly link into stochastic ecosystem models (e.g. De Laender et al., 2015). Such models are able to extrapolate effects found for specific functional groups to the community level, incorporating factors like species interactions and functional redundancy (Rosenfeld, 2002). For GB approaches, examples exist of how to extrapolate direct effects to population level effects. For instance, De Coen and Janssen (2003) have found a strong relationship ($0.88 < R^2 < 0.99$) between the cellular energy allocation biomarker response to several chemicals and population level effects of *Daphnia magna*. However, studies extrapolating effects found on a single species to community level effects remain absent. For IC models, no examples of extrapolations to higher biological levels exist, besides the use of assessment factors.

3.3. A combined approach to predicting sensitivity

Since all the methods discussed in this review have their own strengths and weaknesses, our main concern is not identifying which method results in models with the highest explanatory power, but rather in understanding how the methods can be incorporated into a conceptual framework. Indeed, all studies discussed in this review (Table 1) have demonstrated the ability to predict differences in species sensitivity to a certain extent, although there was not one method that consistently outperformed the others, and all of them seemed restricted in the maximum amount of variation in species sensitivity they could explain. However, studies which combined predictors from multiple mechanistic explanations observed an increased model performance compared to when predictors belonging to only one mechanistic explanation were included. For example, Larras et al. (2014) and Buchwalter et al. (2008) both found that combining TB and RB methods (trophic preference with phylogenetic signal, and body weight with taxonomic family, respectively) explained more variation than either method alone. These findings have found consistent support in further studies (e.g. Ippolito et al., 2012; Poteat et al., 2015).

That combining predictors belonging to different predictor groups leads to better models can be explained by the fact that each of the predictor groups explains a different part of the sensitivity processes as understood under the TKTD framework (Fig. 2). Studies describing species differences in TK parameters (e.g. Buchwalter et al., 2008; Rubach et al., 2012) found that traits like mode of respiration, body size and other morphological traits are good predictors of uptake rates, whilst elimination rates have a very strong phylogenetic signal. We are unaware of any studies that have explored the relationships between GB predictors and TD parameters, but since TD parameters describe processes related to toxicity thresholds inside the organism, the presence, absence, and distribution of chemical receptors are likely to be strong predictors of differences in the TD part of species sensitivity (e.g. as found in Larras et al., 2014). So we can hypothesise that TB approaches are good in explaining the TK part of differences in species sensitivity, whilst GB approaches are good in explaining the TD part of differences in species sensitivity (Fig. 2). Additionally, RB approaches have the potential to represent aspects of both TK and TD processes, because relatedness acts as a proxy for the likelihood of sharing a niche and therefore traits (TK), but also for sharing similar biochemical processes (TD). Therefore, RB predictors can be added to the model to represent sensitivity related processes that are still unknown (Fig. 2). Alternatively, a stand-alone RB analysis can be used to distinguish which taxa are sensitive and tolerant to a specific chemical or MOA. This information can help ease the search for molecular target(s) or traits powerful in describing differences in species sensitivity, since it must be due to genomic or trait differences existing between sensitive and tolerant taxa. Finally, IC models can be used if the MOA of the chemical under study has been extensively studied before, and if the taxonomic coverage of these models is sufficient to determine the potential risk to non-target organisms.

Considering that the best performing models can be found by combining the different methods in a conceptual framework, the different layers (IC, RB, TB, GB) of the TK and TD processes as illustrated in Fig. 2 can be regarded as different levels of a tiered approach, each level introducing more complexity and mechanistic explanation. At the lowest level of this approach, you can find IC models, which can be used for a preliminary hazard assessment. For this, existing IC models should be collected and applied to conduct a preliminary assessment of hazard following a weight-of-evidence approach. Besides evaluating the potential risk to non-target species, the used models should be assessed on their taxonomic coverage and model performance, whose thresholds should be set beforehand. The thresholds of the taxonomic coverage and model performance will depend on the trade-off between the purpose of the modelling effort (i.e. to support priority setting procedures, to supplement the use of experimental data in weight-of-evidence approaches, or to completely substitute the need for experimental data) and the strictness of the regulatory framework that the target compound falls under (some being more conservative than others). At the end of every tier, an evaluation is done to check whether the risks are shown to be negligible or acceptable with reasonable certainty, and whether enough information is available to make a regulatory decision. If the evaluation still indicates a potential risk to certain non-target organisms or further information is required for decision making, continuation to the next tier is necessary.

In the higher levels of this approach, predictor groups are added according to their data availability. First, the most abundantly available and easily accessible data is added to the models: taxonomic relatedness. Model construction is done anew, followed by an evaluation of the risks, taxonomic coverage, and model performance. If necessary,

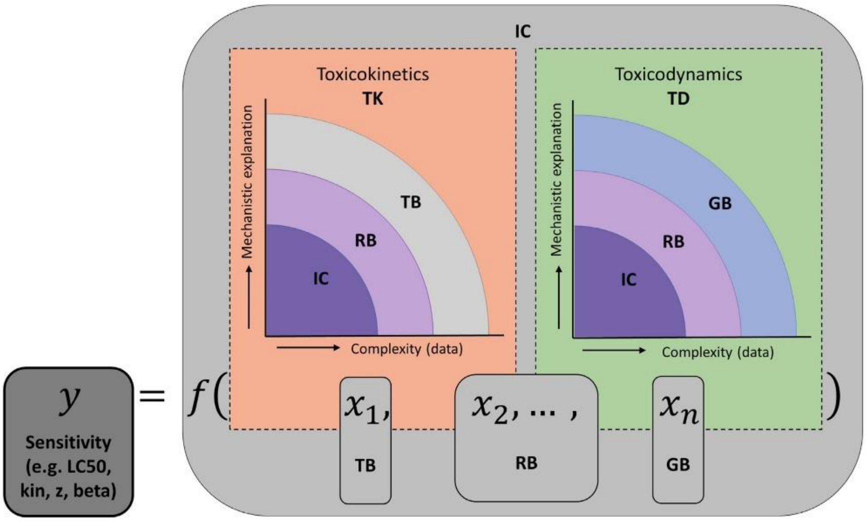

Fig. 2. An abstract visualization of the conceptual framework suggested to combine the different modelling approaches (IC, RB, TB and GB) discussed in this review. The different layers (IC, RB, TB, GB) of the TK and TD processes can be regarded as the steps of a tiered approach, increasing in complexity and mechanistic explanation.

we continue to the next level, in which trait predictors are introduced. For this, a hypothesis-driven approach is used to select sensitivity-related traits. In the case that sensitivity-related traits of the taxa-compound combination are unknown, the previous RB approach can be used to focus research. For instance, the RB approach has distinguished certain taxonomic groups as sensitive or tolerant. A study of the traits belonging with these taxonomic groups can assist in creating hypotheses regarding sensitivity-related traits. If traits data are insufficiently available in existing databases, new traits data can be collected using literature research or measuring the traits in the laboratory. Once sufficient traits data are available, TB-RB models can be constructed, and risk and model evaluation is repeated. In the next and final level of this approach, more mechanistic information can be added to the models by introducing GB predictors. For this, molecular markers important for the MOA of the target compound under study need to be known and available. If this is not the case, the RB approach can be used to focus research, similarly as how this was done for traits. Once sufficient data are available, TB-GB models can be constructed, potentially supplemented with RB predictors to represent any missing molecular markers or traits that are important for describing the sensitivity process. Only when it is still not clear whether the risk conclusion is acceptable after the final risk and model evaluation, execution of experiments following one of the more traditional tiered approaches is necessary.

4. Which statistical considerations are important when extrapolating species sensitivity?

The final feature of predictive models that this review discusses, is the statistical considerations that are important when extrapolating species sensitivity. After all, most modellers are aware that a major part of the modelling outcome is determined by choices made along the modelling process. These choices range from the selection of input data (Section 2), to the method selected for (preliminary) variable selection. Here, we want to discuss modelling considerations that have so far not been discussed in this review, but are main determinants for the modelling outcome.

The first consideration is the omission of data points. Modelling studies often depend on a subset of data available in literature or databases, and, as mentioned in Section 2, model performance is largely dependent on this sub-setting of the input data. Therefore, it is crucial that data are only omitted or included under clear and well-documented circumstances. Data should never be omitted without explanation, as this can lead to the suspicion that outliers were merely removed to improve the model.

The second consideration is the use of confounded predictors. If two predictors are highly collinear, they contribute the same information twice, thus confounding the statistical association and making it more difficult to deduce a mechanistic interpretation (Dormann et al., 2013). Therefore, preliminary variable selection is an important process. Van den Berg et al. (2019) assessed the optimal collinearity threshold for trait predictors, and found an increase in cross-validation error with an increasing collinearity threshold. In general, a collinearity of maximum 70% is allowed, and is found sufficient to keep collinearity under control (e.g. Dormann et al., 2013). Research performed on a GB based approach studied the influence of different preliminary variable selection methods on model performance (Mannheimer et al., 2019). They found that the variable selection method only had marginal effects on Spearman correlations between predicted and measured values, and that as long as the signal to noise ratio is high, the dominant effect will be captured regardless of the preliminary variable selection method. This is to a large extent true for big datasets containing many collinear predictors, which might be the case for GB approaches. For smaller datasets, however, preliminary variable selection methods can have a severe impact on the modelling results. Predictors should in that case be collected deliberately avoiding collinearity, and with clear underlying hypotheses.

The third consideration is that any descriptor value, measured or calculated, can potentially contain errors. Molecular descriptors, for instance, may vary depending on the conformation of molecules and on the software used (Benfenati et al., 2001; Schultz and Cronin, 2003). Traits like size and number of offspring per clutch are known to vary over space (Orlofske and Baird, 2014), and are additionally recognized to alter ecological dynamics through indirect effects (Bolnick et al., 2011). Therefore, the more predictors included in the model, the larger the chance of incorporating errors. Extrapolating the variation associated with predictors is a field not yet satisfactorily explored, but crucial if modelling approaches ever want to take a more dominant place in the risk assessment process (e.g. by means of Bayesian approaches, Wintle et al., 2003). For this to be possible, though, accessibility to raw data is necessary. Proper registration and transparency of test methods used and results generated will help making data-mining approaches more feasible, especially if raw data are organized according to clear standards. Guidelines and standards have been developed for ecotoxicity data (e.g. Kase et al., 2016; Moermond et al., 2016; Society of Environmental Toxicology and Chemistry, 2019), but also for gene expression data the minimum quantity and quality of information required to interpret and verify study results has been defined (Brazma et al., 2001).

The fourth and final consideration concerns overfitting in general. Biological processes consist of complex dynamic interactions in a multi-dimensional system, and non-linear methods have the ability to capture these complex interactions between variables (e.g. Ladroue et al., 2009). However, in a multi-dimensional system these methods tend to incorporate noise leading to overfitting. Alternatively, linear methods are more robust to overfitting, although at the cost of potentially missing important non-linear interactions (Mannheimer et al., 2019). Whether a linear or non-linear method is more suitable depends on the hypothesised relationship between the dependent and independent variables, the number of independent variables available, and on the degree of mechanistic information contained within these independent variables. Regardless, additional measures can be taken to ensure overfitting is avoided. The use of the adjusted R^2 as model selection criterion should, for instance, be avoided, although this rule is still regularly broken (e.g. Rico and Van den Brink, 2015; Rubach et al., 2012; Rubach et al., 2010). This criterion focuses entirely on maximizing fit and completely disregards model complexity, therefore often resulting in models overfitted to the training data. Information criteria that consider both fit and complexity (e.g. Aikaike's Information Criterion) are better suited for selecting a model (Johnson and Omland, 2004), and are therefore recommended. Another crucial approach to avoid overfitting is to perform a model validation step. This can be done by splitting the data in a training and a test set. The model is then fitted to the training data, before being evaluated on the test data. In this way, the model can be evaluated on its predictive power, rather than on its fit. Doing this in a repeated, randomized manner is called cross-validation. However, it is important to realize that a (cross-)validation exercise is primarily feasible when the dataset is sufficiently large. When data are limited, bad validation results do not necessarily indicate an erroneous relationship, and literature might be available to provide support for the found relationship. However, good validation results provide proof that the found relationship is consistent among the available data, and that the model is not performing well merely due to coincidence.

Regardless of the exact choices made on the considerations discussed in this section, it is likely that statistically significant models will be found. However, the outcome and performance of these models does to a large extent depend on the modelling choices made. For this reason, communication of choices made during the modelling process is just as crucial for understanding the modelling outcomes, as are the modelling outcomes themselves. Striving for reproducible research is one way to force modelling choices to be communicated, since being able to recreate the whole process will enable external reviewers to re-run all the steps made. Reproducible research has as additional advantage that methods that have been implemented once, do not require reimplementation multiple times. In this way, we can spend our efforts on using and elaborating on existing work.

5. Concluding remarks

This review provides an overview of the methodologies currently available for extrapolating species sensitivity towards chemical stressors. However, there is not one straight-forward answer to the question '*How can we extrapolate species sensitivity?*'. Indeed, the answer to this question depends on the answers to the sub-questions addressed in this review: i) how can we describe species sensitivity, ii) which independent variables are useful for explaining differences in species sensitivity, and iii) which statistical considerations are important when extrapolating species sensitivity?

Regarding the first question, we show that ERA can primarily benefit from modelling approaches by describing species sensitivity on effects that are ecologically relevant and sufficiently robust such that the data can be used to accurately represent species sensitivity. However, attention should be paid to data heterogeneity, since this strongly influences the reliability of the resulting models. Additionally, the importance of the unit used to describe species sensitivity was discussed, which is primarily important when sensitivity is compared across chemicals, for instance, when data is grouped according to MOA. Ideally, concentrations should be described using molarities, since chemical sensitivity is primarily related to molecular activities. Finally, when deciding on which model is most suitable to answer a specific research question, we should keep in mind that model performance is a function of the number of chemicals and/or organisms that the model covers.

Regarding the independent variables that are useful for explaining differences in species sensitivity, we find that none of the methods discussed in this review result in the best model performance when considered alone. When sufficient toxicity data are available, and the MOA of the chemical is not very specific, IC models are likely to work (e.g. for baseline toxicants with a strong phylogenetic signal). However, as toxicity data for the same chemical is required for the tested and predicted species, IC methods are limited to species frequently used in laboratory testing. Extrapolating to other species therefore requires mechanistic approaches to construct trustworthy models. In that case, a combination of predictors originating from multiple approaches is likely to achieve optimal model performance, since all predictors explain a unique, complementary part of differences in species sensitivity (Fig. 2). For these reasons, we suggest a conceptual framework (Fig. 2), combining predictors describing important traits determining the uptake and elimination of chemicals (e.g. size, respiration mode, exoskeleton-thickness), with the amount of sequence similarity in molecular targets, and relatedness predictors utilised where data for traits and molecular targets are unavailable. This conceptual framework can be considered a tiered approach, where moving up a tier equals moving up in level of complexity and mechanistic understanding of the sensitivity process. We realize that the conceptual framework suggested in Section 3.3 needs to be developed further to enable practical application in regulatory risk assessment. A more detailed, set-by-step framework, supplemented with case studies demonstrating potential practical applications, will be of great importance for moving this field forward.

The final question has perhaps the most straight-forward answer, since regardless of the method selected, significant models can be found. It is, therefore, important that modelling is done in a reproducible way, and that modelling decisions are clearly communicated along with modelling results. To optimise reproducibility, we advise the publication of well-documented scientific code along with scientific studies, as is also in accordance with the good modelling practise as advised by EFSA (2014). This will not only clarify modelling choices, but will also help avoid re-implementing methods that have been implemented before, so that we can spend our efforts on continuing and elaborating on existing work.

So, after answering these three sub-questions, is it now clear how to extrapolate chemical sensitivity across species? For some of the methods discussed in this review, this is indeed straight forward, and in some occasions they have already been used in regulatory risk assessment. For instance, IC models matching model requirements can directly be used in regulatory risk assessment. However, for cross-species extrapolation methods to really find its way into regulatory risk assessment, additional work will have to be done, especially in the area of their uncertainty and

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practical applicability. As briefly has been mentioned before in Section 3.3, the requirements of the modelling effort (e.g. acceptable uncertainty boundaries) will depend on the trade-off between the purpose of the modelling effort (i.e. to support priority setting procedures, to supplement the use of experimental data in weight-of-evidence approaches, or to completely substitute the need for experimental data) and the strictness of the regulatory framework that the target compound falls under (some being more conservative than others). For example, when models are applied to support priority setting, or to supplement experimental data in weight-of-evidence approaches, their use is more indirect. Under these circumstances, experimental data and other information is available, making the extrapolation results not likely to be decisive in the final assessment. However, when the objective is to replace experimental data with modelled data, the risk assessment will heavily rely on the performance of the models, and therefore will require properly validated and applicable models. Especially in the latter case, a firm grip on the uncertainty associated with these models is necessary. Without concrete measures of uncertainty, modelling outcomes will have to be supplemented with something similar to the assessment factors that we considered unspecific and therefore inappropriate for risk assessment purposes.

Considering additional work on the practical applicability of cross-species extrapolation models, the main focus should lie on developing the conceptual framework suggested here in more detail. Working through some case studies will demonstrate how feasible the suggested approach is, and which research fields will need to evolve more before practical implementation becomes possible. For example, which difficulties lie in the application of RB and TB methods to still unknown taxonomic- or trait profiles? Will they indeed be able to accurately predict the sensitivity of natural species assemblages, or will their species coverage remain too low? Considering GB approaches, however promising they sound, will it really become possible to use approaches like this for a wide range of species, or will we get lost in the maze of AOPs, genetic markers, and key events? Finally, the question remains whether the current surge for open science and reproducible research will really turn the field of ecotoxicology into ART (accurate, reliable, and transparent), or that crucial data and information will remain hidden behind walls of journal requirements and regulatory frameworks? It is only after these things become clear, that we will know how we can extrapolate species sensitivity. This would offer opportunities for refining risk assessments, including spatial and temporal consideration of sensitivity, and provide methods for reducing animal testing and the costs associated with them.

Declaration of competing interest

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

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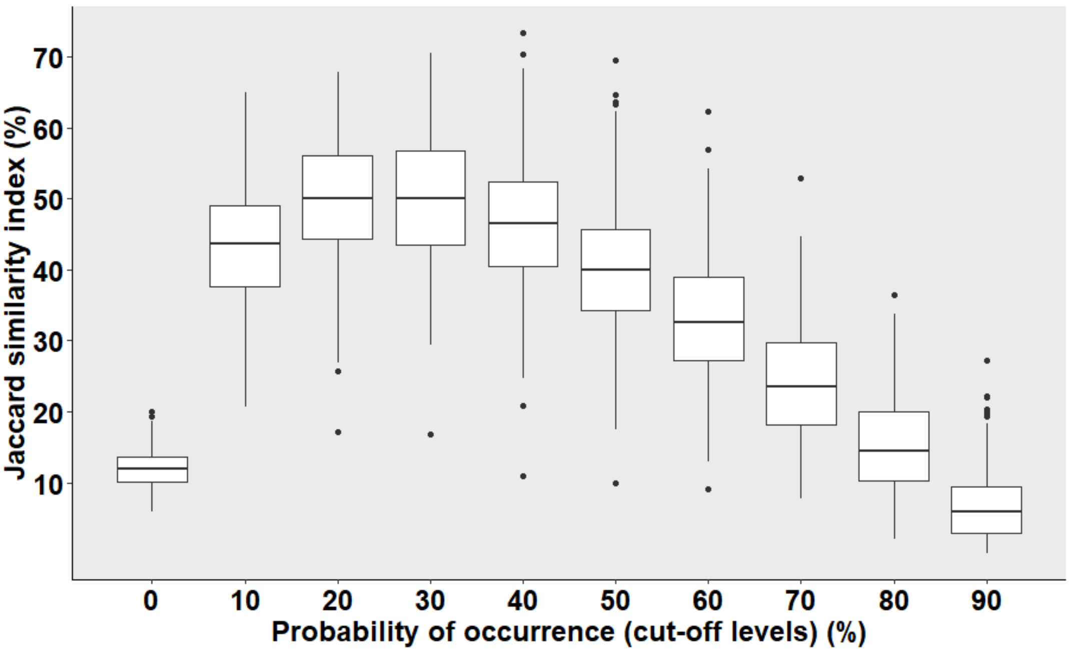

Figure S2.1 Jaccard similarity between observed and predicted freshwater macroinvertebrate assemblages at RIVPACS reference sites

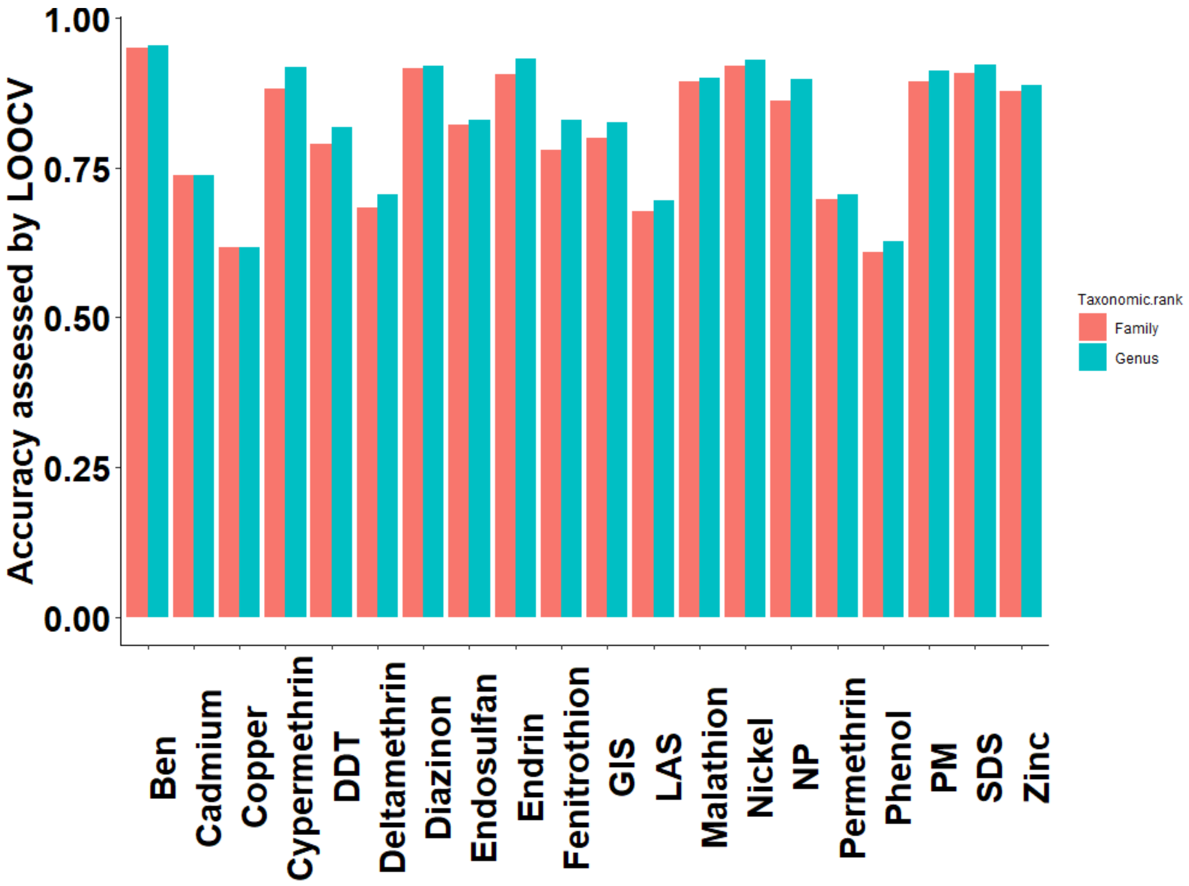

Figure S2.2 The accuracy of hSSD model predictions assessed using Leave-One-Out-Cross-Validation (LOOCV) to 20 study chemicals (provided by Sinclair, T., 2021).

Table S2.1 Descriptive statistics of HC5 values (µg/L) for observed freshwater invertebrate assemblages to 20 chemicals

Chemical	Min	Median	Max	Mean	Max/Min
Ben	3898	64984	143123	65466	36.7
Cadmium	32	372	3373	491	106.4
Copper	22	90	305	96	14
Cypermethrin	0.0008	0.0064	1.9378	0.0113	2546
DDT	1.205	4.367	252.269	5.199	209.4
Deltamethrin	0.001	0.008	18.833	0.022	12556.4
Diazinon	0.553	1.784	563.564	3.135	1019
Endosulfan	0.314	0.915	143.163	1.319	456
Endrin	0.003	0.024	19.497	0.073	7374.6
Fenitrothion	1.274	3.796	180.918	4.563	142
GIS	2834	5519	14239	5639	5
LAS	0.6293	3.2711	9.0063	3.3457	14.3
Malathion	0.2827	2.1911	868.3847	3.8349	3072.1
Nickel	327	2217	10933	2633	33.5
NP	0.273	2.1	25.464	2.386	93.2
Permethrin	0.023	0.137	50.248	0.283	2212.8
Phenol	2.341	8.612	154.365	10.578	65.9
Parathion-methyl	0.866	2.088	218.551	3.065	252.3
SDS	1062	1886	7859	1967	7.4
Zinc	818	1915	5110	1968	6.2

Table S2.2 Descriptive statistics of HC5 values (µg/L) for RICT predicted freshwater invertebrate assemblages to 20 chemicals

Chemical	Min	Median	Max	Mean	Max/Min
Ben	49325	75458	90329	73800	1.8
Cadmium	255	554	1890	650	7.4
Copper	38	56	131	64	3.5
Cypermethrin	0.004	0.007	0.019	0.008	5.1
DDT	1.838	2.483	5.07	2.575	2.8
Deltamethrin	0.003	0.005	0.014	0.005	4.6
Diazinon	1.461	2.037	6.356	2.233	4.3
Endosulfan	0.798	1.286	4.323	1.413	5.4
Endrin	0.027	0.07	0.401	0.086	14.7
Fenitrothion	2.342	3.765	5.551	3.754	2.4
GIS	4828	6257	8161	6310	1.7
LAS	1.956	2.495	3.399	2.579	1.7
Malathion	1.37	2.005	4.512	2.13	3.3
Nickel	1041	2111	7463	2695	7.2
NP	0.509	0.855	2.284	0.883	4.5
Permethrin	0.079	0.124	0.301	0.137	3.8
Phenol	8.331	11.915	26.852	12.846	3.2
Parathion-methyl	1.825	2.618	6.996	2.719	3.8
SDS	1857	2299	2743	2276	1.5
Zinc	1451	2064	2731	2057	1.9

Table S3.1 Number of sites/assemblages in each category of river catchment typologies

Descriptor	Category	Count
Altitude	Lowland	2008
	Midland	310
Size	Very small	684
	Small	1553
	Medium	81
Geology	Calcareous	1251
	Siliceous	1012
	Mixed	32
	Organic	23

Table S3.2 Number of sites/assemblages in each category of land use

Land use	Count
Improved grassland	995
Urban and suburban	473
Arable	410
Woodland	359
Semi-natural grassland	67
Mountain, heath and bog	14

S4.1 The recoding of factor traits

Traits	Original data/categories	Current categories	Recoding
Aquatic stages	0-3	Adult	0 or 1
Dispersal mode	Aquatic passive	non dispersal	0
	Aquatic active	Aquatic passive	1
	Aerial passive	Aquatic passive and active equally	2
	Aerial active	Aquatic active	3
		Aquatic and aerial equally	4
		Aerial passive	5
		Aerial passive and active equally	6
		Aerial active	7

S4.2 Equations of weight calculations

$w1 = 0.5$

$w2 = 1/3 * w1$

$w3 = w1 * \text{Trait value}_{\text{Aerial-Active}} / (\text{Trait value}_{\text{Aerial-Active}} + \text{Trait value}_{\text{Aquatic-Passive}} + \text{Trait value}_{\text{Aquatic-active}})$

$w5 = w1 * \text{Trait value}_{\text{Aquatic-Passive}} / (\text{Trait value}_{\text{Aerial-Active}} + \text{Trait value}_{\text{Aquatic-Passive}} + \text{Trait value}_{\text{Aquatic-active}})$

$w7 = w1 * \text{Trait value}_{\text{Aquatic-active}} / (\text{Trait value}_{\text{Aerial-Active}} + \text{Trait value}_{\text{Aquatic-Passive}} + \text{Trait value}_{\text{Aquatic-active}})$

$w4 = w9 = 0.2 * w3$

$w6 = 0.5 * w5$

$w8 = 1/3 * w7$

$w10 = 0.5 * w5 + 1/3 * w7$

S4.3 The weights of land use classes

Land use classes	Weights
Broadleaf woodland	6
Coniferous woodland	5
Arable	4
Improved grassland	3
Semi-natural grassland	2
Mountain, heath, bog	8
Saltwater	10
Freshwater	1
Coastal	9
Built-up areas and gardens	7