Integrating the effects of climate-change into the agrochemical risk assessment for freshwater ecosystems

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“We sit together, the mountain and I, until only the mountain remains”
Li Bai

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Summary

Climate change and pollution are major drivers of global biodiversity loss and freshwater ecosystems are particularly at risk from diffuse pollution from agrochemical landscapes. The predicted increase in the impact of climate change on weather systems may have important consequences for environmental exposure and ecological impacts of agrochemicals. However, current consideration of the effects of climate change on environmental risk assessments (ERAs) is limited to qualitative reviews or specific case studies. Therefore, there is a need to quantify the effects of climate change on agrochemical exposure and ecosystem sensitivity and integrate findings within ERAs.

This thesis aims to address three major issues relevant to freshwater ERAs: firstly, how spatial and seasonal variation in climate change affects agrochemical fate via the soil matrix considering chemical persistence. Secondly, to assess what effect species composition has on assemblage sensitivity and how this varies by i) chemical type, and ii) the change in composition under climate change. Finally, multi-stressor of chemical and temperature shock was investigated for mitigation by multi-generation acclimation.

The effects of climate change exhibited spatial and seasonal variation with precipitation and chemical degradation being major factors affecting chemical flux into freshwaters. Assemblage composition, including shifts under climate change, significantly affect sensitivity, although the extent of variation is chemical dependent. Temperature influenced reproductive strategy, but multi-stressors and temperature acclimation effects were limited.

The conclusions from each chapter were drawn together by contextualising the results within an ERA framework using case study chemicals. These indicated that the change to risk is spatially variable and beyond existing uncertainty accounted for within ERAs. This demonstrates that application of these results to realistic chemical uses is insightful and that existing ERAs can be adapted to include climate change. This is necessary as this thesis indicated that specific chemicals and locations underwent a large increase in risk.

Contents

CHAPTER 1

Factors affecting agrochemical input and sensitivity to freshwater ecosystems under the influence of climate change

Chapter 1. Introduction

1.1. A changing world

Anthropogenic impacts have altered ecosystems and landscapes throughout human history but human development following the industrial revolution has increased the extent and magnitude of effects, which now occur at a global scale (Ellis et al., 2021). Chemical pollution is one anthropogenic driver of change with global effects; chemical pollutants have been detected from Mount Everest to the Marianas Trench (Dasgupta et al., 2018; Miner et al., 2021). Agricultural land use represents 55% of Earth's land area (Ellis et al., 2010), and increasing intensity and industrialisation of agriculture since the Green Revolution has seen agrochemical use increase rapidly; even recently, between 1990 to 2019 global pesticide use has nearly doubled from 2.3 million tonnes to 4.2 million tonnes (FAO, 2020; Liu et al., 2015). Application of agrochemicals results in environmental exposure beyond the target ecosystem as the chemical is transported. This results in toxic effects of agrochemicals to organisms, ecosystems and the benefits directly and indirectly humans gain from them (ecosystem services) being observed ultimately leading to issue of environmental pollution entering public discourse (Carson, 1962; Carvalho, 2017; Reid, 2005). Ultimately, this led to regulations that calculated the risk posed to ecosystems by considering the quantity of chemical the ecosystem is exposed to and the sensitivity at which an ecosystem and its functioning and services are impaired (Nienstedt et al., 2012). Consequently, an ecosystem services perspective forms an important contextual point when considering agrochemical use and the unintentional exposure of non-target ecosystems, and thus requires the evaluation of how increased agricultural yield weighs against the negative impacts on non-target ecosystems (Daryanto et al., 2018; IPBES et al., 2019; Palm et al., 2014). Going forwards, increasing human population and developing dietary patterns have agrochemical use forecasts which predict continued pollutant pressure over the 21st century (Delcour et al., 2015; UN, 2019). However, agrochemicals will not be the only stressor to ecosystems and services, and the 21st century is set to be one increasingly affected by the rise of climate change as stressor of ecosystems and their biodiversity across the entire planet (Pecl. *et al.*, 2017; Malhi *et al.*, 2020). Indeed, climate change, which currently contributes to the decline of 19.4 % of threatened species (i.e., the 7th largest contributor), is set to increase in importance during the 21st century and to become one the major causes of species extinction due to rising greenhouse gas emissions exacerbating the changes to climate and therefore ecosystems (Maxwell et al., 2016).

Beyond the effects of climate change itself, climate change is expected to alter the risk from extensively studied agrochemicals, such as pesticides, and from emerging chemical pollutants (Balbus et al., 2012; EFSA et al., 2020; Fijalkowski et al., 2017; Watts et al., 2015). For example, direct effects of climate change on ecosystem sensitivity involve multiple stressor effects where chemical sensitivity of receptor organisms is mediated by climatic factors such as temperature stress or water availability(Birk et al., 2020) Other direct effects affect chemical exposure by altering the fate and behaviour of chemical transport to non-target ecosystems (Bloomfield et al., 2006; Gagnon et al., 2016). Climate change may also mediate the effect of chemicals on ecosystems indirectly, affecting chemical exposure by altering the types and amounts of agrochemicals used and their use patterns (Bussi et al., 2018; Delcour et al., 2015; Zimmermann et al., 2017), or altering the sensitivity of the ecosystem indirectly by shifting community composition and hence the presence of different receptor organisms, all with varying chemical sensitivities. Different approaches are used to investigate each of these potential climate change effects, and are discussed in further detail in 1.3 below, although a full consideration of combined risk is needed to integrate each approach into a framework and be adaptable to the range of chemicals, climate effects and ecosystems worldwide.

Freshwater ecosystems cover only 0.8% of the planet's area, but are disproportionally biodiverse, representing around 6% of described species (Dudgeon et al., 2006). Within the UK, riparian ecosystems and their catchments have been heavily impacted with none unaffected by human activity (Maltby et al., 2011; Vaughan and Ormerod, 2012). Assessments of UK rivers indicate that only 14% of monitored river water bodies are in good ecological status while 0% are in good chemical status (Environment Agency, 2020, 2018). While efforts have been made to improve water quality, with pollutant loads to rivers reducing by 70% between 1995-2005, point source chemical pollution still occurs and inputs from agricultural land pose ongoing threats to river ecosystems, and agrochemical use continues to rise (Garthwaite, 2018; Liu et al., 2015). Furthermore, the development of more sensitive analytical detection techniques and fate pathways has uncovered the potential risks from emerging chemical pollutants (Boxall et al., 2003; Sarmah et al., 2006). For example, pharmaceuticals, which were previously undetected in freshwaters and therefore not considered as a pollutant, are now understood to be a potential risk in UK rivers following exposure from sewage systems or from livestock, biofertiliser and irrigation emissions onto agricultural land (Daughton and Ternes, 1999; Gros et al., 2019; Ternes et al., 2007).

While the effects of climate change and chemical pollution occur globally, their effects vary spatially depending on the local freshwater ecosystems, climates, and anthropogenic drivers (Birk et al., 2020; Vaughan and Ormerod, 2014). Within Europe, existing freshwater ecosystems, their condition and their services have been shown to be failing the objectives set for their protection with both chemical pollution and climate change indicating deteriorating trends and targets largely not on track (EEA, 2019). Therefore, to assess and address their impacts, consideration of the risk posed by these stressors and the extent of variation in them is required. Environmental risk assessments (ERAs) provide a method with which to quantify risk to ecosystems from agrochemical application and therefore represent one approach to this (EFSA, 2013a). However, existing consideration of climate change within ERA approaches is limited and has been acknowledged as such from both a scientific and regulatory perspective (Brooks et al., 2013; EFSA et al., 2020; Van den Brink et al., 2018). Consequently, linking the different aspects of climate change and agrochemical pollution together is a major, but necessary, challenge to future proof ERAs and to protect freshwater ecosystems from the negative effects of agrochemical inputs (Reid et al., 2019). This thesis aims to investigate aspects of the exposure and sensitivity of ecosystems to agrochemicals and to quantify the effects of climate change on them by adapting specific aspects of the ERA and integrating them back into the existing ERA framework. In particular, it focuses on quantifying some of the effects of climate change on both exposure and sensitivity within a UK perspective due to the extent of existing freshwater, landscape, chemical and climatic data available (University of Hertfordshire, 2007a, 2007b; Met Office, 2019; Cranfield University, 2021; Environment Agency *et al.*, 2021). The quantification of climate change on risk will be considered simultaneously with the development of a framework for considering the effect of climate change on ERAs that is adaptable to different chemicals, locations and climates of ecosystems potentially at risk.

1.2. Agrochemicals: Context and risk assessment

1.2.1. Emissions & fate

Following introduction to agricultural land, agrochemicals pass through multiple processes to ultimately affect ecosystems and the benefits we derive from ecosystem services, such as water processing, purification and extraction, and pest and disease control (Figure 1.1). Chemicals applied to agricultural land are transported to non-target ecosystems ranging from adjacent land both cultivated and not, local soil ecosystems and bodies of freshwater such as ditches, rivers and ponds, each subject to negative effects on the constituent organisms (Gagnon et al., 2016; Steffens et al., 2015; Wang et al., 2019). Pesticide transport by surface runoff depends on local climate, agricultural practices, topography, soil characteristics, crop type, and pest phenology conditions. To accurately assess the impact of climate change, these factors must be accounted for in a single framework by integrating their interaction and uncertainty. Such negative effects cascade throughout different species through community interactions, impacting the ability of the ecosystem to function and Ultimately this impairment in functioning reduces ecosystem services necessitating consideration of chemicals risk for ecosystem protection (Brauman et al., 2007; Nienstedt et al., 2012; Palm et al., 2014). Exposure of non-target ecosystems occurs following application through transport of the agrochemical via multiple routes including air, soil, water and trophic transfer (Bach et al., 2016; Lorenz et al., 2017). Consequently, there is a need to understand both the inputs and routes of chemical exposure. This has been done in chemical fate field trials which have quantified the movement of chemicals through the soil matrix and into freshwater (Capel et al., 2008; Gros et al., 2019). As both inputs and routes of chemical exposure are sufficiently understood and quantifiable, equations for accurately calculating chemical fate have been discovered. This has led to the development of exposure models to predict the concentration of agrochemicals in freshwater bodies often supported and validated by field data (EFSA, 2013a; EMA et al., 2017; Parker et al., 2007; Schnoor, 1992). These exposure models not only enable the chemical fate, behaviour and transport into freshwater ecosystems to be quantified, but they can also be altered to account for specific scenarios, chemicals and situations (Bach et al., 2016).

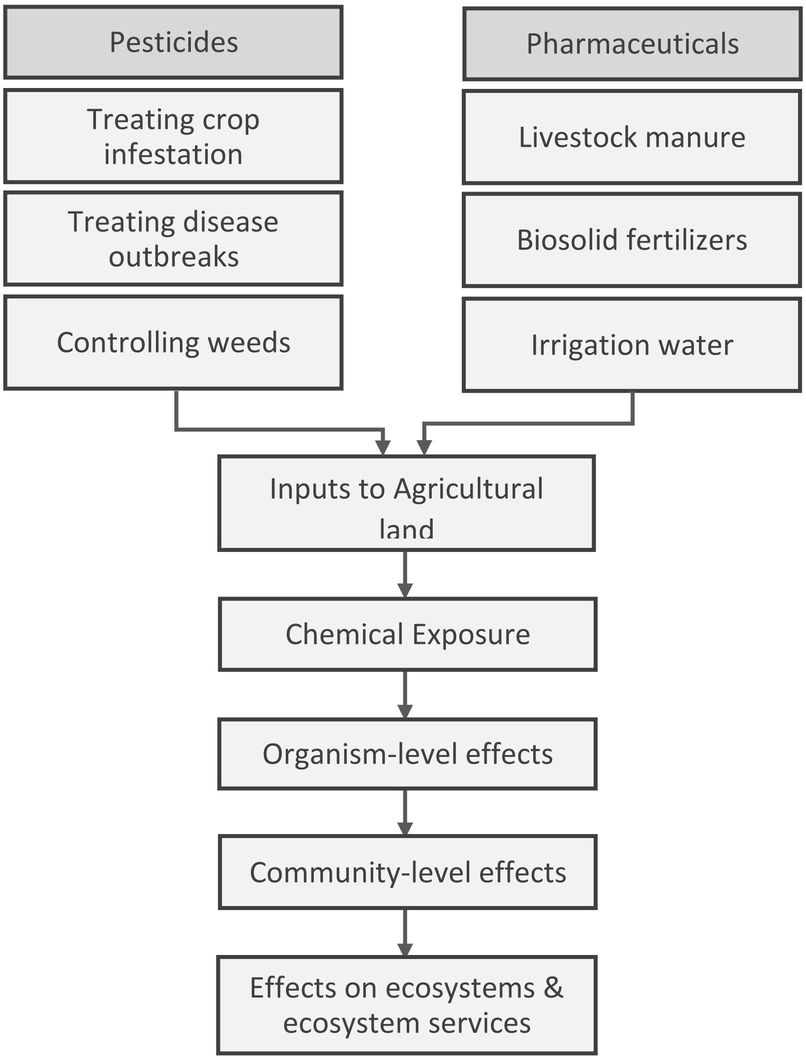

Figure 1.1 Pathway of introduction of agrochemicals to agricultural land and the resultant pathway of effects that cascade onto to ecosystems and the services gain from them

Since 1993, the FOrum for Co-ordination of pesticide fate models and their USe (FOCUS) have attempted to integrate and harmonise the calculation of predicted environmental concentrations of chemicals and agree upon fate models suitable for the ERA (FOCUS, 2021). Consequently, there are a suite of exposure models used in regulatory risk assessment that enable chemical exposure to be considered for specific locations, chemical applications and uses, and meteorology, providing the required input data are available (Bach et al., 2017; Jarvis et al., 1997; Mullins et al., 1993; US EPA, 2016a). In addition to ERA uses, models have been run for a series of realistic and hypothetical location-specific case studies, including watershed level predictions (Wang et al., 2019), with comparison of land-use and chemical fates (Oliver et al., 2012). There have also been efforts to create risk assessment scenarios for chemical exposure including for Europe-wide assessments (Blenkinsop et al., 2008), down-the-drain chemicals discharged directly into freshwaters (Franco et al., 2017) and soil-specific fate assessments (Bach et al., 2017).

However, when considering the impacts of climate change on the effects of agrochemicals on freshwater ecosystems, previous studies either consist of theoretical frameworks without methods to quantify exposure (Biswas et al., 2018; Bloomfield et al., 2006) or highly specific case-study locations (e.g. the fates of persistent-organic pollutants into the Venico Lagoon (Valle et al., 2007) or agricultural runoff into San Joaquin Valley, California (Ficklin et al., 2010)). Climate change will have a multitude of effects on chemical fate pathways, with further consideration of for example chemical transport in floods or chemical dynamics for intermittent waters (Ademollo et al., 2011; Boxall et al., 2009). Consequently a framework that quantifies the impacts of climate change on chemical fate and transport that is applicable across multiple locations and chemicals while accounting for underdeveloped pathways, requires development.

1.2.2. Organisms under agrochemical exposure

Concurrently with the Green Revolution, harmful effects of agrochemicals to organisms were being observed and acknowledgement of these issues entered public discourse (Carson, 1962; Carvalho, 2017). Application of agrochemicals resulted in environmental exposure as the chemical was transported into non-target ecosystems and affected non-target organisms, with effects on freshwaters being particularly noted (Cairns et al., 1972; Pilli et al., 1988). Freshwater organisms may be exposed to agrochemicals through absorption, ingestion or filtering of contaminated water, sediment or food (Schwarzenbach et al., 2006; Walters et al., 2016). Upon exposure, organisms will begin to exhibit toxic effects dependent on the concentration of the chemical. Generally, these effects are categorised by the timeframe of exposure, with acute toxicity occurring over short periods of time (i.e., hours or days) being associated primarily with mortality and behavioural changes. In contrast chronic toxicity occurs over longer periods of time (i.e., weeks or months) either through continual or repeat exposure and is noted for also affecting organism growth, reproduction and development (Ashauer et al., 2006; EFSA, 2013a).

In Europe, an environmental risk assessment has been required for pesticides since 1991 and for veterinary medicines since 1996 (Brauman et al., 2007; Nienstedt et al., 2012). This section describes the approach used to assess risks Regulatory ERAs are typically based on single species toxicity data derived from laboratory tests with standardised test species and protocols (Harrass, 1996). Such studies are limited by their lack of realistic community dynamics and abiotic conditions, but are reliable and repeatable and therefore provide a base with which to extrapolate to natural ecosystems within the assumption of being conservative with risk assessment (EFSA, 2013). Standard test species (e.g. *Daphnia magna*) are selected as they are generally sensitive to chemical toxicants and capable of being reared and tested in a laboratory environment (Craig et al., 2012; Wu et al., 2007). Standard toxicity tests have defined endpoints; most commonly this is the concentration causing 50% effects for acute studies, although other percentiles may be used (as effect concentration x%, ECx, or lethal concentration for x%, LCx), and for chronic studies either the concentration at which no effects were observed (NOEC) or an ECx (where x is typically 10%) are commonly used (ECHA, 2008; EFSA, 2013a). If refinement of the toxicity data is required, additional (higher tier) approaches may be adopted that increase the realism of the studies either by altering the study environment (biotic and abiotic) or altering the chemical exposure regime from a continuous to a variable exposure regime that reflects exposure patterns that occur in the real environment (Boxall et al., 2002; EFSA et al., 2018b). However, despite these methodological alterations, there remains considerable uncertainty when extrapolating from laboratory or semi-field studies to natural ecosystems. These uncertainties increase when accounting for future climate change conditions in the assessment of ecological risk. Where possible, a precautionary approach is used in ERAs in conjunction with assessment factors to address uncertainties associated with extrapolating from standard toxicity tests to potential effects on natural ecosystems (Hickey et al., 2009; Kuemmerer et al., 2019; Metz and Ingold, 2017). Such assessment factors are intended to cover all uncertainties, including from study design, repeatability of studies, and the variation in the sensitivity and exposure across natural ecosystems.

1.2.3. Communities under agrochemical stress

Communities consist of multiple species and the community-level effect of chemical exposure will be a function of toxicity to individual organisms and the consequences of interspecific and intraspecific interactions (Preston, 2002; Relyea and Hoverman, 2006). The structure and composition of communities varies spatio-temporally and this complexity makes fully understanding and predicting the effects of chemical exposure on natural communities difficult. Regulatory ERAs address this complexity by considering broad taxonomic groups of organisms (e.g., invertebrates, fish, algae, microbes) and identifying acceptable levels of risk for each group. The assumption being that, if risk to these major groups is acceptable, then risk for the entire community the risk will also be acceptable (EFSA, 2010; Ibrahim et al., 2013).

The initial tier of ERAs utilises the toxicity endpoint of a single sensitive species in each of the taxonomic groups of interest (EFSA, 2013a). For freshwater risk assessment the major taxonomic groups and species are: invertebrates (cladoceran e.g., *Daphnia magna*), vertebrate (fish e.g., *Oncorhynchus mykiss*), and algae (unicellular green algae e.g., *Raphidocelis subcapitata*). This endpoint is used to assess the entire group with assessment factors used to represent the diversity in toxicity and uncertainty of different species' responses to a chemical. The greater the uncertainty, the larger the assessment factor. Several approaches can be used to make the data package more robust. Uncertainty associated with interspecific variation in sensitivity may be addressed by testing additional species. These data can then be used to generate species sensitivity distributions that more accurately reflect the range of sensitivities in natural ecosystems (Aldenberg and Jaworska, 2000). Uncertainty associated with species interactions and interspecific variation in sensitivity can also be addressed using multispecies studies (e.g., microcosms and mesocosms) that include population interactions and dynamics across a range of scales and stressors (Birk et al., 2020; Juvigny-Khenafou et al., 2021). Mesocosm studies that aim to replicate natural ecosystems exhibit high ecosystem specificity with the reduction of applicability to other community types (Miko et al., 2015). Mesocosm studies are viewed as having the lowest uncertainty when extrapolating to natural ecosystems and accordingly have the smallest assessment factors.

1.3 Implications of climate change on agrochemical risk

1.3.1 Meteorology

Increased greenhouse gases in the atmosphere retain heat, but complex interactions within meteorological systems affect a plethora of climatic conditions beyond warming alone (Lowe et al., 2018; Watts et al., 2015). Consequently, climate change has and will continue to alter hydrological and atmospheric cycles, ultimately affecting all meteorological conditions, each of which can feedback onto other climate conditions (Lucarini et al., 2017). Although complex, improved meteorological modelling has shown that the effects of climate change will vary depending on the time of year (e.g. by season) and spatially down to a local level (e.g. down to 2.6 km scale in the UK, Lowe et al., 2018). In addition a greater frequency of extreme events is projected (Kennedy-Asser et al., 2021; Min et al., 2011). On a global scale, effects from climate change vary; for example temperature changes are more pronounced towards the poles, yet spatial variation exists across all scales and down to sub-national levels (Clem et al., 2020; Maclean et al., 2017). Therefore, the effects of climate change on chemical exposure and effects must be considered within the context of climatically relevant spatial and temporal scales.

For example, the UK climate is warming with the long-term average for 2010-2019 being 0.9°C higher than the long-term average for 1961-1990. The change in long-term, average temperature has been greatest for spring (1.1 °C) and smallest for winter (0.7°C) (Kendon et al., 2020). Climate projections for the UK (Kennedy-Asser et al., 2021; Lowe et al., 2018) suggest that this trend will continue with a greater frequency of warmer weather and hot summers being more common. The increased warming is expected to be greatest in the southern UK. The UK has also become wetter, with the recent decade (2010-2019) being on average 5% wetter than 1961-1990 (Kendon et al., 2019). However, this is seasonally dependent, with climate projections suggesting increased rainfall in the winter, but lower rainfall in the summer (Watts et al., 2015). There will also be an increase in extreme precipitation notably due to convectional rainstorms primarily in spring and summer (Kendon et al., 2014). The effects of climate change on precipitation are more pronounced towards the southern and western parts of the UK. Overall, the projections trend towards a higher frequency of warmer and wetter winters and a greater frequency of hotter and drier summers.

1.3.2 Agriculture under climate change

Climate change will have a multitude of effects on agriculture and on the inputs of chemicals to agricultural ecosystems. Warmer temperatures will affect growth rates and phenology of the crops, but also of the pest species associated with them (Gomez-Zavaglia et al., 2020; Tirado et al., 2010; Ziska and McConnell, 2016), potentially affecting the economic viability of certain crops. Future temperature and precipitation changes will mean that crops will be associated with different profiles of pests and diseases and therefore different pesticide regimes will be required (Bajwa et al., 2020; Teixeira et al., 2018; Zimmermann et al., 2017). These effects of climate change will alter the types of the agrochemicals used, when in the year they are applied and the quantity required to treat crop infestation (Craufurd and Wheeler, 2009; Gericke et al., 2010; McCauley et al., 2018).

Higher temperatures also increase fertilizer breakdown, necessitating an increase in fertilizer application, including of manure and biosolids, and any associated emerging chemical pollutants with them (Di and Cameron, 2004). Decreased precipitation, particularly in summer, will necessitate the use of increased irrigation, potentially introducing further chemicals present in water sources (Gondim et al., 2012; Kinney et al., 2006; Ternes et al., 2007; Woznicki et al., 2015). Increases in the use of treated wastewater for irrigation, in response to water scarcity issues resulting from climate change, will increase the loadings of chemical pollutants to agricultural soils (Carter et al., 2019). These changes will alter the timing and quantity of agrochemical applied and must be assessed alongside emission scenarios for emerging chemical pollutants, such as with veterinary medicines and pharmaceuticals that are not currently considered within ERAs (Brooks et al., 2013; EFSA et al., 2020). Consequently, there is a need to adapt existing methodologies to quantify the effects of shifting and novel chemical emissions.

1.3.3 Fate & behaviour under climate change

The fate and behaviour of chemicals transported into freshwater is highly dependent on the physical properties of the landscape, meteorology and the properties of the chemical itself (Lorenz et al., 2017). The extent to which climate change will affect the quantity of chemicals in freshwater ecosystems is dependent on the pathway of introduction and transportation of the chemical from agricultural land to freshwaters. For example, transport of sprayed chemicals may be affected by changes to wind conditions under climate change. However current regulations restrict spray application under windy conditions and therefore future changes to wind patterns under climate change may alter the opportunities to apply chemicals rather than change the transport of chemicals that are applied (Arvidsson et al., 2011; Silva Maciel et al., 2018).

In contrast, chemical movement through soil and water is highly dependent on meteorology with precipitation being a major trigger for chemical movement and temperature affecting degradation processes (Biswas et al., 2018; Gagnon et al., 2016). For such modes of transportation, assessing how climate change will affect chemical movement is particularly important for assessing ecological risk. For example, wetter winters may lead to more frequent runoff and therefore increased exposure events while warmer temperatures could increase chemical degradation (Kallenborn et al., 2012; Valle et al., 2007). However, research into these pathways must account for spatial and seasonal effects of climate change and chemical application if a realistic understanding of risk is to be calculated. In particular there is need to develop a framework for quantifying by how much climate change may affect risk that can be applied both to current agrochemicals as well as being adaptable to new chemical discoveries and emerging chemical pollutants of potential risk to the environment. For example, chemical fate models used extensively in regulatory frameworks can be used for a range of chemicals and climate conditions by altering the environmentally relevant chemical properties and meteorological inputs.

1.3.4 Freshwaters under climate change

Climate change may alter the physical properties of freshwater ecosystems in multiple ways. River water volume and flow will alter in line with precipitation, with the location specific increase or decrease depending on the season (Henriques et al., 2015; Planton, 2002). Furthermore, an increase in extreme weather events will results in more periods of both flooding and drought with associated short-term changes to volume and flow (Pall et al., 2011). For example, UK climate projections (Lowe et al., 2018; Met Office, 2019) have been used to provide higher resolution projections on how climate change may impact river flows and flood peaks (Kay et al., 2021). Whereas, in general, there is a predicted increase in 20-year return flood peaks with climate change, the changes are generally smaller in the south-east than in the north-west. With respect to flow, low- and mid-range flows are predicted to decrease, but high flows are generally predicted to increase, particularly during winter and in upland areas (Burt and Ferranti, 2012; Hannaford and Buys, 2012). Flow changes vary between catchments partly due to spatial variation in climate change but also because of variation in catchment characteristics that influence the response to climatic inputs, such as soil and riverbed substrate and river width depth and flow.

As climate change increases ambient air temperatures, the temperature of waterbodies also increases, although the exact relationship is dependent on both the physical characteristics of the river and of land and water management strategies. This trend is historical, with records indicating an increase in UK river water temperatures of 0.29°C per decade (Orr et al., 2010). As for future predictions, (Jackson et al., 2018), predicted that a 1 °C increase in summer maximum air temperature would result in between a 0.4 and 0.7 °C rise in maximum water temperature of UK rivers depending on location, with the most sensitive rivers to changing air temperatures being those in the north and north-west.

Multiple chemical properties, particularly degradation in the water column and sediment, affect chemical fate and exposure and will alter under increasing river temperatures (Cooney et al., 1985; Daam and Van den Brink, 2010; Warren et al., 2003). Consequently Szalinska et al. (2021) have incorporated these changes into in freshwater fate models alongside climate projections for a case study watershed. However, rising temperatures will have effects on both how susceptible organisms are to pollutants and their niche compatibility, both affected by their ability to withstand climatic changes (Bruder et al., 2017; Kidd et al., 2014). How those changes influence other organisms and therefore community interactions will then alter the sensitivity of the ecosystem over multiple scales. Full consideration of these interacting factors and how these may be quantified for integration within an ERA framework is required to accurately assess whether ecosystems are at risk and if chemical use should be altered (Brooks et al., 2013; EFSA et al., 2020). The existing research on these is discussed below.

1.3.5 Community under climate change & agrochemical sensitivity

Macroinvertebrate communities

Studies of the potential impact of drought on macroinvertebrate communities have either compared permanent and intermittent rivers (Stubbington et al 2009; Hill et al 2019; Majdi et al 2020) or used mesocosms (Aspin et al 2019). The riverbed and hyporheic zone provide a refuge for some river invertebrates and many species adapted to intermittent rivers have drought resistant life history stages. Habitat fragmentation, loss of connectivity and very low water levels may have major adverse effects on community structure and function. For example, reducing water depth to 1 cm decreased leaf decomposition by macroinvertebrate shredders by approximately 75% (Hill et al 2019).

Long-term monitoring datasets have been used to explore the impact of climate change on river macroinvertebrate communities. A 2.0-2.4 °C increase in water temperature observed over 25 years in Welsh upland rivers was associated with an average 21% decrease in macroinvertebrate abundance (Durance & Ormerod, 2007). However, relationships between increased water temperature and macroinvertebrate community change are not observed in all rivers studied, due to local confounding factors, including improving water quality, masking any temperature effect (Durance & Ormerod 2007, 2009; Floury et al 2013; Vaughan & Ormerod, 2014). Consequently, assessing the effects of climate change must be considered within the ongoing changes to adjacent agricultural practice and chemical regulation and use, and how changes in both climate change and chemical exposure contribute to shifts in macroinvertebrate communities through multiple, differing and potentially opposite mechanisms.

Freshwaters are subjected to multiple stressors, both natural and anthropogenic, and many changes are likely to have occurred within a catchment over several decades. There are relatively few field manipulations of water temperature and laboratory studies tend to focus on the responses of species rather than changes in communities (Leberfinger et al 2010; Briffa et al 2016). Field manipulations include studies that have adjusted the thermal regime of rivers either by heating river water (Hogg et al 1995) or by exploiting the warming of rivers caused by the discharge of heated water from power stations (Majdi et al 2020). These manipulation studies confirm that small changes in temperature (1.5-3.0 °C) can have significant impacts on the abundance and composition of river invertebrate communities. Indeed, within the UK long-term invertebrate monitoring has observed community shifts, and temperature is known to be a factor influencing communities within the River Invertebrate Classification Tool (RICT) which projects the expected macroinvertebrate river communities based upon the characteristics of the river (Environment Agency et al., 2021; Wright, 1994). While RICT is primarily used for ecological water quality assessment, it also has been proposed for other uses including macroinvertebrate community prediction (Clarke et al., 2003; Wright et al., 1998), and could be integrated with other data, for example climate change temperature conditions.

Community level chemical sensitivity

Understanding the community level effects of climate change and agrochemical exposure is complicated by the extensive interactions between species within communities and the spatial and temporal variation in species composition (Morecroft et al., 2009; Woodward et al., 2010). It has been described previously (Section 1.3.4) that community composition and structure will alter under climate change conditions, and has already done so within the UK (Burgmer et al., 2007; Chiu et al., 2017; Durance and Ormerod, 2007). Each member of the community is a contributing factor to the sensitivity of the ecosystem, either through its response to a chemical stressor or any alteration to community interactions (Belanger et al., 2017). Because species vary in their sensitivity to chemicals (Section 1.2.3) any changes to community composition due to climate change will also affect how the entire ecosystem responds to chemical stress, although this may depend on how sensitive the most sensitive members are and the connectivity of that species within the community (Bruder et al., 2017; Craig et al., 2012). The relative sensitivity of species is known to be chemical specific (Cairns, 1986; Hoekstra et al., 1994) and with different species compositions it would be expected that the sensitivities of a community would be different for depending on chemical identity. Furthermore, it has been established that climate change will shift the community composition (Burgmer et al., 2007; Vaughan and Ormerod, 2012; Zettler et al., 2013). Given variation in species sensitivity depends on chemical identity, differing combinations of species within varying natural community compositions would be expected to exhibit variable chemical sensitivities. As climate change will shift macroinvertebrate community compositions and species identity affects sensitivity, climate change may also be expected to affect the sensitivity of macroinvertebrate assemblages by shifting community composition.

As described in Section 1.2.3, regulatory ERAs are based on standardised laboratory toxicity tests and occasionally mesocosm studies. ERAs generate a single sensitivity value quantified as a regulatory acceptable concentration (RAC) for all ecosystems and do not account for temporal or spatial variation in community composition and hence sensitivity (Brock et al., 2006; EFSA, 2013). Thus, the RAC is unrepresentative of the diversity of species present in natural communities and does not capture the variation in chemical sensitivities between species. RACs in current ERAs are derived from a set group of unchanging laboratory test species or mesocosms performed in ambient temperatures. Consequently, how climate change affects community composition and how this will affect risk cannot be assessed using the data available as part of existing ERAs alone.

The major challenge in accounting for changes in species composition when assessing the chemical sensitivity of communities is the lack of toxicity data for most species in the communities of interest. If the sensitivity of untested species could be predicted then existing techniques for assessing the sensitivity of species assemblages, such as species sensitivity distributions, can be applied to generate a sensitivity value of a specific species assemblage and chemical combination (Aldenberg and Jaworska, 2000; Newman et al., 2000). One solution to this is to employ species sensitivity extrapolation methods, which can be used to predict the sensitivity of untested species to specific chemicals. By combining with existing toxicity data, such methods can extrapolate across to untested species; however a range of methods exist including those based on traits, relatedness and genomics, each with their own data demand (van den Berg et al., 2021).

Genomics based methods can compare the genetic basis of biochemical pathways and enzymes of species using the molecular target site of chemical toxicity through adverse outcome pathways and therefore represent the most accurate method of extrapolation (Ankley et al., 2010; LaLone et al., 2013). However, genomics-based methods require the adverse outcome pathway to be fully understood and the sequenced genome of each species, and thus have a large data requirement meaning these methods are more suitable for the smaller number of species higher up food chains (e.g. fish, mammals, birds), rather than diverse groupings such as macroinvertebrates (Farmahin et al., 2012; Fedorenkova et al., 2010). Trait based methods have a lower data requirement that only requires major traits of the untested species be known (e.g. body size, diet, location within freshwater) and has been applied to pre-defined communities (Van den Berg et al., 2019). While the coverage of trait databases is building, they have incomplete coverage of macroinvertebrate species. Most notably they are dominated by Copeopod and Cladoceran taxa (87.0%, or 341 of 392 macroinvertebrate species in Hébert et al., 2016; Usseglio-Polatera et al., 2000) leaving many taxonomic groups, especially outside Crustacea, poorly represented. In contrast, UK freshwater invertebrate species are extensively sampled and identified with complete taxonomic structure (Wright et al., 1996). This leads to relatedness-based methods being the most broadly applicable currently to macroinvertebrate communities of the UK (noting the advantages that currently undeveloped methods combining traits and relatedness may offer), and could be adapted for use with current, historic and projected macroinvertebrate assemblages.

1.3.6 Multi-stressor effects on organisms

Warming is one of the main predicted impacts of climate change and both increased temperature and chemical exposure may result in adverse effects on organisms (Noyes and Lema, 2015). Higher temperatures reduce the oxygen content of water and hence increase ventilation rates of aquatic organisms, which in turn may increase the uptake and movement of the chemical into the organism, potentially worsening toxic effects (Op de Beeck et al., 2017; Pereira et al., 2019). However, increased temperatures also increase the degradation of chemicals and the metabolic rates of poikilotherms, potentially increasing the depuration of absorbed toxicants thereby reducing risk (de Beeck et al., 2017; Norhave et al., 2014; Pereira et al., 2019). The effect of changing temperature on organism chemical sensitivity represents the most extensively studied direct effect of climate change on organism sensitivity. However, multi-stressor effects at the organism level are complex with the effects of temperature on an organism and its internal metabolic processes varying between species and chemicals (Nieto et al., 2016; Rathore and Khangarot, 2002; Willming et al., 2013). However, organism status is an important factor, and multigenerational effects of evolution and long-term acclimation and adaptation of organisms exposed to multiple stressors are observed (Contador et al., 2014; Macaulay et al., 2021; Tran et al., 2019). Consequently, understanding how temperature and chemical sensitivity changes for the diversity of species present in natural ecosystems is a challenge and requires investigation from the population scale to molecular pathways (Knillmann et al., 2013).

Most multi-stressor studies have investigated sudden short-term temperature changes (de Beeck et al., 2017; Malaj et al., 2016; Op de Beeck et al., 2018). Such findings from temperature shock experiments may provide insight into the combined effect of chemical exposure and heat waves, but it is less certain how the findings from these studies relate to the combined effects of chemical exposure and warming events that occur over the lifetime of multiple generations of a species Long-term effects of climate change, such as shifts in mean annual river temperature, occur gradually over decades (Watts et al., 2015). This timeframe will span multiple generations of macroinvertebrates providing an opportunity for species to acclimate or adapt to the changing conditions. While a few studies have investigated the effect of short-term acclimation and toxic effects over multiple generations (Bae et al., 2016; Silva et al., 2020), there is a major knowledge gap over the effect of gradual temperature acclimation of multiple generations on chemical sensitivity.

1. Aim and objectives

The aim of this thesis is to explore the implications of climate change for the exposure and sensitivity of freshwater ecosystems to agrochemicals, focusing on the pathways shown in Figure 1.2. This aim is addressed by considering different aspects of the environmental risk assessment (ERA) under climate change conditions before contextualising with chemical case studies in chapter 5.

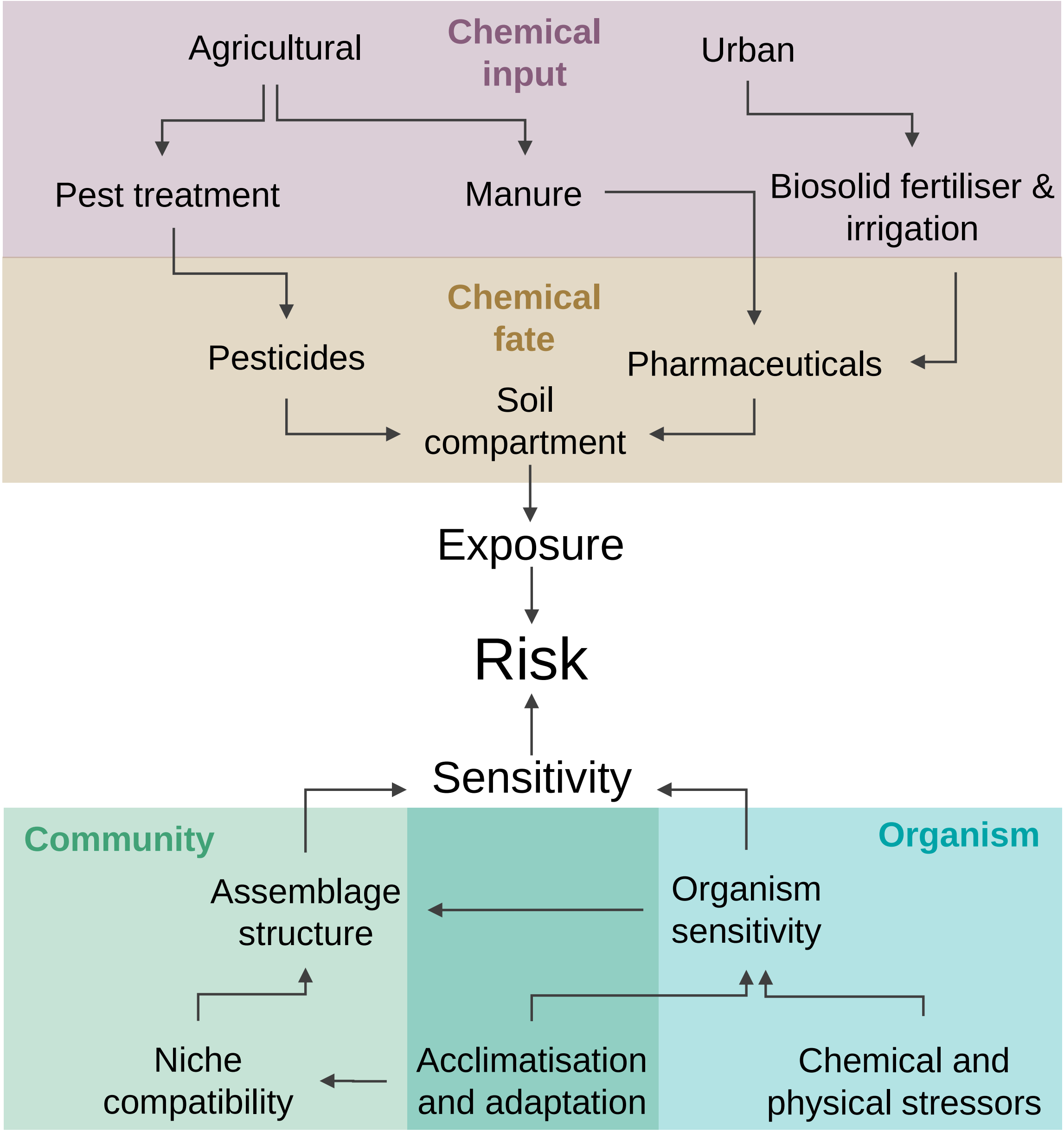

Figure 1.2 Aspects from the environmental risk assessment for chemicals considered within this thesis upon which climate change can interact to affect risk

Chapter 2 addresses how climate change interacts with chemical flux from agricultural land to freshwater and utilises UKCP18 data from the UK Met Office and the PRZM fate modelling program to quantify flux into freshwaters. The chapter considers the application of 24 pesticides and pharmaceuticals to six locations across each of the climate groups within England & Wales and its main objectives are:

- Establish how temperature and precipitation (meteorology expected to alter under climate change) will affect chemical flux via soil for hypothetical chemicals with varying persistence in soil
- To what extent are the relationships established in the previous objective relevant to actual climate conditions, chemical properties and sites under baseline and future climate change
- Assess how the season of application for a chemical affects chemical flux under climate change conditions given the different degradation rates of chemicals
- Quantify the effect of climate change on chemical flux via soil for agrochemicals applied under realistic timings

Chapter 3 addresses assemblage sensitivity utilising the RICT model and reference data set with the hSSD model to predict toxicity values for species without toxicity data for both baseline and future communities. The associated objectives are:

- Run the hSSD model for the collated toxicity datasets and evaluate the ability of the hSSD model to accurately predict sensitivity of taxa
- Assess to what extent the sensitivity between assemblages varies and the variation in sensitivity patterned across chemicals with different toxic modes of action
- Assess whether the sensitivity of an assemblage relates to the physical landscape characteristics of where that freshwater body where the assemblage is located
- To quantify how the shifting of community composition under climate change alters the assemblage sensitivity

Chapter 4 considers gradual changes to organism sensitivity under climate change by a case study of how the growth and reproduction of *Daphnia* gradually acclimatised to different temperatures is affected by copper exposure. The laboratory experiment aims to reflect the gradual changes expected from climate change rather than the short period of acclimatisation and temperature shock common to most toxicity studies. The associated objectives are:

- Determine to what extent does temperature acclimation affect organism reproductive parameters and reproductive strategy of organisms, and are the same patterns observed following temperature shock
- Calculate endpoints for reproductive parameters for organisms experiencing rises in temperature, and does acclimation mitigate the multi-stressor effect
- Evaluate whether the magnitude of thermal shock affects the relationship of the multiple-stressor effects on reproduction

Chapter 5 sets to draw a broad conclusion over the effect of climate change on agrochemical risk by considering case study ERAs accounting for the implications from the previous chapters as well as integrating the previous finding into a general framework of how climate change could be considered within an ERA. It does so by considering three case study chemicals: glyphosate, a chemical investigated in Chapters 2 and 3; copper which was investigated in Chapter 3 and 4, and a theoretical new chemical to show how the framework can be adapted beyond the agrochemicals used within this thesis.

CHAPTER 2

Impacts of weather and chemical persistence on freshwater flux through soil under climate change conditions

Chapter 2. Impacts of weather and chemical persistence on freshwater flux through soil under climate change conditions

2.1. Introduction

2.1.1. Meteorology, climate change and chemical fate

When considering the fate and behaviour of chemicals applied to agricultural land, localised weather represents a major factor affecting movement, and in particular, precipitation represents the largest determinant of transport as it triggers and directly moves chemicals through the soil matrix (Hutson, 1993; Lewan et al., 2009; Nolan et al., 2008). When considering the exposure of surface waters, the major routes of transport are via spray drift and through soil, with soil based transport tending to be the predominant route (Bach et al., 2016; Zhang et al., 2018). Spray drift is highly wind dependent, and relatively unaffected by other meteorological conditions, and as such agrochemical application is frequently limited if local wind speeds are too high (Arvidsson et al., 2011; Gil et al., 2015; Silva Maciel et al., 2018).

In contrast, transport of chemicals through the soil matrix into freshwaters is driven by water and is highly affected by multiple meteorological conditions. Temperature, humidity and wind speed affect evapotranspiration and therefore soil moisture content, while precipitation introduces water (and any agrochemical solute) to the soil saturating it. Precipitation is highly important to chemical fate as it causes water flow in saturated soil triggering runoff of the chemical dissolved in the solvent or in erosion of soil particles that the chemical has bound to (Bach et al., 2016; Winton and Weber, 1996). In addition to the precipitation events, other meteorological factors impacting chemical fate are ambient air temperature, which affects chemical degradation (Matthies and Beulke, 2017; Valle et al., 2007), and the water content of the soil, which influences the amount of precipitation required to cause water flow (Lewan et al., 2009).

While an increase in temperature retention by greenhouse gasses is the ultimate cause of climate change, complex alterations in atmospheric movements and the water cycle will result in climate change affecting multiple meteorological factors beyond temperature, including precipitation and wind movements (Lowe et al., 2018; Stocker, 2014). Changes in temperature, precipitation and wind movements, in turn affect humidity, evaporation rates and solar flux via feedback networks (Bony et al., 2006; Cess, 1976). Many of the meteorological factors that will alter under climate change are also important for determining chemical fate (Table 2.1). From this, climate change will potentially alter flux into freshwater, posing a potential for both an increase and decrease in edge of field flux. Consequently, there is a need to evaluate the combined impact of all these factors which could affect chemical fate to fully assess the effect of climate change on chemical exposure.

Table 2. 1 Summary of chemical fate affecting factors of the agricultural land and its associated meteorology. How such factors may be affected by climate change conditions is described and the likely effect of how climate change may affect the quantity of chemical flux reaching freshwater ecosystems predicted. Note that effects are simply increases (+) or decreases (-) and do not indicate the expected magnitude of change.

Factor	Fate process affected	Effect of climate change	Climate change process	Effect on chemical exposure	References
Climate – temperature	Degradation	+	Higher temperatures increase the kinetic energy of chemical reactions, including degradation reducing the quantity of chemical in the soil system to be transported	-	Biswas et al., 2018; Cáceres et al., 2008; Cavoski et al., 2008; Delnat et al., 2021; Ficklin et al., 2010; Kookana et al., 2010; Matthies and Beulke, 2017; Noyes et al., 2009; Valle et al., 2007
Climate – winter precipitation	Runoff, erosion	+	Increased winter precipitation directly increases the amount of water in the soil compartment increasing soil saturation and the volume of precipitation required to trigger runoff and erosion events	+	Bloomfield et al., 2006; Dunn et al., 2012; Ficklin et al., 2010; Oliver et al., 2012
Climate & extreme weather – summer meteorology & drought	Runoff, erosion	+	Decreased summer precipitation, increased temperatures and increased solar flux from reduced cloud cover, reducing the amount of water in the soil compartment by direct input and evaporation meaning greater precipitation is required for saturation and triggering of flow	-	García-Prieto et al., 2012; Hrdinka et al., 2012; Miralles et al., 2012; Mosley, 2015; Whitehead et al., 2009
Extreme weather – precipitation	Runoff, erosion	+	More frequent and larger water surges through the soil compartment, particularly during summer from convection, trigger large runoff and erosion events	+	Enanga et al., 2016; Hrdinka et al., 2012; Hudak and Banks, 2006; Mayes et al., 2021; Whitehead et al., 2009

Soil – fissurisation	Runoff, erosion	+	Warmer temperatures increase occurrence and length of soil fissures permitting more flowthrough	+	Correa et al., 2019
Crop – drilling	Interception	Later	Warmer temperatures permit the drilling of crops later resulting in less crop interception	+	Craufurd and Wheeler, 2009; Pawel Marcinkowski and Piniewski, 2018; Olesen et al., 2012; Zimmermann et al., 2017
Crop – maturation	Interception	Earlier	Higher temperatures mature crops faster, covering more soil and increasing interception	-	
Crop – harvest	Interception	Earlier	Earlier harvesting results in longer period of exposed soil	+	
Irrigation	Application rate, runoff, erosion	+	Reduced summer precipitation and increased drought rates may necessitate use of irrigation. This may also introduce chemicals from treated water to freshwater	+	Gondim et al., 2012; Woznicki et al., 2015

In addition to the varied effects of climate change on flux into freshwaters, both existing climates and the effects of climate change exhibit spatial and temporal variation on global (Ruosteenoja et al., 2003) and local scales (Kendon et al., 2020; Lowe et al., 2018). This results in climate change altering both the general climate of the area as well as an increase in the frequency of extreme weather events, including extreme rainfall and storm events (Hirabayashi et al., 2008; Min et al., 2011; Ohba and Sugimoto, 2019) and of periods of drought and heatwaves (Hirabayashi et al., 2008; Stott, 2016). Consequently, any consideration of the risk posed by agrochemicals and how this will alter under climate change conditions must consider the local effects of meteorology.

While climate change can affect the transport of agrochemicals through soil directly by changing meteorological parameters (Berg et al., 2017; van der Putten et al., 2016), the behaviour of agrochemicals themselves and how they interact with the soil matrix under climate change conditions must also be considered (Steffens et al., 2015; Szalinska et al., 2021; Valle et al., 2007). This is because the combination of chemical properties, climate and soil determine fate. Climate change effects depend on specific soil and agrochemical properties resulting in variation in fate within and between agrochemicals in response to climate change (Kookana et al., 2010; Lammoglia et al., 2018). A summary of the expected results of climate change on soil and chemical properties is presented in Table 2.2. Given that effects of chemical properties on flux are not independent of chemical type and interact with meteorology, there may also be an interaction between effects of climate change and chemical properties. Consequently, considering how climate change will affect chemical flux with chemical properties will require isolating specific properties of interest that are most impactful.

Degradation of the chemical, by microbial activity, hydrolysis, photolysis or spontaneous breakdown within the soil matrix represents the largest ultimate fate of agrochemicals (Jackson, 2004; Xuan et al., 2008). Thus, the DT_{50} (reported as degradation rate in days for the chemical to reach half of its initial mass in soil at 20°C in standard laboratory studies) of a chemical is a major factor for influencing flux. However, degradation reaction rate is temperature dependent and therefore likely to increase under climate change (Cáceres et al., 2008). As both the chemical's DT_{50} value and the environmental temperature represent how persistent the chemical will be in the soil matrix, the increased temperatures from climate change may increase degradation rate, reducing the quantity of chemical present leading to decreased chemical flux (Cavoski et al., 2008; Matthies and Beulke, 2017).

In contrast, precipitation-induced chemical transport is affected by multiple chemical properties. Highly soluble, low molecular weight chemicals are more easily moved by water flow. In contrast, chemicals that bind strongly to organic matter (high k_{OC}) will therefore bind more strongly to organic matter in soil reducing movement by runoff. Any erosion and movement of the soil itself can transport large quantities of such high k_{OC} chemicals. Although several environmental, soil and meteorological factors affect chemical fate, chemical flux into freshwaters is driven by two factors: the persistence of the chemical in the soil matrix (a function of chemical degradation rate and temperature) and the occurrence of extreme precipitation events (Nolan et al., 2008; Steffens et al., 2015; Webster et al., 2004).

Table 2.2 Comparison of major environmental flux relevant chemical and soil properties, and how they may alter chemical flux under the meteorological changes to temperature and precipitation

Property		**Expected effect on exposure**		**References**
		Increased temperature	Increased precipitation	
Chemical properties	DT_{50}	Decrease, due to faster degradation	Predominantly unaffected	Cáceres et al., 2008; Cavoski et al., 2008; Kookana et al., 2010; Ma et al., 2004; Matthies and Beulke, 2017; Noyes et al., 2009; Valle et al., 2007; Wolt et al., 2002
	k_{OC}	Increase, due to decreased binding to soil enabling increased mobility	Increase, more flushing of chemicals bound to carbon	Farenhorst et al., 2009; Oliver et al., 2012; Paasivirta et al., 1999; Wolt et al., 2002
	Molecular weight	Predominantly unaffected	Increase, more flushing of low-mobility heavy chemicals	Mangas et al., 1998
	Solubility	Increase, greater concentration can be dissolved	Increase, more fluid to dissolve into	Paasivirta et al., 1999; Wolt et al., 2002
Soil properties	Bulk density	Increase in due to drying and compaction, reduces water holding capacity of the soil	Increase, saturated ground triggers runoff more rapidly	Correa et al., 2019; Ma et al., 2004; Wolt et al., 2002
	Organic carbon percent	Increase, due to decreased chemical binding soil enabling increased mobility	Predominantly unaffected	Cáceres et al., 2008; Conant et al., 2011; Farenhorst et al., 2009

2.1.2. Agrochemical fate and freshwaters in the UK

Agrochemicals are a vital component of food production and farming systems within the UK, but the transport of such agrochemicals beyond the field can have adverse effects on biodiversity, with freshwaters adjacent to agricultural land representing an ecosystem often exposed and shaped by agrochemical pollution (Lorenz et al., 2017; Malaj et al., 2014; Nordborg et al., 2017). When considering chemical transport through soil, adjacent freshwaters represent high risk ecosystems subject to pollutant exposure with chemical flux causing toxic effects to freshwater species (Berger et al., 2017; Ceschin et al., 2021; Reid et al., 2019).

Agrochemicals fall into a series of broad groups relating to their use. For example, pesticides represent a broad collection of chemicals including both organic compounds and metals and are grouped by their target organism e.g. fungicides, insecticides, herbicides, while pharmaceuticals see input in manure, irrigation water and biosolid fertiliser following their use in livestock and humans (Fijalkowski et al., 2017; Pollard and Morra, 2018; Wu et al., 2010). Given these differing uses, the time of the year when application to agricultural land occurs will vary depending on the chemical. The timing of chemical application is important as the UK exhibits seasonal climates with precipitation and particularly temperature varying throughout the year. Consequently, the timing of applications will match that of the crop and pest phenology (Bajwa et al., 2020; Rhodes and McCarl, 2020a).

For example, in the UK, rising temperatures and changes to precipitation and humidity will increase crop susceptibility and spread of fungal infections in spring and summer (Lasram et al., 2010; West et al., 2012a), and favour the growth of weed plants necessitating earlier herbicide application. For insects, the population and range of agricultural insect pests has increased with pest outbreaks occurring earlier in the year (Kattwinkel et al., 2011; Ladányi and Horváth, 2010). However, climate change has also increased the numbers of specific insect natural predators and parasitoids (Thomson et al., 2010). These changes have been associated with both increases and decreases to insecticide use depending on the type and location of crop grown. Equally, the shifting in crop phenology will mean application of pre-drilling insecticides will move to match later crop drilling while herbicide desiccants are being applied earlier matching the shift to earlier summer harvesting times (Craufurd and Wheeler, 2009; Gericke et al., 2010; Olesen et al., 2012).

More recent understanding of emerging chemical pollutants and chemical fate pathways have also highlighted the risk posed by pharmaceuticals entering agricultural systems via livestock manure or biosolids as fertiliser or water used for irrigation (Michael et al., 2013; Wu et al., 2010). Fertilizers are applied in autumn and spring prior to crop emergence and during major growth phases (Liu et al., 2015; Sarmah et al., 2006), while irrigation will occur during periods of drought, the frequency of which is set to increase under climate change (Gondim et al., 2012; Woznicki et al., 2015). While climate change will have impacts on the timings and application of agrochemicals, there is also a need to understand how the effects of climate change will affect the transport of such chemicals into freshwaters if full consideration of the change to risk climate change poses to ecosystems is accounted for and mitigated.

In addition to climate varying throughout the year, there are also spatial differences in the climate across the UK. Broadly the UK climate is associated with a North-South temperature gradient and an East-West precipitation gradient with higher temperatures occurring further south and greater precipitation in the West (Kendon et al., 2020). Furthermore, there are coastal-inland differences, with coastal areas having their temperatures buffered by the sea resulting in a decreased range of temperatures compared to inland areas. Another spatially varying aspect that can affect chemical fate is the soil and landscape properties: for example, the extent and slope of a field can increase water and soil movement (Morselli et al., 2018). Across the UK there are a wide range of soil types each exhibiting different structures and compositions across their component horizons, with a comprehensive dataset available for the UK as LandIS: The Soils Guide (Cranfield University, 2021). Loamy soils tend to have higher organic carbon content leading to different flux patterns particularly for chemicals with high k_{OC} (Farenhorst et al., 2009). Broadly both sandy and loamy soils tend to permit water flow while clay soils restrict flow. In addition, clay based soils in the UK are commonly drained leading to an additional route of exposure for freshwater ecosystems (Bach et al., 2017; EFSA, 2013a).

How some soil properties that may alter flux under climate change are shown in Table 2.2. Given the large number of landscape, cropping and soil parameters, and how they interact with multiple chemical properties, on top of assessing spatial effects of climate and climate change, the impacts of spatial variation on flux are varied and complex. Thus there is need to isolate factors for consideration by keeping others constant with the ultimate goal of developing scenarios for encompassing the range of risks that spatial variation covers (Blenkinsop et al., 2008; Vernier et al., 2017).

The risk posed by pesticides to freshwater ecosystems is highly regulated within the UK and is assessed within an environmental risk assessment framework (EFSA, 2013a). For pesticides, existing regulation focuses on predicting probabilistic chemical flux based upon models integrating meteorological conditions, chemical properties, and soil and landscape characteristics that have been standardised into generic scenarios. The climatic condition from multiple years is used for several example locations that capture reasonable representative scenarios. Given standardised climatic scenarios are used when calculating fate ERAs and the effect that changing meteorology has on flux, the effect of climate change could drastically alter the quantity of chemical flux and ultimately freshwater chemical exposure. Currently, consideration of how climate change can affect flux has predominantly focused on case study areas that may not be representative of the risk posed under climate change conditions, although there is increasing acknowledgement of the issue beyond academic and research sectors (Biswas et al., 2018; EFSA et al., 2020; Ma et al., 2016; Valle et al., 2007).

In contrast to pesticides, other chemical groups such as pharmaceuticals, do not have the same regulation and environmental risk assessment performed. Furthermore, where there is consideration of agriculturally relevant pharmaceuticals, this generally focuses on the adjacent topic of human health and anti-microbial resistance rather than ecotoxicity (Boxall et al., 2003; Wolstenholme et al., 2004). Consequently, the ecotoxicological risk posed by pharmaceuticals is largely unconsidered and associated with uncertainty, although increasing research on the effects of climate change and pharmaceuticals is being performed (Balbus et al., 2012; Boxall et al., 2009). Therefore, there is a need to develop a methodology that considers the risk for pharmaceuticals and other emerging chemical pollutants in addition to pesticide risk.

2.1.3. Aim and objectives

The aim of this chapter is to consider the effect of climate change on agrochemical fate to UK freshwaters. While multiple meteorological conditions affect chemical fate, two are notable for being the largest contributors to change in fate: precipitation as the trigger of chemical movement and temperature as a major factor of degradation rate (Biswas et al., 2018; Elrashidi et al., 2013). This is because both have extensive effects on chemical flux and are expected to change extensively and spatio-temporally under climate change conditions (Bloomfield et al., 2006; Hawkins and Sutton, 2011). For example, across England, year-round average temperatures are expected to rise by 3.51 °C with temperature extremes increasing by 4.73 °C representing a 24% and 45% increase respectively (Met Office, 2019). Equally for precipitation, maximum precipitation is set to increase by 6.27mm (22% increase), while overall mean precipitation and the number of precipitation days exhibit much less change (1.6% and 1.0% decrease respectively). However, this does not reflect the spatial and seasonal differences under climate change which also affect flux. Ultimately, any consideration of the effect of climate change on chemical flux must be both relevant to and suitable for existing pesticide ERAs while being adaptable and generic enough to consider the risk for pharmaceuticals and other emerging chemical pollutants.

Assessing the effect of climate change on chemical fate can be split into two broad topics of interest: firstly, how the seasonal effects of predicted climate change, particularly precipitation, will alter chemical fate and secondly, what is the relationship between the spatial differences expected under climate change and varying properties of agrochemicals. Therefore, the following objectives and specific research questions have been explored:

1. Establish the relationship between how meteorological parameters that will alter under climate change (temperature and precipitation) will affect flux for a series of hypothetical chemicals with varying persistence in soil
    - Does the relationship between precipitation and flux deviate from a 1:1 relationship?
    - Does the degradation of chemical over time mean that increasing the delay to large precipitation events (and therefore time for degradation to occur) reduce flux?
    - Is the temperature-degradation rate relationship non-linear and variable among chemicals?

2. Establish if the predicted relationships investigated above hold for actual and climatically divergent agricultural sites under both baseline and future climate conditions

    - Does the amount of precipitation that triggers major chemical flux events increase as DT_{50} increases, and to what extent is there climatic spatial variation?
    - Does the delay to the precipitation that triggers major chemical flux events increase as DT_{50} increases, and to what extent is there climatic spatial variation?

    - Given the results of the two above, does the amount of chemical flux from major events increase as DT_{50} increases, and to what extent is there climatic spatial variation?

    - Does the quantity of chemical flux in extreme chemical flux events increase under climate change, and does this vary spatially?
    - Does the degradation rate-flux relationship change under climate change conditions and to what extent is there climatic spatial variation?

3. Consider to what extent does the season of application affect the change in chemical flux under climate change conditions, and how does this vary spatially, and by the persistence of the chemical in soil.

    - Are there variable differences between the change in chemical flux over degradation rate between sites and additionally from differing seasons of application within a site?

- Given the effect of higher DT_{50} values on the delay and size of precipitation event, does the variation of chemical flux between seasons decrease with increasing DT_{50}?
- Does the season of application, and the meteorological events linked to season, affect the relationship between change in chemical flux and the delay to or precipitation amount in the event that triggered it? How does this relationship vary depending on chemical degradation rate?

4. Quantify the effect of climate change on flux via soil with actual agrochemicals with realistic application timings, and is the change in flux patterned by chemical persistence across different sites

Approach to answer research questions

A core requirement to address the research questions is a method to quantify chemical fate from agricultural soil compartments to freshwater. When considering methods for calculating chemical transport into freshwater via runoff and erosion several models have been developed (Jarvis et al., 1997; US EPA, 2016a). Since their initial development in the 1980s, chemical fate models for soil transport have been validated by field trials and have been refined to improve their fate predictions (Bach et al., 2016; Carsel et al., 1985; EFSA, 2006). The predicted chemical exposures from these models have been and continue to be used within ERAs to assess chemical flux to freshwaters via soil and to approve chemicals for use. One such model, PRZM (Pesticide Root Zone Model) calculates edge of field exposure for chemicals transported via runoff and erosion and predicts all the necessary information to address the research questions of this chapter.

PRZM was first developed in 1984 to calculate chemical fate from agricultural land to freshwater (Carsel et al., 1985; Wagenet and Rao, 1990). However, the applicability of the model led to the adoption of PRZM within regulatory environments (EFSA, 2006; US EPA, 2020). A diagram of the inputs and process of PRZM is presented in Figure 2.1. The PRZM model calculates chemical input, transport within the soil matrix and eventual exit from the system by degradation, to freshwater or to groundwater. PRZM calculates input into freshwater from both erosion and runoff and does so daily. In addition, it provides the option to export and water flow and chemical quantity remaining within the modelled compartments (soil at cm divided depths, surface and on crops) to track chemical movement and status.

Importantly, PRZM can accept custom daily climate data, enabling climate change projections to be read into the model and their effects predicted. Because the climate data can be taken at the daily level, extreme localised rainstorm and heat-wave events can be represented in the model that would not be captured with coarser timescales. Other timed events can also be simulated, particularly crop phenology, dates of chemical applications and irrigation. For chemicals, PRZM incorporates degradation rate as half-life (DT_{50}) for multiple contexts including within soil, as utilised in this study, in addition to foliar, water column, photolysis and aquatic sediment. Furthermore, the range of environmentally relevant properties used by PRZM to calculate chemical fate includes the molecular mass, solubility in water, Henry's coefficient and organic carbon partitioning coefficient of the chemical.

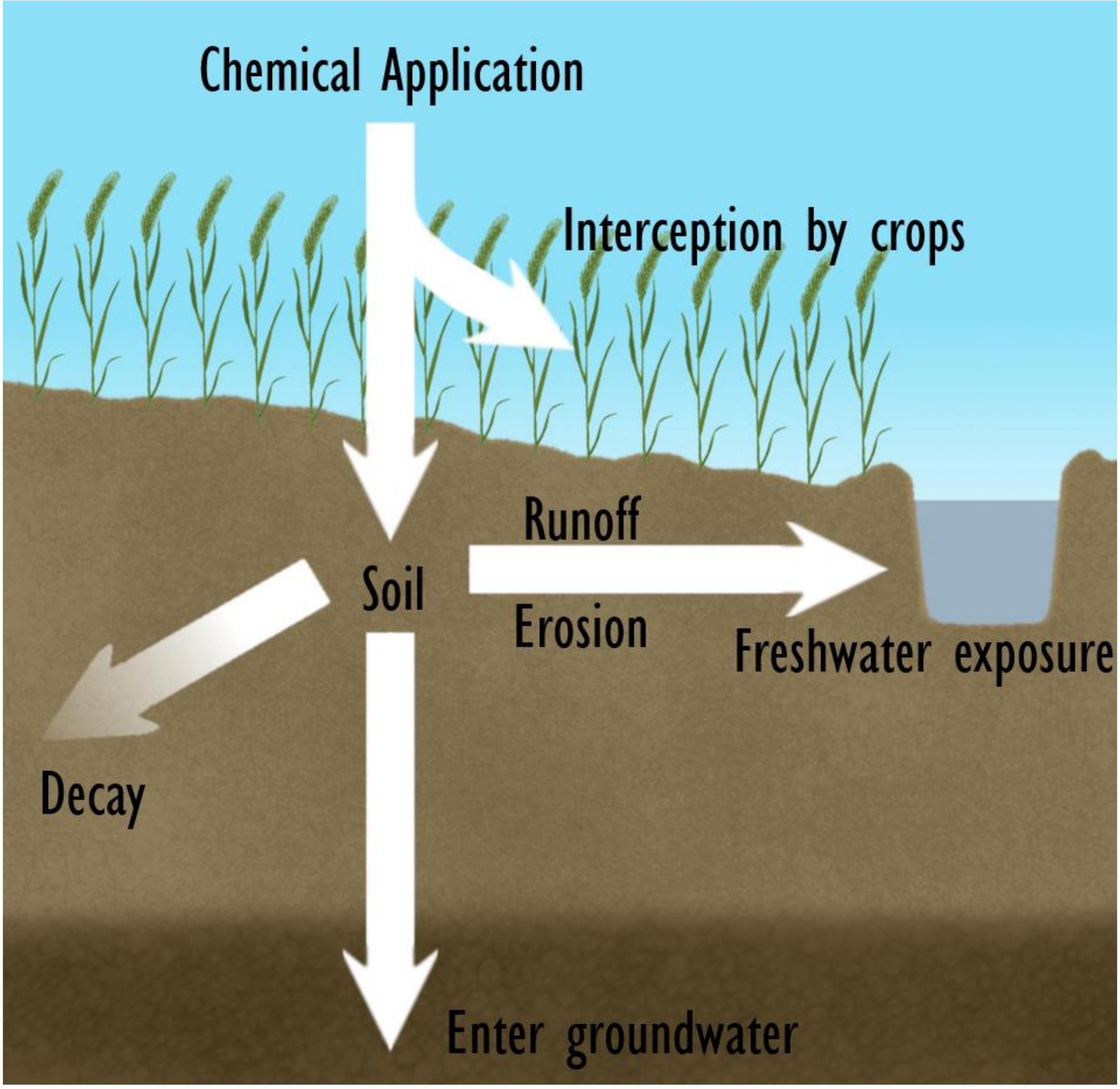

Figure 2.1 Diagram of chemical transport including movement through the soil matrix modelled by the Pesticide Root Zone Model (PRZM)

2.2. Methodology

2.2.1. Overview

To address the research questions, three stages of investigation were necessary. First, changes in chemical flux under different meteorological conditions and interactions between chemical degradation and persistence in soil were established. Hypothetical climate data and hypothetical chemicals were used to establish relationships between:

1. Size of precipitation on edge of field chemical flux
2. Delay to precipitation event and persistence in soil (as a function of soil degradation rate) on edge of field chemical flux
3. Air temperature and soil degradation rate on chemical persistence in soil

Secondly, climate change projections were used to evaluate whether the patterns investigated in the first objective hold for actual chemical, climatic and landscape data. This was investigated using scenarios with actual chemical properties applied to spatially variable climates in England and Wales with comparisons between sites, and baseline (1980-2000) and future end of century (2060-2080) conditions.

Third, the importance of seasonality in the effects of climate change and local meteorology were investigated. Given that meteorology affects chemical transport, chemical flux would be expected to vary depending on when in the year a chemical was applied. As site, season of application and chemical degradation rate were all considered, complex interactions were expected, so analysis focused on the relationship between season of application and chemical degradation rate.

Finally, the results from the third objective were contextualised by being combined with the actual application time of the various agrochemicals to quantify how climate change will increase or decrease in percentage the 90th percentile edge of field chemical flux under future conditions compared to baseline climates.

2.2.2. Data collection and processing

PRZM requires a suite of data from across geographic, agronomic and chemical studies (Table 2.3). Each of the major data groups is associated with their own set of references depending on the type and ubiquity of data. The collection process and justification for each group is described in further detail below.

Table 2.3 Data collected and used to run PRZM as part of chemical exposure modelling

Data group	Parameters	Reference
Meteorology	Temperature (mean, minimum, maximum), Precipitation, Solar radiation, Wind speed at 10m, Pan-evapotranspiration	Met Office, 2019,
Soil	Soil layers, density, Saturation (minimum, maximum), Organic carbon content, Soil erodibility	Cranfield University, 2021; Hallett et al., 2017
Landscape	Slope, Field runoff, Field area, Slope length-gradient, Farming practice (slope, contour or strip)	EFSA, 2013; Mullins et al., 1993
Crop	Crop type, Date of: emergence, maturation, and harvest; Root depth, Canopy cover, height, and holdup; Interception factor, Area of field cropped, Tillage method	Craufurd and Wheeler, 2009; EFSA, 2013; Olesen et al., 2012
Chemical	K_{OC}, DT_{50} in: water column, benthic, aqueous photolysis, hydrolysis, in soil, and on foliage; Molecular weight, Vapour pressure, Solubility, Henry's constant, Date(s) of application, Quantity of application	EFSA, 2013; University of Hertfordshire, 2007a, 2007b

Chemical data

Chemicals were selected to provide a range of soil DT_{50}, molecular mass, organic carbon partition coefficient (k_{OC}) and solubility values representative of agrochemical pollutants. Chemicals selected were primarily across two classes: pesticides and pharmaceuticals, with preference given to the most commonly applied chemicals. This was to both represent multiple groups of agrochemical pollutants and to ensure full coverage of the chemical property space. For example, pharmaceuticals had half-lives in soil of up to an order of magnitude less than pesticides (Figure 2.2).

Chemical properties were collected from three sources: EFSA reports on the chemical, papers investigating the environmental properties of the chemical, and the Pesticide/Veterinary Properties Database (University of Hertfordshire, 2007a, 2007b). Only chemicals that had the full suite of environmental properties to run PRZM and that expanded the chemical space were selected. Twenty-four chemicals were selected (14 pesticides and 10 pharmaceuticals) and full chemical properties are presented in Table A2.1.

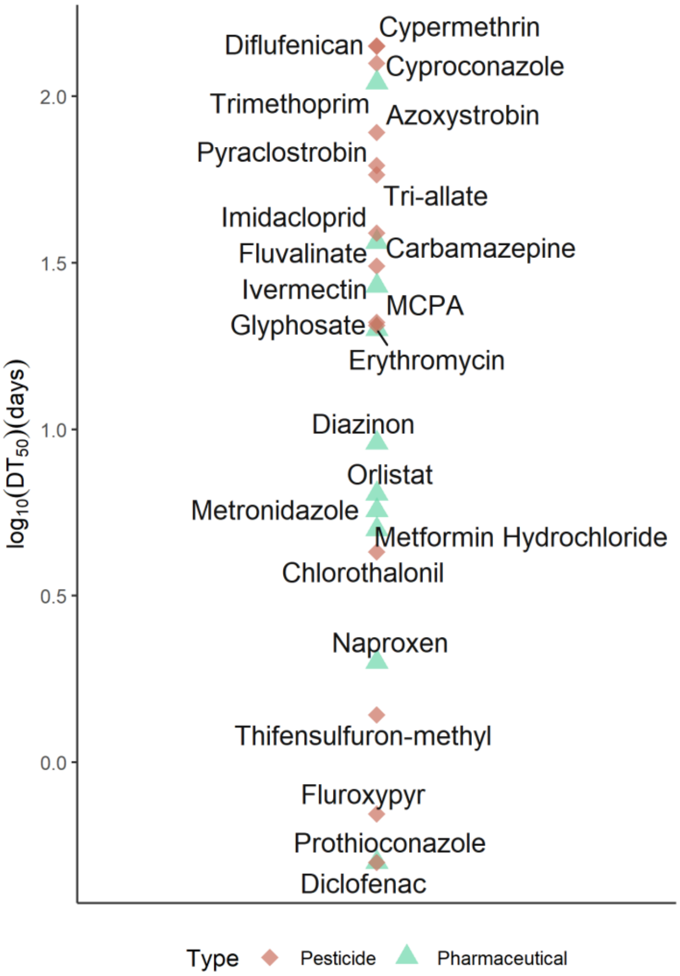

Figure 2.2 Comparison of the soil degradation rates (DT_{50}) in days for the 24 chemicals over a log base 10 scale

Climate data

United Kingdom Climate Projections simulated by the UK Met Office in 2018 (UKCP18) were used in this study (Met Office, 2019). Met Office projections represent the most extensive set of localised climate projections for England and Wales and are based on historic climate measurements collected from weather stations since the 1940s. UKCP18 projections, which utilise probabilistic projections and convection-permitting meteorology, run from 1980-2080 and have a resolution of 5 km (Met Office Hadley Centre, 2017; Met Office UKCP, 2018). High resolution climate projections capture high impact events that would be averaged at larger resolutions, such as localised heavy rainfall, making them particularly relevant to chemical transport. However, these high-resolution projections have some limitations: probabilistic limits are not available and they were only projected for the RCP 8.5 climate scenario and therefore the most extreme outcome. While probabilistic projections would be preferable for assessing the variation of climate change impacts, data were only available at the coarser 12km scale, therefore missing localised extreme rainfall events, and did not include two variables essential for running PRZM: surface wind speed and net long wave flux.

To accurately run PRZM the meteorology file must represent the range of meteorological events projected under climate change. However, it must also avoid taking the results from a single extreme or outlying year unrepresentative of future climate conditions. Consequently, baseline and future climate scenarios were created from 20-year time steps from the 50th percentile value taken from the central unperturbed version of the ensemble of projections the Met Office produced for these data. Daily data was subsetted from 1980-2000 for the baseline scenario and 2060-2080 for the future scenario.

Site data

Sites were selected according to the UK climate districts as defined by the UK Met Office (Figure 2.3). In total, six sites were selected, one from each of the districts across England and Wales. The UK climate districts are represented in Figure 2.3 and fall across a north-south temperature gradient and the east-west precipitation gradient with cooler temperatures associated with northerly sites and reduced precipitation at easterly sites. The location in each climate district was selected to ensure that they are representative of both the climate district as a whole and the meteorological gradients of the England and Wales while ensuring that the selected sites were not too geographically close to one another.

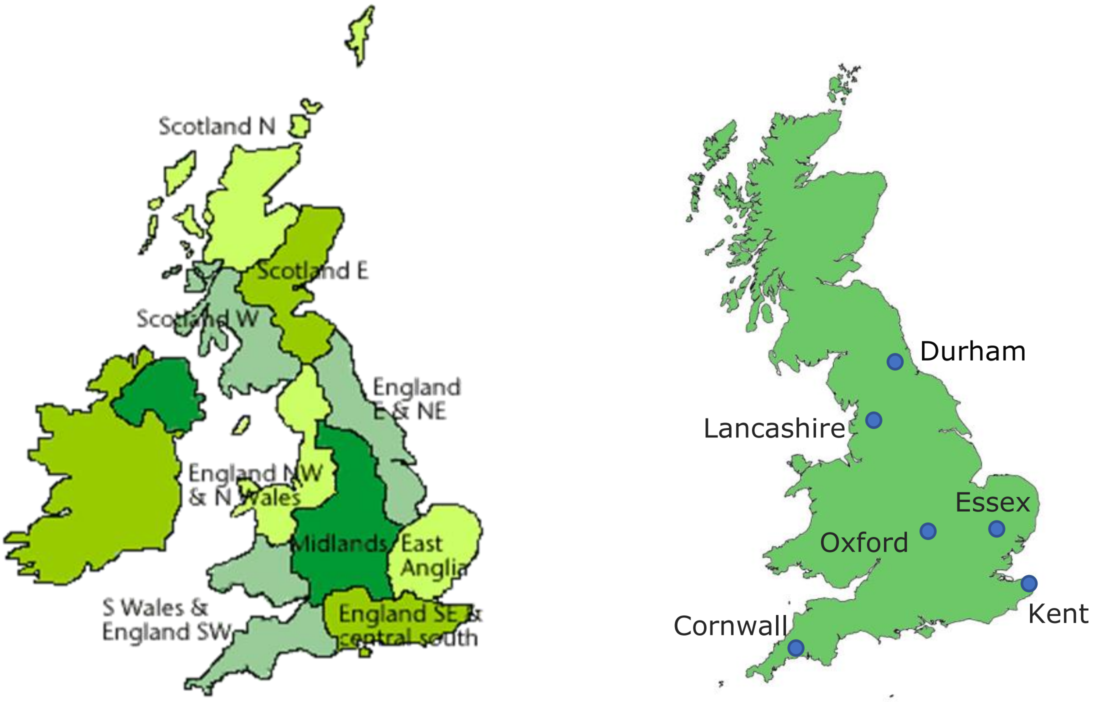

Figure 2.3 UK climate districts as defined by the UK (image adapted from Met Office, 2021) compared to the six sites selected for investigation in this study

2.2.3. PRZM setup and running

The US EPA fate modelling suite PWC (Pesticide Water Calculator, version 2.001) contains the latest version of PRZM (version 5.0+) within the program and was run for PRZM outputs only. When saving the PRZM outputs, the following calculated values were included in the output (in zts format): runoff volume, erosion volume, mass of chemical in runoff, mass of chemical in erosion, daily volatilisation of the chemical, leaching into groundwater, decay in soil profile, chemical mass remaining in soil profile and the chemical mass on foliage. The zts formatted files were then converted to csv format within R.

To address the meteorology-degradation relationships, PRZM was run using the hypothetical chemical, an azoxystrobin clone with customised soil degradation values of 0.5, 2.5, 12.5, 62.5, 312.5 days and a hypothetical weather file altered to investigate each weather parameter of interest on the standard site, with no crop modelled to avoid the crop dynamics being an additional factor affecting the relationship. For investigating the precipitation-flux relationship, precipitation amounts of 1, 2, 4, 8, 16 and 32mm occurred 4 days after chemical application and the total amount of edge of field chemical flux totalled. The delay-degradation-flux relationship was measured by running the hypothetical chemical suite for meteorology files with a single precipitation event of 10 mm occurring at 1, 4, 9, 23, 58, 144 or 360 days after each hypothetical chemical was applied. The temperature-degradation relationship was investigated by measuring the amount of chemical degraded by the day after each hypothetical chemical application under different meteorology files with no precipitation and different temperatures of 0 to 40 °C in 5 °C steps.

To assess the effects of climate change on chemical fate, PRZM was then run for the 24 chemicals across each of the 6 sites and for 4 different application times of spring, summer, autumn and winter (on the 1st of April, July, October and January respectively). Edge of field chemical flux was totalled from calculated chemical flux from both erosion and runoff. From these files, the effects of chemical degradation rate, site specific meteorology and seasonality could be assessed depending on how the PRZM outputs were processed and analysed.

2.2.4. Analysing PRZM outputs

Weather conditions and chemical fate considering degradation

All statistical analysis were performed within R with processing and visualisation supported by the tidyverse package(Wickham et al., 2019). To address the first objective, meteorology-degradation relationships with the hypothetical chemicals and set weather files were plotted and analysed. As it is necessary to isolate the effects of degradation on flux under differing meteorological conditions, a series of hypothetical chemicals were established where all the chemical parameters were kept constant while the DT_{50} value was altered. This was done because all other chemical properties did not affect the degradation of the hypothetical chemical and therefore were not necessary to investigate the hypothesis. While the values of chemical properties kept constant were unimportant, they are required to run PRZM. As the identity of these values was not important, all non-degradation chemical properties were cloned from those of azoxystrobin as it was the first chemical alphabetically.

The relationships were fitted based upon the expected interactions among chemical fate and meteorology or chemical properties from the literature (EFSA, 2008; Ma et al., 2004; Nolan et al., 2008; Wolt et al., 2002). For precipitation and flux, this was a linear model, while both the degradation over time plots and temperature degradation plots had logarithmic curves fitted. As chemical degradation occurs in a natural logarithmic fashion, the remaining chemical in the soil (relevant to the effects of delay on flux) were fitted with curves fitting the following equation:

$$y = a + r\, ln(x)$$

Where y is the chemical flux, a is the static coefficient representing the flux, r is the gradient coefficient representing the shape of the decay curve and x is the days after application (>0).

In contrast, the effect of temperature on chemical degradation in soil is of exponential form with a Q10 value a representative ratio of chemical degradation given a 10°C rise (EFSA, 2008). This informed the fitting of curves to the temperature-degradation relationships within PRZM for the landscape and soil characteristics used within this study. Consequently, the following equation was used:

$$y = a\, e^{(rx)}$$

Where y is the degradation rate, a is the static coefficient representing the intercept, r is the gradient coefficient which alters the shape of the exponential curve and therefore Q10 value, and x is the temperature of the chemical undergoing degradation.

Relationships between flux, precipitation and degradation for agrochemicals

All other objectives dealt with flux into freshwater meaning a suitable threshold value for assessing flux was required. The edge of field 90th percentile chemical flux (EFF_{90}) was calculated across the 20-year periods of each the baseline and future climate scenarios. This 90th percentile was selected as it is generally considered to cover reasonable worst-case scenario resulting from meteorological and subsequent chemical fate conditions and has precedence for both pesticides and pharmaceuticals (Cunningham, 2006; EFSA, 2014).

The relationship of EFF_{90} and meteorology (precipitation that triggered the EFF_{90} event, and the time after application that the EFF_{90} event occurred) from soil degradation rate was assessed using linear models for each site and for baseline and future climates using generalised linear models.

The interactions between site, climate change scenario and degradation rate were investigated with 3-way ANCOVA. From this, Tukey's HSD test with Sidak correction were used to compare the effect of different sites' climate on the DT_{50}-EFF_{90} relationship, including for the delay to and size of precipitation event that triggered the EFF_{90}. The Tukey's HSD test with Sidak correction was separately performed for both baseline and future climate conditions and then compared.

To investigate the direct effect of climate change on chemical flux, a paired t-test was performed on the data from all chemicals applied in all seasons but each site considered separately. Bonferroni correction was applied to account for the paired t-test being run for each of the six sites.

Effects of seasonal application and DT_{50} on flux under climate change

In contrast, site and season are associated with different meteorological patterns that both influence EFF_{90} together. Degradation effects on the change to EFF_{90} under climate change were non-linear in response to meteorology, meaning different patterns between the sites and season of chemical application. Instead, the inter-seasonal variation of EFF_{90} under climate change was compared based upon the degradation rates of the chemical to address the third objective.

Variation was calculated on a chemical basis for the variation in flux (EFF_{90}) between different seasons of application at the same site. As site was separated at this point, the relationship of variation and DT_{50} was investigated using ANOVA with Tukey's HSD test with Sidak correction used to compare the relationships in a pairwise fashion between sites.

Effects of climate change on flux for agrochemicals and application times

As the final objective focused on actual chemicals with realistic application dates, the change in flux under climate change conditions was quantified as a change of the EFF_{90} of the future climate as a percentage of the EFF_{90} of the baseline climate, with percentages >100% representing an increase in flux under climate change conditions, <100% a decrease, and 100% itself no change under climate change.

2.2. Results

2.2.4. Climate change on UK weather

For the six sites in England and Wales, the effects of climate change on meteorological conditions were site, season and parameter dependent. In England & Wales, mean daily temperature exhibits a north-south gradient with southerly sites having higher mean daily temperatures. Under climate change conditions (Figure 2.4), the effects on mean daily temperature were consistent across sites, with an increase in temperature throughout the year that was more pronounced in the summer and autumn months (3.7°C to 5.0°C), compared to spring and winter (2.1°C to 2.8°C). In contrast, maximum daily temperature, representative of extreme climate conditions, exhibits greater variation between sites and the seasonality of the temperature change. Projected increases were reduced in the westerly (5.4°C Lancashire and Cornwall 4.2°C) sites compared to other locations (range of 8.6°C to 10.0°C).

Precipitation in England & Wales Mean occurs throughout the year although precipitation is generally lowest during late summer. While seasonal variation is generally minimal across sites compared to other global climates, northerly (Lancashire and Durham) and westerly (Lancashire and Cornwall) sites exhibit greater variation with wetter winters and dryer summers than other sites. Future climate conditions tend to exacerbate the seasonal differences in mean daily precipitation resulting in wetter winters (17.3% to 42.7%) and dryer summers (-16.2% to -35.6%) across all sites, with less change in spring and autumn. Maximum precipitation exhibited greater monthly variation for both baseline and future scenarios. Consequently, when in the year the maximum precipitation event occurred was different for each site and changed under future climate conditions. Generally, maximum precipitation increases in summer under climate change for most sites (13.9% to 67.4%), except for Cornwall (-11.4%) and Durham (-33.1%). Notably, maximum spring precipitation in Oxfordshire sees an increase of 103% under future conditions, much greater than any other sites (next largest is Essex with a rise of 28.1%). Equally Essex exhibited a much larger increase to maximum precipitation in Autumn (58.7%) than other sites (next largest is Kent with an increase of 8.0%)

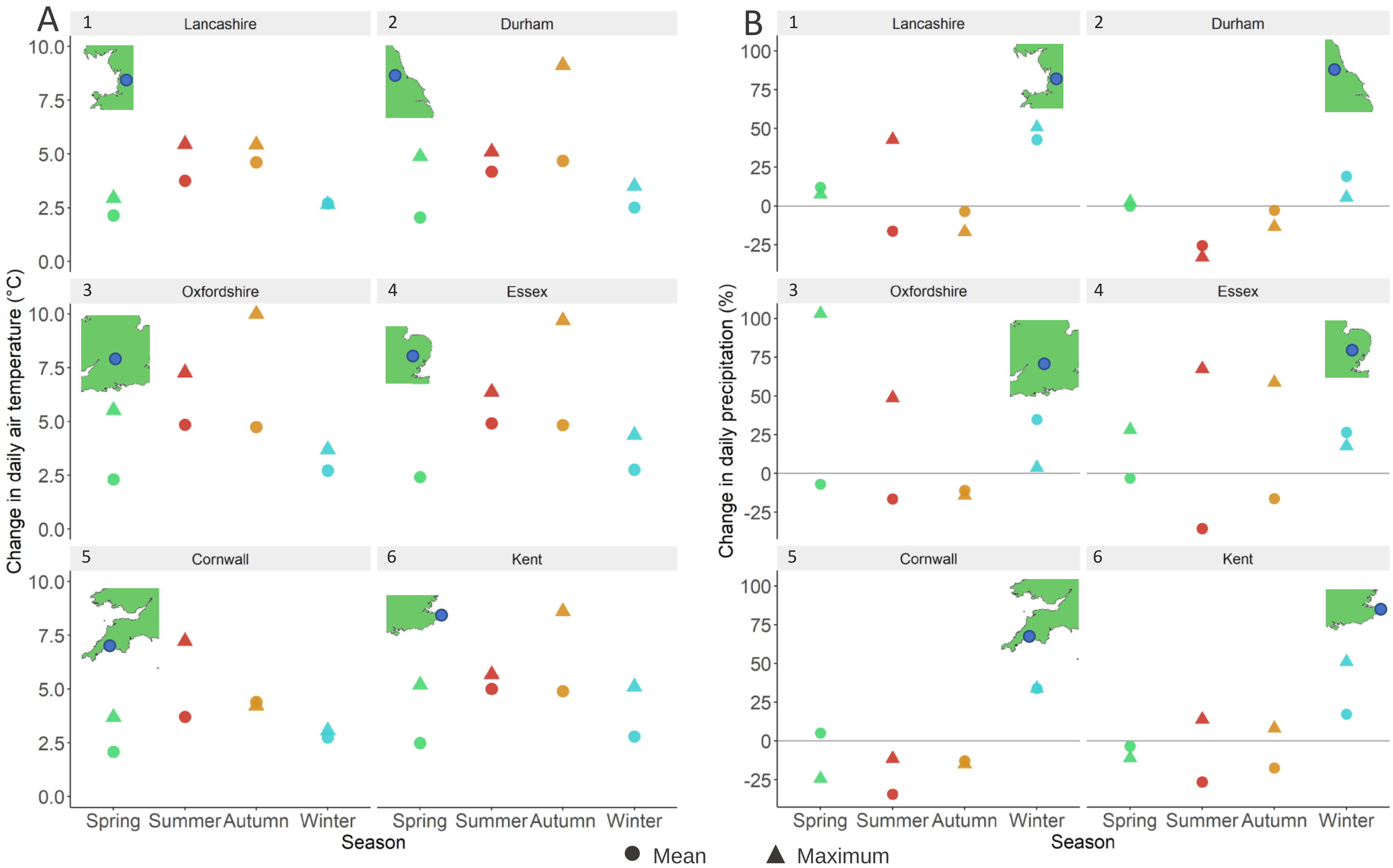

Figure 2.4 Effects of climate change on mean and maximum daily A) air temperatures (absolute change) and B) precipitation (percentage change) across each season, between baseline (1980-2000) and future (2060-2080) UKCP18 climate scenarios for six UK sites representing different UK Met Office climate regions (A1-6 & B1-6 respectively). Mean daily values across the season are shown as dots while maximum daily values for the whole season are triangles

2.3.2. Weather and chemical fate

Precipitation quantity and flux

The amount of precipitation directly increases edge of field chemical flux (Figure 2.5). Following a lag phase for precipitation values below 4mm, chemical flux increases linearly with precipitation (gradient of 0.00326 mg ha^{-1} mm^{-1}, $p < 0.01$, df = 5). The maximum single precipitation event across all sites and time scenarios was 94.4 mm while the mean precipitation value that triggered the 99th percentile chemical flux events across baseline and future scenarios was 50.1mm (sd = 1.89, range = 12.6-94.4 mm). Credible precipitation and chemical flux events are represented would not be expected to deviate from the relationship exhibited in Figure 2.5.

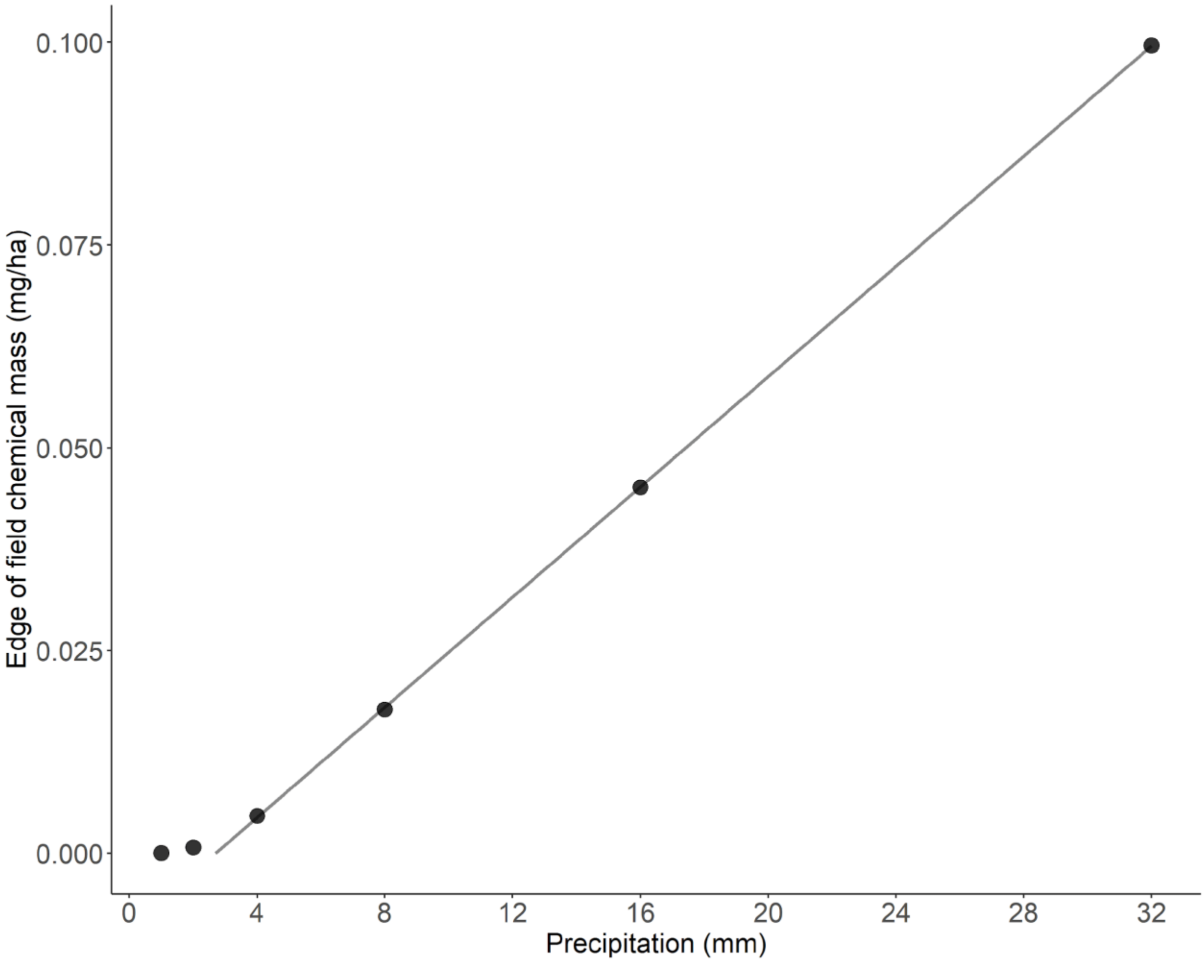

Figure 2.5 Relationship between individual precipitation (mm) events and edge of field chemical flux (mg/ha) for the generic hypothetical site using precipitation values along an exponential scale. Precipitation events occurred the day following the application of the generic hypothetical chemical with slow degradation (DT_{50} of 312.5 days) at 1 kg/ha

Precipitation delay, degradation and flux

Edge of field chemical flux reduced in a logarithmic relationship as the delay to a precipitation event increased (Figure 2.6). This was true for all DT_{50} values of the chemicals meaning that the edge of field flux is proportional to the decreasing quantity of chemical remaining within the soil matrix due to degradation. Hypothetical chemicals with lower DT_{50} values (0.5, 2.5 and 12.5 days) resulted in negligible chemical flux before the end of a year due to near complete degradation, while slower degrading chemicals (62.5 and 312.5 days) remained within the soil matrix and could be liable to transport beyond the year timeframe where chemical application may occur again leading to accumulation in the soil. Natural logarithmic models fitted to the flux curves in Figure 2.6 varied depending on their degradation rate, with the coefficients shown in Table A2.2. The static factors (a) for the lowest DT_{50} values of 0.5 and 2.5 days (a of 0.003 and 0.016 respectively) result in a much lower initial flux than from the higher DT_{50} values (a of 0.024 to 0.026). In contrast the gradient factor (r) for a DT_{50} value of 0.5 days is lower than all other DT_{50} values (r of -0.0007 compared to r of -0.0028 to -0.0042) reflecting the different shape of the decay curve for this DT_{50} value.

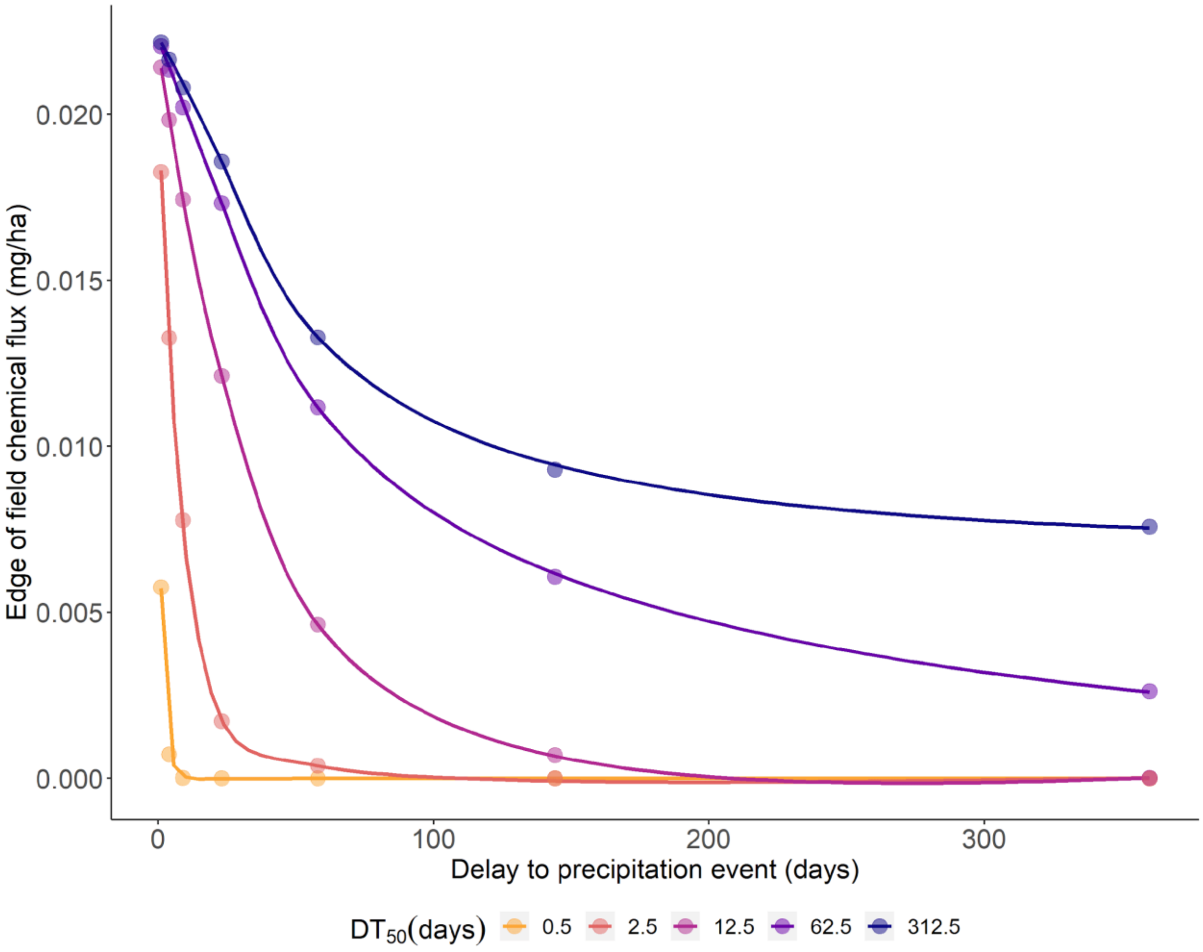

Figure 2.6 Effect of increasing the delay to a precipitation event after application of a chemical to the resultant edge of field chemical flux. The hypothetical chemical was altered with differing degradation values

Temperature and degradation

For all DT_{50} values chemical degradation rates increase as temperature increases, although the hypothetical chemicals have different relationships of temperature and degradation. For most DT_{50} values the rate of change to degradation increases with temperature increases, equivalent to the degradation rate increasing with an exponential relationship to temperature increases (Figure 2.7). The relevant coefficients calculated for these standardised degradation curves and their respective Q10 values are presented in Table A2.3. For chemicals with increasing DT_{50} , the calculated Q10 converges to 1.77 for the soil and landscape parameters tested. While the 0.5 day DT_{50} hypothetical chemical exhibits the inverse relationship to increasing temperature, this is due to increased depletion of the chemical from higher temperature degradation. This results in a large enough reduction to chemical capable of undergoing degradation that degradation rate within the soil matrix decreases. It is the effect of reduced chemical to undergo degradation that reduces the Q10 value for more rapidly degrading chemicals.

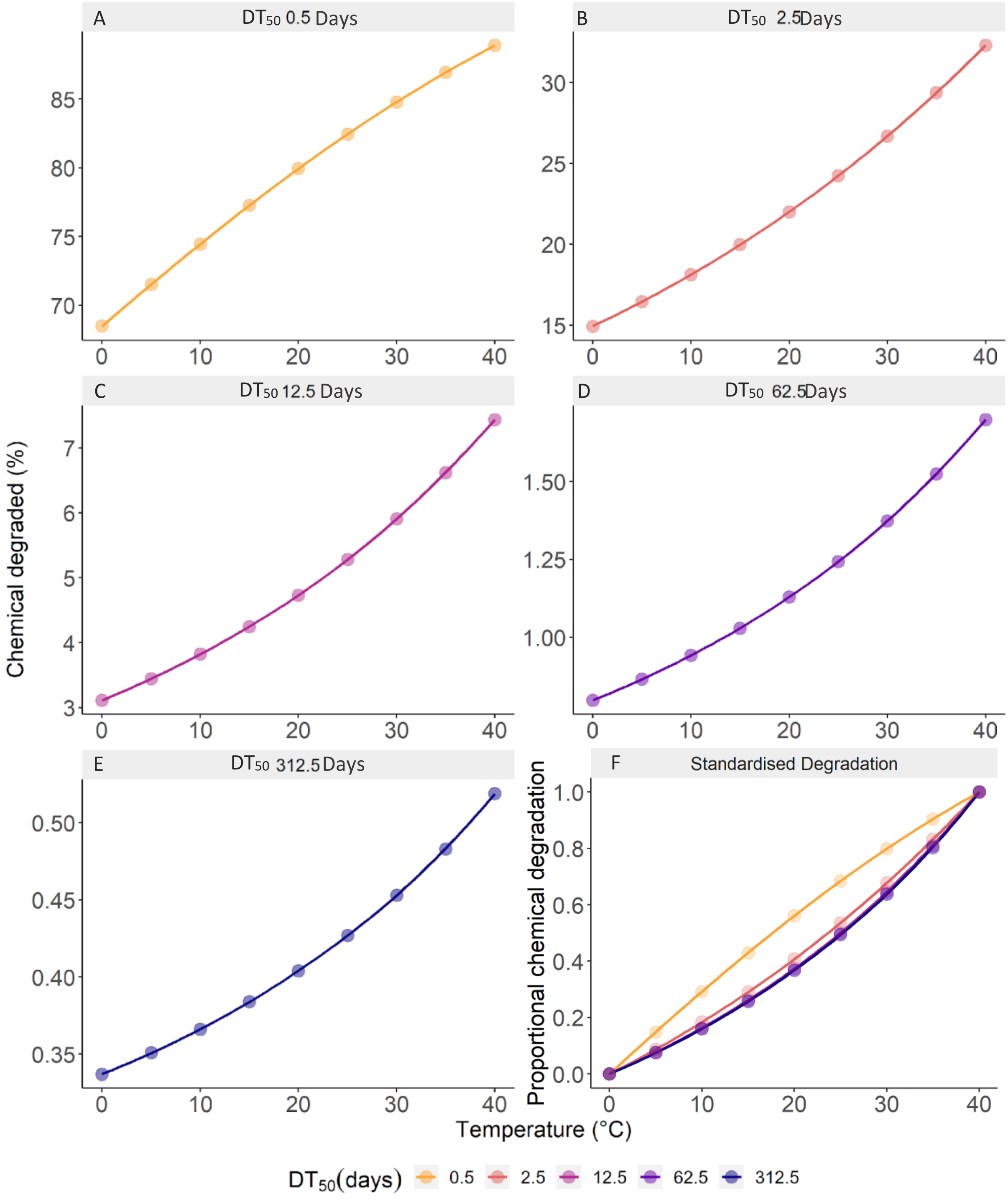

Figure 2.7. Effects of increasing air temperature on chemical degradation rate for five hypothetical chemicals with a range of degradation rates (A-E DT_{50}) including a plot where the percentage of chemical degraded was standardised according to the minimum and maximum percentages degraded for each chemical (F)

2.3.2. Interactions of climate change and chemical properties on fate

Chemical persistence and extreme flux events

Relationship of DT_{50} and the delay to and size of precipitation triggering 90th percentile flux

For the 24 chemicals with environmental properties tested in PRZM, grouping all sites over both baseline and future conditions as the DT_{50} of the chemical increased, the size of threshold precipitation events triggering the edge of field 90th percentile chemical flux (EFF_{90}) also increased (log-transformed linear model, $p < 0.001$, linear model $R^2 = 0.71$). The equivalent consideration for how the delay to the threshold precipitation events triggering the 90th percentile edge of field chemical flux varied by DT_{50} found a similar relationship (log-transformed linear model, $p < 0.001$, linear model $R^2 = 0.46$).

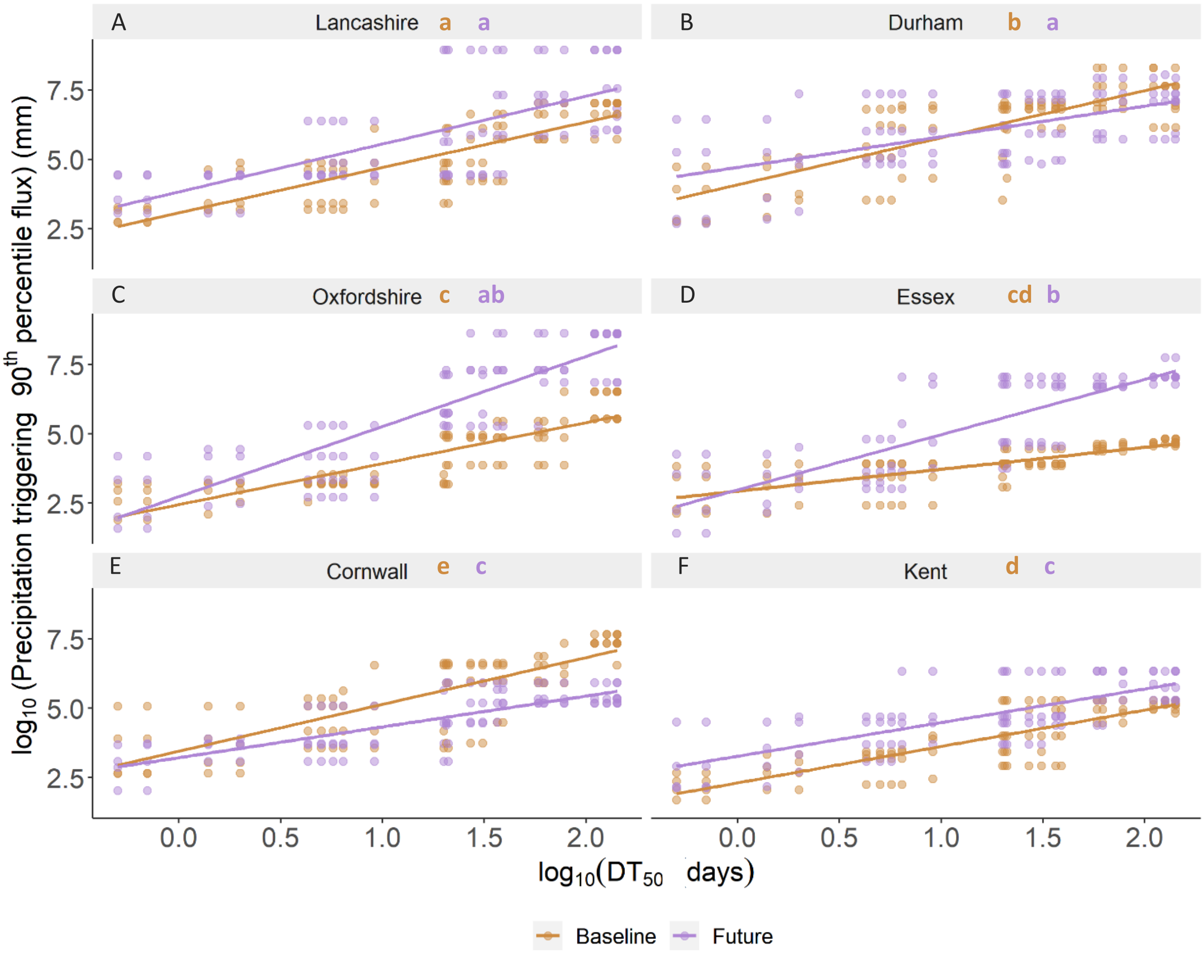

Figure 2.8 Relationship between how the size of threshold precipitation events triggering the EFF_{90} varies by chemical soil DT_{50} across 6 sites (panels A to F) in England and Wales and all seasons of chemical application when predicted within PRZM. Shared lowercase letters following the site indicate pairwise comparisons between sites that are not significantly different ($p<0.05$) following a Tukey's HSD test with Sidak correction where colour represents a baseline (orange, 1980-2000) or future (purple, 2060-2080) climate

While all sites and climate scenarios exhibited positive relationships between delay and degradation, there is spatial variation between sites in this relationship over both baseline and future climate conditions, as shown by the orange and purple letters in Figure 2.8. For baseline conditions the central and south-eastern locations of Oxfordshire, Essex and Kent all share a non-significantly different DT_{50}-precipitation relationship, while all other sites exhibited steeper and had significantly different relationships. In contrast, under the future climate scenario, the grouping is more homogenous following a north south gradient. A full table including the individual pair-wise comparisons is available in Table A2.5A and Table A2.5B.

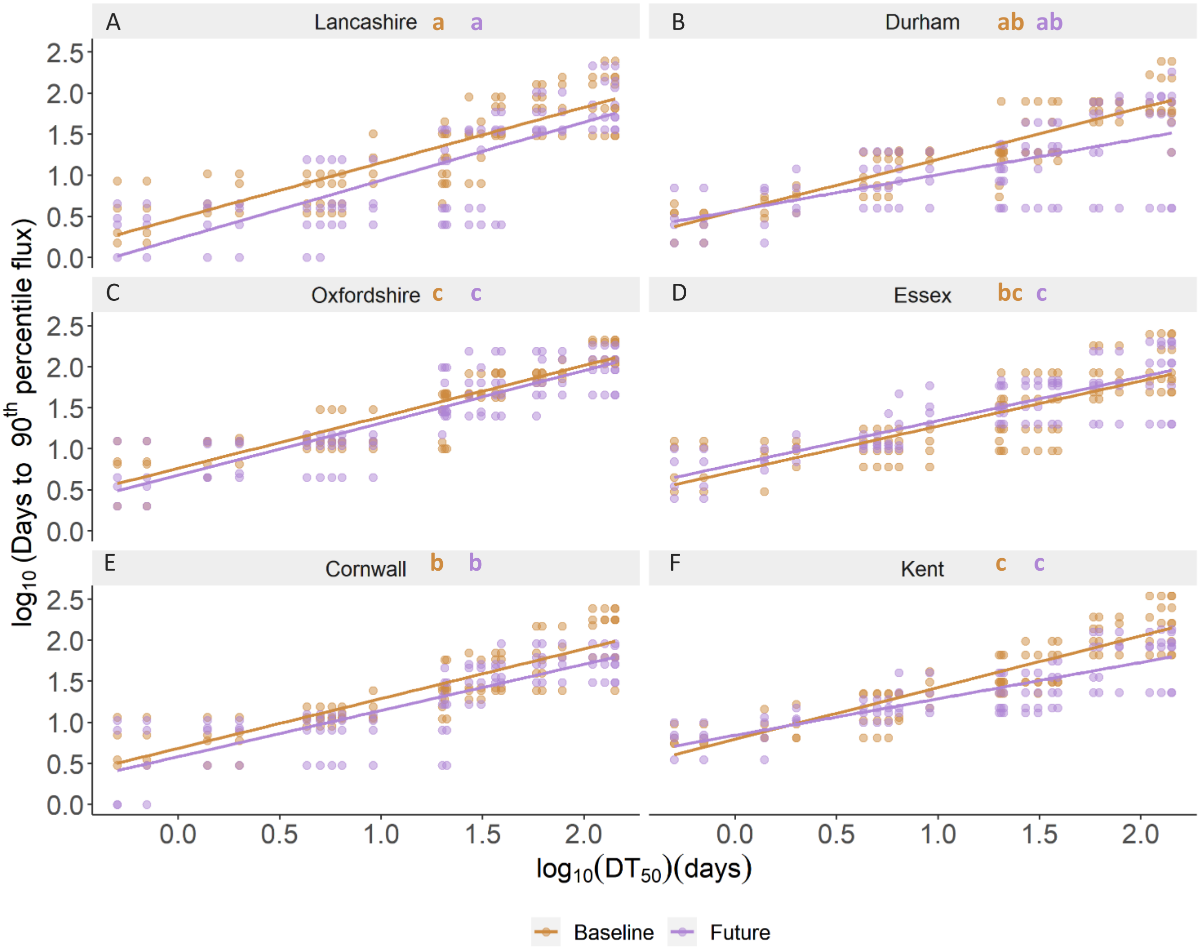

Figure 2.9 Relationship between how the delay to threshold precipitation events triggering the EFF_{90} varies by chemical soil DT_{50} across 6 sites (panels A to F) in England and Wales and all seasons of chemical application when predicted within PRZM. Shared lowercase letters following the site indicate pairwise comparisons between sites that are not significantly different ($p<0.05$) following a Tukey's HSD test with Sidak correction where colour represents a baseline (orange, 1980-2000) or future (purple, 2060-2080) climate

Again, although all sites and climate scenarios exhibited positive relationships, there is spatial variation between sites Figure 2.9. This manifests with a broadly northern group typified by Lancashire, and a central and southeast group with Oxford and Kent with the other sites falling between these two. Specific p-values for pairwise comparisons are shown in Table A2.5C and Table A2.5D. However, in contrast to the DT_{50}-precipitation relationship, the DT_{50}-delay relationship exhibited little change in the groupings of sites between baseline and future climates with only Essex becoming more distanced from the Durham and Cornwall relationships. Additionally, both Durham and Kent see significant shallowing of the DT_{50}-delay relationship under climate change conditions associated with increased frequency of extreme precipitation events ($p = 0.003$ and 0.032 respectively, df = 2303, 2280, 3-way ANCOVA).

Relationship between DT_{50} and 90th percentile flux

For the 24 chemicals with environmental properties tested in PRZM, grouping all sites over both baseline and future conditions as the DT_{50} of the chemical increased the edge of field 90th percentile chemical flux (EFF_{90}) also increased (log-transformed linear model, $p < 0.001$, linear model $R^2 = 0.65$). There was spatial variation between the sites for the DT_{50}-EFF_{90} relationship as shown from pair-wise comparisons (Figure 2.10), and these groupings differed to those for the DT_{50}-delay and DT_{50}-precipitation (Table A2.5E and Table A2.5F, compared to A-D). For baseline climates three patterns formed; this was a western group (Lancashire and Cornwall), and a central and southeast group (Oxfordshire, Essex and Kent), with Durham significantly different to all others. However, under climate change this shifted to a southern group composed of Cornwall and Kent, and a central group of Oxfordshire and Essex, but with Essex was no longer significantly different from Lancashire. Again, Durham was significantly different to all other sites.

Climate change impacts on flux

The overall effect of climate change on EFF_{90} was assessed with paired t-tests investigating whether flux altered under climate change conditions over the 24 chemicals and 4 seasons of application. Such tests were performed separately each site, and indicated spatial variation between them. The central and southeast sites of Oxfordshire, Essex and Kent all exhibited significant increases in flux under climate change conditions while Cornwall exhibited a significant decrease (all $p < 0.001$, df = 91, paired t-test). In contrast, the northern sites of Lancashire and Durham exhibited no significant difference in flux between baseline and future conditions ($p = 0.88$ and $p = 0.982$ respectively, df = 91, paired t-test).

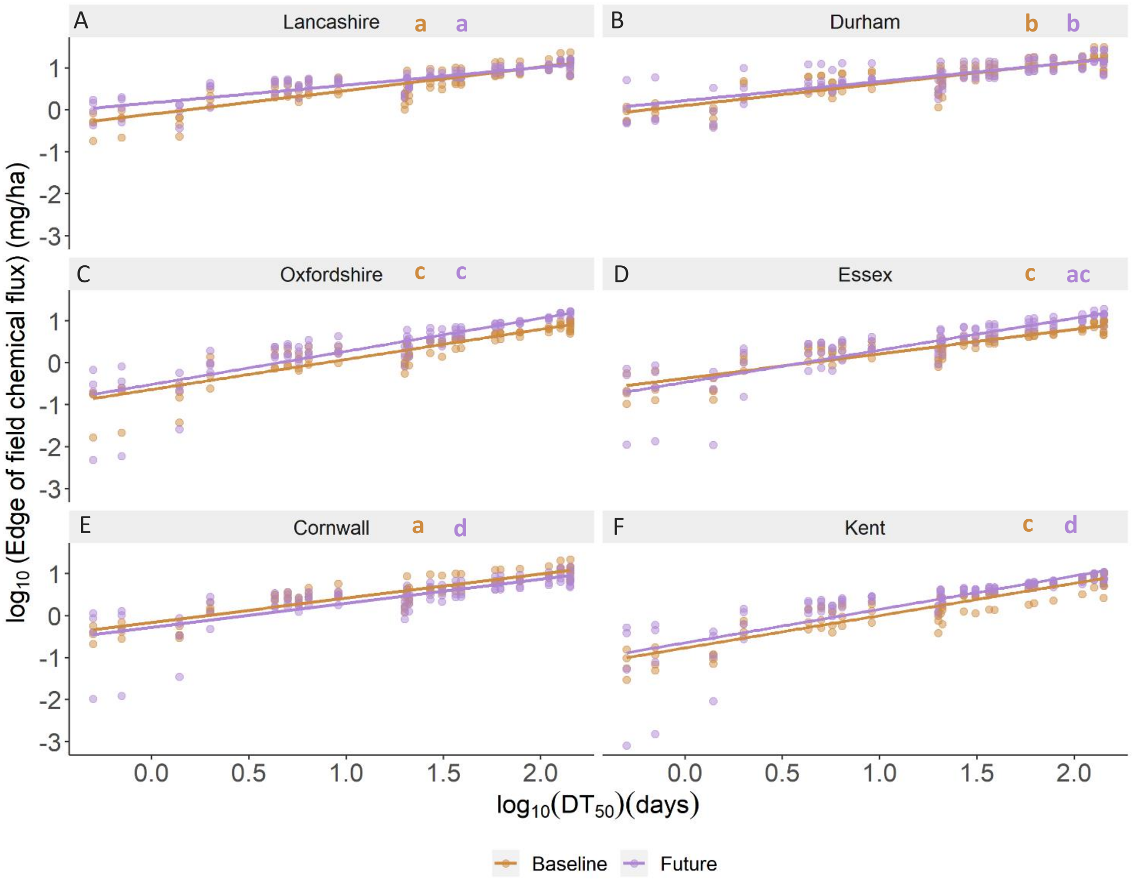

Figure 2.10 Relationship between how the threshold precipitation event that triggered the EFF_{90} varies by chemical soil DT_{50} across 6 sites (panels A to F) in England and Wales and all seasons of chemical application when predicted within PRZM. Shared lowercase letters following the site indicate pairwise comparisons between sites that are not significantly different ($p<0.05$) following a Tukey's HSD test with Sidak correction where colour represents a baseline (orange, 1980-2000) or future (purple, 2060-2080) climate

2.3.3. Season and degradation on flux

When comparing how the percentage of edge of field 90th percentile chemical flux (EFF_{90}) changes under the climate change scenario, all of site, season of application and the degradation rate of the chemical (DT_{50}) affect the percentage change. This can be seen in Figure 2.11 where the effect of increasing DT_{50} has differing effects of change to flux for each site and season combination. Qualitatively, the pairings of Oxfordshire & Essex, and Cornwall & Durham appear most similar, but even then, differences are notable, particularly in autumn for Oxfordshire & Essex, and in spring for Cornwall & Durham. In contrast, Kent is notable for the maintaining of a large percentage increase to flux for summer applications beyond equivalent DT_{50} values of all the other sites.

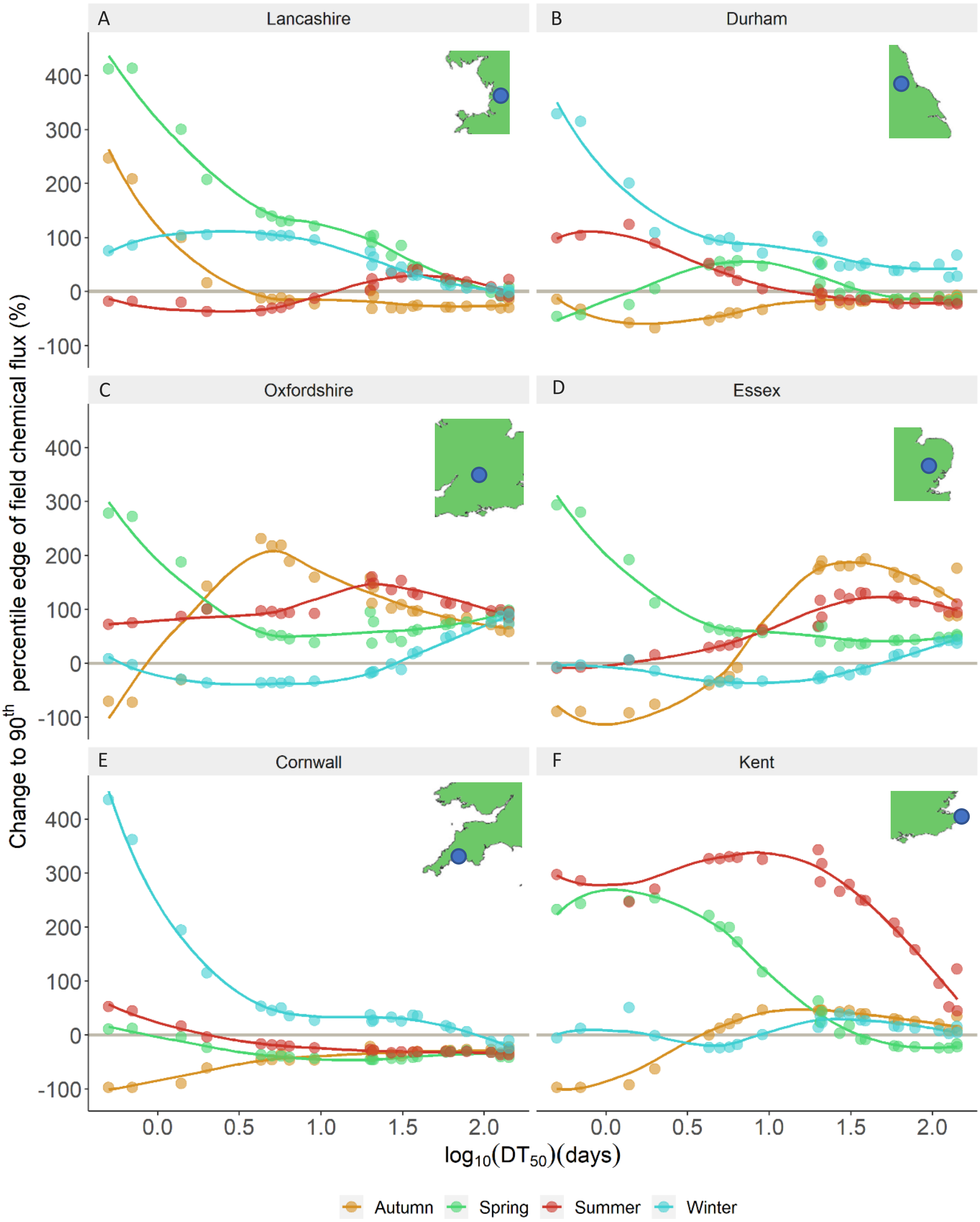

Figure 2.11 The percentage change to the EFF_{90} due to future (2060-2080) climate conditions compared to the baseline scenario (1980-2000) considering the effects of 24 chemicals with differing degradation rates. The effect of seasonal differences to weather under climate change was considered with applications of the chemical being modelled for each season. Lines between and are not indicative of any model fit but rather to show how change in chemical flux changes over the differing DT_{50} values

One aspect common across all sites is the reduced effect of the season of application on the percentage change to edge of field 90th percentile chemical flux (EFF_{90}) as the DT_{50} of the chemical increases. This is demonstrated in Figure 2.12 by the reduction in variation of flux between seasons for increasing DT_{50} values ($p < 0.001$, linear model, $R^2 = 0.71$). The results from pair-wise comparisons between the sites indicate that all sites exhibit a similar relationship between the extent of seasonal variation in chemical flux depending on the degradation rate of the chemical.

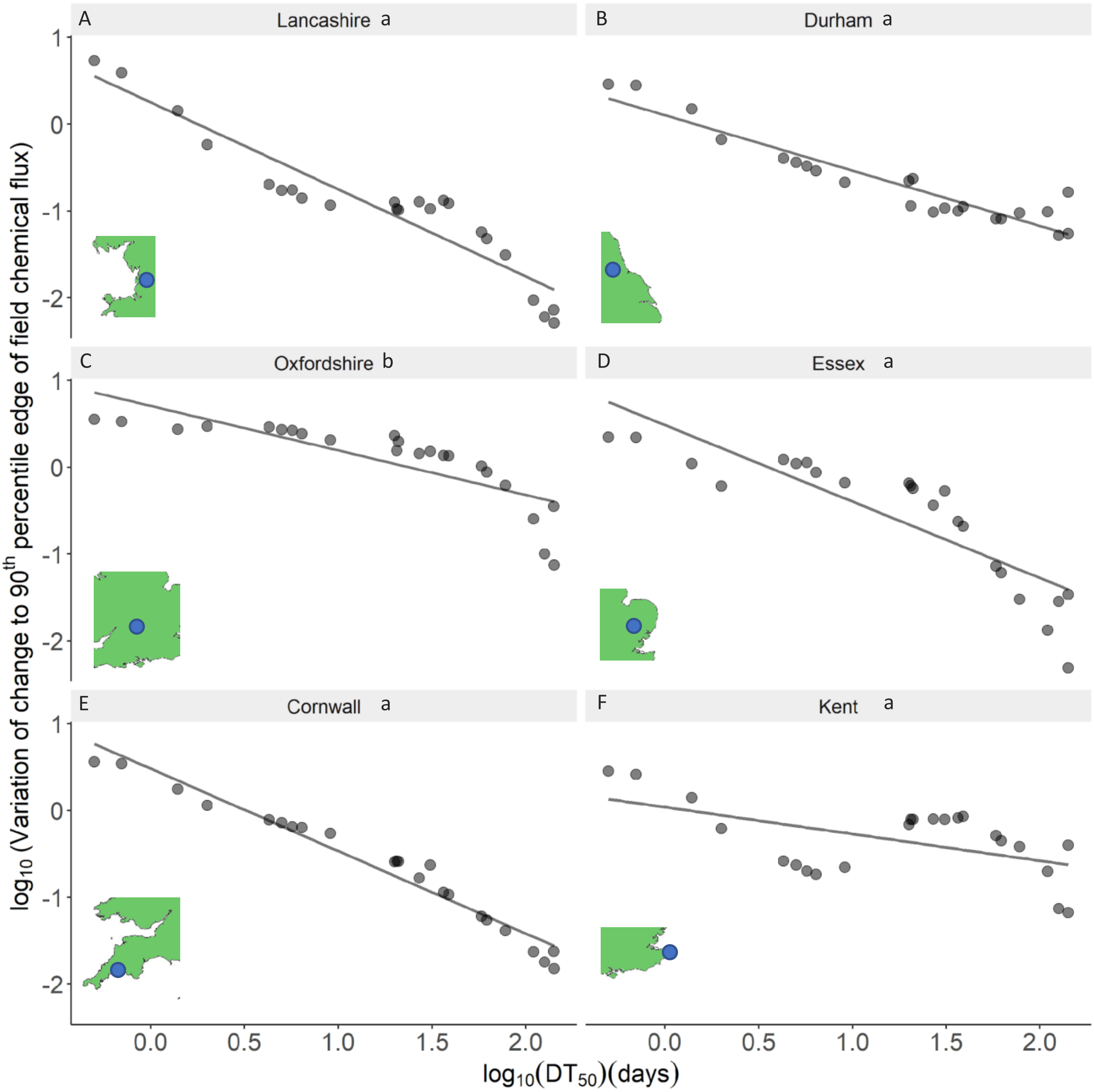

Figure 2.12 Variation (σ^2) between different seasons of chemical application for the percentage change in EFF_{90} between baseline (1980-2000) and future (2060-2080) scenarios across the degradation rate range of the chemicals. Shared lowercase letters following the site indicate pairwise comparisons between sites that are not significantly different ($p<0.05$) following a Tukey's HSD test with Sidak correction

2.3.4. Effects of climate for realistic chemical application scenarios

Pesticides have specific times of application so they are most efficacious against their target organisms. As pests life histories are variable among plants, animals, fungi and microorganisms, the timing of pest infestation and therefore season of pesticide application vary. However, most commonly application tends to occur during major crop growth periods (spring and summer) when conditions are also favourable for pest growth and few applied in winter. This is reflected in the application times for the pesticides investigated within this study (Table 2.4). Generally, there is a large variation in the effect of climate change on edge of field 90th percentile chemical flux (EFF_{90}), with percentage changes ranging from -97% to 249%. Chemicals applied in Cornwall always exhibited a decrease in EFF_{90} while aside for those which rapidly degrade ($DT_{50} < 5$ days), all chemicals exhibited an increase in Essex and Oxfordshire. Season of application affected both the direction and magnitude of change, but this depended on the site. For example, fluvalinate and imidacloprid have similar DT_{50} values (31 and 38.9 days respectively), but are applied in different seasons (summer and autumn). Consequently, when applied in Kent imidacloprid increases by 249%, while fluvalinate increases by just 18%. In contrast when applied in Durham, fluvalinate sees a 9% rise while imidacloprid sees a decrease of 23%. These changes to EFF_{90} mean that the reasonable worst-case quantity of chemical entering freshwater bodies is enough to alter the outcome of existing ERAs. Consequently, the effects of climate change on the shift to chemical flux is significant and requires consideration at a spatial and chemical specific level, including accounting for when in the year the chemical is applied.

Table 2.4 Effect of climate change on EFF_{90} for 14 different pesticides applied under realistic application times in accordance with good agricultural practice across six sites representing the climatic groups of England & Wales. All values at 100% or below represent no change or an decreasing in EFF_{90} and are coloured green. Values >100% represent an increase in flux under climate change and become increasingly red as the change in flux increases

Chemical	**Chemical**	**DT_{50} in soil (days)**	**Season**	**Change in 90th percentile edge of field flux under climate change conditions over 20 years (%, 1980-2000 to 2060-2080)**					
				Cornwall	Durham	Essex	Kent	Lancashire	Oxfordshire
Prothioconazole	Fungicide	0.5	Spring	-97	-14	-89	-97	248	-71
Fluroxypyr	Herbicide	0.7	Spring	-97	-33	-90	-97	209	-73
Thifensulfuron methyl	Herbicide	1.39	Spring	-90	-58	-92	-92	100	-31
Chlorothalonil	Fungicide	4.29	Spring	-46	-54	-40	0	-12	231
Pyraclostrobin	Fungicide	62	Spring	-29	-15	159	28	-29	80
Azoxystrobin	Fungicide	78	Spring	-27	-12	155	25	-27	84
Cypermethrin	Insecticide	141.3	Spring	-22	-7	176	35	-15	75
Glyphosate	Herbicide	20.5	Summer	-46	15	40	24	91	37
MCPA	Herbicide	21	Summer	-46	52	70	43	104	77
Fluvalinate	Insecticide	31	Summer	-45	9	38	18	85	40
Cyproconazole	Fungicide	126	Summer	-40	-17	47	-25	-9	95
Imidacloprid	Insecticide	38.9	Autumn	-31	-16	129	249	40	127
Tri-allate	Herbicide	58.2	Autumn	-31	-23	124	207	24	112
Diflufenican	Herbicide	141.8	Winter	-25	29	44	4	-2	91

Currently, pharmaceuticals primarily see introduction onto crops within manure and biosolid fertiliser use which is associated with application in autumn and spring for winter wheat. As for pesticides, the effects of climate change are highly spatially variable and chemical specific for pharmaceuticals with variable percentage changes to edge of field 90th percentile chemical flux (EFF_{90}) of -97% to 343% with distinct patterns forming over both site and chemical degradation rate. Autumn application in Kent see the largest rise of 96 – 343%, but this pattern is not seen for Kent spring application which exhibit both decreases (up to -97%) and increases (up to 46%). Cornwall see universal decreases in $EFF_{90,}$ aside from the rapidly degrading diclofenac in Autumn, while Oxfordshire sees universal increases aside from diclofenac in spring. Patterns of DT_{50} are qualitatively visible for some site and season combinations, such as a positive correlation of DT_{50} and EFF_{90} in Essex, both autumn and spring, while the reverse relationship is observed for autumn applications in Durham.

Table 2.5 Effect of climate change on EFF_{90} for 10 different pharmaceuticals applied under realistic application times as fertiliser either in autumn or spring across six sites representing the climatic groups of England & Wales. All values at 100% or below represents no change or an decreasing in EFF_{90} and are coloured green. Values >100% represent an increase in flux under climate change and become increasingly red as the change in flux increases

Chemical	**DT_{50} in soil (days)**	**Season of fertiliser application**	**Change in 90th percentile edge of field flux under climate change conditions over 20 years (%, 1980-2000 to 2060-2080)**					
			Cornwall	Durham	Essex	Kent	Lancashire	Oxfordshire
Diclofenac	0.5	Autumn	53	99	-9	297	-18	72
Naproxen	2	Autumn	-4	89	16	270	-37	100
Metformin Hydrochloride	5	Autumn	-19	38	32	327	-31	96
Metronidazole	5.7	Autumn	-19	37	33	330	-30	92
Orlistat	6.4	Autumn	-21	20	39	329	-23	94
Diazinon	9.1	Autumn	-24	5	61	326	-13	92
Erythromycin	20	Autumn	-27	5	69	343	2	147
Ivermectin	27	Autumn	-33	-16	128	266	34	136
Carbamazepine	36.5	Autumn	-32	-16	131	250	40	131
Trimethoprim	110	Autumn	-33	-17	104	96	9	97
Diclofenac	0.5	Spring	-97	-14	-89	-97	248	-71
Naproxen	2	Spring	-61	-68	-76	-63	16	143
Metformin Hydrochloride	5	Spring	-46	-48	-33	13	-15	217
Metronidazole	5.7	Spring	-36	-39	-24	21	-12	219
Orlistat	6.4	Spring	-46	-40	-8	30	-16	189
Diazinon	9.1	Spring	-47	-33	64	46	-23	160
Erythromycin	20	Spring	-21	3	174	45	3	158
Ivermectin	27	Spring	-36	-21	181	43	-30	102
Carbamazepine	36.5	Spring	-32	-18	188	39	-27	97
Trimethoprim	110	Spring	-27	-9	132	20	-25	72

2.4. Discussion

The aim of this chapter was to assess the effect of climate change on the edge of field agrochemical flux the via soil matrix while considering the spatial variation in local climates and the persistence of agrochemicals in soil. The impact of future weather patterns on seasonal variation in the edge of field flux of 24 different agrochemicals was investigated for six sites across different climatic zones of England and Wales. This study had four main objectives: first to assess how climate parameters and chemical degradation affect flux, secondly to see if these relationships hold using the properties of actual chemicals and accounting for spatial variation of climates and future projections, third to assess how the season of application influences the effects of climate change in accordance with chemical degradation, and finally to quantify the effects of climate change on flux accounting for actual agrochemical uses and application times.

2.4.1 Factors influencing chemical flux under climate change

Meteorological parameters, chemical degradation and flux

UK climates

Using the climate data for the six specified UK sites representative of the UK Met Office climate zones for England and Wales, 5km scale UKCP18 data were extracted and processed for visualisation and inputting into PRZM (Met Office, 2021, 2019). The UK is set to undergo seasonally variable climate change with hotter dryer summers and warmer wetter winters with an overall increase in extreme events, both floods and droughts, throughout the year (Lowe et al., 2018). However, visualisation of the UKCP18 data here also revealed where there was spatial variation in the effects of climate change. For example, with mean daily temperature, the westerly sites of Lancashire and Cornwall exhibited more marginal increases in maximum autumn temperatures compared to other sites, while the changes to maximum precipitation were more varied between sites and seasons affecting the precipitation events triggering chemical flux. In contrast, the rise in mean temperature was relatively consistent between sites, although the increase was more pronounced in summer and autumn (3.7°C to 5.0°C across all sites and both seasons) than spring and winter (2.1°C to 2.8°C across all sites and both seasons), thus degradation rates would be expected to increase year-round, but particularly so in summer and autumn.

Processes underlying flux and degradation

Chemical flux is established to be primarily determined by the size and frequency of precipitation, and here both within examples using hypothetical chemicals, sites and climate conditions to specifically test the effects of size of precipitation event, delay to precipitation event for differing DT_{50} values and how temperature affects degradation in soil for differing DT_{50} values. These supported the existing laboratory data on degradation mechanics which also find logarithmic models of decay and quantity of chemical to undergo transport including for pharmaceuticals (Lammoglia et al., 2018; Pollard and Morra, 2018; Xuan et al., 2008), and the results are comparable to prior outputs from PRZM (Farenhorst et al., 2009). However, the calculated Q10 of 1.77 was lower than the standard of 2.2 used in ERAs (EFSA, 2008). Given the variation in soil properties that can affect the Q10, in particular organic matter and water holding capacity, this may not be unexpected and was within the range of Q10 values from laboratory studies (Cavoski et al., 2008).

Seasonality of climate change and chemical flux

Within the UK, it has been projected that the effects of climate change vary throughout the year, with temperatures rising more in summer than winter, and summer becoming dryer and winter wetter. In reality this has been demonstrated to be more nuanced by spatial variation in the effect of climate change on local climates (section 2.4.2.1). As climate conditions affect chemical transport, the effects of climate change on chemical transport and flux would be expected to change seasonally too. Equally, the climate and effect of seasons with climate change will both vary spatially. Consequently, seasonal effects on flux were expected to vary extensively across different UK climate zones, which was present in the results and shows the complex influence of seasonality. This supports previous studies which exhibit strong patterning in chemical fate depending on the season of chemical sampling and occurs across multiple freshwater ecosystems (Johnson et al., 1997; Varty et al., 2021). Both spatial and chemical identity, particularly around the degradation rate in soil affected the seasonal impact on the change in flux. This emphasises the complex relationship between these factors and the difficulty in extrapolating the effects of climate change from single site studies. However, some broad characteristics common across all sites and seasons could be observed, most notably that the effect of season of application made less difference to the change in flux for slower degrading chemicals. This relationship was common across all sites and has implications when considering how the timings of chemicals may shift or how new chemicals may respond under future conditions, particularly for the care required when timing the application of rapidly degrading chemicals so application does not result in chemical flux (Gros et al., 2019).

2.4.2. Implications & applications

Considering climate change with agrochemical exposure

Increasing realism: Agrochemicals & application times

By considering realistic application times for different chemicals the actual effects of climate change on chemical flux were quantified and placed into a context that could inform chemical use and risk to non-target ecosystems. The need for this has been previously highlighted by both regulatory and scientific perspectives (Brooks et al., 2013; EFSA et al., 2020). It should be noted that within PRZM pesticides and pharmaceuticals are treated equally with all chemicals based off their environmental properties (Mullins et al., 1993). The only difference with regards to fate is the timing of application which contextualises pharmaceutical input to match that of manure or biosolid fertiliser application in spring and autumn (Gogos et al., 2012; Gros et al., 2019). Across all sites and chemicals, climate change causes a wide range of effects, from decreases of up to 97% to increases of 343% compared to the baseline scenario. Previous studies investigating the effects of climate change on chemical fate are limited either by lack of scalability or being fit to specific water systems. Szalinska et al., (2021) has shown an increase of 75% for six metals at a watershed level in 2046–2055 under RCP 8.5 conditions while Valle et al. (2007) focused on chemical degradation and distribution following input predicted decreases in chlorinated polyaromatic hydrocarbons but increased mobility under higher temperatures. However, when considering these realistic application times within an ERA perspective the large changes, both positive and negative, to chemical flux would drastically affect the outcome of ERAs. Equally the extent of spatial variation from climate alone mean that any ERA should either consider the effects of climate change on a spatially explicit scale, or create representative worst case scenarios to be run in a manner similar to the spatially explicit soil and climate scenarios used for FOCUS surface water modelling in ERAs (Fabrega and Carapeto, 2020; Hommen et al., 2010; A. S. Pereira et al., 2017). Overall, the effects of climate change on chemical flux have been shown to be a necessary point of consideration if the future risk to ecosystems from agrochemicals is to be accurately assessed.

Accounting for phenology

Regarding how the timings of agrochemical input may shift in response to climatic change will depend on how the phenology of agricultural crops and their pests requiring chemical application shift under climate change conditions. For example, the effect of climate change to the timing of commonly grown crops such as winter wheat has been investigated with aspects such as emergence, maturation and harvest shifting by 14-28 days by the end of the century (Craufurd and Wheeler, 2009; Paweł Marcinkowski and Piniewski, 2018; Olesen et al., 2012). This in turn will require altering the timing of fertiliser and pre-emergence herbicide use to optimise crop yield (Zimmermann et al., 2017). Equally, some pests are expected to track their phrenology to that of crops, leading to shifting times for chemical application (Bajwa et al., 2020). Furthermore, the climatic conditions may change the profile and magnitude of negative effects, and therefore the type and quantity chemical used to address the pest. For example hotter, dryer summers may increase the susceptibility and spread of fungal diseases to wheat crops (Rhodes and McCarl, 2020b; West et al., 2012b) necessitating increased use of fungicides at times of the year not currently applied. Adapting the methods used within this study may help address the possible risk these timing or chemical shifts may pose, noting a greater focus on faster degrading chemicals that are more liable to seasonal variability.

Integration and expansion within fate research

Future developments of climate projections

The UKCP18 climate data used here were high resolution 5km scale which can accurately project the occurrence and magnitude of localised extreme weather events which are associated with the largest chemical fluxes, and therefore important to realistically represent future risk (Lowe et al., 2018; Met Office, 2019). However, these projections are only available for RCP 8.5, which is the business-as-usual high emissions scenario. However, RCP 8.5 is currently viewed as an extreme scenario with little to no climate change mitigation, and therefore not the most likely climate change scenario (Christensen et al., 2018; Van Vuuren et al., 2011). Thus, these results accurately capture convectional precipitation dynamics but represent a worse case climate change scenario and therefore should be protective of future risk. However, PRZM is limited in that it can only accept daily data but the UKCP18 5km scale data is available down to hourly time periods (Carsel et al., 1985). Using another regulatory fate model, such as MACRO, could expand upon the work here and incorporate hourly data (Jarvis et al., 1997).

Building upon fate research and regulation

While the above results can provide some insight into potential changes to chemical risk, there are limitations that were necessary as part of the methodology to isolate the effects of climate and chemical. However, existing research into fate, agricultural and soil dynamics could be integrated with the work presented here to improve the realism and expand the scenarios that could be represented with this methodology. Notably the soil, landscape and cropping was kept consistent throughout the sites, but there exists great heterogeneity across the UK for which data already exists and could be directly integrated into this research (Cranfield University, 2021; Garthwaite, 2018). For example, soil is a major factor affecting chemical fate and behaviour and would have interacted with chemical properties, most notably k_{OC} (Farenhorst et al., 2009; Oliver et al., 2012; Paasivirta et al., 1999). Equally different crops would have differing phenologies, pests, root structures and interceptions and each will have their own shifts under climate change conditions, all of which can be accounted for within PRZM (Bajwa et al., 2020; Craufurd and Wheeler, 2009; Squire et al., 2016).

Equally, the downstream processes from edge of field flux occur in the freshwater body and can alter the quantity and location of the chemical. These include the degradation or transport of the chemical out of the freshwater body, and the partitioning of the chemical into river sediment (Bach et al., 2016; Gavrilescu et al., 2015). This may enable exposure of the chemical to new ecosystems and organism groups (e.g. downstream water bodies and sediment dwelling organisms). Considering dynamics within freshwater bodies also permits the consideration of chronic risk. This is because long-term chemical exposure can be calculated rather than just acute peak concentrations associated with edge of field flux (Pereira et al., 2017). Just as models such as PRZM consider fate dynamics and transport in soil, extensively researched models exist for chemical fate in freshwater; TOXSWA, a regulatory model for chemical fate in freshwater bodies, can take the outputs directly from PRZM and are combined together pesticide ERAs to form the modelling suite of SWASH (Adriaanse, 1996; Te Roller et al., 2015).

In contrast SWAT is a combined soil and water fate model that functions at the watershed level rather than the field-scale explored here (Di Luzio et al., 2002; Wang et al., 2019). Indeed, Szalinska et al. (2021) used SWAT to consider the effects of climate change on fate at the watershed level projecting increases in metal loadings by 75%, while noting the specificity of these results to the location modelled (Carpathian basin, Hungary). Consequently, while the results here may prove indicative of overall trends of chemical fate into freshwater ecosystems, the major advancement here is the adaptability of this method to different chemicals and climates, which can be directly integrated with other models such as TOXSWA as done in existing ERAs (Van den Berg et al., 2015), expanding the consideration of climate change to freshwater fate represents a logical next step for contextualising the effects of climate change on chemical flux presented here.

Overall, this study has demonstrated a functional methodology from which the change to agrochemical risk from climate change can be quantified and built upon by including real chemical agricultural practices policies for regulation could be determined. For example, rapidly degrading chemicals ($DT_{50} < 2$ days), often pharmaceuticals can see drastic rises under climate change associated with an increase in rainfall events following application that can be mitigated if there is no precipitation immediately following application. An example regulation would be “avoiding the use of manure or biosolid fertilizer within the three days prior to predicted precipitation events”. Furthermore, the spatial aspect of risk has been highlighted, with specific sites, such as Cornwall tending to have the largest decrease in percentage risk for most chemicals, while those applied in Oxfordshire tend to see an increase regardless of season of application. Consequently, a spatially differential risk assessment could restrict or allow application on a site and seasonal basis, like existing regulation restricting the application of specific insecticides in spring during flowering time to avoid ecotoxicological exposure to bees and other pollinators that is high risk at that time of year. Beyond consideration of edge of field flux, contextualising the result by simulating dynamics of the freshwater ecosystem could improve the realism of the risk assessment. Existing freshwater fated models exist, but would need adapting to future climate change conditions, but can be integrated to provide a full ecosystem perspective on chemical exposure (US EPA, 2016a; Wang et al., 2019). To summarise, this study has shown how localised climate projections can be adapted to existing ERAs and landscape characteristics to predict how climate change can alter the flux of various agrochemicals. These results highlighted the need to assess the effects of climate change accounting for spatial variation in climate change itself which resulted in divergent changes in flux for the same chemical with different climate data. Equally, the method here has shown how PRZM can be tailored to a wide range of climate, chemical, crop and location scenarios.

CHAPTER 3

Assessing the effect of taxonomic composition on chemical sensitivity at an assemblage scale

Chapter 3. Assessing the effect of taxonomic composition on chemical sensitivity at an assemblage scale

3.1 Introduction

3.1.1 Sensitivity on scales above the organisms

Because species vary in their geographical distribution and life histories, the species composition of communities varies in space and time in response to biogeographic factors (Cleland et al., 2013; Legendre et al., 2005; Silvertown et al., 1994). The sensitivity of species to chemical pollutants also varies and their relative sensitivity is chemical specific (Cairns, 1986; Craig et al., 2012). Consequently, the sensitivity of spatially-varying communities to chemical pollutants is also expected to differ (Relyea and Hoverman, 2006). Yet current chemical environmental risk assessments (ERAs) have no option to consider the spatial differences in taxa and to what extent this affects the sensitivity of the ecosystem (EFSA, 2013a; Hickey et al., 2009). Consequently, ERAs fail to account for spatial variation, only aiming to acknowledge this uncertainty by using assessment factors (ECHA, 2008).

Issues of the ERA at an assemblage scale

Existing ERAs consider sensitivity from species from toxicity tests but are associated with two issues relating to the species tested. Firstly, ERAs for regulated chemicals are generic and are applied over broad taxonomic groups (e.g. fish, macroinvertebrates, algae). A single regulatory acceptable concentration for each group is used irrespective of any spatial variation in the sensitivity of potentially exposed communities (Vaal et al., 2000; Verschoor et al., 2011). Secondly, testing of species is limited compared to the diversity of species exposed in nature (Liess and von der Ohe, 2005; Lorenz et al., 2017). For example, in the RICT reference site dataset which is meant to be reflective of the macroinvertebrate diversity of the UK in minimally impacted rivers has recorded 617 species (Wright, 1994). In contrast the agrochemical tested on the most species in the publicly-collating ECOTOX database is malathion with 176 unique species from a global distribution with uneven taxonomic testing (e.g. 16 species were tested in the *Aedes* genus, US EPA, 2021). Consequently, there is a need to assess to what extent differences in assemblage composition among natural ecosystems has on the variation in sensitivity and whether such variation has any bearing on the outcome ERAs, improving realism and making the protection of ecosystems spatially explicit.

Accounting for the sensitivity of multiple species within an assemblage has been investigated using empirical approaches such as mesocosm studies that include species expected to be found in natural habitats or statistical approaches such as species sensitivity distributions (SSDs) that collate toxicity data from multiple laboratory studies (EFSA, 2013a). However, neither approach is primarily used to address the relationship between community composition and sensitivity to chemical exposure. Instead, mesocosm studies are designed to incorporate biotic interactions and community dynamics in the assessment of risk (Boyle and Fairchild, 1997). While mesocosm studies include some natural species and can account for some community dynamics, they are limited by the not fully representing interspecific and food web interactions, notably missing the top trophic level provided by fish, and the migration and recolonisation of natural river ecosystems once perturbed (Van den Brink et al. 2005; Preston, 2002). In addition they are time and capital intensive and therefore only performed for most chemicals' ERAs as a refinement of unacceptable risk at lower tiers of the ERA (Boxall et al., 2002). Furthermore, this impracticability means that research into the effects of assemblage composition on sensitivity between mesocosms is limited with studies focusing on the dynamics of one community (King et al., 2016; Rohr and Crumrine, 2005).

In contrast, SSDs are used to represent risk to a theoretical universe of potentially exposed species and calculate a hazard concentration value (HC_x), the chemical concentration for which a specific percentage (x%) of species are affected (Wagner and Løkke, 1991). The data requirement for SSDs is sufficient toxicity endpoints (recommended at least eight, minimum five) for a chemical to fit a suitable (log-normal distribution, EFSA, 2013a). For SSDs, the availability of toxicity data limits their use for spatially variable communities. This is because toxicity tests are performed on limited numbers of species compared to the diversity in natural ecosystems, and those that are suitable for laboratory testing tend to be unrepresentative of species in natural ecosystems (Liess and von der Ohe, 2005; Lorenz et al., 2017). While SSDs can be applied broadly and altered to be spatially explicit (Posthuma et al., 2019), they remain limited in scope within ERAs by a limited species pool and therefore are unreflective of natural assemblages. Equally from a chemical identity perspective, Verschoor et al., 2011 has shown that SSDs for different metals vary and exhibit spatio-temporal variation in risk, although this was due to differences in chemical-environment interactions rather than community structure which was the same across sites.

Another issue is that SSDs assume that the sensitivities of species are independent and that for a species, its sensitivity ranking for one chemical is uninformative to another (Aldenberg and Jaworska, 2000). This means an SSD assumes that the identity of the species tested does not matter. Yet, the non-exchangeability of chemical sensitivity between species has been shown (Craig et al., 2012). This means some species tend to be more sensitive to chemicals than others, and closely related species share chemical sensitivity due to relatedness being correlated with increased sharing of genetics and traits for chemical sensitivity. Consequently, because different combinations of taxa can be used in SSDs they can reflect different spatial and temporal scales of natural assemblages. The limited taxonomic diversity of toxicity data mean the SSD produced is generic and used to represent all communities (Belanger et al., 2017). This means the question of whether the sensitivity profile varies between assemblages beyond the coverage of a generic SSD and how chemical identity affects such variation is not considered and requires investigation.

Given the potential for species composition to influence sensitivity at the assemblage level (eg a set proportion of species exhibiting toxic effects as with a hazard concentration value, HC_x), the range of communities play an important role for the variation in assemblage sensitivity. Within the UK the RICT (River Invertebrate Classification Tool) model is used as a measure of ecological water quality by predicting expected macroinvertebrate communities for an unimpacted freshwater river given the landscape characteristics of a specific river (Environment Agency et al., 2021; Wright, 1994). However, the model itself was built upon an extensive reference dataset of 835 minimally impacted sites (those experiencing only minor changes to river structure, watershed and pollution since pre-industrial times) in the UK and reflects how the variation in river typologies and properties influence the macroinvertebrate community (Davy-Bowker et al., 2006; Wright et al., 1996). Additional to spatial variation, climate change, in particular temperature, is known to influence the macroinvertebrate communities of the UK (Durance and Ormerod, 2007; Vaughan and Ormerod, 2012). Consequently, climate change over the 21st century could affect sensitivity by shifting community composition. Again, RICT can be adapted to use different river parameters, including temperature, permitting the effects of climate change on macroinvertebrate communities to be predicted (Armitage, 2000). This means that the diversity in assemblage composition which varies both spatially due to freshwater landscape and typology, and temporally due to the effects climate change, will need i) a way of assessing assemblage sensitivity, and ii) a consideration to what extent variation in assemblage sensitivity is down to taxonomic composition.

Methods for predicting taxa sensitivity

One approach capable of addressing both the genericism of ERAs and the lack of toxicity data for species in natural ecosystems is to predict the toxicological sensitivity of untested species and integrate these results into spatially explicit ERAs (van den Berg et al., 2021). There are multiple methods for predicting assemblage sensitivity and each has differing data requirements, explanatory power for the predictions, and limitations and assumptions, but none are currently employed within risk assessments (Baird and Van den Brink, 2007; Craig, 2013; Guenard et al., 2011; US EPA, 2016b). The calculation of a value of sensitivity is dependent on how the methodology utilises environmental and ecosystem data. For example, two common protection goals, i) ecosystem functioning because of the traits of organisms (e.g. shredders and decomposers), and ii) the genetic and taxonomic diversity of the communities themselves can each be protected using predicted sensitivities from examples of these predictive methodologies (Loreau and Mazancourt, 2013; Nienstedt et al., 2012). These are reflected in two of the predictive methods, trait-based and relatedness-based which are discussed in further detail in the next paragraph. Genomics-based methods represent a highly accurate methodology for species prediction by basing sensitivity prediction on the genetic basis of biochemical pathways and enzymes of species using the molecular target site of chemical toxicity. However to know the molecular pathways, genomics-based methods require genetic sequencing of every taxon to be predicted. Although sequencing of macroinvertebrates is improving, sequencing of the genetics underlying the relevant target site is limited for most species (Fedorenkova et al., 2010; LaLone et al., 2013).

Traits based predictive methods work on the assumption that organisms exhibiting similar traits will have similar chemical uptake (e.g. diet, oxygenation mechanism, location within freshwater) and depuration (e.g. body size, lipid content, metabolic rate) will therefore exhibit similar chemical sensitivity (Rubach et al., 2010; Van den Berg et al., 2019). In contrast relatedness-based methods use the fact that closely related species tend to exhibit similar sensitivities to chemicals by acting as a proxy for the extent of shared sensitivity-influencing factors (e.g. body size, metabolic pathways, target receptors, Blomberg et al., 2003). This occurs as closely related species share more of their genetics and niches, affecting traits, compared to distally related species. This is due to a shared distance from a common ancestor resulting in similar biochemistry and phenotype and therefore a shared susceptibility to specific chemicals (Guenard et al., 2011; Malaj et al., 2016). Ideally phylogenetics would be used as the basis of relatedness. However, the phylogenetic tree for UK macroinvertebrates remains unresolved and so it is not possible to be used currently, although advancements in sequencing mean that this is becoming increasingly resolved (Guenard et al., 2014).

The data requirements for trait-based methods are a list of relevant traits for the predicted taxa (Baird and Van den Brink, 2007) and while macroinvertebrate trait databases are available and improving, coverage of freshwater macroinvertebrate taxa is incomplete, for example coverage of Crustacea and Insecta is good but more limited for less studied groups such as Mollusca and Annelida (Hébert et al., 2016; Rubach et al., 2010; Usseglio-Polatera et al., 2000). In contrast, relatedness-based methods that utilise taxonomic ranking and structure have known relatedness relationships of UK freshwater macroinvertebrates making such methods suitable for predicting sensitivity (Craig, 2013).

Hierarchical species sensitivity distributions (hSSDs)

Hierarchical species sensitivity distributions (hSSDs) are a relatedness-based predictive model that integrates taxonomic information to predict toxicity endpoints for a group of species with known taxonomy permitting the fitting of SSDs (Craig, 2013, and described in the methods below). The hSSD method is based upon hierarchical taxonomy and the establishment of the following from toxicity data: firstly, that there is a sensitivity tendency of a species and that these tendencies vary between species making species non-exchangeable, and secondly that interspecies correlation occurs with the strength of correlation decreasing with relatedness such that the uncertainty surrounding taxa sensitivity predictions will vary based on the relatedness of available toxicity data. This is in contrast to existing SSDs in that chemical identity will determine if a taxon is sensitive to that chemical or not, and therefore assemblage composition will affect the structure of the SSD curve on a chemical basis, a limitation noted for SSDs in current ERAs (Belanger et al., 2017). Acknowledging this, the hSSD method uses a Bayesian approach of different hierarchical model relationships depending on the degree of taxonomic relationship between the toxicity data and predicted taxa to calculate a sensitivity value, then collates the predictions for multiple taxa to calculate a HC_5 value representing assemblage sensitivity. More detail on the hSSD method is presented in Appendix A3.1.

Considering the existing literature above, the identified research gaps this chapter aims to address are to what extent do species assemblages vary in their sensitivity to chemicals and secondly, how is this variation affected by species composition and chemical toxic mode of action. Sensitivity prediction methods, in particular the relatedness-based hSSD method, can approach this by predicting sensitivity values for taxa with described taxonomy noting that the predictive capability of the model improves with a greater quantity of and more closely related toxicity dataset for the predicted taxon. It has been noted how such methods can improve upon the genericism of existing SSD methods, enabling spatially specific predictions of assemblage sensitivity. Furthermore, providing the effects of climate change on community composition can be projected, predictions for climate change affected assemblages could be calculated and compared to baseline predictions enabling some insight into the effects of climate change on the sensitivity of freshwater ecosystems. Contextualizing within an ERA perspective, this method could be used to inform locations where existing risk assessments are over or under protective, both currently and in a climate change affected future, avoiding excessive mitigation or highlighting areas that currently are or will undergo environmental degradation but are not considered within current ERAs.

Consequently, the suitability of addressing these two issues with the hSSD method here by posing the following questions: 1) Given that organism sensitivity varies depending on the chemical, does the sensitivity of a species assemblage vary with the chemical and its mode of action? 2) Given communities vary spatially and have different species compositions, does the calculated sensitivity threshold decrease in accordance with sensitive species presence? 3) How will the shift in taxa composition from climate change affect assemblage sensitivity compared to baseline conditions?

These questions were addressed by: 1) Considering the magnitude of variation and the correlation co-efficient of predicted values for common species between different chemical and mode of action groupings, 2) Comparing the calculated assemblage sensitivity values for different natural assemblages and linking the patterns in sensitivity to sensitive taxa and river typology, 3) Predicting the expected assemblages at a series of locations under baseline and future climate conditions and comparing the change in sensitivity between the same location for a series of agrochemicals.

3.1.2 Hypotheses and Objectives

1. Run the hSSD model for the collated toxicity datasets and evaluate the ability of the hSSD model to accurately predict sensitivity of taxa

    How strong is the correlation of using a leave one out analysis based on the toxicity datasets alone where taxonomic distances vary?

    How strong is the correlation between predicted and laboratory sensitivities for taxonomically closely related species (ie. members of the same genus and family) where similar sensitivity values would be expected?

2. Assess to what extent the sensitivity between assemblages varies and is the variation in sensitivity patterned across chemicals with different toxic modes of action

    Over how many orders of magnitude are the differences in assemblage sensitivity?
    Do chemicals with modes of action targeting specific taxa (eg insecticides) exhibit a greater range in predicted assemblage sensitivities?

3. Assess whether the sensitivity of an assemblage relates to the physical landscape characteristics of where that freshwater body and its macroinvertebrate assemblage is located

    To what extent does site typology relate to the type of taxa present?

    Are the most sensitive assemblages linked to typologies associated with taxa particularly sensitive to that chemical?

4. To quantify how the shifting of community composition under climate change alters the assemblage sensitivity

    By how much does assemblage composition change under climate change?

    Does the shift in assemblage from climate change increase mean assemblage sensitivity, and is this pattern the same for all chemical types?

    Does the shift in assemblage from climate change increase the range of assemblage sensitivities, and is this pattern the same for all chemical types?

3.2 Methods

3.2.1 The hSSD Model

Natural assemblages contain a large number of species, only a few of which will have toxicity data for any specific chemical. To generate a species sensitivity distribution (SSD) for a specific assemblage, predicting the toxicant sensitivity of untested species is therefore usually necessary. The hSSD model was developed to assess the toxicant sensitivity of an assemblage that contains species whose sensitivity to the focal chemical is unknown (i.e. no toxicity data). It also accounts for the inherent uncertainty surrounding laboratory toxicity testing (EFSA et al., 2018a, (Forbes and Calow, 2002; Hickey et al., 2012). The hSSD model uses the EC_{50} (effect concentration affecting 50% of individuals of that species) values as a measure of toxicant sensitivity. The hSSD model used within this thesis is based on Craig (2013) and is model version 122 as this includes all taxonomic ranks (kingdom, phylum, class, order, family, genus, species) but not any ranks between these levels (e.g. subphylum or superclass). Consequently, a description of the model and how it was adapted to the toxicity data used in this thesis, and how the model predicts taxa and assemblage sensitivity is included below.

At the simplest level, an EC_{50} value (y) of a species (j) sensitivity to a chemical (i) considering the uncertainty of laboratory testing (k), a single EC_{50} can be calculated using Equation 1.

$$y_{ijk} = \alpha_i + \beta_j + \delta_{ij} + \varepsilon_{ijk} \qquad \text{Equation 1}$$

Where y_{ijk} is the predicted EC_{50} value, α_i represents the “true” average toxicity of the chemical *i* to a species across all species, β_j the tendency of species *j* to be more or less sensitive on average to chemical exposure, δ_{ij} the interaction factor of chemical *i* and species *j* representing per species chemical effects and ε_{ijk} the inter-test variation when performing a laboratory toxicity study *k* featuring chemical i and species *j*, representing the measurement error from performing toxicity tests.

While δ_{ij} is assumed to normally distributed for different species *j*, the shape of the distribution would be expected to be different for differing chemicals. For example, the range of sensitivities for a general narcotic would be less than for a highly specific insecticide. Consequently, a scaling factor (φ) for chemical *i* is incorporated into the hSSD model to represent this variation between chemicals (Equation 2).

$$y_{ijk} = \alpha_i + \beta_j + \varphi_i \delta_{ij} + \varepsilon_{ijk} \qquad \text{Equation 2}$$

Factor α_i was assumed to be represented by the inputted toxicity data and calculated from a normal distribution of all available EC_{50} values for chemical j in format $N(\sigma^2 \alpha)$ with N representing the normal distribution and σ the standard deviation of the available toxicity endpoints (α) for chemical j. In contrast, ε_{ijk} the inter test variation was modelled using a t distribution due to test variation tending to have one extreme tailed distribution as previously demonstrated from analysis of toxicity datasets (Craig et al., 2012). Consequently, the distribution for ε_{ijk} can be summarised as $t(\sigma^2\varepsilon)$ with t representing the t-distribution and σ the standard deviation of what would be expected to be the outcome from differing toxicity tests.

The sensitivity tendency of species (β_j) and the chemical-species interaction (δ_{ij}) can be further broken down to reflect the taxonomic structure of species and allow extrapolation from known species sensitivities and interaction factors from more distally related species at a higher taxonomic rank (Equation 3a and Equation 3b). This permits the hSSD model to predict sensitivity values even when toxicity studies on that specific species are unavailable. Nine taxonomic ranks were incorporated into the hSSD model: species, genus, family, class, superclass, sub-phylum, phylum, kingdom (numbered 1 to 9 in Equation 3a and 3b) to align with the range of taxonomic ranks covered by invertebrate species.

$$\beta_j = \beta_{1\,(j)} + \beta_{2\,(j)} + \cdots + \beta_{9(j)} \qquad \text{Equation 3a}$$

$$\delta_j = \delta_{1\,(ij)} + \delta_{2\,(ij)} + \cdots + \delta_{9(ij)} \qquad \text{Equation 3b}$$

Where all members at a specific taxonomic rank n (ie. 1 through 9) are incorporated to each $\beta_{n\,(j)}$ and $\delta_{n\,(ij)}$ factor. Each individual taxonomic ranking component is sampled from a normal distribution of inputted data in the format $N(\sigma^2\beta_n)$ or $N(\sigma^2\delta_n)$ with N representing the normal distribution and σ the standard deviation in for data at taxonomic rank n.

3.2.2 Chemical selection and processing

To run the hSSD model, chemicals with acute toxicity data were collated for the most taxonomically diverse selection of species possible. Chemicals were only selected for inclusion if they had suitable acute endpoints from at least 10 different taxa and represented at least 80% of the major invertebrate phyla found in UK freshwater ecosystems. The criteria for suitable endpoints were that they must be calculated EC_{50} or LC50 values for mortality or immobility from aquatic exposure via the medium. While the study must be aquatic, freshwater, brackish or marine species across a global distribution were considered acceptable as Maltby *et al.* (2005) indicated that taxonomic identity was an important factor influencing HC_5 calculated values, but the geographic distribution and test media of the species was not. Where laboratory test guidelines are available (e.g. OECD 202 for *Daphnia*, OECD (2004)), studies should ideally follow them without alteration to the media or test set-up. Any media concentration regime was acceptable, although preference was given to renewal or flow through studies as a consistent concentration is more likely to be maintained. The time frame for the study endpoint must be between 1-7 days, although ideally 96h endpoints were taken, or 48h for species where 96h studies are not performed as standard. This was done to increase the number of studies where the toxic effects would occur over the same timeframe. While both measured and nominal concentrations were considered acceptable, measured concentrations were taken in preference where available as they represent a more accurate exposure of the test organism.

Datasets were collated from two sources, the toxicity datasets previously collected for Maltby et al., 2005, and the publicly available US EPA ECOTOX database (US EPA, 2018). The chemicals included in Maltby et al., 2005 were supplemented with additional datapoints from the ECOTOX database. Endpoints from the ECOTOX database that were outliers compared to other values or were potentially from studies with multiple-stressors or non-standard methodologies had the original studies consulted to ensure suitability for inclusion. From these 40 chemicals were collected and grouped into three main types depending on their chemical properties and toxic effect on macroinvertebrates; metals formed one group, while organic chemicals were separated out based on whether they had a mode of action that specifically targeted a macroinvertebrate group, of which insecticides were the only group. All other organic chemicals were not specifically acting to one group of macro invertebrates and have been treated as general narcotics.

For the hSSD model to read the chemical data, each taxa needed the current Linnean taxonomy for each of the ranks included in the hSSD model (kingdom, phylum, subphylum, superclass, class, order, family, genus, species). To ensure consistent taxonomy when filling in the taxonomic ranks the taxize package for R was used to complete the taxonomy using taxa and classification as listed in the NCBI database (Chamberlain and Szöcs, 2013; NCBI, 2021). The taxonomy for each file was then checked with the taxonomy database within the hSSD model and corrected to ensure the two align and would in intelligible by the hSSD model. Any taxa with toxicity data at a higher rank than species was assumed to be a generic taxon in that rank rather than removing that toxicity endpoint. To make these data readable by the hSSD model the previously blank lower taxonomic ranks were replaced with Unknown (x rank) where x is the taxa and rank the taxonomic rank which is known (e.g. Unknown (Coleoptera family) where a toxicity endpoint for Coleoptera was available only at the family level of classification).

3.2.3 Testing the hSSD model

Prior to use of the hSSD model for species sensitivity prediction, the model needed validation with the toxicity datasets collected above to ensure that the model would produce suitable output from these data. Firstly, a whole dataset leave one out analysis was performed to the following method. A taxon from the toxicity dataset was removed and the hSSD model run to predict a sensitivity value for the removed taxon from the other toxicity data. This was repeated for each taxon in the dataset. However, the distance in taxonomic ranks between each left out taxa and the remaining toxicity data varies. Consequently, due to the rank distance related uncertainty included in the hSSD model, the analysis will include predictions over both close relatedness e.g. shared genus, with low uncertainty factor, and distally related e.g. over phylum, where the expected strength of correlation is much weaker. While this analysis gives insight into the general predictive nature of the hSSD mode over broad taxonomic coverage, the inclusion of an uncertainty factor obscures the predictive power of the model alone.

To address this, a secondary leave one out analysis was performed with a different methodology that predicted over consistent taxonomic distances. Again, each taxon in the dataset for a chemical was isolated but was converted into a generic taxon that shared a genus with the isolated taxon in the same form for where lower taxonomy is not known; for example, the species *Daphnia magna* would become Unknown (*Daphnia* genus). The hSSD model was then run for the generic genus taxa. If multiple members of the same genus were within the toxicity dataset for that chemical, the hSSD model was run for a taxon with that generic genus once. The strength of correlation between the actual toxicity value for that genus (with a geometric mean taken for multiple values in the same genus) and the prediction from the hSSD model was measured with Pearson’s method and was calculated independently for each chemical.

3.2.4 Assemblage selection and processing

To apply the hSSD model to UK freshwater ecosystems, a comprehensive list of macroinvertebrate assemblages representative of differing ecosystems across the country is required. The River Invertebrate Classification Tool (RICT) is a tool featuring a model to classify water quality based on macroinvertebrate biotic indices. The modelling aspect of RICT was constructed from a series of 835 reference sites across the UK reflecting a range of macroinvertebrate assemblages from the local climatic and landscape characteristics in rivers minimally impacted from anthropogenic action. Due to being minimally impacted they are representative of how chemicals would affect an otherwise unaffected macroinvertebrate assemblage. Again, the taxize package linked to the NCBI database was used to complete the taxonomy which was then aligned with the taxonomy database within the hSSD model and blank taxonomic ranks filled with the same Unknown (x rank) format.

3.2.5 Running the hSSD model

A single list of all taxa present in the 835 reference sites was collated from the RICT reference dataset, while a separate list was collated for all taxa predicted to occur at these sites under either baseline or future climate conditions based on the output of the RICT model with altered temperature values. The hSSD model was then performed for 6000 runs of the Monte-Carlo sampler for each taxon in these lists for all chemicals, and one run of the hSSD model generates a predicted EC_{50} value for each taxon in the list. The first 1000 runs were discarded to ensure convergence of the Monte-Carlo sampler. The next 5000 runs of the hSSD model were retained and for each taxon a geometric mean of these values calculated to give a predicted EC_{50} value for each taxon. The master list of predicted EC_{50} values was the subsetted for each reference, baseline and future assemblage to generate a list of species and EC_{50} values for each of the 40 study chemicals, which were then used to calculate assemblage level sensitivity.

The hazard concentration affecting 5% of taxa (HC_5) is an established measure of assemblage sensitivity and is calculated by determining the species sensitivity distributions (SSDs, ECHA, 2008; Raimondo et al., 2008). A lognormal distribution was fitted to the predicted EC_{50} values for the component taxa of each site to generate assemblage-specific SSDs. The 5th percentile for the SSD was calculated and represents the HC_5 for that assemblage. Confidence intervals for HC_5 values were generated using parametric bootstrapping using the method described by (Grist et al., 2002). For each assemblage, a random value was generated from the original SSD distribution, then fitted with the original EC_{50} values to a new lognormal distribution followed by HC_5 calculation. This process was repeated 1000 times and the HC_5 value from each forming a normal distribution with the 50th percentile value forming the HC_5 value for that assemblage and the 95% intervals of the bootstrapped values derived to give the confidence intervals of the HC_5.

3.2.6 Variation in chemical sensitivity across reference sites

A total of 33,400 assemblage-specific HC_5 values were generated (i.e. 835 sites by 40 chemicals). To ensure comparability between chemicals, the HC_5 for each site and chemical combination was standardised using the 50th percentile HC_5 value for that chemical by dividing all assemblage HC_5 values by the 50^{th} percentile assemblage HC_5 for that chemical. The distribution of predicted HC_5 values across all chemicals (33,400 HC_5 values) was visualised using a violin plot and the magnitude of difference of HC_5 values across all sites and chemicals was calculated considering the range in the standardised HC_5 values. To compare between different chemical types across the range and distribution (here taken as difference between 95% and 5% quantiles of the HC_5 values), the properties of the HC_5 distribution for each chemical were compared. The ranges and distributions for each chemical were calculated and then grouped by chemical type. To analyse whether ranges and distribution differed between chemical types, a one-way ANOVA featuring chemical type as the factor and Tukey tests used to distinguish between chemical types.

3.2.7 Influence of typology on assemblage composition and chemical sensitivity

To visualise the link between typology and assemblage composition, a non-metric multi-dimensional scaling (NMDS) plot was fitted to presence/absence data for the assemblages of 835 sites grouping taxa at the order rank. Bray-Curtis was the distance metric used and the NMDS attempted 20 fittings with goodness-of-fit being measured as stress with the aim of minimising stress. Loadings were calculated for each taxonomic order with adjustment for the frequency of each order such that higher frequency increases loading strength. Typologies were assigned to each site based upon those recorded in the RICT reference database (Wright, 1994), and visualised across two dimensions with adjusted loadings according to typology.

The Water Framework Directive categorises typology as being based upon the altitude, catchment area size and geology of the river sampled (Davy-Bowker et al., 2006). Each of these is subdivided into different categories: altitude above sea level into low (< 100 m), medium (100-1000m) and high (> 1000 m), catchment area into very small (<10m^2), small (10 - 100 km^2), medium (100 - 1000 km^2), and large (> 1000 km^2), and finally geology into calcareous, silicious or organic. Typologies have seen use within the WFD as the type and quantity of taxa expected in river ecosystems are associated with specific river typologies. The RICT reference sites have been categorised into each WFD category as part of inclusion in the database (Wright, 1994). For each chemical type, to analyse the link between typology and the most sensitive sites, a Chi squared test was used to compare the proportion of typologies represented across the 5% most sensitive assemblages for each chemical type and the overall proportion of each typology represented by the RICT reference sites.

3.2.8 Effects of climate change on assemblages

RICT is used for assessing water quality by comparing the sampled macroinvertebrate assemblage from a site to the theoretical expected macroinvertebrate assemblage that would be present in optimal water quality conditions as predicted by RICT (Wright et al., 1998). RICT does so by comparing the landscape and climate characteristics from the sampled site and to the properties of the reference sites. RICT then predicts the assemblage for the sampled site probabilistically for each taxon based upon on the similarity of landscape and climate characteristics between the sampled and reference sites. For reference sites more in common with the sampled sites, the taxa that were present at the reference sites are more probable in the sampled site. Therefore, RICT can also be used to predict the expected macroinvertebrate assemblage for a given set of climate and landscape characteristics.

When RICT predicts the assemblage, it utilises a series of landscape and climatic characteristics of the site. Under climate change conditions, landscape parameters intrinsically associated with the site such as river geology (as calcareous, siliceous and organic) and altitude will not be expected to change. Thus, of the parameters used by RICT, mean air temperature and discharge are the two categories that are influenced by the climate. RICT uses four discharge categories each an order of magnitude apart. Projected changes to mean river flow across various river catchments across the UK are expected to be between a -8.9% to +2.2% for UKCP18 projections for the A1B emission scenario, supporting similar findings for projections from UKCP09 datasets (Kay et al., 2021, 2020; Prudhomme et al., 2012). This change is far less than the order of magnitude difference between discharge categories used by RICT. Consequently, the change to mean air temperature will be the largest effect of climate change on RICT.

Projected mean air temperatures for the UK are produced by the UK Met Office (Lowe et al., 2018). The latest version of the United Kingdom Climate Projections (UKCP) are from 2018 (with revisions in 2019) are known as UKCP18 and are the climate projections used in this study (Met Office, 2019). The UKCP18 dataset is available at a variety of time periods and resolutions. To reflect the local climate of each reference sites and to match the climate data used within Chapter 2, UKCP18 data at the 5km resolution was used. The 5km resolution uses projections for the RCP 8.5 pathway and mean temperature projections between 1980-2000 (baseline) and 2060-2080 (future) were used matching the scenarios in Chapter 2. Each of the 835 sites were linked to the nearest UKCP18 5km grid square and the daily mean air temperature data across the scenario period extracted and then averaged annually to form the baseline and future air temperature value for each site. Mean daily air temperature at 10m was used as this was the value used in constructing RIVPACS, the original model underlying RICT.

Using these UKCP18 values, the built-in air temperature value for the reference sites was replaced with the respective baseline or future temperature and then run within RICT. RICT predicted the probability of taxa being present at the site and a cut off probability of occurrence of 0.5 was used in accordance with the cut off originally used within RICT (Wright et al., 1996). By repeating this process for each of the 835 reference sites, 1670 macroinvertebrate assemblages for each site under baseline and future conditions was formed. To fill the necessary taxonomic rankings for running the hSSD model, the NCBI database was used for macroinvertebrate taxonomy with the taxize package (Chamberlain and Szöcs, 2013). This completed missing taxonomic ranks from the taxa RICT predicted with any remaining blank taxonomic ranks filled with the format Unknown (x rank) where x is the taxa and rank the taxonomic rank which is known (e.g. Unknown (Coleoptera family) where a toxicity endpoint for Coleoptera was available only at the family level of classification).

3.2.9 Assemblage composition under climate change and chemical sensitivity

When accounting for the effect of climate change on assemblage composition it was necessary to quantify by how much macroinvertebrate composition shifts under climate change (using the RICT predictions from 3.2.8). Jaccard similarity (Jaccard, 1912) was calculated to compare assemblage composition at each of the 835 sites under baseline and future climate conditions. The Jaccard Similarity (j) was calculated as below, where two assemblages have the number of taxa A and B, and A∩B is the number of taxa in common between the two assemblages.

$$j = \frac{A \cap B}{A + B - A \cap B}$$

The hSSD model was then applied to the baseline and future assemblages for each chemical to calculate HC_5 values according to the same methodology as the RICT reference sites (see 3.2.5, 6000 runs with first 1000 burned, SSD fitted with a log-normal function)

To compare the assemblage sensitivity (as HC_5 values) of baseline and future assemblages for one chemical, a paired t-test was used including the HC_5 values from each site. This was repeated for all chemicals, the results from which were then grouped by chemical type (i.e. insecticides, metals and narcotics). To analyse the effect of climate change on the change to HC_5 between baseline and future sites and whether this varied depending on chemical type, a two-way ANOVA incorporating both climate change and chemical type was performed, with Tukey tests to compare the chemical types. These analyses were performed for both the mean change in HC_5 and the distribution (here taken as difference between 95% and 5% quantiles of the HC_5 values) of HC_5 values across sites.

3.3 Results

3.3.1 Chemical and assemblage taxonomy with model predictions

Using the chemical selection methodology described in 3.2.2, 40 chemicals were considered suitable with all having between 12 and 177 suitable toxicity endpoints depending on the chemical across phyla coverage of between 82.3% and 98.2% of the RICT reference dataset. A full breakdown of the toxicity data on a per chemical basis is presented in Appendix Table A3.1

The hSSD model was constructed with the following relationship; as the taxonomic distance between the taxon to be predicted and the nearest taxonomic rank for which toxicity data is available increases, the strength of correlation between the prediction and toxicity data decreases and the uncertainty surrounding the prediction increases. A comparison of the overlap in taxonomy of the assemblages at 835 reference sites compared to the toxicity datasets for the 40 chemicals is presented in Figure 3.1 for different taxonomic levels (a full breakdown on a chemical type and individual chemical basis is presented in Table A3.1). At the phylum level, at least 93% of phyla in reference assemblages were represented in toxicity data sets for metals (98%), insecticides (95%), and narcotics (93%). The phyla not represented in toxicity data sets were those most infrequently tested and were also rarely recorded within the RICT reference dataset such as Tardigrada, Porifera and Cnidaria. The proportion taxa to be predicted that overlapped with the taxa in the toxicity data decreased at lower taxonomic ranks (Figure 3.1), with only 6-10% of the 635 taxa in the reference sites sharing a genus with the toxicity data (mean across chemicals grouped by type, for individual chemicals the range was 0.1 – 22.5%).

The hSSD model was first tested with a leave one out analysis using the toxicity datasets from 40 chemicals. For each of the 40 chemicals, the EC_{50} value for each taxon in the toxicity dataset was predicted from the EC_{50} data for other taxa in that dataset. The Pearson R^2 value calculated between the actual and predicted toxicity data ranged between 0.4 to 0.5 across all taxonomic ranks (i.e. species-specific predictions based on toxicity data for taxa in the same genus, family, order, class or phylum). A second leave one out analysis, using family and genus data only in the extrapolation, resulted in an R^2 value of 0.83 across all chemicals for species-specific predictions based on taxa in the same family and 0.85 for species-specific predictions based on taxa in the same genus.

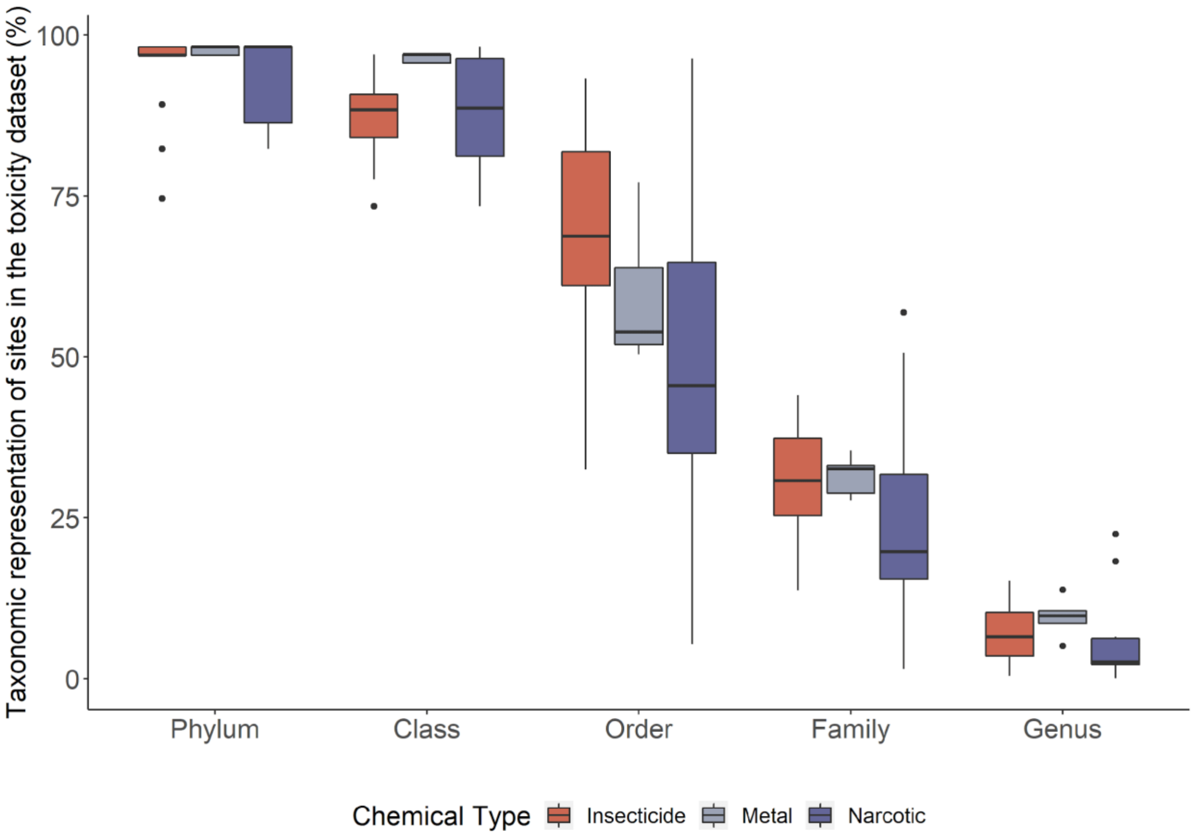

Figure 3.1 Mean percentage overlap at differing taxonomic ranks between taxonomy in the reference 835 sites and toxicity data for 40 chemicals grouped by chemical type (with the number of chemicals in each type being 24 insecticides, 5 metals and 11 narcotics). Boxplots representing the 25th, 50th and 75th percentile across all combinations with whiskers representing the media plus 1.5 times the inter-quartile range

3.3.2 Distribution of chemical sensitivity across reference sites

The 122 version hSSD model was used to calculate the EC_{50} values for all species present at the reference sites, then specific HC_5 values were calculated for each of the 835 site-specific assemblages and for each of the 40 study chemicals. Assemblage-specific HC_5 values were standardised across different chemicals by dividing by the 50th percentile HC_5 for that chemical and the distribution of the resulting 33,400 HC_5 values is presented in Figure 3.2. Most sites fell around the 50th percentile (ie. a standardised $\log_{10}$[predicted HC_5] of 0 in Figure 3.2), although the range if HC_5 values among sites was large, with different assemblages having HC_5 values of over 3 orders of magnitude apart. There was a positive skew to this range with the upper tail for sites with high HC_5 extending further beyond the 50th percentile than sites with smaller HC% values. On a per chemical basis, the distribution of HC_5 values for specific chemicals and their chemical type without standardisation of the HC_5 is presented in the appendix (Figure 3.3).

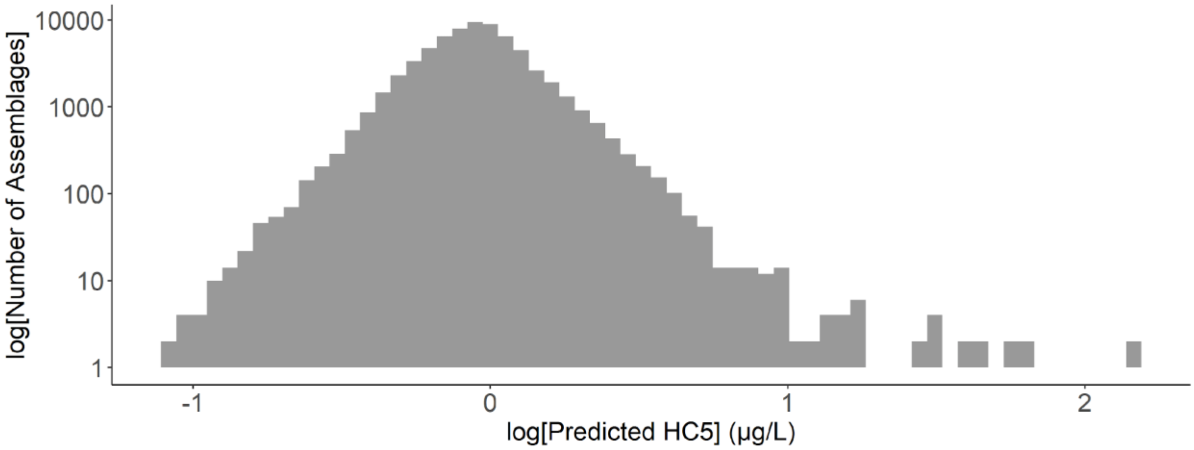

Figure 3.2 Distribution of 33,400 predicted HC_5 values from across 835 reference sites in the UK and 40 chemicals presented on a base 10 logarithmic axis. As species and therefore assemblages have chemical-dependent sensitivity, the site HC_5 values have been standardised by dividing by the 50th percentile site HC_5 for that chemical.

The proportional range of HC_5 values (maximum HC_5 divided by the minimum HC_5) varied depending on chemical type. Insecticides, which have a toxic mode of action targeting insect species, exhibited a significantly greater range of HC_5 values than narcotics, which exhibit general toxic effects (Figure 3.4, F value = 30, DF = 2, 37, p = <0.001). On average, the proportional range of HC_5 values for assemblages at references sites varied by a factor of 25.2 for insecticides, much greater than the factor of a factor of 2.68 for metals and a factor of 2.51 for narcotics.

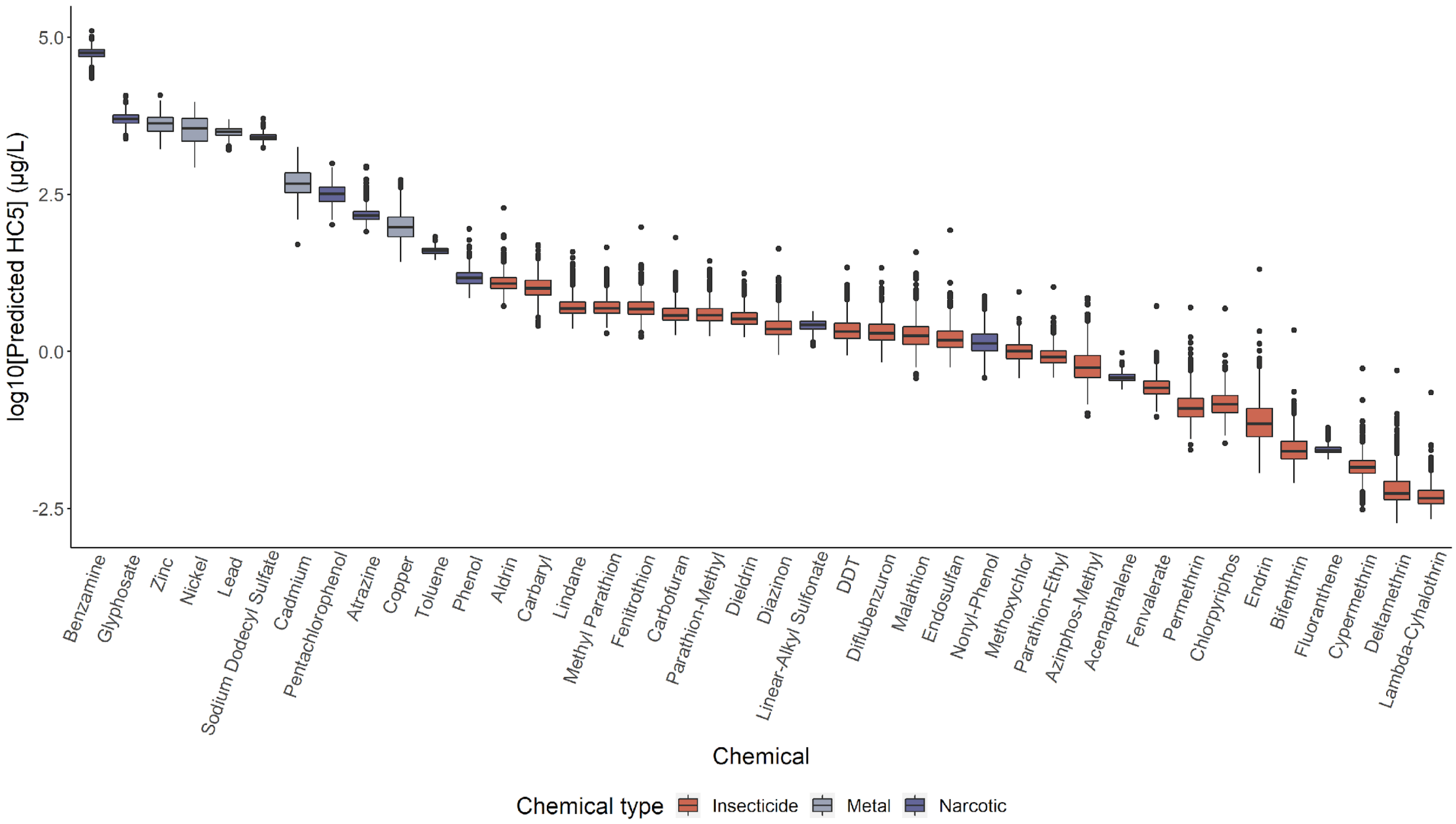

Figure 3.3 Comparison of the calculated HC_5 values across the 835 RICT reference sites for 40 different chemicals. Chemicals have been coloured by chemical type representing their grouping and effect on macroinvertebrate taxa

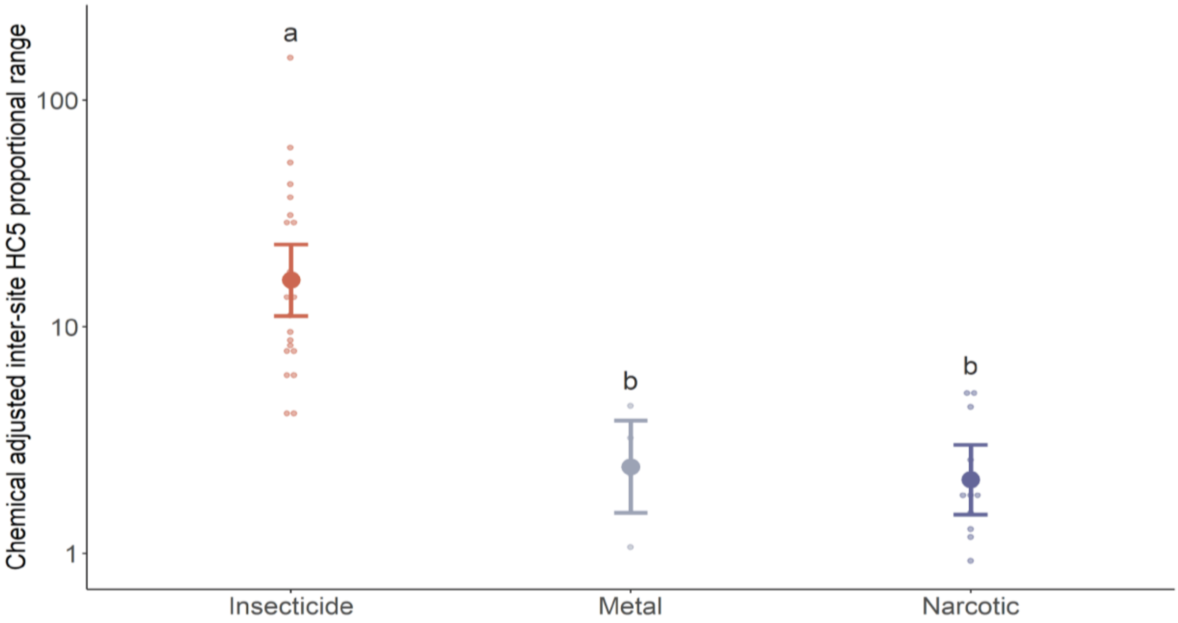

Figure 3.4 Proportional difference between the predicted HC_5 values for the maximum and minimum range across site-specific assemblages across sites where the proportional range is the maximum HC_5 divided by the minimum HC_5. Letters denote results of pair-wise comparisons (Tukey's HSD test) and means values sharing the same letter are not significant different ($p < 0.05$). Results have been presented on a logarithmic base 10 scale with the mean represented as the central point with 95% confidence interval error bars while values for individual chemicals are represented as smaller points

3.3.3 Influence of typology on assemblage composition and chemical sensitivity

Reference sites differ in the composition of their macroinvertebrate assemblage in accordance with the physical properties of the site included in the Water Framework Directive (WFD) as presented in Figure 3.5. The NMDS plots in Figure 3.5 show the ordination of 835 RICT reference sites based on the presence of macroinvertebrate families in each site-specific assemblage. Sites are colour-coded to indicate WFD classifications of river geology, altitude above sea level and river catchment size. The NMDS plot of site-specific assemblages had Spearman correlation of 0.867, and the loadings collated at the order rank are shown on the plot. In general, loadings were more strongly aligned with the NMDS1 axis than the NMDS2 axis, and the NMDS1 axis was the primary differentiator between sites. Major Insecta orders including Plecoptera, Odonata and Ephemeroptera among others, and the worm order Lumbriculida were associated with negative NMDS1 values. In contrast most soft bodied invertebrate orders, such as Mollusca (including Bivalva and Gastropoda) and leeches (Arhynchobdellida and Rhynchobdellida) with positive NMDS1 values. Positive NMDS1 values were also associated with a different set of Arthropod taxa including Isopoda Crustacea, Trombidiformes (freshwater mites) and multiple Insecta clades: Hemiptera, Lepidoptera, Megaloptera, and Neuroptera.

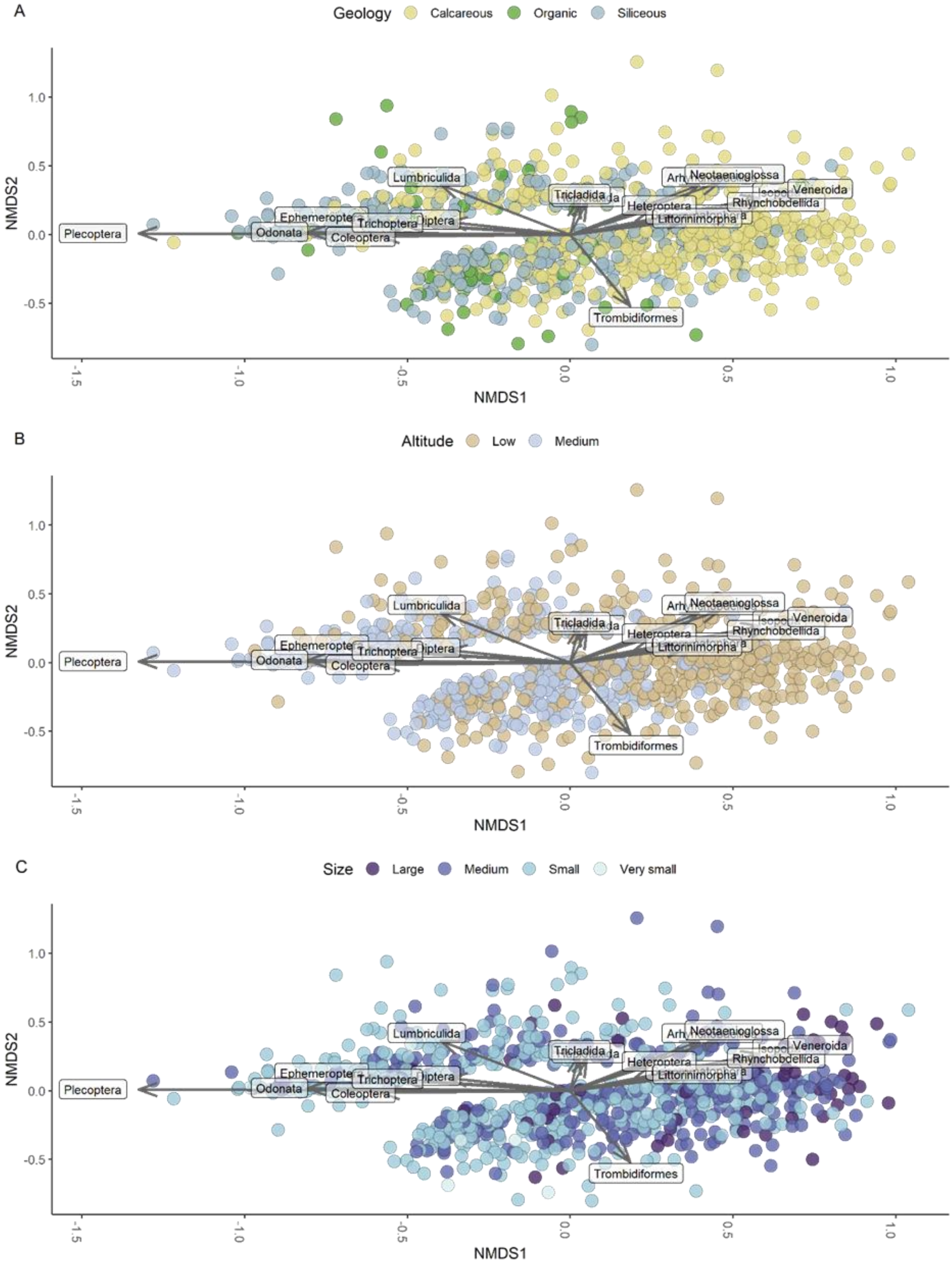

Figure 3.5 Ordination of the 835 RICT reference sites based upon family level assemblage composition with non-metric dimensional scaling across two axes with arrows demoting loadings at the order level. Typologies have been separated out based upon the classification according to Water Framework Directive with the three component categories of riverbed geology (A), altitude (B, ≤100m above sea level low, > 100m above sea level medium), and river catchment size (<10m^2, 10 - 100 km^2, 100 - 1000 km^2, > 1000 km^2 from very small to large respectively)

All the typological categories of geology, altitude and catchment size exhibited differentiation in the NMDS, particularly along the NMDS1 axis (Figure 3.5). Figure 3.6 shows that the midpoint for organic sites at all altitudes, and both calcareous and silicious sites at medium altitude was at NMDS1 values of below one, while lowland (<100m altitude) calcareous sites had their midpoint at a positive value of 0.31 and was significantly higher than all other typologies. Low silicious sites had a midpoint of 0.00 and again, were significantly different to all other typologies. River catchment size is partially autocorrelated with altitude; upland sites are nearer to river source and therefore have both smaller catchment sizes and are at higher altitudes compared to the large drainage basins associated with lowland rivers. Supporting this, catchment size increased positively along the NMDS1 axis. Consequently, these patterns of typology align with the effect of taxonomy on the NMDS scales. Smaller upland rivers and those with silicious and organic geologies tend to have more of specific Insecta taxa such as stoneflies (Plecoptera), mayflies (Ephemeroptera), beetles (Coleoptera) and caddisflies (Trichoptera) than large lowland and calcareous rivers which are more associated with other Insecta species (e.g. Heteroptera), Isopoda Crustacea, and Mollusca taxa.

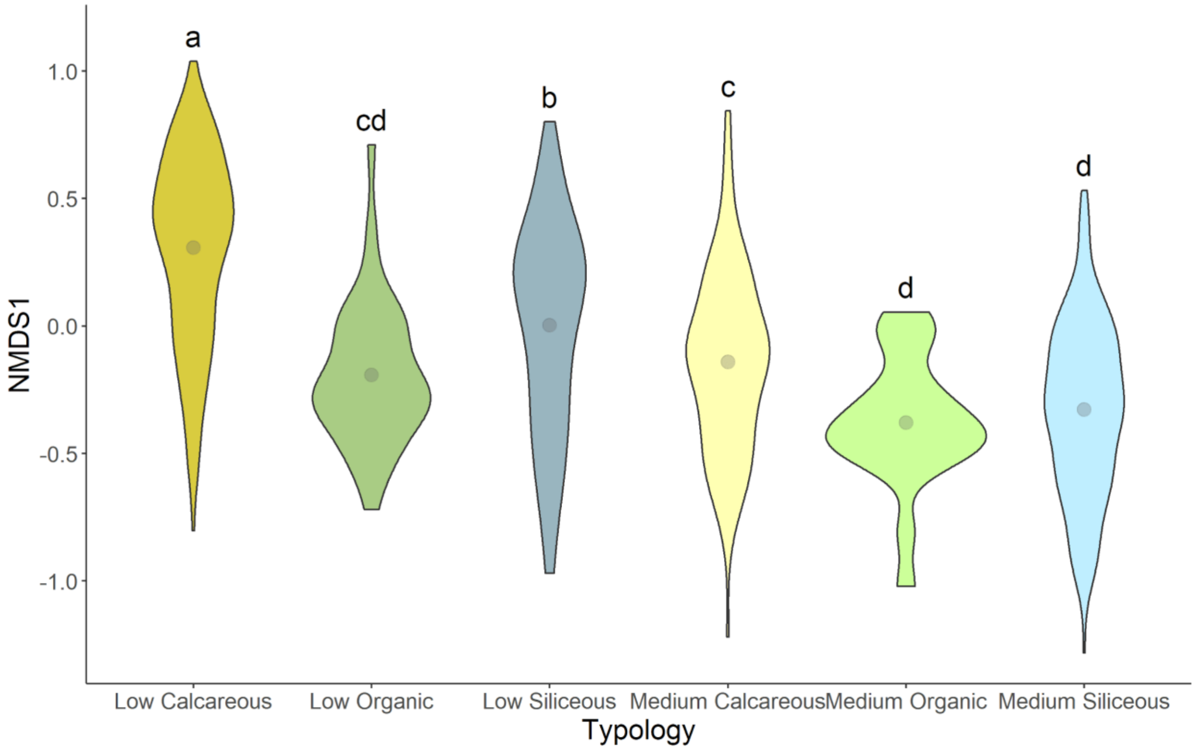

Figure 3.6 Violin plots indicating the distribution of assemblages and their associated NMDS1 scores based upon ordination at the Order level for the taxonomy of 835 RICT reference sites separated by typology with the midpoint of each displayed as a point in the violin plot. Letters denote results of pair-wise comparisons (Tukey's HSD test) and means values sharing the same letter are not significant different ($p < 0.05$). Typologies have been separated out based upon the classification according to Water Framework Directive with the three component categories of riverbed geology and altitude (≤100m above sea level low, > 100m above sea level medium)

When considering the most sensitive assemblages for each chemical (here the 5% most sensitive), the proportion of sites belonging to each typology (where typology is one of six combining the WFD geology and altitude classification) varied depending on chemical type (Figure 3.7B). Compared to the proportion of these typologies in the reference sites (Figure 3.7A) there were significant differences in the 5% most sensitive assemblages for metals (X-squared = 115, df = 5, $p < 0.001$) and narcotics (X-squared = 24, df = 5, $p < 0.001$). In contrast there was no significant difference between the proportion of typologies in the 5% most sensitive sites for insecticides and the proportion of typologies in the RICT reference sites (X-squared = 2.9, df = 5, $p > 0.99$). Metals were associated with low calcareous sites where they were proportionally more of the sensitive sites (77%) than for both insecticides (38%) and narcotics (52%). Concurrently, proportionally fewer of the most sensitive sites were of medium silicious typology for metals (4%) than for insecticides (25%) and narcotics (22%). For some typologies, comparatively few of the 835 reference sites were of these typologies (e.g. medium organic and low organic composed only 4 and 14 sites or 2.5% and 4.5% of total sites respectively in the RICT reference dataset) meaning drawing conclusions for such typologies is difficult.

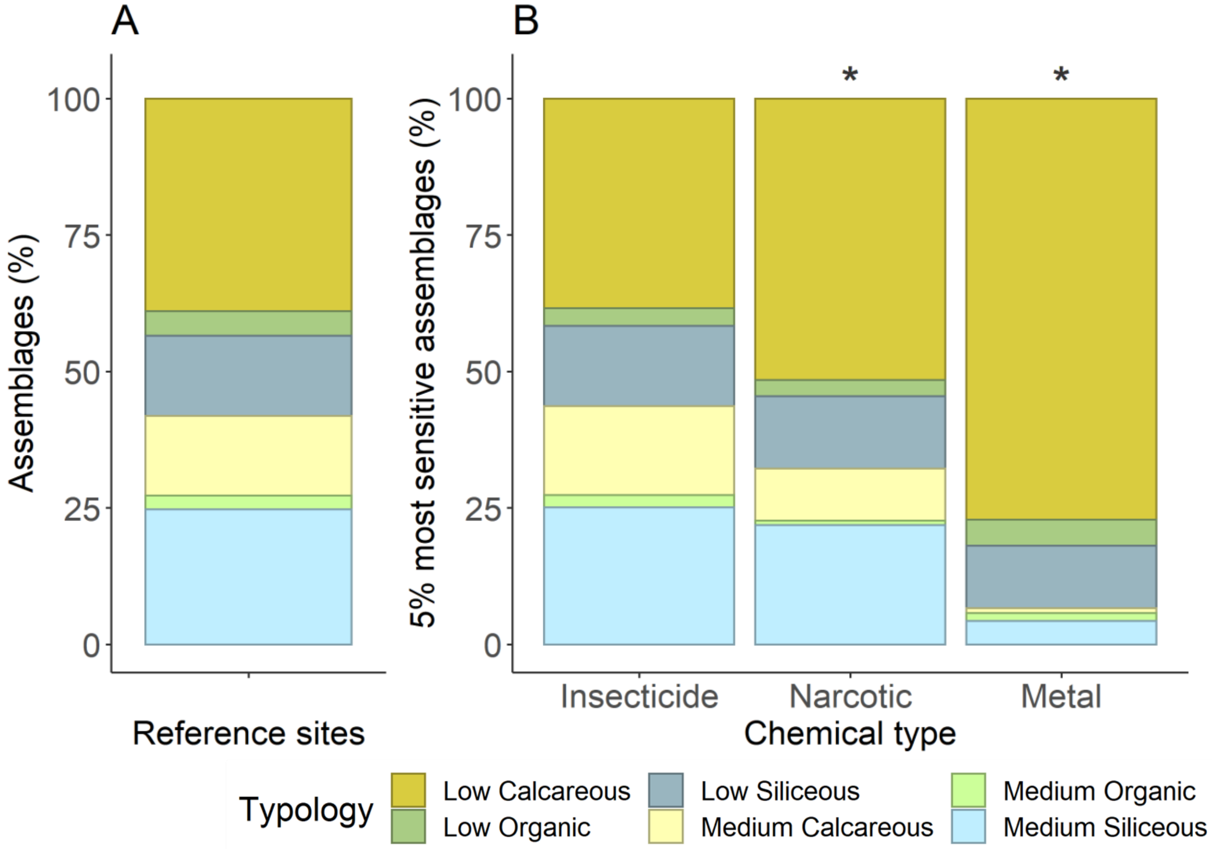

Figure 3.7 Plots displaying the typological distribution of the sites associated with A, each assemblage from the RICT reference sites and B amongst the 5% most sensitive assemblages based on the predicted HC_5 values for each chemical grouped by chemical type. Typologies were defined based on geology and altitude three component categories of riverbed geology, altitude (≤100m above sea level low, > 100m above sea level medium) On B, * indicate chemical types where the typologies from the sites of the 5% most sensitive assemblages are significantly ($p < 0.05$) different to the proportion of assemblages associated with the site typologies of the RICT reference sites

3.3.4 Effects of future climates on assemblages and their chemical sensitivity

Running RICT with the reference site data but with meteorological characteristics altered resulted in two scenarios for each site, a baseline and future assemblage. The amount of change in taxonomic composition was not the same over all sites, as shown in Figure 3.8 where Jaccard similarity indicated how similar the assemblage compositions are, and Jaccard similarity decreases with an increase in temperature change between baseline and future conditions (linear model, gradient = -0.16, df = 835, 834, t value = 16.9, p value < 0.001). There are notable peaks at similarities of 0.3 and 0.5 not associated with any typology (ANOVA, df = 5, F value = 1.91, p value = 0.09) likely corresponding to thresholds within the RICT model.

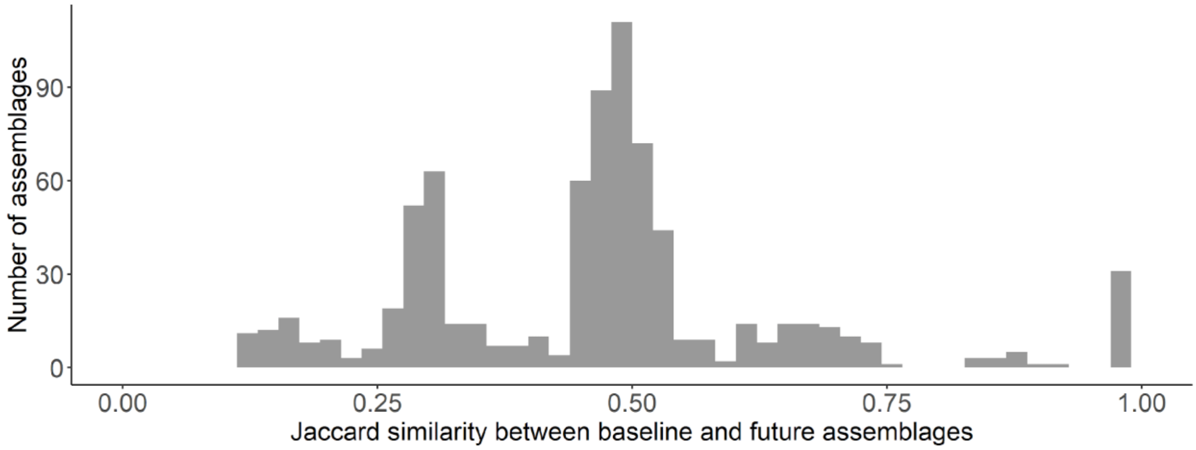

Figure 3.8 Jaccard index measuring similarity between the assemblage composition of the future scenario compared to the assemblage composition of the baseline scenario for each of the 835 reference sites, where a Jaccard index of 0 is total dissimilarity and 1 is total similarity. Both baseline and future assemblages were predicted by RICT utilising UKCP18 climate data for mean daily temperature and range at a 5km scale for each site

The shift in taxonomic composition also altered the sensitivity of the sites as the predicted HC_5 values changed across chemical types. Considering the mean HC_5 across each site for all chemicals (Figure 3.9A), when grouping by chemical types both insecticides and narcotics exhibited a significant increase to mean HC_5 of 16.1% and 2.7% respectively and therefore an average decrease in site sensitivity due to future assemblage composition compared to baseline assemblages (t value = 45, DF = 19031, p < 0.001 and t value = 12.25, DF = 8722, p < 0.001 respectively). There was no significant change in the mean HC_5 between the baseline and future scenarios for metals (2.7% increase, t value = 1.54, DF = 3964, p = 0.12). However, there was a significant difference between the mean HC_5 value for insecticides and narcotics with assemblages shifting to become increasingly less sensitive to insecticides than narcotics (F value = 3.78, DF = 2, 37, p = 0.032).

In contrast, the range between the 5% least and most sensitive sites is set to significantly decrease under climate change for all chemical types, with the 50th percentile range for insecticides, metals and narcotics having a decreased range of 32%, 27% and 16% respectively (Figure 3.9B). Although this pattern was common across all chemical types, insecticides exhibited a significantly greater decrease to this range than narcotic chemicals (F value = 7.93, DF = 2, 37, p = 0.014).

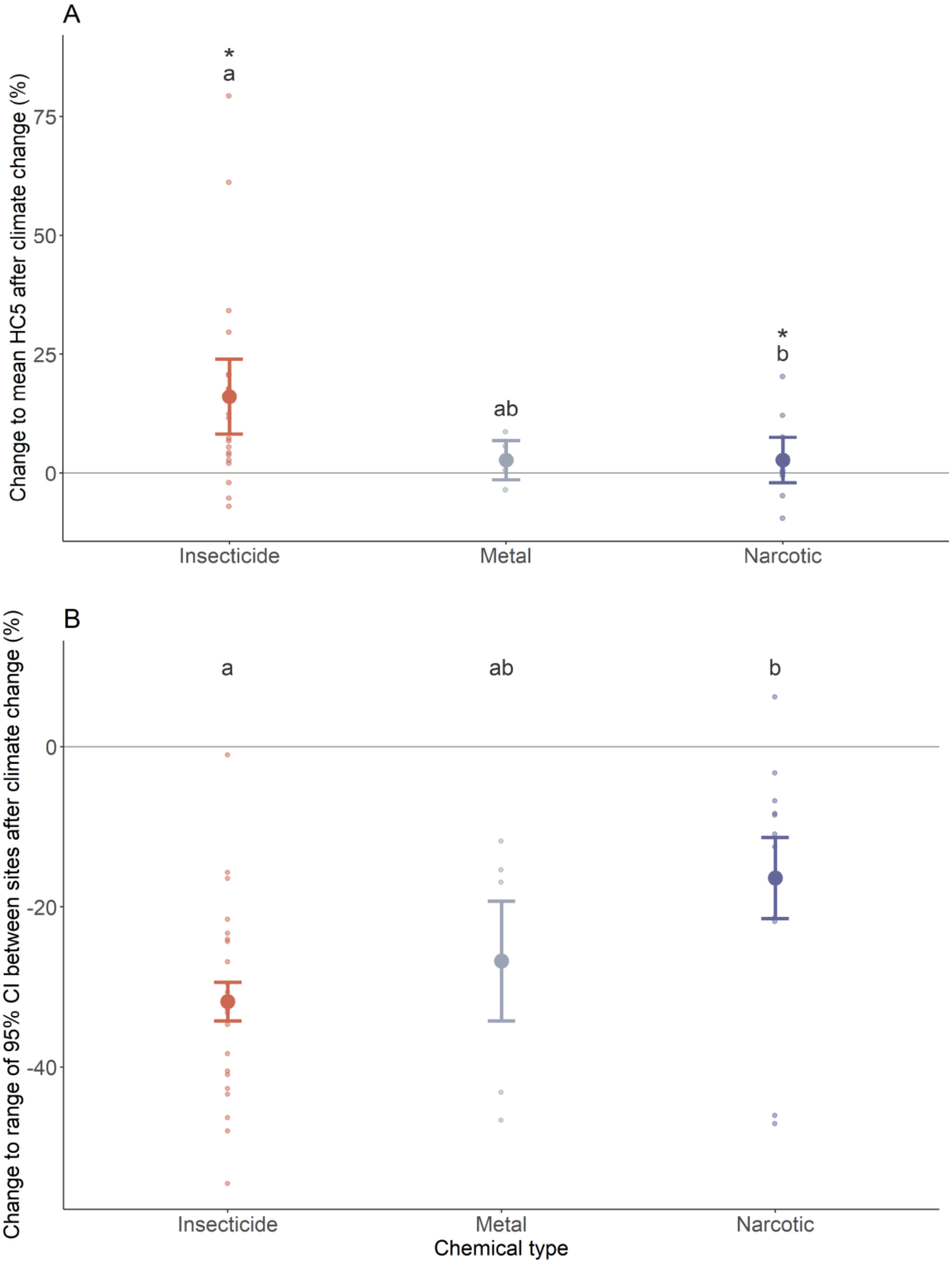

Figure 3.9 *The percentage change to A, the mean HC_5 across sites and B, the change range between the 5% lowest and highest HC_5 values for each chemical grouped by type when predicting HC_5 values for assemblages predicted under future climate conditions (2060-2080) compared to baseline conditions (1980-2000). The results have been grouped by chemical type with letters denoting results of pair-wise comparisons (Tukey's HSD test) and means values sharing the same letter are not significant different ($p < 0.05$). For plot A, * denote chemical types where the change to HC_5 was significantly (<0.05) different from 0%, representing a change to mean HC_5 from shifting assemblage composition. For B, all chemicals were significantly (<0.05) different from 0%*

3.4 Discussion

Spatial and temporal variation in the taxonomic composition of stream macroinvertebrate communities is well established and based upon records on macroinvertebrate samples in the UIK dating back to 1980s (Bradley and Ormerod, 2001; Vaughan and Ormerod, 2012). Such communities change in composition due to environmental stressors, such as pollutants or changes in temperature, changing species' niche compatibility and interspecific interactions, driving population changes, migration and colonisation. On the other hand, understanding of how species composition translates into ecosystem sensitivity to chemicals is poorly understood (Gessner and Tlili, 2016). Any consideration of the threat posed by chemicals to natural species assemblages faces a major challenge; toxicity data are only available for a limited number of species meaning the sensitivity of each of the diverse species in natural ecosystems is unknown. Therefore, there is a need to develop and improve approaches capable of extrapolating toxicity data from tested species to multiple untested species in assemblages of interest. In this chapter, the hSSD model has been used to predict the sensitivity of untested species in natural assemblages and to investigate how variation in the taxonomic composition of natural assemblages influences their sensitivity to chemical pollutants.

To capture the likely effects of agrochemicals on natural ecosystems, 835 minimally impacted sites (those experiencing only minor changes to river structure, watershed and pollution since pre-industrial times) in the UK formed the base assemblages for predicting sensitivity (Wright et al., 1996). To represent the suite of agrochemicals, toxicity datasets for 40 chemicals, grouped by chemical type and effect on invertebrate taxa, were investigated to establish whether the variation in sensitivity would affect the outcome of Environmental Risk Assessments (ERAs) and if there was any linkage between the spatially variable typological properties of the site and therefore assemblage and chemical sensitivity. Furthermore, a reinterpretation of the RICT model, usually used for assessing water quality, has been used to predict the effect of rising temperatures associated with future climate change on invertebrate composition. While this option for the RICT model has been previously highlighted (Armitage, 2000), it has not seen applied use and thus this research represents a novel reinterpretation of RICT. By combining predicted changes to taxonomic composition with species-specific toxicity predictions from the hSSD model, a novel use for RICT has been found. This is as a way to quantify how taxonomic composition changes under climate change affect the sensitivity of natural ecosystems.

3.4.1 Model functionality and applicability

The hSSD model provides a way to predict chemical-specific sensitivity values for taxa without toxicity data (i.e. untested taxa for the focal chemical) that can be integrated into an SSD for the entire assemblage and used to calculate an assemblage-specific HC_5 value for a specific chemical. The predictive power of the model is a function of the toxicity dataset for each chemical to the assemblage being predicted, namely the taxonomic distance being extrapolated over, and the quantity and extent of concordance of the toxicity data at each taxonomic rank. Although the hSSD model has was developed and tested for functionality and statistical robustness (Craig, 2013), this study is the first time it has applied to naturally occurring assemblages. Consequently, additional analyses were performed to evaluate the robustness of the hSSD model. Two types of leave-one-out analyses were performed; the first predicted a sensitivity value for each taxon in the toxicity dataset using all other toxicity datapoints and resulted in a of correlation > 0.4 for all chemicals reflecting the expected relationship of taxonomic distance and sensitivity from taxonomically diverse sensitivity datasets (Craig, 2012). The second leave-one-out analysis tested extrapolations where a high degree of correlation would be expected ie. a taxonomic distance of the family and genus level between the toxicity data to the predicted species. Predictions to taxonomic data separated at the at the family and genus level resulted in mean correlations of 0.83 and 0.85 respectively across all chemicals demonstrating the functionality of the model and applicability to species in natural ecosystems.

The hSSD model is only one approach to addressing prediction of species toxicity, but while genomics-based or trait-based methods may be able to provide greater insight into assemblage sensitivity, both methods are limited by their data requirements not covering the genomes and full trait profiles for the 637 taxa recorded in the RICT reference data (Blomberg et al., 2003; Guenard et al., 2014; van den Berg et al., 2021). Thus, given the ubiquity of organism taxonomy, the major data requirement for the hSSD model is for a wide range of taxonomic data that covers a sufficiently broad range of the phyla, classes and families to be predicted making the hSSD method easier to apply than other predictive methods. For extensively researched chemicals with a broad taxonomic coverage the hSSD model provides a useful method for predicting assemblage sensitivity but has limited utility for data-poor chemicals such as newly synthesised pesticides or emerging chemical contaminants (US EPA, 2018). This limitation has been shown within the datasets; using the selection criteria described in 3.2.5 (10 acute EC_{50} endpoints for at least 10 unique taxa and coverage of at least 80% of the phyla represented in the RICT dataset) there are no pharmaceuticals with taxonomically rich enough datasets to run the hSSD model and therefore provide reliable sensitivity predictions.

While the use of taxonomy as the basis of sensitivity predictions allows the application of the hSSD to a diverse set of macroinvertebrates, there are limitations: taxonomic ranks represent differing levels of phylogenetic distance and therefore genetic similarity between different invertebrate clades, yet rank is being used as a discrete proxy for genetic similarity of chemical sensitivity (Barraclough, 2010; Pfrender et al., 2010). Secondly the genetic difference between taxa does not necessarily relate directly to sensitivity, with some sensitivity influencing traits (e.g. body size, lipid content) being highly labile between members of the same genus (Blomberg et al., 2003; Rubach et al., 2010). Regarding the former, while phylogenetic methods exist, but have poor applicability to natural ecosystems as the phylogenetic tree for all macroinvertebrates has not been resolved (Guenard et al., 2014; Malaj et al., 2016). This means that the extent of genetic similarity between many macroinvertebrate taxa is currently unknown, although improvements to mass sequencing may permit this method in the future and supplant taxonomy utilising relatedness-based methods of predicting species sensitivity. Regarding the second point, there are cases of closely related species e.g. those separated at the genus level and even intraspecific phenotypic differences, that exhibit drastically different sensitivities to the same toxicant, due to the traits of the organisms that are not currently accounted for in traits-based methods (van den Berg et al., 2019). Again applicability to a wide range of macroinvertebrates is an issue as trait knowledge is a limiting factor with many non-arthropod clades being incomplete, although this is improving (Hébert et al., 2016; Rubach et al., 2010). Ideally, an integrated predictive methodology combining the hSSD with other sensitivity prediction methods, such as genomics-based and trait-based approaches may provide the most suitable and accurate method of predicting species sensitivity (van den Berg et al., 2021). However, given the early stage of research into predictive sensitivity, a proof of application for the hSSD model has been demonstrated here that can be built upon.

3.4.2 Assemblage sensitivity considering chemical type and site typology

The predicted HC_5 values for each site-specific assemblage demonstrate that chemical sensitivity varies between assemblages, and that the magnitude of this variation is influenced both by type of chemical being studied and the river typologies of the sites being compared. The range of assemblage sensitivity (measured as chemical-adjusted HC_5) averaged out over all chemicals as a factor of 16.1, although this varied greatly by chemical identity. While some chemicals, such as tolulene exhibited little difference in sensitivity (factor of 0.9), 21 chemicals varied by a factor greater than 5, and 15 chemicals had sensitivities vary by orders of magnitude (factor of >10) including endrin with largest factor of difference at 154. This extent of between assemblage variation in sensitivity means the location of chemical exposure is important as specific locations may be more prone to chemical exposure, supporting existing literature on the site variability on the effects of chemical pollution (Beketov et al., 2009; Berger et al., 2017), while also providing a quantitative and chemical specific sensitivity value. Contextualising in an ERA perspective, over half the chemicals investigated would exhibit greater sensitivity between sites than covered by the assessment factor of 5 recommended for ERAs based upon SSDs to account for spatial variation, among other sources of uncertainty (ECHA, 2008; EFSA, 2013a).

Moving beyond pan-chemical considerations, the range in assemblage sensitivity varies between chemical types. Some chemicals target particular groups of invertebrate species (i.e. specifically acting chemicals) whereas other have a broader spectrum of activity (i.e. generally acting chemicals). In this study, insecticides, which specifically target arthropods, are notable for having significantly larger between-assemblage variation in sensitivity than other chemicals studied (i.e. metals and narcotics). This supports the hypothesis postulated in the Introduction that assemblages will exhibit a greater range in sensitivity for specifically acting chemicals as the differences in taxonomic composition will in the sensitive taxa in the assemblage determining the HC_5. This result represents the first broadscale investigation of how assemblage composition can affect sensitivity; Posthuma et al. (2019) have calculated SSDs for 1760 chemicals and over 22,000 waterbodies but only considered variation in chemical fate dynamics without any consideration on how spatial variation in assemblage composition affects the SSDs and therefore sensitivity. In contrast, Birk et al. (2020) investigated multistressor effects, including agrochemical toxicity, on a series of 33 mesocosm and 36 watershed basins. While taxon-sensitivity indices were used within the study, these were to inform biodiversity as a response to multiple stressors rather than a factor driving variation in sensitivity to agrochemicals. Thus, the hSSD methodology of predicting and assessing assemblage specific HC_5 could represent nuanced assessment of agrochemical risk not currently given due consideration.

Given that it has been established that variation in assemblage sensitivity (HC_5) that is dependent on the taxonomic composition of assemblage, any environmental factors that influence assemblage composition could therefore also influence chemical sensitivity. River typology, defined in terms of altitude, geology and catchment size, has been shown to be an important determinant of UK macroinvertebrate assemblage composition (Davy-Bowker et al., 2006; Townsend et al., 2003). Landscape typology acts as a factor influencing assemblage composition, with patterns between taxonomy and geology, and the linked variables of altitude and catchment size observed. Investigating how the link related to agrochemical sensitivity, the hypothesis that assemblage sensitivity would also vary between river typologies was supported. Assemblages sensitive to metals and narcotics were more abundant in low altitude calcareous rivers compared to the proportion of reference sites with this typology. Low altitude calcareous sites were associated with Isopoda Crustacea and soft bodied taxa such as Hirudinea (leeches), and Mollusca. Molluscs are known to be particularly sensitive to metals, such as copper (Bjerregaard et al., 2015; Verschoor et al., 2011). In contrast the distribution of assemblages sensitive to insecticides was not significantly different to the proportion of each typology in the RICT reference dataset. Insecta and Crustacea are highly sensitive to insecticides and therefore drive the sensitive end of the SSD shape and therefore greatly influence the HC_5 (Forbes and Calow, 2002). However, unlike other invertebrate clades which are associate only with zero to positive NMDS1 values, Insecta and Crustacea taxa are ubiquitous in UK freshwater ecosystems irrespective of typology, albeit with different clades being found at positive and negative NMDS1 values. This also meant that the sensitive insecticide taxa are likely to be present across NMDS1 scales irrespective of the river typology.

3.4.3 Accounting for climate change on assemblage composition

The implications of climate change for freshwater communities has been investigated and the combined influences of changing climate and water quality have been observed on broad large scale patterns (EFSA et al., 2020; Pearson et al., 2016; Vaughan and Ormerod, 2014). Equally, Chiu et al., (2017) investigated a specific Californian watershed as a case study area on the effects of climate change on invertebrate communities and their response to chemical contamination. However, currently no methodologies exist for quantifying the consequences of climate-induces changes in assemblage composition on chemical sensitivity that can be adapted to different chemicals and sites. This was addressed here by using the RICT model to predict site-specific assemblages under baseline (1980-2000) and future (2060-2080) climate conditions using site-specific temperature predictions from UKCP18 RCP 8.5 and then comparing the predicted HC_5 values between the baseline and future assemblages.

The RICT model is primarily used to calculate the water quality of a location based on the presence of freshwater invertebrate species given the physical parameters of the site (Clarke et al., 2003). However, by effectively running the model using altered physical parameters, the model can be used to predict species compositions rather than assess quality (Armitage, 2000). There are some limitations of using RICT for these purposes not encountered during the normal use of the RICT model, particularly where temperatures rise beyond those in any of the reference sites. Following extreme climate change, some locations will have moved their meteorology beyond any of the reference sites in the model resulting in increased uncertainty over the results. Moving forwards, with the WFD directive seeing use across multiple countries, including those who currently experience climate conditions comparable to future UK river sites, integration of such sites with the RICT model could prove useful for evaluating shifts in community composition under climate change (Kochskämper et al., 2016; Wilby et al., 2006).

The similarity in the predicted baseline and future assemblages at the 835 reference sites varied between 0.13 and 1.0 (ie. no change), with the median similarity being exactly 0.5. Future changes in assemblage composition were associated with decreases in the sensitivity of assemblages to chemical exposure (i.e. increase in HC_5), although this was only statistically significant for assemblages exposed to insecticides or narcotics. The quantification of the effects of climate change on chemical sensitivity from assemblage composition represent a novel application little investigated; Chiu et al. (2017) noted little to no change in the SPEAR index in agricultural land and an increase in some upland streams. However, the SPEAR index is not chemical specific and could not be used to compare the change in risk on a per-chemical basis as considered here (Beketov and Liess, 2008; Liess and von der Ohe, 2005).

The results from this chapter are that the shifts to assemblage composition from climate change will have relatively limited effect on the site sensitivity with a slight increase in median site tolerance across all chemical types. However, it should be noted that there was a corresponding decrease to the range of sensitivities between sites by varying percentages (45 – 106%) for all chemicals, and therefore an decrease to the proportion of sites below the 5% most sensitive sites under baseline conditions not reflected in the change to the 50th percentile HC_5. While the overall increase in the median tolerance of assemblages to chemicals under climate warming was common across all chemical types, at the individual species level, many studies have reported additive or synergistic effects between warming and chemical exposure (Cuco et al., 2016; Janssens et al., 2017; Khoma et al., 2021; Nieto et al., 2016; Rathore and Khangarot, 2002; Silva et al., 2020; Tran et al., 2018), which may counteract the increase in chemical tolerance due to compositional changes.

3.4.4 Applied use of the hSSD model

The use of the hSSD model to assess assemblage specific sensitivity has relevance to status of freshwaters and water quality, including under climate change scenarios. Biological monitoring remains important in the assessment of pollution, and will remain relevant for where long-term chemical exposure is expected to occur, but the hSSD model provides an opportunity to highlight locations with taxonomic compositions vulnerable to specific chemicals of interest and pre-empt necessary action (Camargo, 1994; Vernier et al., 2017). In addition, chemicals themselves may influence communities, so complex interactions may occur as chemical risk is alleviated or alters but climate change worsens (Berger et al., 2017; Vaughan and Ormerod, 2012). This also highlights the need for forecasting and for regular invertebrate sampling to identify both sites affected by chemical pollution and climate change, but also those who have undergone shifts in community structure and are now vulnerable to different chemical types.

One use of the hSSD method could be as a refinement within the ERAs for chemicals in the same way that SSDs themselves are used as a refinement with sufficient toxicity data (EFSA, 2013). However, the advantage of the hSSD method is increased realism compared to SSDs alone given the same number of toxicity data available. This is due to considering the sensitivity of natural taxa and reflecting variation in taxonomic composition between sites the toxicity data that exiting SSDs do not reflect (Newman et al., 2000; Posthuma et al., 2019; Raimondo et al., 2008).

The results here show that the range of sensitivities between site-specific assemblages goes beyond the current extrapolation factors used in ERAs when risk is considered (usually 5 for an SSD, and 10 or 100 for chronic and acute data alone respectively, EFSA, 2013). Furthermore, this is chemical dependent with chemicals with specific toxic modes of action, such as pesticides targeting specific organisms, are often those undergoing extensive regulation already and exhibit larger ranges in assemblage sensitivity (a mean factor of difference in the range of 25.2 for insecticides, compared to 2.68 for metals and 2.51 for narcotics). Consequently, the results here show that by not considering risk on a per-site basis, current risk assessments may be both not conservative enough for some specific sites and chemicals, and concurrently too conservative for others.

This study has demonstrated the potential for river typologies to be used to inform assemblage sensitivity, permitting a spatially variable risk assessment that can target locations with typologies associated with the most vulnerable taxonomic compositions. This could enable both a triage of at-risk sites based upon typology, as well as forming the basis of hypothetical risk scenarios with differing taxonomic compositions that would need to be passed for acceptable risk, enabling a spatially variable risk assessment. Existing freshwater risk assessments primarily account for spatial variation in risk from exposure with limited consideration of how biota influence sensitivity and therefore risk (EFSA, 2013a; Franco et al., 2017). While the toxicity data requirement of a diverse toxicity dataset to run the hSSD method may currently limit use to chemicals with taxonomically diverse datasets, one proposal could be for integration with ERAs as a higher tier refinement. To use the hSSD method within an ERA a suite a taxonomic ranks (e.g. Insecta, Crustacea, all major macroinvertebrate phyla) covered within the toxicity dataset could be specified that would be sufficient for the hSSD model to accurately run.

The results here show how the hSSD model can be used to predict assemblage sensitivity, and have assessed how climate change influences assemblage sensitivity across different chemical types. Chemicals that target specific organisms (e.g. the insecticides investigated here) exhibit the greatest spatial variation in macroinvertebrate communities and represent a risk not currently covered by existing ERAs. Additionally, the relevance of river typologies and their associated macroinvertebrate assemblages is noted, for example with for lowland calcareous sites being vulnerable to metal pollutants. However, these observations form one aspect of the complexity surrounding the sensitivity aspect of chemical risk. For climate change, the shifts to assemblage composition from rising temperatures will on average not change or increase the tolerance of an average site to chemical pollution depending on the chemical.

While taxonomic composition informs one aspect of ecosystem sensitivity, it fails to account for community and population level dynamics, in particular food webs and interspecific interactions (Brose et al., 2019; Kidd et al., 2014). Furthermore, these dynamics themselves are affected by climate change independently of chemical pollution (Woodward et al., 2010), and understanding the multiple stressor conditions of chemical pollution and climate change add an additional layer of complexity. However, the hSSD method has shown to be a useful and novel tool for predicting assemblage risk of natural ecosystems that has previously not been investigated and paves the way for integration into a tiered ERA, for considerations of water quality, or with RICT and theoretical community and organism level consideration, the first step in a conceptual framework of the effects of climate change on ecosystem sensitivity.

CHAPTER 4

Multi-stressor impacts in a warming world: the importance of multigenerational thermal acclimation

Chapter 4. Multi-stressor impacts in a warming world: the importance of multigenerational thermal acclimation

4.1 Introduction

Freshwater ecosystems are potentially at risk from multiple chemical, physical and biological stressors (Reid et al, 2019), whose combined effects on individuals may have consequences for community structure and functioning (Birk et al., 2020; Vaughan and Ormerod, 2012). Climate change and pollution have been identified as major threats to freshwater biodiversity (IPBES, 2019) but the effects of temperature shock and toxic effects start by acting on the organism scale on acute and chronic timeframes. Negative effects on organisms go on to affect population and community dynamics, ultimately reducing ecosystems services., for example, knockout of organic matter decomposition following pollution events (Fent et al., 2006; Giller et al., 1998; Sokolova and Lannig, 2008). However, the co-occurrence of temperature stress and chemical exposure has a combined effect that can be described as antagonistic, additive or synergistic depending on whether the negative effects on the organism are less than, equal to or greater than those of each stressor independently.

4.1.1 Interactions and mechanisms of temperature and toxicants

To investigate the existing research on the multi-stressor effects a literature search was performed using standardised search strings to capture ecotoxicological studies that investigate the interactions between temperature and chemical toxicity on invertebrate species. The search aimed to accounting for different types of macroinvertebrate taxa and climate change effects (e.g. heat wave, temperature rise, intermittent flow). Studies were filtered based on their relevance to the topic and must contain both temperature and chemical toxicity effects. In addition, they must be relevant to UK freshwater ecosystems meaning any marine studies or freshwater from non-relevant ecosystems were not considered. These studies cover a wide range of temperature increases, taxonomic and chemical groups, although the most common temperature increase studied was 3-5°C, which is the mean increase expected in the UK by the mid-end of the century based on the current climate projections (Lowe et al., 2018). From this search 35 suitable studies were considered, and the breakdown of study taxa, chemical, multi-stressor effect and endpoint type is presented in Figure 4.1.

Previous studies investigating multi-stressor effects indicate additive or synergistic effects of higher temperatures on toxicant sensitivity (Bae et al., 2016; Boeckman and Bidwell, 2006; C. M. S. Pereira et al., 2017). Synergistic interactions between climate change variables (including temperature) and toxic chemicals may be a result of the effect of chemical exposure on an organism’s response to climate change (i.e. toxicant-induced climate change sensitivity, TICS) or the effect of climate change on an organism's chemical sensitivity (i.e. climate change-induced toxicant sensitivity, CITS) (Hooper et al, 2013; Moe et al; 2013). Antagonistic temperature-chemical interactions may be a result of a temperature-induced change in exposure either via increase degradation rate of toxicant in the environment, increase detoxification, sequestration, or depuration the toxicant within the organism (toxicokinetics), or increase growth rate and body size resulting in dilution of internal toxicant concentrations (de Beeck et al., 2017; Verheyen and Stoks, 2020).

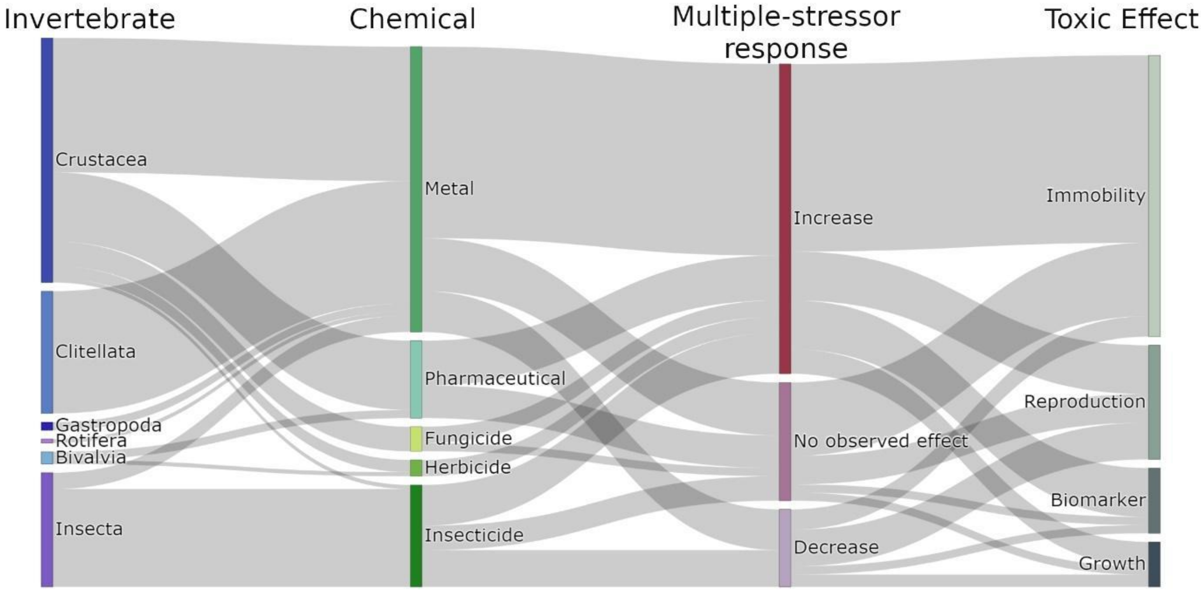

Figure 4.1 Flow chart collating 119 multi-stressor toxicity endpoints under temperature change collected from a systematic review into the multistressor effects of climate change on macroinvertebrate chemical sensitivity Endpoints are separated proportionally based upon four factors: invertebrate taxa tested at the class rank, chemical type grouped according to metal or pesticide target organism, multi-stressor response observed and the toxic endpoint calculated within the study

One limitation of the existing research investigating the combined effects of temperature stress and chemical toxicity is that the temperature change organisms experience is in the form of temperature shock or short-term (i.e. less than one generation) acclimation periods to temperature change. In natural ecosystems the effects of climate change are gradual, leaving time for organisms to acclimatise and adapt to different temperature regimes. Bae et al., (2016), Haap et al., (2016) and Tran et al., (2018) all investigated combined temperature and chemical stress on reproductive and growth parameters over two to four generations. However, these studies investigated temperature and chemical stressor co-occurrence, and therefore not representing any CITS effects from temperature altering chemical sensitivity. Furthermore, all studies were performed over a small number of generations (maximum 3) therefore not reflecting any potential long-term phenotypic changes in organisms that may mitigate the effects of temperature change. In contrast, another approach is to sample test taxa from populations exposed to differing temperature regimes either spatially (Dinh Van et al., 2014; Op de Beeck et al., 2017) or temporally (Cuenca Cambronero et al., 2018). However, these are associated with two caveats: firstly, adaptations to environmental factors other than just temperature will have occurred that may also affect organism sensitivity, and secondly that the populations undergo sexual reproduction and therefore genetic adaptation as well as phenotypic acclimation occurs. Consequently, there is a need to establish whether similar results would occur from multi-generational asexual laboratory temperature acclimation alone.

4.1.2 Thermal acclimation and multi-stressor effects

Short-term temperature changes are known to affect the functioning, life history and reproduction of organisms, for example with increased temperatures elevating biochemical stress responses and triggering life-stage changes and metamorphoses (Clark and Peck, 2009; Hofmann and Somero, 1995; Kroiher et al., 1992; Morgan and Jangoux, 2002). However, many organisms exhibit some level of adaptability to environmental changes, including temperature, with acclimation and evolutionary adaption occurring over multi-generational timescales (Brans and De Meester, 2018; Clarke, 1987; Reger et al., 2018). The impacts of temperature changes on organisms are therefore a function of thermal history, physiological acclimation and genetic adaptation. It is therefore hypothesised that organisms would exhibit reduced effects of temperature change (e.g. changes to generation time, time to first brood, reproductive investment) when acclimated to that temperature over multiple generations (Hypothesis 1).

The reproductive strategy exhibited by an organism can vary depending on environment, and temperature is one factor that influences how an organism invests resources in reproduction. For invertebrates, increasing temperatures have been associated with shorter generation times observed in all cases, and specifically increased fecundity at a less mature, smaller adult size (Brans and De Meester, 2018), a greater short-term reproductive investment (Clarke, 1987) and early maturation (Morgan and Jangoux, 2002). Chen and Stillman, (2012) demonstrated that *Daphnia* acclimatised at 25°C and 30°C exhibited little metabolic variation after 6 generations compared to those cultured at the original temperature of 15°C, but time to first brood decreased as acclimation temperature increased. Considering multi-stressor effects, with multi-generation phenotypic adjustment from acclimation, the reproductive strategy would be expected to alter towards that of the acclimatised temperature strategy. For example, it may be expected that higher temperatures lead to shorter generation times meaning investment in a smaller number of larger offspring at first brood (Hypothesis 2).

Most multi-stressor studies have either not acclimated study organisms to the test temperature or tested temperature change and chemical stress over multiple generations together. However, some have investigated the effects of short term acclimation on chemical sensitivity; Silva et al. (2020) acclimated *Daphnia* to different temperatures with a acclimation period of neonate to adult then introduced glyphosate testing for acute effects with acclimation being protective of the synergistic effects of increased temperature and glyphosate exposure. Moller et al. (1994) instead investigated the effect of two-month temperature acclimation on snails, followed by cadmium exposure. Again, acute toxicity was measured and increasing temperatures increased toxicity, although acclimation appeared to worsen the effects of cadmium exposure. If Hypothesis 1 is correct, and temperature does affect reproduction (Hypothesis 2) then it follows that multi-generation temperature acclimation will mitigate temperature-toxicant interactions (Hypothesis 3), noting that existing literature on the topic is both limited and has mixed outcomes relative to this hypothesis.

The effects of increasing temperature on an organism and their metabolic processes is non-linear with organisms reaching thermal maxima after going beyond a point of increased biochemical and metabolic rate (Dallas and Rivers-Moore, 2012a; Ernst et al., 1984; Williams et al., 2012). Thus, it would be expected that larger temperature increases are associated with greater multi-stressor effects than expected for a smaller temperature rise alongside exposure. Thus it is hypothesised that CITS will be greater for organisms experiencing larger temperature increases. Furthermore, the magnitude of CITS is expected to be greater for organisms acclimated at the higher temperatures as greater temperature increases are expected to be associated more stress to the organism and its ability to metabolise toxicants (Hypothesis 4).

4.1.3 Approach, and hypotheses and objectives

The importance of multigenerational acclimation to temperature change on temperature-toxicant interactions was investigated using *Daphnia magna* as the model system and copper as the exemplar toxicant. *Daphnia magna* is a common member of the zooplankton community in many lentic freshwater ecosystems, it is well suited to laboratory conditions, has a short generation time and is generally more susceptible to chemical stressors than other tested invertebrate species (Craig et al., 2012). *Daphnia* are extensively used within a regulatory environment as the standard invertebrate species being tested for both lethal (i.e. immobility) and sublethal (e.g. reproduction) effects (EFSA, 2013a; OECD, 2012, 2004). Copper is released into environments from mining and industrial activities and from agricultural land, where sources include pesticides and biosolid fertilizer applications, with known effects on freshwater ecosystems (de Oliveira et al., 2004; Gogos et al., 2012; Howard et al., 2015). Furthermore, as copper is a metal ion in solution it does not degrade; this means that degradation can be ruled out. However, copper concentrations in the test solutions may change from uptake by and binding to organic matter (e.g. *Daphnia,* algal food, waste) or reacting and precipitating out of solution. *Daphnia* from a single clone were acclimated to different temperatures over multiple generations before exposing organisms from each acclimation temperature to copper at different test temperatures. The acclimation and test temperatures were 15 °C, 20 °C and 25 °C and the specific predictions tested were:

- Do *Daphnia* exhibit reduced effects of temperature when acclimated to that temperature over multiple generations?
- Does multigeneration temperature acclimation shift reproductive strategy between number of offspring and offspring size such that higher temperatures are associated with fewer, but larger offspring? Does combined temperature shock and copper exposure alter this relationship?
- What are the effects of temperature shock on the sensitivity of macroinvertebrates exposed to copper and does multigeneration temperature acclimation mitigate the effect of CITS on copper sensitivity?
- Are multistressor effects greater for larger magnitudes of temperature increase (i.e. acclimation at 15°C tested at 25°C) when compared to a temperature increase at lower temperatures (i.e. acclimation at 15°C tested at 20°C)?

4.2 Methods

4.2.1 *Daphnia* culture

Daphnia magna were obtained from the clonal line maintained at the University of Sheffield (Clone S-1, the historic line from Sheffield, UK, (Baird et al., 1991). These *Daphnia* had been maintained under complete darkness in a temperature-controlled room at 20°C, with feeding every other day with 1 mL of algae solution (*Chlorella* species, culture sustained under complete light conditions at 20 °C in Ebert medium) and weekly media changeovers in 1L plastic vessels. Aside from temperature and use isolated test vessels per individual, the test conditions were maintained within those recommended in OECD 211 (OECD, 2012) throughout the study. They are explained in further detail along with the methods for the acclimation process and copper exposure tests below. The media used for culturing *Daphnia* was a hard ASTM (American Society for Testing and Materials) solution enriched with 4 mL marinure/L with a hardness of 170 mg $CaCO_3$ /L and a pH of 7.4 (ASTM, 1980).

4.2.2 Acclimation process

Acclimation began with 75 individuals from a clonal line that had exhibited no signs of stress, including atypical behaviour, limited mobility, discolorations, developmental defects or ephippia in their prior generations. Groups of five individuals were placed in approximately 260 mL glass jars with 250 mL of ASTM media and a set of five jars were placed in a temperature-controlled room and in two temperature-controlled cabinets, all of which were set at 20^{o}C ±1°C in complete darkness. As before, feeding occurred every other day, with media changes every week with hard ASTM media. Jars were checked daily and all first brood neonates and any dead *Daphnia* were removed and discarded. All other neonates were isolated into new 260 mL glass jars with 250 mL of ASTM media to form the next generation. Once at least 25 neonates were ready from the next generation, the previous generation were maintained under the same conditions but not used for testing then disposed of once the next generation produced neonates. Following a one-week acclimation period at 20 °C, the temperature one of the temperature-controlled cabinets was decreased by 0.5°C a week and the other was increased by 0.5 °C a week. Weekly 0.5°C temperature changes to the temperature-controlled cabinets occurred over a ten-week period until they reached the final acclimation temperatures of 15 and 25 °C. The temperature-controlled room was maintained at 20°C throughout in darkness.

4.2.3 Copper concentrations

Six concentrations of copper were selected across a range that would cover a value above the immobility EC_{50} at 25°C (34 µg Cu/L (Bae et al., 2016)) down to concentrations that would ensure that sub-lethal effects (including behavioural, growth and reproductive) would be triggered without the death of the test organisms. The test concentrations were 6, 9, 13, 20, 45 and 68 µg Cu /L and a control without any added copper. Hydrated copper (II) sulfate (from Timstar Laboratories, 40.0% copper content in ion form) was used as the source of copper ions and stock solutions were created by dissolving this 105compound in fresh ASTM solution. Test solutions were created by serial dilutions from a stock of 20 mg Cu/L, itself diluted from a 1 g Cu/L stock solution. The studies were semi-static with weekly renewals of test solutions prepared from stock solutions that were recreated on a weekly basis.

Copper samples were taken from the stock solutions, the initial and final test concentrations with a sample taken of the fresh test solutions at each concentration and sample taken from each test vessel at the end. Following filtration through a 0.45 µm filter, 10 mL samples were fixed in solution using 0.1 mL of concentrated nitric acid grade >99%, and stored in darkness and under refrigeration at 4°C. The nitrified samples for the stock solutions were then analysed via spectrophotometric analysis using a Perkin Elmer Optima 7300 V. This instrument analysed the concentration of copper using five wavelengths of 213.6, 222.5, 222.8, 224.7 and 327.4 nm using a 2mg/L Yttrium internal standard.

4.2.4 Toxicity test design

Tests to assess the toxicity of copper to *Daphnia* were performed according to OECD 211 (OECD, 2012) where possible, although test duration was extended beyond 21 days to account for slower reproduction and generation times at 15°C. Except for the test temperatures and concentrations of copper, test conditions were as for the acclimation period (i.e. complete darkness, with feeding every other day and weekly media changeovers). Food was the same algae culture as for the acclimation period. Toxicity tests were performed with *Daphnia* from all three lines (15, 20 and 25 °C) exposed to all three test temperatures (15, 20 and 25 °C) in a Latin square format, resulting in nine acclimation x test temperature combinations. Triplicate copper toxicity tests were performed for each acclimation x test temperature combination. For each toxicity test (acclimation-test temperature replicate), seven non-first brood neonates (i.e. <24 hours old) were each placed in a 60 mL jar and exposed to 50 mL of ASTM with no additional copper added (control) or to 50 mL of copper spiked ASTM (for each of the following concentrations: 6, 9, 13, 20, 45 and 68 µg Cu/L).

4.2.5 Measurement and parameters

Dissolved oxygen concentration and pH of the initial and final test solutions was measured with a YSI Professional Plus probe capable of measuring both parameters. Temperature was recorded daily for each temperature-controlled room or cabinet. *Daphnia* were checked daily to observe and measure toxic effects. The following parameters were investigated during the experiment: immobility (no movement including after gentle stimulation with a pipette), time to first reproduction, number in first brood, size of parent at first brood, size of neonates at first brood and the presence and size of any ephippia. *Daphnia* were checked for toxic effects (immobility, atypical behaviour, limited mobility, discolorations, developmental defects or ephippia) and neonates daily. Tests were run for 30 days if a full first brood of *Daphnia* had occurred, otherwise the test was extended to 40 days to enable more time for a first brood to occur.

The size of adults and neonate *Daphnia* was measured from photographs. Photographs taken with a USB camera attachment with 30 times magnification and the photographs measured with the program Measuring Body Length developed by Agatz et al., (2015). Body length was defined as the distance from the top of the head above the compound eye down to the base of the apical spine and average of three measurements taken (Comber et al., 1993; Green, 1956). *Daphnia* were measured at death where possible and on the day of every brood with neonates or ephippia being photographed with the parents when present. Each brood was treated as separate, and each brood defined by the presence of neonates when no neonates had been observed on the prior day, thereby grouping neonates on consecutive days together.

4.2.6 Data analysis

Data were analysed within R and three different analyses of reproductive parameters were performed based upon the research questions. Here the reproductive parameters used were time to first reproduction, size of parent at first brood, and the reproductive investment of the adult. Reproductive investment was calculated as the number of neonates multiplied by the mean size of neonates in that brood. The first analysis tested the effects of acclimation temperature on reproductive parameters and compared them to how temperature shock affected reproductive parameters. The second analysis assessed the relationship between how acclimation and test temperature alongside chemical exposure affected the reproductive parameters. Finally, a calculation of how acclimation and test temperature affected the calculated EC_{50} values for each reproductive parameter was performed.

The effects of acclimation and shock test temperature were compared by comparing how the reproductive parameters changed between acclimation-test temperature combinations using a two-way ANOVA with differences between combinations statistically compared with Tukey's honest significant difference tests. Reproductive investment was calculated as an index of the number of offspring multiplied by average neonate size, and the shifts between neonate size and number were assessed as for the reproductive parameters with a two-way ANOVA with differences between combinations statistically compared with Tukey's honest significant difference tests.

Direct toxic effects were considered using the shifts in reproductive parameters and reproductive strategy (increasing number of neonates against increasing neonate size) under increasing copper exposure for the acclimation-test temperature combinations relevant to investigating whether acclimation mitigated effects and whether magnitude of change was relevant. Due to the large number of combinations affecting toxicity (acclimation temperature, test temperature, chemical concentration, endpoint type) a MANOVA was performed to avoid replicating repeat statistical analysis and inappropriately low p values.

Dose response curves were fitted to acclimation-test temperature combinations across all replicates with non-linear log-logistic models. Dose response curves and effect concentrations (EC_x) were calculated and visualised using the drm and ED functions of the drc package respectively which calculates dose response curves for the data; curve fitting was fitted to a four-parameter log-logistic function (Noel et al., 2018; Ritz, 2010). Comparisons directly between EC_{50} values for different acclimation-test combinations were the result of two categorical factors, acclimation and test temperature. Consequently, as for investigating the effects of temperature on reproductive parameters, to investigate the effect of acclimation-test temperature combinations on EC_{50} for each parameter two-way ANOVA with differences between combinations statistically compared with Tukey’s honest significant difference tests were applied.

4.3 Results

4.3.1 Experimental parameters

Across all the controls (acclimation and test temperature the same and no additional concentration of copper) there was an overall mortality of 8.3%. This was due to one mortality for a *Daphnia* acclimated and tested at 20°C. All other control *Daphnia* did not appear to exhibit toxic effects throughout the study.

The temperature of the temperature-controlled rooms and cabinets did not vary by more than ±1°C throughout the study except for one 23.9°C recording for 25°C acclimation temperature. Temperature ranges were 14.0°C to 15.6°C for 15°C acclimation, 20.1°C to 20.3°C for 20°C acclimation and 23.9°C to 24.7°C for 25°C acclimation. All temperatures were within the ±2°C recommended within OECD 211 (OECD, 2012). Dissolved oxygen content of the test jars ranged between 88.6% to 102.7% throughout, well above the OECD 211 (OECD, 2012) recommended minimum oxygen concentration of >3 mg/L (roughly >40% oxygenation). For pH values recorded, the range was between 7.72 to 8.30 across all jars, within the ranges of and pH 6-9 and with a difference of less than 1.5 throughout the test recommended within OECD 211 (OECD, 2012).

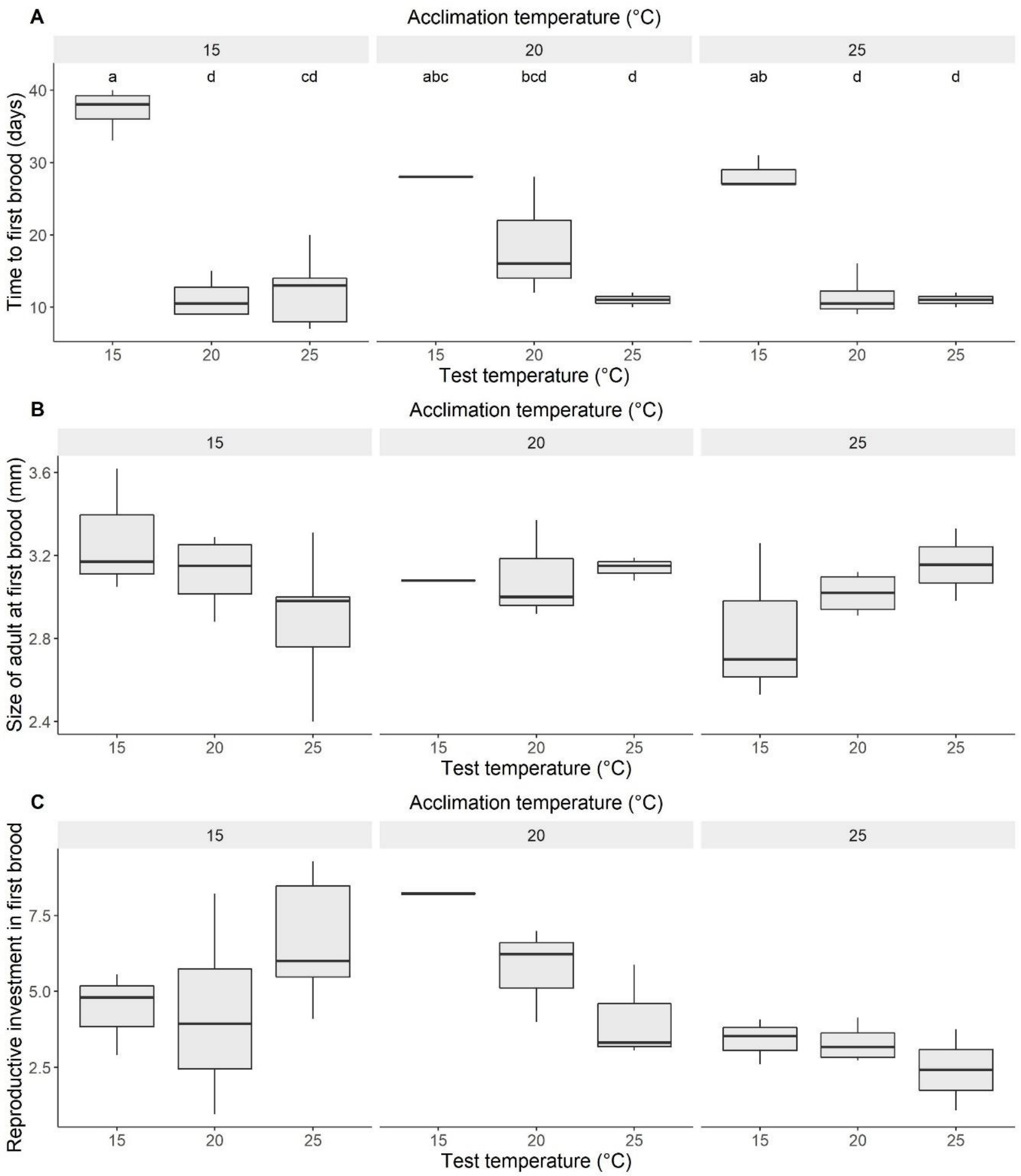

Figure 4.2 Effects of shock temperature change (test temperature) on the reproductive and growth parameters of control *Daphnia* (no copper exposure) acclimated over multiple generations to 15°C, 20°C or 25°C (acclimation temperature). A, the time to first brood, B, adult size at their first brood and C, the reproductive investment of the adult in the first brood calculated as a measure of both the number and size of neonates. Boxplots represent the 25th, median and 75th percentiles, with whiskers the minimum and maximum values. Letters denote results of pair-wise comparison tests; values sharing the same letter are not significantly different ($p > 0.05$), for B and C no significant differences were observed.

The stock solutions of 20 mg Cu/L (from which test solutions were created), and 1 g Cu/L (from which the 20mg/L solution was created) underwent duplicate spectrophotometric analysis. The analysis across the five wavelengths were 96% and 98% of the nominal for the duplicate analysis on the 20 mg Cu/L solutions, and 96% and 94% of the nominal for the duplicate analysis of the 1 g Cu/L solutions.

4.3.2 Reproductive parameters

Temperature on reproduction

Multigenerational temperature acclimation affected the reproductive performance of *Daphnia,* including before copper exposure. Temperature acclimation resulted in the *Daphnia* acclimatised at 15°C being on generation 8, while those acclimatised at 20 °C and 25 °C being on generation 11 and 12 respectively at the end of the 36-week acclimation period, indicating an increase time between generations. Within the study itself, for the controls the time to first brood was significantly affected by the test temperature (DF = 2, F = 72.8, p <0.001) and notable for the interaction between acclimation and test temperature (DF = 4, F value = 4.0, p value = 0.066), but not affected by acclimation temperature alone (DF = 2, F value = 0.009, p value = 0.99). Figure 4.3A shows these patterns and highlights that acclimation and testing at 15°C was significantly different to *Daphnia* tested at higher temperatures regardless of acclimation.

Regarding the size of adults at first brood in the controls (Figure 4.3B), there was no significant effect of acclimation temperature (DF = 2, F value = 0.5, p value = 0.61) or test temperature (DF = 2, F value = 0.25, p value = 0.78), and no significant interaction between the two (DF = 4, F value = 1.56, p value = 0.23). In contrast, for the reproductive investment of the controls (Figure 4.3C), there was a significant effect of acclimation temperature (DF = 2, F value = 4.2, p value = 0.03), although no significant effects of either test temperature alone (DF = 2, F value = 0.13, p value = 0.88) or the interaction of acclimation and test temperature (DF = 4, F value = 2.1, p value = 0.11) were observed.

Shifts to reproductive strategy

Although significant changes to reproductive investment were observed depending on the acclimation temperature, reproductive investment here was a measure of neonate size and number of neonates. Considering the number of neonates at first brood in the controls, there was a significant effect of acclimation temperature (DF = 2, F value = 3.6, p value = 0.046) and the interaction of acclimation temperature and test temperature (DF = 4, F value = 3.22, p value = 0.034), with no significant effects for test temperature (DF = 2, F value = 0.03, p value = 0.97). In contrast the only significant effect on neonate size was the interaction between acclimation and test temperature (DF = 4, F value = 3.6, p value = 0.02), with no significant effects for either acclimation temperature (DF = 2, F value = 0.25, p value = 0.78) or test temperature alone (DF = 2, F value = 2.1, p value = 0.15). As shown in Figure 4.3, the only significant multi-comparison difference between acclimation-test temperature combinations was for the size of neonates of *Daphnia* acclimatised at 25°C and tested at 25°C being larger than those acclimatised at 25°C and tested at 20°C.

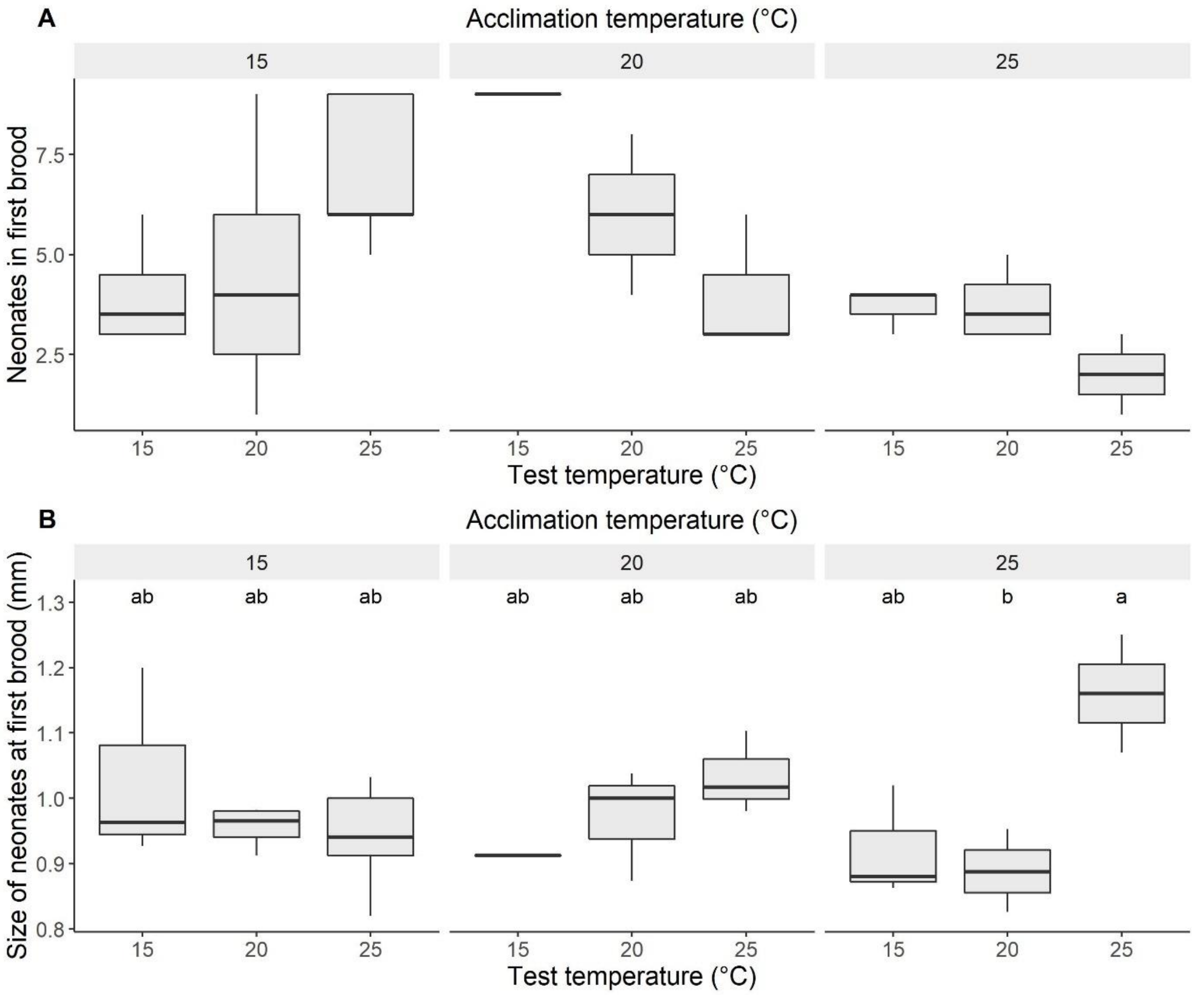

Figure 4.3 Effects of shock-term temperature change (test temperature) reproductive investment parameters for control *Daphnia* (no copper exposure) acclimated over multiple generations to 15°C, 20°C or 25°C (acclimation temperature). A, first brood neonate count, B, mean neonate size in the first brood. Boxplots represent the 25th, median and 75th percentiles, with whiskers the minimum and maximum values. Letters denote results of pair-wise comparison tests; values sharing the same letter are not significantly different ($p > 0.05$)

4.3.3 Effects of temperature on copper toxicity and endpoints

Temperature acclimation and toxicity

When assessing the multi-stressor effects of copper and temperature, any temperature shock was considered in comparison to the *Daphnia* acclimated and tested at the same temperature (i.e. acclimation-test temperature combinations of 15-15°C, 20-20°C and 25-25°C). A full set of calculated EC_{50} values is presented in the Table A4.1 of the appendix. Notably, the *Daphnia* acclimatised at 20°C and tested at 20°C were the least sensitive *Daphnia* tested. For these *Daphnia* the calculated EC_{50} values for all reproductive parameters ($EC_{50\text{ time to 1st brood}}$ = 41.1 µg/L, $EC_{50\text{ size of adult at 1st brood}}$ = 45.3 µg/L, $EC_{50\text{ reproductive investment in 1st brood}}$ = 42.9 µg/L) were significantly less sensitive than all other acclimation-test temperature combinations (except for the endpoint of adult size at first brood for *Daphnia* acclimatised at 15°C and tested at 20°C). In contrast, between *Daphnia* acclimatised at 15°C and tested at 15°C and *Daphnia* acclimatised at 25°C and tested at 25°C there was no significant difference in sensitivity in the endpoints for size of adult at first brood (EC_{50} of for 15-15°C and for 25-25°C) and the reproductive investment (EC_{50} of for 15-15°C and for 25-25°C). However, *Daphnia* acclimatised at 25°C and tested at 25°C resulted in a significantly more sensitive endpoint (EC_{50} of 2.8 µg/L) for the time to first brood compared to those *Daphnia* acclimatised at 15°C and tested at 15°C (EC_{50} of 17.2 µg/L).

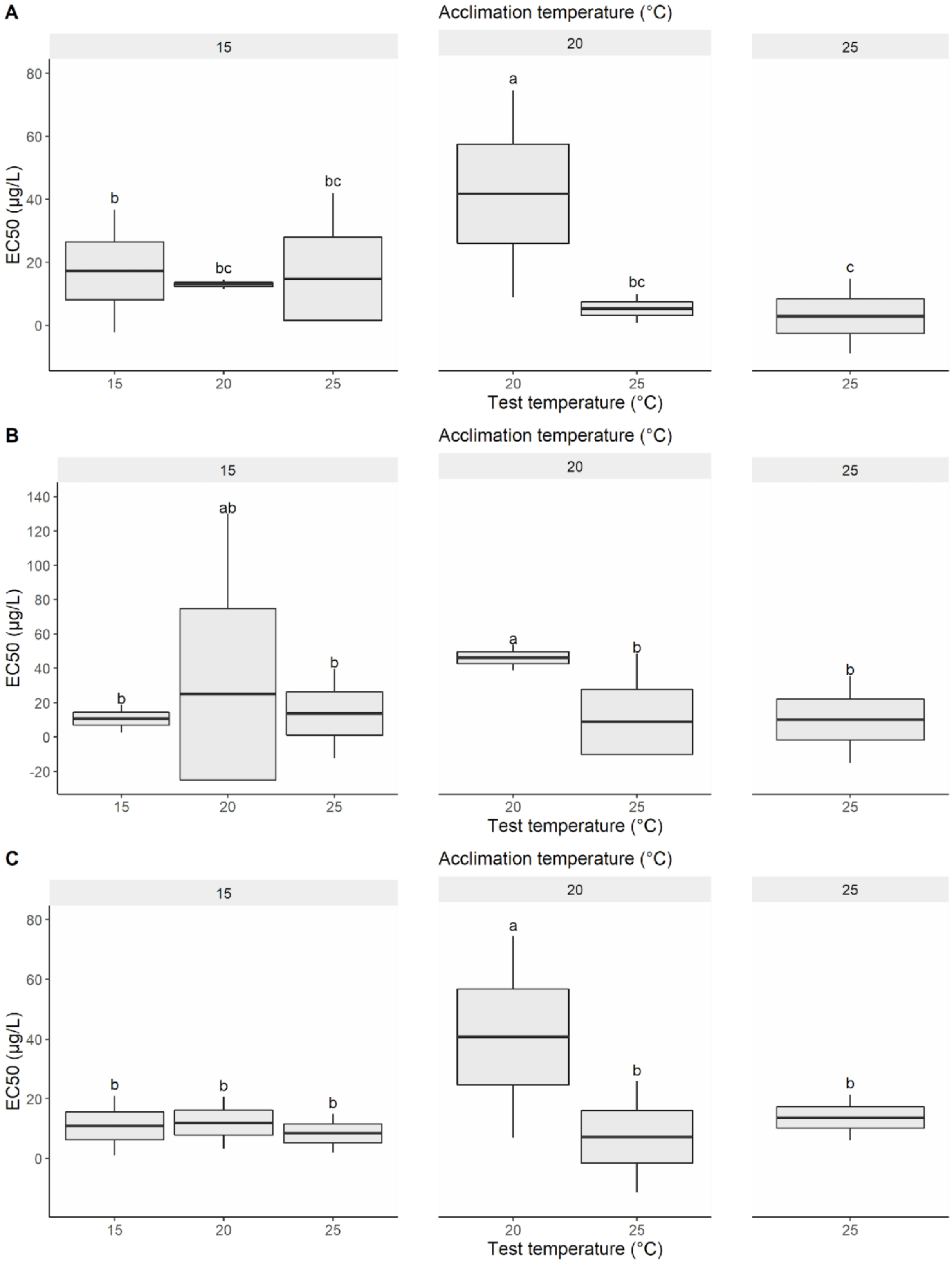

Figure 4.4 Effects of shock temperature increase (test temperature) on the reproductive and growth endpoints (EC_{50}) of *Daphnia* exposed to copper following acclimation over multiple generations to 15°C, 20°C or 25°C (acclimation temperature).Plots are A, the time to first brood, B, adult size at their first brood and C, the reproductive investment of the adult in the first brood calculated as a measure of both the number and size of neonates. Boxplots represent the 25th, median and 75th percentiles, with whiskers the minimum and maximum values. Letters denote results of pair-wise comparison tests; values sharing the same letter are not significantly different ($p > 0.05$)

Three sets of *Daphnia* experienced a shock rise in temperature that could be directly compared to *Daphnia* acclimatised and test tested at the same temperature. *Daphnia* acclimatised at 15°C and tested at 20°C, and *Daphnia* acclimatised at 20°C and tested at 25°C both experienced a shock temperature rise of 5°C alongside copper exposure. A 10°C shock temperature rise was experienced by *Daphnia* acclimatised at 15°C and tested at 25°C.

Comparing a shock 5°C rise to test temperature for *Daphnia* acclimatised at 15°C did not increase sensitivity compared to those *Daphnia* acclimatised at 15°C and tested at 15°C indicating no effect of heat shock on sensitivity. In contrast, shock 5°C rise to test temperature for *Daphnia* acclimatised at 20°C saw a significant increase in sensitivity for all reproductive parameters compared to those *Daphnia* acclimatised at 20°C and tested at 20°C. However, given there is no significant difference when comparing the EC_{50} values between *Daphnia* acclimatised at 20°C and tested at 25°C (ie a shock temperature rise of 5°C) and *Daphnia* acclimatised at 25°C and tested at 25°C (acclimation to the higher temperature), it cannot be concluded that there is an effect of heat shock on sensitivity.

The 10°C rise is a greater thermal increase than the other *Daphnia* that only experienced a 5°C rise in temperature. However, none of the reproductive endpoints considered here indicated that a larger temperature rise was associated with a significant increase of sensitivity (Figure 4.4). Equally, the *Daphnia* acclimatised at 15°C and tested at 15°C did not have any significantly different endpoints compared to those *Daphnia* acclimatised at 15°C undergoing a shock rise in temperatures.

4.4 Discussion

Most previous studies investigating the toxicity of copper to *Daphnia* at different temperatures use non-acclimated animals (i.e. temperature shock). Climate change is not necessarily associated with temperature shocks alone, but the gradual increase in freshwater temperatures compared to baseline conditions (Watts et al., 2015). Given the effects of long-term acclimation currently have limited consideration, there is a need to assess how multi-generational temperature acclimation can influence the multi-stressor response to temperature increase and chemical exposure. Consequently, the aim of this study was to investigate importance of multigenerational acclimation to temperature change on temperature-toxicant interactions. In particular, it addressed the following hypotheses:

1. Organisms would exhibit reduced effects of temperature on reproductive parameters when acclimated to that temperature over multiple generations
2. Higher temperatures lead to an investment in a smaller number of larger offspring

3. Multi-generation temperature acclimatisation will mitigate temperature-toxicant interactions reducing the effect of CITS
4. The effects of a given temperature increase on CITS will be magnitude dependent such that a greater change to chemical sensitivity will occur for greater temperature increases

The effects of copper exposure and temperature increase on *Daphnia magna* reproduction was used to investigate these hypotheses.

4.4.1 Effects of temperature acclimation and shock

Organisms have the capacity to acclimate to changing temperatures, and gradual temperature increases have been demonstrated to mitigate the stress from heat shock (impairment of body and metabolic functions) associated with temperature increases over short periods of time (Ernst et al., 1984; Moulton et al., 1993, Paul et al., 2004). Indeed, gradual acclimation can increase the threshold temperature at which stressor effects begin to occur (Dallas and Rivers-Moore, 2012b). Previous studies have demonstrated that *Daphnia* acclimated to higher temperatures, including over multiple generations (Lamkemeyer et al., 2003), had increased metabolic rate, physical movements and swimming rates (Paul et al., 2004), increased growth rates (Chopelet et al., 2008) and shorter generation times (Chen and Stillman, 2012). Thus changes to reproductive parameters alter the organism's ability to respond to stress and undergo recovery when population decreases from stressor events occur (Woodward et al, 2016).

Gradually acclimatising a single *Daphnia* clone to different temperatures over 8-12 generations resulted in significant changes in reproductive parameters. A gradual 5°C decrease from the long-term culturing temperature of this clone (i.e. 20°C) resulted in an increase in time to first brood and increase in reproductive investment but no change in adult size, whereas a gradual 5°C increase resulted in no change in the time or size at first reproduction but did cause a significant reduction in reproductive investment.

4.4.2 Shifts to reproductive strategy

An increase in acclimation and test temperature from 20°C to 25°C resulted in a significant reduction in reproductive investment. However, given reproductive investment here was a measure of the number and size of neonates, the reduction in reproductive investment was the result of a shift in reproductive strategy. *Daphnia* acclimatised and tested at 25°C increased their average size of neonates at the expense of a smaller number of neonates in total for the first brood. This occurred alongside significantly faster times to first brood than those tested at 15°C. Previous studies have also reported significantly longer times to first brood for *Daphnia* maintained at lower temperatures (Chen and Stillman, 2012; Im et al., 2019), although an increase in temperature has not previously been associated with a change to the number of neonates (Im et al., 2019).

4.4.3 Acclimation and mitigation of temperature and chemical stress

With regards to temperature as a combined stressor with copper exposure, broadly speaking the results here indicate limited multi-stressor effect of copper and temperature increase. Aside from *Daphnia* acclimatised at 20°C and tested at 20°C which are discussed in more detail below, the only significant change in sensitivity was the EC_{50} for time to first brood where *Daphnia* acclimatised at 25°C and tested at 25°C were significantly more sensitive than those acclimatised at 15°C and tested at 15°C. The second objective explored here was with regards to the magnitude of temperature change on multi-stressor effects of copper and temperature. *Daphnia* acclimatised at 15°C experienced temperature shocks of 0°C, 5°C and 10°C, but there was no significant difference in sensitivity between these *Daphnia* for all reproductive endpoints tested. As discussed above, in this study temperature appears to have had little effect on *Daphnia* sensitivity to copper, and larger magnitudes of thermal change did not significantly affect the calculated EC_{50} values.

Notably the *Daphnia* acclimatised at 20°C and tested at 20°C were consistently the least sensitive to copper exposure for all reproductive parameters and was the case even after *Daphnia* had been acclimatised to differing temperatures over many generations. Existing literature has observed similar patterns before. Lamkemeyer et al. (2003) did not investigate chemical toxicity, but did acclimate *Daphnia* over multiple generations and discovered that the optimum temperature for *Daphnia* was between 16.2 and 19.5°C for *Daphnia* acclimatised at 20°C and between 21.4 and 21.7°C for those acclimatised at 30°C indicating an optimum temperature around 20°C. Similar results were observed by Chen and Stillman (2012) where the optimum temperature for *Daphnia* metabolism was found to be around 20°C.

However, two previous studies have investigated the effect of copper on *Daphnia* after short-term (2 generations) temperature acclimation (Hochmuth et al., 2016; Pereira et al., 2017). The results presented here are consistent with those of Hochmuth et al. (2016), who reported that *Daphnia* reproduction (number of neonates) was less sensitive to copper when tested at 19°C (compared to 15°C and 23°C). This contrasts with the results from Pereira et al. (2017), which reported that *Daphnia* reproduction (number of neonates) was most sensitive to copper when tested at 15°C and that there was no difference in sensitivity between *Daphnia* tested at 20 and 25°C. This may be reflective of this clonal line being well adapted to laboratory testing at 20°C, and 20°C has been shown to be the metabolic optimum for *Daphnia* in previous studies (Chen and Stillman, 2012; Lamkemeyer et al., 2003). From an applied perspective, *Daphnia* are tested in ecotoxicological tests at 20°C by standard (OECD, 2012, OECD, 2004), but the applicability of such studies may need to be reconsidered under the context of a warming planet given the effects that a 5°C increase would have on the sensitivity of *Daphnia* and other test organisms to chemical exposure.

4.4.4 Implications and limitations

The main thrust of this research was to investigate multiple stressor effects of temperature and chemical exposure. Consequently, the controls here were designed in number of replicates and format to match those exposed to copper, meaning the results from the controls may not be reflective or have enough power to represent the outcomes of a study designed to test the effects of acclimatisation and temperature shock alone. Furthermore, when considering the effect on macroinvertebrates, one natural route of improving the realism of the experiment to simulate natural day night temperature cycles as has been done for other invertebrate species and has been shown to be a major factor that can affect organism sensitivity (Delnat et al., 2021, 2019; Willming et al., 2013).

Overall, where suitable, the results from the controls here support the previous findings on changes to temperature and *Daphnia*. Testing *Daphnia* at 15°C consistently led to significantly slower times to first brood than *Daphnia* tested at higher temperatures in line with the findings from other studies (Chen and Stillman, 2012; Im et al., 2019). Otherwise, no significant conclusions could be drawn for the other reproductive and growth parameters. However, the influence of both test and acclimatised temperature affected the reproductive strategy; higher temperatures, both during acclimation and testing saw an investment in a smaller number of larger offspring earlier on in the *Daphnia's* life stage. Equally, for a multi-stressor perspective the results here show limited effect of warming on *Daphnia* sensitivity, with the exception of *Daphnia* cultured at 20°C, which may have relevance for the use of *Daphnia* as a model ecotoxicological species for climate change conditions.

CHAPTER 5

Agrochemicals and climate change effects on freshwaters: implications for environmental risk assessment

Chapter 5. Agrochemical and climate change effects on freshwaters: implications for environmental risk assessment

5.1 Introduction

The aim of this thesis was to evaluate how climate change will alter the risk agrochemicals pose to freshwater ecosystems, taking England and Wales as a case study. The thesis focused on the transport of agrochemicals from field to ecosystem via soil, the sensitivity of and shift in composition of macroinvertebrate assemblages and the effect of long-term adaptation on the response of organisms to multiple stressor exposure. This chapter aims to draw together these results by i) exploring the implications of climate change on different components of the environmental risk assessment (ERA) process; ii) based on the results from i), develop a framework that incorporates climate change into the ERA, and iii) illustrated the application of the framework for some case study compounds to explore how the risks of agrochemical pollution for these chemicals might alter in the future due to climate change. This chapter first discusses the main findings of the preceding chapters, and then proposes ERA framework that integrates these findings and that accounts for the effects of climate change on agrochemical risk. Figure 5.1 outlines how the research described in Chapters 2 to 4, links to the ERA framework. The proposed framework is then applied to potential real-life scenarios with three case study risk assessments (glyphosate, copper and a theoretical new chemical) that provide both a practical application of the research within this thesis while noting the data requirements for integration with existing ERAs.

5.2 Main findings

5.2.1 Interaction between climate change and chemical flux

The effect of climate change on localised climatic patterns in 2060-2080 was considered for the edge of field chemical flux for 24 agrochemicals and compared to baseline (1980-2000) values using the high resolution 5 km^2 UKCP18 data (Met Office, 2019). Broadly, climate change projections are for warmer wetter winters and hotter dryer summers, with an increase in extreme precipitation events, although this expected to be spatially variable across the UK (Lowe et al., 2018). Consequently, the effects of climate change on chemical flux was evaluated across six sites representing the climatic zones of England and Wales (Met Office, 2021). Chemical transport in soil is affected primarily by the size of precipitation events that trigger chemical movement, and secondarily by the quantity of chemical in the soil matrix when precipitation events occur, which itself is determined by the persistence of the chemical (Biswas et al., 2018; Elrashidi et al., 2013). The persistence of a chemical is determined by the degradation rate of the chemical, which is influenced by temperature (Cavoski et al., 2008; FOCUS, 2006; Matthies and Beulke, 2017).

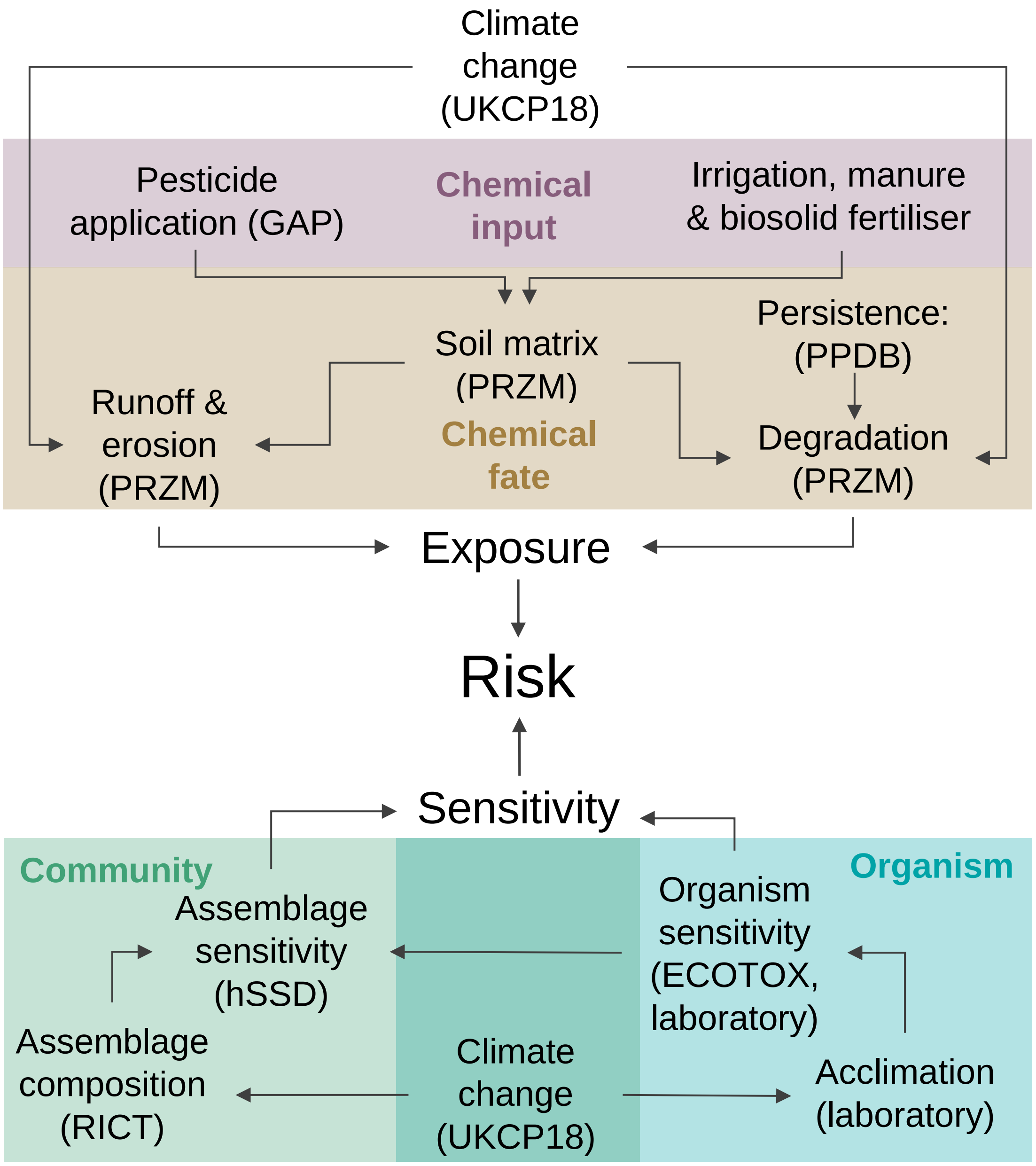

Figure 5.1 Flow diagram of how climate change was integrated into the ERA for considering the exposure and sensitivity aspects of the risk with the databases and models utilised for each aspect shown in brackets: UKCP18 (Met Office, 2019), Pesticide Root Zone Model (PRZM, Carsel et al., 1985; US EPA, 2016), Pesticide Properties Data Base (PPDB, University of Hertfordshire, 2007), River Invertebrate Classification Tool (RICT, Environment Agency et al., 2021), hierarchical Species Sensitivity Distribution (hSSD, Craig, 2013), ECOTOX database (US EPA, 2021)

As climate change-induced alterations in precipitation patterns and temperatures vary in space and time, and soil characteristics vary spatially, it was expected that the site, season of application and chemical identity would all influence the effects of climate change on chemical flux from soil to surface waters. This was supported in the results where range in effects of climate change (in 2060-2080) on edge of field flux differed markedly between sites (e.g. for prothioconazole from -97% in Cornwall to

+248% in Lancashire of baseline (1980-2000) edge of field chemical flux), and seasons of application (e.g. for diclofenac in Kent from -97% in Kent to +248% in Lancashire of baseline (1980-2000) edge of field chemical flux).

To consider how these different factors would influence flux, the commonly used fate model PRZM was used to quantify the change in flux accounting for the effects of climate change (Carsel et al., 1985). The underlying hypothesis was that compared to baseline conditions, persistent chemicals with slow degradation rates exhibited less seasonal variation and generally exhibited decreases in flux, due to increased degradation, under future climate conditions, which was supported by the decrease in variation of the effects of climate change for slowly degrading chemicals. Where flux increased for slowly degrading chemicals, this was due to an increase in the intensity of rainfall events under future climate conditions. In contrast, rapidly degrading chemicals (DT_{50} < ~5 days) were highly dependent on the weather following application and flux varied greatly between sites; with the largest changes between baseline and future conditions due to precipitation events occurring days after application. Thus, intensity of precipitation events change under future climates and therefore the probability of exposure of freshwater ecosystems also change in line with the probable climate conditions post application.

By using the high resolution UKCP18 data at the 5km scale, highly localised extreme weather events, that drive chemical flux, cannot be discerned at coarser scales, and so could be captured when modelling change of flux at a site specific level (Kennedy-Asser et al., 2021). Given the different climatic zones of the UK that were represented by selected sites, the effects of climate change were found to be both site and season specific. Site, season of application and chemical identity were all factors affecting flux and with edge of field flux being the result of complex interactions among all three. However, distinct patterns between chemical persistence and change to flux under climate change were observed across all agrochemicals, although this was more obvious for pharmaceuticals as chemicals with a range of DT_{50} values were applied at the same seasons and sites. Equally the variation in flux from site and season of application emphasised the importance ensuring chemicals are modelled with accurate application times and locations, and as shown within chapter 2, quantification of spatial variation of climate change on a per-chemical basis is possible.

Overall, this chapter has shown how meteorology under the effect of climate change will significantly alter agrochemical flux into freshwaters. The magnitude and direction of change in flux relates to chemical degradation, forming distinct patterns on a site and season of application specific level. Furthermore, chapter 2 has highlighted how climate projections can be integrated with spatially explicit fate models to quantify how climate change conditions will affect chemical flux into freshwater ecosystems.

5.2.2 Assemblage sensitivity

The biodiversity of natural ecosystems means that the community composition varies spatially, and the non-exchangeability of chemical sensitivity between species (Craig, 2012) mean some species tend to be more sensitive to specific chemicals than others. Given both of these, it is postulated that variation in the taxa composing an ecosystem will mean that the sensitivity of that ecosystem is also expected to vary (Schmitt-Jansen et al., 2008). However, climate change conditions are also set to shift the composition of freshwater ecosystems (Durance and Ormerod, 2007; Niggebrugge et al., 2007). The relative sensitivity of species is chemical specific and is driven by the mode of action of the chemical, for example Arthropoda to tend to be the most sensitive macroinvertebrates to insecticide exposure (Maltby et al., 2005). Consequently, chemicals were grouped into three types in chapter 3, insecticides, metals and narcotics.

Thus chapter 3 aimed to assess the extent that spatial variation in the composition of freshwater macroinvertebrate assemblages influences assemblage sensitivity and whether this varied by chemical type. Equally, how the shift in assemblage composition under climate change would affect assemblage sensitivity on top of existing spatial variation was also investigated. This research considered the response of macroinvertebrate assemblages from 835 minimally impacted sites (those that underwent macroinvertebrate sampling for the RIVPACS reference database (Wright et al., 1996) that underlies RICT) to 40 chemicals under baseline (1980-2000) and future (2060-2080) climate conditions. An issue was that the sensitivity of most natural taxa have not been studied in toxicity tests and remain unknown. To address this a hierarchical species sensitivity model (hSSD, Craig, 2013) was used to predict macroinvertebrate assemblage sensitivity for each chemical while the multivariate model River Invertebrate Classification Tool (RICT, Wright et al., 1996) was used to predict expected assemblage composition under future conditions.

The 40 chemicals studied were grouped into three chemical types: insecticides, metals and narcotics. It was hypothesised that different assemblage compositions would have differing sensitivities to the chemicals and that variation of assemblage sensitivity would be greatest for chemicals that target a specific group of macroinvertebrates (e.g., Insecta and closely related Arthropoda clades such as Crustacea for insecticides). This was supported by the results that indicated that there was a greater magnitude of range between both the maximum and minimum HC_5 values for the insecticides compared to metals and generally acting chemicals, here classified as narcotics.

Secondly, assemblage composition aligns to river typology (Davy-Bowker et al., 2006) and given assemblage composition has been shown to affect sensitivity in the previous results from this chapter it was hypothesised that specific typologies may be associated with toxicant prone or tolerant assemblages. Here the results indicated that the 5% most sensitive sites for both narcotics and metals was composed of a different proportion of site typologies compared to the reference sites. Large lowland calcareous sites tended to be the only locations with snails, mussels and leeches, and such soft-bodied taxa are known to be highly sensitive to metals such as copper (Bjerregaard et al., 2015; Verschoor et al., 2011). This was reflected in the results with 77.1% of the most sensitive sites for metals being low calcareous typology compared to 38.9% of the RICT reference sites. In contrast for insecticides, Insecta and Crustacea are highly sensitive to insecticides and therefore drive the assemblage sensitivity when SSDs are fitted to insecticide toxicity data (Forbes and Calow, 2002). The ubiquity of Arthropoda meant sensitive taxa for insecticides could be found across all typologies equally.

Although these two hypotheses do not directly address the effect of climate change on assemblage sensitivity, these results address another pertinent question for environmental risk assessments: to what extent is there spatial variation in the sensitivities of assemblages? Current risk assessments do not take spatial variation in the species composition of exposed ecosystems into account explicitly. Spatial uncertainty, along with other uncertainties, are lumped together and covered within a single assessment factor and are applied to Tier 1 ecotoxicity data and HC_5 values, but the results here show that for some chemicals the range of HC_5 values goes beyond the recommended assessment factors for HC_5s of three to six depending on the quality and quantity of toxicity data (ECHA, 2008; EFSA, 2013a). If between-site variation in species composition has a pronounced effect on assemblage sensitivity, any effect of climate change on composition will therefore affect sensitivity. Moreover, because the meteorological consequences of climate change vary spatially, the impact of climate change on species composition and hence assemblage sensitivity may also vary spatially.

The hypothesis that shifts in species composition due to climate change would result in a change in assemblage sensitivity (i.e. HC_5 value) was investigated by using RICT to predict the macroinvertebrate composition of 835 sites under baseline and future temperature conditions and comparing their HC_5 values for 40 chemicals. On average, assemblages were less sensitive under future climate scenarios although the magnitude of change compared to the HC_5 of baseline assemblages varied between chemical groups: 16% decrease for insecticides, 2% decrease for narcotics. Within chemical groups there were examples of chemicals that exhibited increased sensitivity (e.g., benzamine (9.5% increase), dieldrin (5.3% increase) and cadmium (3.5% increase)) and some chemical with particularly large decreases in assemblage sensitivity (e.g., bifenthrin (34% decrease), deltamethrin and permethrin (61% decrease), all pyrethroid insecticides, (79% decrease).

Overall, this chapter has developed and applied sensitivity predictive methods to quantify risk at the assemblage level for a range of natural ecosystems. The results have shown that the spatial variation in community composition influences assemblage sensitivity. However, this variation is currently not accounted for in existing ERAs outside of assessment factors (that also aim to account for other sources of uncertainty). Yet the results here show that the variation in sensitivity between natural assemblages is greater than these assessment factors for some chemicals making the ERA not protective of natural ecosystems. Furthermore, the effects of climate on community composition will affect assemblage level sensitivity resulting in both increases and decreases to sensitivity depending on the chemical meaning a chemical specific assessment of future risk is necessary.

5.2.3 Multi-generation adaption and organism sensitivity

In addition to influencing assemblage sensitivity by changing species composition, climate change may also affect the sensitivity of individual species to chemical exposure. Most studies investigating the effect of warming on chemical sensitivity have been heat shock experiments with limited temperature acclimation of test organisms. However, warming due to climate change will occur gradually providing an opportunity for species to adapt or acclimate to new temperature regimes. The hypothesis that gradual, multi-generation temperature acclimation will influence the effect of heat shock on chemical toxicity was investigated using the Cladocera, *Daphnia magna,* exposed to copper.

Daphnia neonates*,* originally cultured at 20°C, were allocated into three cultures, which had the target temperatures of 15, 20 and 25°C. Culture temperature was changed gradually by 0.5°C a week until the target temperatures were reached. Temperature acclimatised *Daphnia* were then maintained at their target temperature until used in a chronic toxicity study. The toxicity study was modelled on OECD 211 with an extended study duration to account for slower growth and reproduction at 15°C (OECD, 2012).

Over the course of acclimation, generation times began to increase with higher temperatures with *Daphnia* acclimatised to 15°C being on generation 8 at test start, compared to generation 11 and 12 for 20°C and 25°C respectively. This was supported in the results from the test itself where *Daphnia* tested at 15°C had significantly longer times to first brood than those tested at 20°C and 25°C. Another hypothesis tested was that *Daphnia* would shift reproductive strategy depending on temperature with higher temperatures associated with faster maturation and smaller reproductive investment per brood (Bonada et al., 2007; Contador et al., 2014). This hypothesis was supported by *Daphnia* acclimated and tested at 15°C exhibiting longer times to first brood than those at 20°C and 25°C, while those at 25°C had lower reproductive investment, primarily caused by shift in strategy for a reduced number of larger neonates.

The hypothesis that a temperature shock increases the sensitivity of *Daphnia* to copper, was not well supported given the results from Chapter 4. In particular, the only *Daphnia* that were significantly less sensitive for the reproductive parameters tested were those acclimatised at 20°C and tested at 20° (EC_{50} values of 41-45 µg/). Otherwise, temperature shock did not appear to affect calculated EC_{50} values of copper toxicity, including when the magnitude of temperature rise was greater (10°C against 5°C or no temperature change). Previous studies have investigated the multi-stressor effects of copper and temperature to *Daphnia*, but without multi-generation acclimation. However such studies, including those that investigated reproductive effects, found that increasing temperatures tended to increase copper toxicity with either additive or synergistic toxicity (Bae et al., 2016; Boeckman and Bidwell, 2006), although some *Daphnia* and copper studies, including two investigating reproductive effects, indicated little change in toxicity due to temperature supporting the results here (Cuco et al., 2016; Hochmuth et al., 2016; C. M. S. Pereira et al., 2017). One explanation for these results is that the optimum temperature for *Daphnia* is near 20°C; Lamkemeyer et al. (2003) and Chen and Stiller (2012) did not investigate chemical toxicity, but did acclimate *Daphnia* over multiple generations . Both discovered that the optimum temperature for *Daphnia* tended towards a value around 20°C. However, acclimation did shift the optimum temperature slightly towards the acclimation temperature.

The results from Chapter 4 indicate that the multi-stressor effects of copper and temperature increase to *Daphnia* did not indicate an increase in *Daphnia* sensitivity to copper, noting the similarities and differences observed in the literature. However, both temperature acclimation and shock temperature change affected the reproductive parameters alone. These changes included a shift in reproductive strategy associated with fewer larger offspring in a shorter time to first brood for those acclimatised and tested at higher temperatures. Such results may still impact sensitivity, for example larger organisms are known to be more tolerant of chemical exposure. However, the tolerance of *Daphnia* acclimatised at 20°C and tested at 20° compared to other temperatures may have important consequences when considering climate change in ecotoxicological studies, as altering the test temperature would be associated with a change in chemical sensitivity regardless of any multi-generation acclimation.

5.3 Application of findings to environmental risk assessment

5.3.1 Framework for considering climate change

The results presented in Chapters 2 to 4 demonstrate that climate change can influence both the exposure and sensitivity of freshwater organisms to chemicals and therefore affect the outcome of ERAs. However, the effect of climate change is both chemical and site dependent meaning that any proposed ERA framework should consider spatial variation in environmental and ecological factors in addition to chemical properties. Existing ERAs have spatially specific scenarios for considering chemical exposure, although the results of Chapter 2 have shown that the effects of climate change on chemical flux are substantial and spatially variable. Therefore in light of climate change, such scenario sites may need to be reconsidered to account how the future change in meteorology will affect the risk posed by chemical exposure. This could be done either by updating such sites with future projections or selecting additional locations particularly prone to increases in risk under climate change conditions to act as climate change scenarios. Equally, when assessing freshwater sensitivity there is no specific consideration of spatial variation in species composition; instead an assessment factor is used to represent all sources of uncertainty including variation of species sensitivity across different sites (ECHA, 2008; EFSA, 2013a).

A spatially specific framework accounting for climate change that includes all the aspects considered in this thesis is shown in Figure 5.1. The framework includes both the elements of risk assessment covered within this thesis, and the models, methods and databases necessary to quantify them. Figure 5.1 shows how each aspect of agrochemical exposure and ecosystem sensitivity considered in this thesis can be drawn together to calculate risk. The effects of climate change explored in this thesis are divided into i) the effects of temperature and precipitation on chemical degradation and the resultant change in runoff and erosion on chemical flux, ii) how temperature may shift the taxonomic composition of natural ecosystems and how such a shift in taxonomic composition affects assemblage sensitivity and iii) how long-term temperature acclimation has notable or negligible effects on the results of laboratory ecotoxicity testing, upon which endpoints used for calculating ecosystem sensitivity are based.

Note, this framework, and the case studies used to illustrate it, represent only a sub-set of the considerations required for a complete picture of risk under climate change. Ideally, a more complete risk assessment would also account for other routes of exposure (spray drift, drainage and groundwater), chemical fate processes within freshwaters, recovery of both species and ecosystems, and community and food web dynamics (Moe et al., 2013; Schmitt-Jansen et al., 2008; Stahl et al., 2013). However, considering that some of these are not accounted within existing ERAs that do not consider climate change, considerable research effort would be required to integrate them into the ERA. Furthermore, while the extent of spatial variation of the effects of climate change on sensitivity and exposure has been demonstrated, such spatial variation reflects only two aspects of the risk assessment. Equally only one taxonomic group assessed in ERAs, macroinvertebrates, was investigated here, while for chemical fate in-field flux only considered one soil and crop type. Furthermore, these spatially variable effects of climate change would be additional to any existing variation among sites. Noting these limitations, the next section explores how a proposed framework of Figure 5.1 could be used to consider the change in ERA risk for three agrochemicals: glyphosate, copper and a theoretical new chemical.

5.3.2 Case study: Glyphosate

Glyphosate is a non-specific systemic herbicide and plant desiccant and represents the single most used herbicide in the UK representing 21% of herbicide use on arable land (Garthwaite, 2018). This case study assumes a 1.44 kg glyphosate/ha pre-harvest summer application on winter cereals to treat broad-leaved weeds and couch grass prior to combining the crop (EFSA, 2015). The effects of climate change on glyphosate flux were calculated for suitable locations for growing winter wheat in the UK (Chapter 2, Table 2.4). The six locations selected within Chapter 2 have cognate river locations to the RICT reference sites nearby that were used in Chapter 3 so that the invertebrate community would be reflective of one receiving input for the agricultural land.

One additional factor that can affect chemical risk is changes to chemical input, effectively the amount and frequency of glyphosate application. Climate change conditions will increase overall weed growth, a shift to agricultural patters and herbicide resistance is set to be a continuing problem over the 21st century that may necessitate shifts in glyphosate use (Dayan, 2019; Helander et al., 2012; Ziska, 2020). However, studies so far predominantly indicate that increases in temperature do not reduce the efficacy of glyphosate to several weed species, including where there was increased growth from elevated CO_2 concentrations, and forecast use on wheat is projected to stay approximately the same (Bajwa et al., 2020; Iqbal et al., 2021; Jabran and Doğan, 2018; Rasche, 2021).

The nearest site combinations were calculated on the minimum trigonometric distance in latitude and longitude of the 835 RICT reference sites to each of the 6 sites in Chapter 2 in turn; the range of distances between sites ranged between 10.58km to 26.74km. Thus, it is possible to consider how the change in flux at each site could be related to the assemblage-specific sensitivity of an ecologically similar nearby river that could potentially undergo exposure. The effect of climate change on flux and assemblage sensitivity for the six sites is presented in Table 5.1.

Table 5.1. Effect of climate change on chemical flux and assemblage sensitivity and the overall effect on the risk quotient for glyphosate applied to winter wheat across six sites representative of the different climatic zones of England and Wales. Percentages here represent the change to edge of field chemical flux and calculated HC_5 under future climate conditions (2060-2080) compared to the baseline (1980-2000) with overall risk being calculated multiplicatively from these values. The locations used in Chapters 2 and 3 were compared to find the nearest RICT reference site to each site selected for flux prediction to ensure that the assemblage sensitivity would be calculated for an assemblage of a similar ecological profile and change in temperature under climate change

Location	Climate zone	Increase of flux (%)	Increased sensitivity of assemblage (%)	Overall increase of risk (%)	Ratio of change to risk
Cornwall	S Wales & England SW	+136	+21	+185.6	1:2.86
Durham	England E & NE	+4	+5	+9.2	1:1.09
Lancashire	England NW & N Wales	-33	+23	-17.6	1.21:1
Kent	England SE & central south	-32	+5	-28.1	1.39:1
Essex	East Anglia	-44	+23	-31.1	1.45:1
Oxfordshire	Midlands	-56	-21	-65.2	2.88:1

The existing ERA upon which glyphosate is approved concluding a low risk to freshwater organisms with the highest FOCUS step 2 PEC_{sw} values for all the representative uses, and with macroinvertebrates not being the organisms driving the risk (EFSA, 2015). In this case study the change to flux is the largest influencer on the change in the overall risk with value of -56% to +136% compared to only a shift of -21% to +23% in assemblage sensitivity. Despite this, the changes to the risk predicted in Table 5.1 emphasise the difference spatial variation has on the outcome of environmental risk assessments and how both exposure (as edge of field flux) and receptor sensitivity (as assemblage HC_5) can combine to affect overall risk. Considering the effects of climate change on both and receptor sensitivity, between the most and least sensitive sites (Cornwall and Oxfordshire respectively) there is a factor of 8.2 difference between the change to the risk quotient under future climate conditions.

At an organism level, there are a limited number of studies performed on macroinvertebrate species investigating the multiple stressor effects of temperature increase and glyphosate exposure. Silva et al. (2020), investigated the effect of short-term temperature acclimation (from neonate to adult) of the cladoceran *Ceriodaphnia silvestrii* on their acute sensitivity (as mortality) to glyphosate compared to a shock temperature change. In general, increasing test temperature increased sensitivity to glyphosate.

Overall, the effects of climate change on the risk posed by glyphosate in light of the calculation and literature presented here are quantifiable and exhibit distinct spatial variation resulting in both increase and decreases to risk under climate change. The most recent UK and EU aquatic risk assessment for glyphosate was resolved as low risk to all aquatic organisms without any higher tier refinements (EFSA, 2015). Given that the sensitivity of aquatic macroinvertebrates is not driving the risk assessment, the effects of climate change may not alter the outcome of the environmental risk assessment for glyphosate. However, even without the effects of climate change, the output from the hSSD model on the RICT reference sites indicates that there is a 4.8-fold difference in sensitivity between different assemblages. This nears the most conservative assessment factor of 5 for an equivalent SSD of the toxicity data, which is intended to cover all sources of uncertainty, including spatial variation in sensitivity (ECHA, 2008; EFSA, 2013a). Consequently, the question of how well assessment factors cover existing environmental risk assessments is raised.

5.3.3 Case study: Copper

The use of copper as a pesticide is primarily within organic farming practices as a fungicide and general bactericide (Garthwaite, 2018). Although copper is used for treating fungal and bacterial infestations, the toxic effects of copper to many organisms are acknowledged, and soft-bodied invertebrates organisms, such as annelid worm and molluscs are particularly sensitive (Bjerregaard et al., 2015; Verschoor et al., 2011). When copper is applied as a pesticide it is in the form of dissolved copper salts such as copper sulphate, copper oxychloride, copper hydroxide or copper oxide, however all share the same risk assessment within the UK, currently based on prior EU authorisation (EFSA et al., 2018; HSE, 2021). As copper is a metal, its fate and behaviour in soil is different to that of organic chemical compounds. For example, while its speciation might change, copper does not degrade and many fate models have lower suitability for predicting its transport compared to organic compounds. Current environmental risk assessment is based upon FOCUS modelling although the limitations of using such a methodology and the associated uncertainties are acknowledged (EFSA, 2013b; E. F. S. EFSA et al., 2018).

Copper is considered to be of high risk to aquatic macroinvertebrates (EFSA et al., 2018) and therefore the results from Chapters 3 and 4 are combined to consider how future climate conditions may affect the sensitivity of freshwater macroinvertebrate assemblages to copper exposure. Given the limited suitability of PRZM for accurately predicting metal fate and the fact that copper is approved for use in orchards and grapevines, rather than cereals, it was not used as a study chemical in Chapter 2.

Future climate change had little effect on the average sensitivity of 835 macroinvertebrate assemblages to copper: overall there was a 2.2% decrease in sensitivity. However, when considering the spatial variation of the effect of climate change, changes in sensitivity ranged from a 139% increase in the HC_5 value to 58.8% decrease, equivalent difference in ratio of 1:3.4 between the two. In addition, the effect of raising temperatures by 5°C for *Daphnia* acclimatised at 20°C (i.e., temperature used in the standard for toxicity tests for *Daphnia*: OECD 202 and OECD 211 (OECD, 2012, 2004)) was associated with an increase in copper toxicity on size of adult (by a factor of 5.5) and reproductive investment at first brood (by a factor of 5.4). *Daphnia* acclimated at 25°C still exhibited an increase in sensitivity compared to those acclimated and tested at 20°C, but the magnitude of effect was reduced to 4.5 for adult size and 3.2 for reproductive investment and must be considered within the caveat that *Daphnia* appear to have an optimum temperature near 20°C that may not be present in other organisms (Lamkemeyer et al., 2003). Overall, the effect of future climate change on increasing the sensitivity of macroinvertebrates to copper is greater at an organism level than from shifts in assemblage composition. However, as some sites will experience large increases in sensitivity due to composition change and another increase in sensitivity when moving away from *Daphnia* tested at 20°C, an overall increase in sensitivity for some sites under climate change is expected. The outcome of the existing ERA for copper already has macroinvertebrates on the boundary of unacceptable risk (EFSA et al., 2018). Consequently, any increases to sensitivity from climate change could result in the overall conclusion of unacceptable risk from copper.

From a chemical input perspective, shifting spring and summer temperature and precipitation patterns under future climate projections favour fungal infestation and may therefore require an increase in the use of copper-based pesticides (Bajwa et al., 2020; Rhodes and McCarl, 2020a; West et al., 2012a). However, accumulation of copper in the field is already restricted to 6 kg copper ha^{-1} $year^{-1}$ (EU Commission, 2002), and increased application rates would need to remain within these limits unless there was a change in regulation. All the assumptions of future copper use, however, are reliant on the continued approval of copper. Given the results presented here, an overall increase in sensitivity to copper may be expected under future climate conditions. Consequently, a future environmental risk assessment for copper may find fewer or no agricultural uses with acceptable risk.

5.3.4 Case study: Theoretical new chemical

While the case studies for copper and glyphosate demonstrate the application of the framework to existing chemicals, this case study assesses how it may applied to new pesticide active substances or emerging chemical pollutants relevant to agriculture, such as pharmaceuticals applied to agricultural land within irrigation water, manure and biosolid fertiliser (Chefetz et al., 2008; Fijalkowski et al., 2017; Pollard and Morra, 2018; Wu et al., 2010).

Spatial variation in climate change projections mean that location should be considered within the ERA. Consequently, either likely areas of exposure or generic scenario sites applicable for the entire UK need to be selected, which would permit the use of localised climate data (e.g., the 5km resolution UKCP18 data to run any exposure assessment (Met Office, 2019). In a manner similar to existing ERAs, specific fate scenarios could be created for the risks of climate change on fate, as demonstrated here within Chapter 2 that covers the major climatic zones of England and Wales. By also incorporating spatial variation in soil (e.g. using the LAND IS database, Cranfield University, 2021) the assessment can tailored to accurately represent a specific site. Equally for considering the local assemblage, RICT and the landscape parameters of a nearby river can be used with the same UKCP18 data to predict baseline and future assemblages to assess assemblage sensitivity (Environment Agency et al., 2021).

To assess the effect of climate change on chemical flux and to run through the risk assessment framework shown in Figure 5.1, first the quantity of the chemical applied should be known. New pesticides will have proposed application rates as part of their approved GAP (Good Agricultural Practice), which indicates when, how, in what quantity and on what crop the chemical may be applied. However, methods for calculating input of chemicals via irrigation water, manure and biosolid fertiliser require development (Fijalkowski et al., 2017). Even without any specific exposure rates, PRZM can still be used with a generic application rate kept consistent between the base and future climate scenarios to assess the effects of climate change as was done here with all chemical being applied at 1 kg/ha.

To run PRZM a suite of environmental chemical properties must be known, including various degradation rates, k_{OC}, solubility in water, vapour pressure and Henry's constant, which may be available in existing databases (University of Hertfordshire, 2007a, 2007b). However, as shown in Chapter 2, persistence is a large determinator of flux and knowing the degradation rate of the chemical in soil and the season of application alone means that a chemical could be compared with the outputs from chemicals with similar properties and approximate effects estimated.

Regarding sensitivity, the hSSD model can address the question of spatial or climate change variation in assemblage and therefore assemblage sensitivity but running the model does require a toxicity dataset of sufficient taxonomic richness for the chemical of interest. Ideally such a toxicity dataset would need to be composed of at least 20 different species covering all the major phyla of macroinvertebrates relevant to UK freshwater ecosystems (Annelida worms, Mollusca, Platyhelminth worms and Arthropoda (including Insecta and Crustacea). At an organism sensitivity level some indication of the multi-stressor effects of temperature and chemicals could inform future change in sensitivity. It could be envisaged that *Daphnia* could be cultured to different temperatures and toxicity tests run on the individuals acclimatised to each of the relevant temperatures, rather than just the 20°C cultures which are currently standard.

5.4 Conclusion

5.4.1 Uncertainties, limitations and recommendations

While this thesis has addressed the effects of climate change on agrochemical risk to freshwaters, the scope of potential impacts goes beyond what has been considered here. Consequently, the uncertainties and limitations surrounding the method and results from each chapter have been discussed below along with recommendations for mitigating and improvement upon these in future research.

Within Chapter 2, spatial variation considered both current and future climates. However, other landscape parameters, particularly soil, can drastically affect exposure (Biswas et al., 2018). Equally the chemical property of k_{OC} can become a determining factor of fate depending on the soil (Farenhorst et al., 2009; Wolt et al., 2002). Furthermore, the effect on flux was only considered for fields growing winter cereals, and the known impacts of climate change on crop phenology (eg. later drilling and earlier maturation and harvest) were not considered (Craufurd and Wheeler, 2009; Olesen et al., 2012). However, given different soils, crops and their phenologies can be directly inputted into PRZM, this research could be customised to represent unconsidered or updated ERA scenarios. This improves the spatial explicitness and adaptability of this research to changes in crop economics, viability and phenology (Cranfield University, 2021). Equally, within the UK fields can be drained, particularly on clay soils, representing an additional route of exposure via the soil not considered here as clay soils were not modelled (Brown and van Beinum, 2009). However, PRZM is not capable of modelling drain flow, therefore necessitating the use of additional fate models, such as MACRO, that can be run to calculate drainflow and gain a more complete picture of exposure (Jarvis et al., 1997). Finally, fate dynamics in freshwater were not considered, although PRZM itself forms one of a series of integrated models used within ERAs to predict soil and water fate (TOXSWA in FOCUS-SWASH, (Te Roller et al., 2015; Van den Berg et al., 2015)). As there is already direct links between PRZM and TOXSWA, assessing the effects of climate change on freshwater fate represents a logical next step.

While the hSSD model has shown that it can predict species sensitivity, there are two major limitations surrounding the use of taxonomy as the basis of sensitivity extrapolation: firstly that taxonomic ranks represent differing levels of phylogenetic distance (Barraclough, 2010), and secondly that some sensitivity influencing traits are highly labile (Blomberg et al., 2003; Rubach et al., 2010). Both mean that taxonomy may misrepresent the similarity of sensitivity between two taxa. One solution to this would be a holistic approach integrating the results from multiple sensitivity predictive methods (van den Berg et al., 2021). While the traits database remains incomplete for macroinvertebrates, it is being built upon and is increasing in its taxonomic coverage (Hébert et al., 2016; Usseglio-Polatera et al., 2000). Thus, integrating with the hSSD model with trait-based approaches of predicting chemical sensitivity may result in a more accurate predictor of species sensitivity. Equally, while RICT can be used for predicting communities, it is limited by the range of sites in its reference database to the UK. Consequently, predictions become increasingly uncertain as temperatures rise beyond those of the reference sites (Clarke et al., 2003). Improving upon this would require integrating water framework directive data from warmer locations (Wilby et al., 2006).

While Chapter 4 investigated the effects of rising temperatures and multi-generational acclimation on chemical sensitivity for a single species, Chapter 3 has explicitly shown that there is interspecific variation in sensitivity. Therefore, there is a question on how representative the results from Chapter 4 are of how thermal change would affect chemical sensitivity when extrapolating to all macroinvertebrates. Given that the species and chemical investigated were *Daphnia magna* and copper, both have already been extensively studied (Bae et al., 2016; Hochmuth et al., 2016; Pereira et al., 2017). Thus these results are therefore representative of the first tier of ERAs where *Daphnia magna* is the most frequent species tested. Still, Chapter 3 has highlighted the need for toxicity data on as wide a range of taxonomically diverse species as possible to improve predictions. This may work hand-in-hand with assessing the effects of acclimation and toxicity on a wider range of species, as in the methodology of Chapter 4 which tested species under both standard laboratory (20°C, OECD, 2004) and increased temperatures.

With regards to case studies considered within this chapter, the effect of climate change on input has been contextualised within each case study, but not explored within this thesis. Reviews have considered how climate change may shift timing of chemicals and the amounts applied to treat the changing pest phenologies (Delcour et al., 2015; Tudi et al., 2021) and how droughts may increase irrigation (Woznicki et al., 2015). A natural next step would be to integrate a method for quantifying how climate change affects chemical use and application rate. However, predicting future use is innately uncertain; economics and agricultural subsidies shift the viability of planting different crops and using the chemicals applied to them, regulation may see the banning or restriction of currently applied agrochemicals, while future technology and research may uncover new chemical active substances or discover emerging chemical pollutants of relevance to ecosystems (Christensen et al., 2018; Gavrilescu et al., 2015).

5.4.2 Conclusion

Overall, this thesis has integrated the effects of climate change on the risk posed to freshwater ecosystems by building upon the existing body of scientific knowledge and environmental risk assessment (ERA) with modelling and laboratory data. Primarily this thesis has demonstrated how climate change can alter environmental risk by shifting agrochemical exposure and ecosystem sensitivity. This resulted in both increases and decreases to risk depending on the specific location and agrochemical in question. One recurring result present within Chapters 2 and 3 is the importance of spatial variation both now and in the future that arises from variation in climates and community compositions respectively. Thus, a major novel finding of this thesis is that for freshwater ecosystems, both agrochemical exposure via soil and assemblage sensitivity of macroinvertebrates are spatially explicit. The variation in risk shown in the results here can increase risk beyond the uncertainty included within existing ERAs. This means risk is currently being underestimated for some site and chemical combinations and may become worse under climate change.

As demonstrated above, this thesis has shown how multiple aspects of the ERA can be further investigated and refined to account for the effects of climate change. This has been practically applied to existing chemicals which could alter the conclusions of their respective ERAs as well as define the package of data required to consider a similar process for another chemical. An ERA which can functionally consider the effects of climate change can inform and refine future agrochemical use while providing suitable protection of ecosystems and their services. However, the work here only approached three aspects of the ERA; future steps adapting and integrating additional aspects will permit a full picture of the change. This will involve a wider range relevant aspects, as discussed in 5.4.1. As constructing a comprehensive environmental risk assessment system requires knowledge of meteorology, chemistry, agronomy, soil science, toxicology, ecology and economics, an interdisciplinary approach is required to accurately capture the effects of climate change is. However, any system developed should be simple enough to be utilised and rolled-out within industrial, water quality-assessment and regulatory environments to make assessment of the effects of climate change on agrochemical risk accessible.

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Appendices

Appendix A2 for Chapter 2

Table A2.1 Relevant environmental properties of 24 chemicals. 14 pesticides and 10 pharmaceuticals. Chemical properties were sourced from the relevant of the Veterinary Substances DataBase (University of Hertfordshire, 2007b) or the Pesticide Properties Database (University of Hertfordshire, 2007a) for pharmaceuticals and pesticides respectively.

Chemical	Target	Time of Application	DT$_{50}$ Soil (day)	kOC	Solubility (mg/L)	Molecular weight	Vapour pressure	Henry's coefficient	DT$_{50}$ Water (day)	DT$_{50}$ Sediment (day)
Azoxystrobin	Fungicide	Spring	78	427	6.7	403.4	1.10E-10	7.40E-09	1000	205
Carbamazepine	Pharmaceutical	Autumn	36.5	419.08	17.7	236.27	1.80E-07	1.08E-07	100	328
Chlorothalonil	Fungicide	Spring	4.29	1288	0.81	265.9	7.62E-05	0.025	1.97	1000
Cypermethrin	Insecticide	Spring	141.3	89	93	416.3	2E-05	5E-05	22.2	1000
Cyproconazole	Fungicide	Summer	126	130031	0.00121	291.8	2.6 E-05	2.6E-05	1000	1000
Diazinon	Pharmaceutical	Autumn	9.1	609	60	304.35	9.01E-05	6.25E-8	4.3	10.4
Diclofenac	Pharmaceutical	Autumn	0.5	833	2.37	296.15	6.14E-08	4.73E-12	5	8
Diflufenican	Herbicide	Winter	141.8	1989	0.05	394	4.25E-06	7.40E-09	31.7	214
Erythromycin	Pharmaceutical	Autumn	20	10	2000	733.93	2.10E-25	5.42E-29	17.63	6.8
Fluroxypyr	Herbicide	Spring	0.7	19550	0.009	367.3	1.35E-06	0.027	38.1	38.1
Fluvalinate	Insecticide	Summer	31	750746	0.00103	502.9	9.10E-11	1.2E-04	1.96	87.32
Glyphosate	Herbicide	Summer	20.51	15844	10500	169.07	1.31E-05	2.1E-07	67.74	67.74
Imidacloprid	Insecticide	Autumn	38.9	242	514	255.7	7E-07	6.50E-11	168.58	27.1
Ivermectin	Pharmaceutical	Autumn	27	12600	4.1	874.7	1.5E-09	4.80E-26	2.9	15
MCPA	Herbicide	Summer	21	24	29390	214.65	1.4E-04	5.50E-07	141	1000
Metformin Hydrochloride	Pharmaceutical	Autumn	5	1693	1060000	165.66	7.60E-05	7.64E-16	20.3	14
Metronidazole	Pharmaceutical	Autumn	5.7	38	11000	171.15	3.10E-07	1.70E-11	385	74.5
Naproxen	Pharmaceutical	Autumn	2	349	15.9	230.26	1.90E-06	3.39E-10	14	7.7
Orlistat	Pharmaceutical	Autumn	6.4	49937	0.0919	495.74	9.77E-14	1.13E-09	18.9	18.9
Prothioconazole	Fungicide	Spring	0.5	1765	0.3	344.3	4E-07	3E-05	1	2.8
Pyraclostrobin	Fungicide	Spring	62	9304	1.9	387.8	2.6 E-06	5.3E-06	28	28
Thifensulfuron-methyl	Herbicide	Spring	1.39	9	2240	387.4	5.20E-09	3.25E-08	22.8	22.8
Tri-allate	Herbicide	Autumn	58.2	4301.4	4.1	304.7	0.012	0.89	103.8	68.2
Trimethoprim	Pharmaceutical	Autumn	110	301	400	290.32	9.90E-09	2.40E-14	20	75

Table A2.2 Coefficients for the static coefficient (a) and gradient coefficient (r) of logarithmic models base e fitted to the degradation of hypothetical chemicals with varying DT_{50} in a hypothetical soil over a 360-day period

DT_{50} (days)	**a**	**r**
0.5	0.003	-0.0007
2.5	0.016	-0.0034
12.5	0.024	-0.0042
62.5	0.026	-0.0036
312.5	0.025	-0.0028

Table A2.3 Coefficients for the static coefficient (a) and gradient coefficient (r) of exponential models fitted to the degradation of hypothetical chemicals with varying DT_{50} over a temperature increase from 0°C to 40°C

DT_{50} (days)	**a**	**r**	**Q10**
0.5	0.204	0.042	1.52
2.5	0.125	0.053	1.71
12.5	0.109	0.057	1.76
62.5	0.106	0.057	1.77
312.5	0.107	0.057	1.77

Table A2.4 Calculated p-values from the inter-seasonal variation in EFF_{90} between the six sites from the Tukey's HSD test with Sidak correction. Sites with differing letters were statistically significant (P<0.05) from each other

	Lancashire	Durham	Oxfordshire	Essex	Cornwall	Kent
Lancashire	a					
Durham	0.995	a				
Oxfordshire	<0.001	<0.001	b			
Essex	0.999	0.938	<0.001	a		
Cornwall	0.996	0.908	<0.001	0.999	a	
Kent	0.965	0.759	<0.001	0.998	0.999	a

Table A2.5 Calculated p-values for the size (A & B) and delay (C & D) to the precipitation event triggering the EFF_{90} (E & F) from baseline (A, C & E) and future (B, D & F) climate scenarios between the six sites from the Tukey's HSD test with Sidak correction. Sites with differing letters were statistically significant ($p<0.05$) from each other

Baseline (1980-2000)

Future (2060-2080)

p-values for log10 (Precipitation triggering 90th percentile flux)

A	Lancashire	Durham	Oxfordshire	Essex	Cornwall	Kent
Lancashire	a					
Durham	<0.001	b				
Oxfordshire	<0.001	<0.001	c			
Essex	<0.001	<0.001	0.080	cd		
Cornwall	0.002	<0.001	<0.001	<0.001	e	
Kent	<0.001	<0.001	0.048	0.999	<0.001	d

B	Lancashire	Durham	Oxfordshire	Essex	Cornwall	Kent
Lancashire	a					
Durham	0.896	a				
Oxfordshire	0.848	0.220	ab			
Essex	0.001	<0.001	0.061	b		
Cornwall	<0.001	<0.001	<0.001	<0.001	c	
Kent	<0.001	<0.001	<0.001	<0.001	0.824	c

p-values for log10(Days to 90th percentile flux)

C	Lancashire	Durham	Oxfordshire	Essex	Cornwall	Kent
Lancashire	a					
Durham	0.992	ab				
Oxfordshire	<0.001	<0.001	c			
Essex	0.012	0.067	0.065	bc		
Cornwall	0.027	0.130	0.031	0.999	b	
Kent	<0.001	<0.001	0.967	0.005	0.002	c

D	Lancashire	Durham	Oxfordshire	Essex	Cornwall	Kent
Lancashire	a					
Durham	0.829	ab				
Oxfordshire	<0.001	<0.001	c			
Essex	<0.001	<0.001	0.999	c		
Cornwall	0.044	0.542	<0.001	<0.001	b	
Kent	<0.001	<0.001	0.535	0.778	<0.001	c

p-values for log10 (Edge of field chemical flux)

E	Lancashire	Durham	Oxfordshire	Essex	Cornwall	Kent
Lancashire	a					
Durham	<0.001	b				
Oxfordshire	<0.001	<0.001	c			
Essex	<0.001	<0.001	0.941	c		
Cornwall	0.549	<0.001	<0.001	<0.001	a	
Kent	<0.001	<0.001	0.996	0.720	<0.001	c

F	Lancashire	Durham	Oxfordshire	Essex	Cornwall	Kent
Lancashire	a					
Durham	<0.001	b				
Oxfordshire	0.012	<0.001	c			
Essex	0.068	<0.001	0.992	ac		
Cornwall	<0.001	<0.001	0.004	<0.001	d	
Kent	<0.001	<0.001	<0.001	<0.001	0.869	d

Appendix A3 for Chapter 3

Table A3.1 Breakdown of the percentage taxonomic coverage of various toxicity datasets collated from the US EPA ECOTOX database compared to the freshwater taxa present across all 835 RICT reference sites grouped by chemical type

	Number of	Coverage of RICT reference taxa in chemical toxicity dataset (%)				
Chemical	**taxa**	Phylum	Class	Order	Family	Genus
Insecticides	**Average 68**	**94.6**	**87.2**	**67.6**	**30.6**	**7.1**
Aldrin	26	82.3	77.6	47.9	17.3	2.7
Azinphos-Methyl	30	96.9	85.3	68.2	28.7	6.5
Bifenthrin	29	74.6	73.4	71.5	25.9	7.9
Carbofuran	24	96.9	85.9	35.4	20.3	3.6
Carbaryl	141	96.9	95.7	91.1	41.2	3.3
Cypermethrin	81	96.9	90.7	60.8	28.9	7.4
Chlorpyriphos	137	98.2	90.8	69.4	33.9	3.7
DDT	109	98.2	90.8	88.9	35.9	12.9
Diflubenzuron	49	98.2	85.1	65.2	30.2	9.6
Dieldrin	40	89.2	79.6	61.1	24.6	4.8
Deltamethrin	81	96.9	89.5	63.8	32.7	11.1
Diazinon	60	98.2	97.0	75.8	37.0	10.1
Endrin	59	98.2	93.4	78.2	40.6	13.3
Endosulfan	71	96.9	89.5	85.2	29.1	4.0
Fenitrothion	120	98.2	90.8	90.2	40.6	6.6
Fenvalerate	21	82.3	77.6	32.5	13.7	0.4
Lambda-Cyhalothrin	45	89.2	79.6	61.8	31.3	13.9
Lindane	70	96.9	85.9	81.6	38.5	15.2
Malathion	176	98.2	97.0	93.2	44.1	12.5
Methyl Parathion	39	98.2	87.2	36.6	20.3	2.9
Methoxychlor	35	96.9	87.3	66.0	25.6	2.8
Parathion-Ethyl	62	96.9	81.1	78.7	34.4	7.0
Parathion-Methyl	38	98.2	90.8	37.6	21.4	3.1
Permethrin	90	98.2	90.8	82.7	39.6	5.6
Metals	**Average 47**	**97.7**	**96.4**	**59.4**	**31.6**	**9.6**
Cadmium	80	98.2	97.0	53.9	35.5	13.8
Copper	59	96.9	95.7	77.1	32.6	9.8
Nickel	26	98.2	97.0	63.8	27.7	5.1
Lead	24	96.9	95.7	50.4	28.8	8.6
Zinc	47	98.2	97.0	52.0	33.1	10.5
Narcotics	**Average 44**	**93.0**	**87.4**	**50.3**	**25.4**	**6.1**
Acenapthalene	12	89.2	73.4	31.5	14.5	2.4
Atrazine	27	82.3	81.1	45.5	17.9	2.6
Benzamine	25	98.2	85.1	49.3	25.2	4.1
Fluoranthene	40	96.9	95.7	49.3	23.9	2.3
Glyphosate	35	98.2	88.6	39.0	19.7	6.0
Linear-Alkyl Sulfonate	40	98.2	97.0	87.1	50.6	22.5
Nonyl-Phenol	35	98.2	88.6	32.9	16.2	0.4
Pentachlorophenol	69	98.2	97.0	80.1	38.3	6.5
Phenol	157	98.2	98.2	96.3	56.9	18.2
Sodium Dodecyl Sulfate	26	83.6	75.3	5.4	1.5	0.1
Toluene	23	82.3	81.1	37.2	14.8	2.1

Table A3.2 Leave one out analysis with the strength of correlation between actual toxicity data endpoints and the predicted values when predicting the toxicity from all other toxicity datapoints and across the taxonomic ranks of family and genus

	Correlation between toxicity data and prediction		
Chemical	**Toxicity dataset**	**Family**	**Genus**
Aldrin	0.424	0.695	0.703
Acenapthalene	-0.158	0.737	0.776
Atrazine	0.464	0.920	0.933
Azinphos-Methyl	0.643	0.897	0.906
Benzamine	0.688	0.907	0.920
Bifenthrin	0.823	0.950	0.954
Carbofuran	0.344	0.906	0.942
Carbaryl	0.808	0.892	0.903
Cadmium	0.491	0.738	0.738
Chlorpyriphos	0.609	0.882	0.919
Cypermethrin	0.620	0.800	0.854
Copper	0.357	0.617	0.618
DDT	0.487	0.797	0.847
Diflubenzuron	0.785	0.906	0.910
Dieldrin	0.259	0.684	0.705
Deltamethrin	0.316	0.789	0.817
Diazinon	0.746	0.916	0.920
Endrin	0.580	0.905	0.932
Endosulfan	0.428	0.821	0.829
Fluoranthene	0.238	0.798	0.809
Fenitrothion	0.633	0.838	0.884
Fenvalerate	0.583	0.931	0.948
Fluoranthene	0.422	0.780	0.830
Glyphosate	0.369	0.800	0.827
Linear-Alkyl Sulfonate	0.425	0.677	0.696
Lambda-Cyhalothrin	0.565	0.847	0.872
Lindane	0.727	0.844	0.852
Malathion	0.766	0.894	0.900
Methyl Parathion	0.714	0.894	0.913
Methoxychlor	0.795	0.946	0.952
Nickel	0.479	0.920	0.930
Nonyl-Phenol	0.398	0.862	0.898
Parathion-Ethyl	0.750	0.876	0.891
Parathion-Methyl	0.845	0.945	0.954
Lead	0.541	0.743	0.747
Pentachlorophenol	0.420	0.776	0.793
Phenol	0.547	0.609	0.626
Permethrin	0.352	0.697	0.705
Sodium dodecyl sulfate	0.587	0.908	0.922
Toluene	0.458	0.839	0.853
Zinc	0.377	0.878	0.889

Appendix A4 for Chapter 4

Table A4.1 Calculated EC_{50} values for the different reproductive endpoints tested within this study for Daphnia acclimatised and tested at 15°C, 20°C or 25°C . EC_{50} values were calculated form dose response curves fitted with non-linear log-logistic models

Endpoint parameter	Acclimation Temperature (°C)	Test Temperature (°C)	Change in temperature (°C)	Estimate EC_{50} (µg/L)	Standard Error
Time to first brood	15	15	0	17.2	9.2
	15	20	5	13.0	0.7
	15	25	10	14.7	13.2
	20	20	0	41.1	15.6
	20	25	5	5.1	2.2
	25	25	0	2.8	5.6
Adult size at first brood	15	15	0	10.6	3.8
	15	20	5	24.9	49.8
	15	25	10	13.7	12.6
	20	20	0	45.3	3.5
	20	25	5	8.3	18.6
	25	25	0	10.1	12.0
First brood reproductive investment	15	15	0	10.9	4.7
	15	20	5	11.9	4.2
	15	25	10	8.4	3.2
	20	20	0	42.9	16.7
	20	25	5	8.0	9.2
	25	25	0	13.3	3.6