The transfer and ecological effects of xenobiotic pollution in freshwater ecosystems

Thesis submitted for the degree of Doctor of Philosophy

by

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“*As crude a weapon as the cave man's club, the chemical barrage has been hurled against the fabric of life – a fabric on the one hand delicate and destructible, on the other miraculously tough and resilient, and capable of striking back in unexpected ways.*”

Silent Spring, Rachel Carson (1962)

Summary

1. The diversity of synthetic, xenobiotic chemicals reaching the wider environment has increased rapidly over the past century. The nature and severity of their ecological effects in freshwater systems, however, remains poorly understood, even for the so-called 'legacy' pollutants. These persistent, bioaccumulative and toxic compounds still risk having negative effects at all levels of biological organisation long after their initial release into the environment.
2. The ability to determine the ecological risks posed by persistent pollutants remains restricted due to: (1) reliance on standardised toxicology testing on individuals in the laboratory; (2) limited understanding of how spatial and biological variation alters potential ecological effects at population, community and food web levels; and (3) poor knowledge of how pollutant bioaccumulation and biomagnification translate to effects in natural ecosystems.
3. Through global, catchment and reach-scale empirical assessments, this thesis investigated spatial and biological variation, trophic transfers and ecological risk in freshwater ecosystems associated with persistent xenobiotic pollutants (polychlorinated biphenyls [PCBs], polybrominated diphenyl ethers [PBDEs] and organochlorines [OCs]).
4. The transfer, accumulation and magnification of persistent pollutants were related to site-specific environmental conditions, biological traits, food web structure and chemical characteristics, and were sufficient for widespread, hazardous levels of contamination. Across river systems, pollutant body burdens could be linked to putative structural and functional effects that appeared to be networked through food webs.
5. Overall, these data indicate the importance of natural processes in influencing the potential effects of persistent pollutants in freshwater ecosystems. Risk assessments that incorporate the variation present in natural systems are required to improve understanding of the role of xenobiotic pollutants in global environmental change across freshwater ecosystems.

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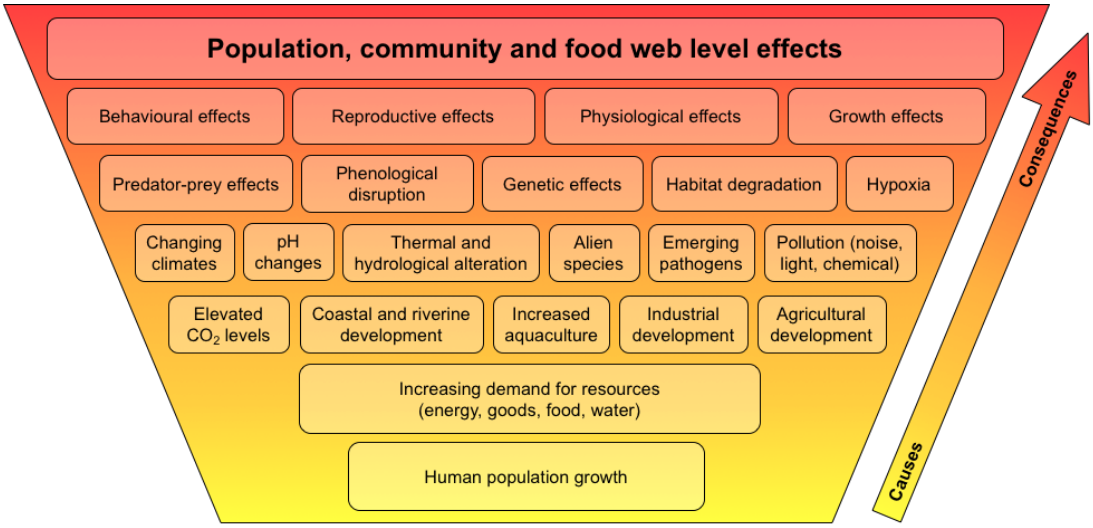
Chapter 1: General Introduction



1.1. Background

Freshwater ecosystems are under threat from a range of stressors (Dudgeon *et al.* 2006; Reid *et al.* 2018), which individually, or in combination, can exert pressures on their biological communities (Ormerod *et al.* 2010). A wide range of human-induced environmental changes affect freshwater ecosystems, including; climate change (Vörösmarty *et al.* 2000), urbanisation (Grimm *et al.* 2008), agricultural intensification (Stoate *et al.* 2009) and water abstraction (Bunn & Arthington 2002). The biological implications of these alterations are variable, ranging from the formation of novel ecosystems and species interactions (Hobbs *et al.* 2006), through to the complete collapse of biological communities and reductions in the provision of ecosystem services (Dobson *et al.* 2006). While the nature and severity of effects reflect the frequency and intensity of anthropogenic activities (Chester & Robson 2013), abiotic and biotic factors mediate the extent to which anthropogenic disturbances cause significant ecosystem-specific responses that are general or specific to any given environment (Nõges *et al.* 2016).

Freshwater ecosystems appear particularly at risk from global environmental change, with greater declines in biodiversity than either their terrestrial or marine counterparts (WWF 2018). This sensitivity stems from a combination of three factors. Firstly, freshwaters are disproportionately exposed to multiple, severe environmental and anthropogenic stressors, resulting from the implicit dependence of human populations on freshwater resources (Strayer & Dudgeon 2010). Secondly, freshwater ecosystems are hotspots of biological diversity – reflecting large species richness per unit area or water volume (Strayer & Dudgeon 2010). Third, freshwater organisms appear particularly sensitive to environmental change due to the unique environmental conditions present in these systems, for example the restricted oxygen availability in the subaqueous environment (Forster, Hirst & Atkinson 2012). With increases predicted in the anthropogenic pressures placed on freshwater resources (Bunn 2016), the biodiversity and functioning of freshwater systems is at considerable risk (Davis *et al.* 2015).

Water quality is an area of significant concern for the conservation of freshwater biodiversity (Martinuzzi *et al.* 2014), arising from pollution by both

organic and inorganic chemicals (Schwarzenbach *et al.* 2006, 2010). As an example, over 7,700 chemicals are currently produced and used in significant volumes globally (US EPA 2018a), with many of these synthetic chemicals entering and subsequently threatening freshwater systems (Schwarzenbach *et al.* 2010; Bernhardt, Rosi & Gessner 2017). The distribution and quantity of chemicals in the environment, however, is both spatially and temporally variable (Malaj *et al.* 2014). In any given freshwater ecosystem, the cocktail of chemical pollutants is a product of contemporary releases of current-use and emerging chemicals (e.g. personal care products, pharmaceuticals, systemic pesticides) as well as the recirculation of persistent chemicals (e.g. brominated flame retardants, organochlorine pesticides) (Kortenkamp 2007). Although improvements in the treatment of wastewaters has reduced the levels of gross pollution across freshwaters and led to widespread recovery of biological communities (Vaughan & Ormerod 2012), mixtures of xenobiotic chemicals remain pervasive and continue to generate significant potential for ecological effects across large regions of the globe (Bernhardt, Rosi & Gessner 2017).

Mixtures of xenobiotic chemicals can have a significant deleterious effect on freshwater biota (e.g. Alexander *et al.* 1988; Colborn, vom Saal & Soto 1993; Arnold *et al.* 2014), demonstrated by experimental and empirical assessments of exposure to a diverse suite of xenobiotic compounds to food web components, including benthic microbial biofilms (Lawrence *et al.* 2005; Rosi-Marshall *et al.* 2013), phytoplankton (Hense *et al.* 2003; Knapp *et al.* 2005), macroinvertebrates (Damásio *et al.* 2011; Tlili *et al.* 2012; Morrissey *et al.* 2013a), fishes (Rodgers-Gray *et al.* 2001; Jobling *et al.* 2002a; Lange *et al.* 2011) and semi-aquatic passerine birds (Ormerod & Tyler 1990; Ormerod, Tyler & Jüttner 2000; Morrissey *et al.* 2013b). Yet there has been only limited research at higher levels of biological organisation, specifically populations, communities and food webs. Initial studies at these broad ecological scales have nevertheless demonstrated significant adverse effects on the structure and function of ecosystems, ranging from alterations in population dynamics to complete food web collapse (Kidd *et al.* 2007, 2014). These studies, however, provide only limited knowledge of the transfers, fluxes and effects of xenobiotic compounds in freshwater environments. Greater understanding of

such ecological processes is required to determine accurately the ecological risk presented by xenobiotic pollutant mixtures in natural systems. Knowledge gaps about the emergent effects on freshwater ecosystems are surprisingly large even for otherwise well-known legacy pollutants such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorines (OCs).

1.2. Aims and research questions

The overarching aim of this study was to use an ecosystem approach to determine the distribution, quantity, trophic transfer and cascading ecological effects of xenobiotic, legacy, pollutants in freshwater ecosystems. The thesis has been divided into several chapters – intended to be publishable scientific papers focusing on the interactions between organisms and pollutants across food webs, networks and ecosystems. Data were collected through systematic literature reviews, primary data collection and secondary datasets, ranging in spatial coverage from individual habitats (e.g. rivers in South Wales) to freshwater ecosystems across the globe. The main objectives were to:

1. Investigate the spatial distribution of xenobiotic pollutants across freshwater ecosystems at a range of physical and ecological scales
2. Examine the transfer pathways of xenobiotic pollutants through the trophic levels of freshwater food webs
3. Assess the ecological risk associated with xenobiotic pollutants in freshwater ecosystems

1.3. Study region (South Wales, United Kingdom)

Primary data were collected over a series of different river systems in South Wales, United Kingdom (see Figs. 4.1, 5.1, 6.1 and 7.1). Sample sites were located across three hydrological catchments, the Taff, Usk and Wye, selected to provide contrasting land cover (urban, industrial, pastoral and arable land uses), sources of pollution and biological communities.

The Taff catchment is dominated by urban and industrial land cover (~20%), but also has a history of heavy industry, with coal mining and associated spoil heaps previously active along large lengths of the river systems (Scullion & Edwards 1980). Potentially dominant sources of xenobiotic pollutants in river

systems across the Taff catchment include sewage effluent, wastewater from industry, combined sewer overflows, road drains and diffuse urban run-off. Sites across this catchment were selected to provide a gradient of urban land cover (0.5–21.1%), yet all sites were expected to represent the environmental conditions indicative of highly urbanised river catchments.

The Usk catchment is characterised by lower levels of urban land cover (~7%) distributed more widely across the catchment and composed of intermediate to small conurbations. Within the catchment there is also proportionally higher levels of both pastoral and arable agriculture (~14%), in comparison to urban land cover (~5%). The likely sources of pollution present across this catchment include, but are not limited to; diffuse agricultural run-off, illicit use of banned pesticides (mainly pastoral, e.g. dieldrin), recirculation of historical pollutants (river sediments), field drains, urban run-off and sewage effluent. Sample sites across the Usk catchment represent systems characterised by a mixture of both agricultural and urban land cover.

The Wye catchment is dominated by high levels of agricultural land cover across significant regions of the catchment (~30%). This catchment has the lowest levels of urban cover of the sites studied (~2%), dominated by small conurbations with limited industry and municipal wastewater treatment facilities. The sources of pollution that are likely to arise across the Wye catchment include agricultural run-off, illicit use of banned pesticides (mainly arable, e.g. hexachlorobenzene), field drains, recirculation of historical pollutants (soils and river sediments), sewage effluent and urban run-off. The sites sampled across this catchment are selected to represent systems characterised by agricultural activity, interspersed with low levels of urban cover.

Although sample sites were situated in catchments characterised by specific combinations of land use and anthropogenic activities, each site had unique combinations of water quality, biological communities and pollution sources. Specific environmental conditions (e.g. hydrology, geomorphology, water quality), land cover variation, and pollution sources across sites and catchments are described in more detail, where relevant, in the individual chapters.

1.4. Chapter structure

Each chapter contributes to the research aims detailed above, and the background, aims and objectives are as follows.

Chapter 2: Xenobiotic pollutants and endocrine disruption in aquatic ecosystems

Knowledge of emergent pollutant effects beyond individual organisms is crucial for accurate environmental risk assessments and effective management. Although a considerable amount of research has been conducted at the level of individual organisms, this review highlights the importance of assessing xenobiotic pollution and endocrine disruption at higher levels of biological organisation, including populations and food webs. The synthesis presented in this review provides context for the subsequent primary and secondary data chapters.

Chapter 3: A global assessment of the ecological risks from xenobiotic pollutant bioaccumulation in freshwater ecosystems

Data on the distribution of xenobiotic pollutants at the global scale are scarce. This chapter provides a systematic review and meta-analysis to assess the global distribution of 26 bioaccumulative and high-risk endocrine disrupting and legacy xenobiotic pollutants in freshwater ecosystems. This global spatial analysis sets out to understand the distribution and severity of the ecological risk associated with the bioaccumulation of these compounds. The data collated within this chapter support the more spatially explicit and detailed assessments in the following chapters.

Chapter 4: Biomonitoring and spatial variation of persistent xenobiotic pollution across river ecosystems in South Wales

The contemporary focus on current-use and emergent chemicals means that the distribution and quantity of legacy pollutants is relatively poorly understood. The chapter provides an assessment of spatial variation in both the environmental concentration and bioaccumulation of several persistent xenobiotic pollutants; PCBs, PBDEs and OCs. By aiming to understand spatial variation in pollutants, a basis for the biological and chemical drivers of pollutant-biota interactions across food webs (Chapters 5 and 6) is provided.

Chapter 5: Transfer pathways of persistent xenobiotic pollution within a river food web

The pathways through which different groups of persistent organic pollutants (POPs) accumulate in food webs is poorly understood, with significant potential for differential partitioning, and thus exposure, within the compartments of natural food webs. This food-web assessment describes compound-specific transfer pathways of persistent xenobiotic pollutants (PBDEs, PCBs and OCs) in a river system. The relationship between trophic transfers, biological traits and pollutant accumulation, as well as magnification, are examined, laying the foundation for understanding differences in the levels of accumulation and magnification in Chapter 6.

Chapter 6: Trophic magnification of persistent xenobiotic pollutants across river ecosystems in South Wales

Studies in aquatic and terrestrial ecosystems have indicated the influence of food web structure on the transfer and magnification of pollutants across ecosystems. Here, a catchment-scale field-assessment of bioaccumulation and trophic magnification investigates the broad scale controls on pollutant transfers, whilst also identifying variation in potential ecological risk posed by persistent xenobiotic pollutants across South Wales. The chapter assesses whether variation not explained by the biological characteristics, or traits, of individual organisms (Chapter 5) is related to the broad-scale structure of river food webs (connectivity, chain length, link density) or variation in the environment conditions at sample sites. The ecological effects of the observed accumulation and magnification (Chapters 4, 5 and 6) are assessed across multiple river ecosystems in following chapter.

Chapter 7: Ecological effects of persistent xenobiotic pollutants across river ecosystems in South Wales

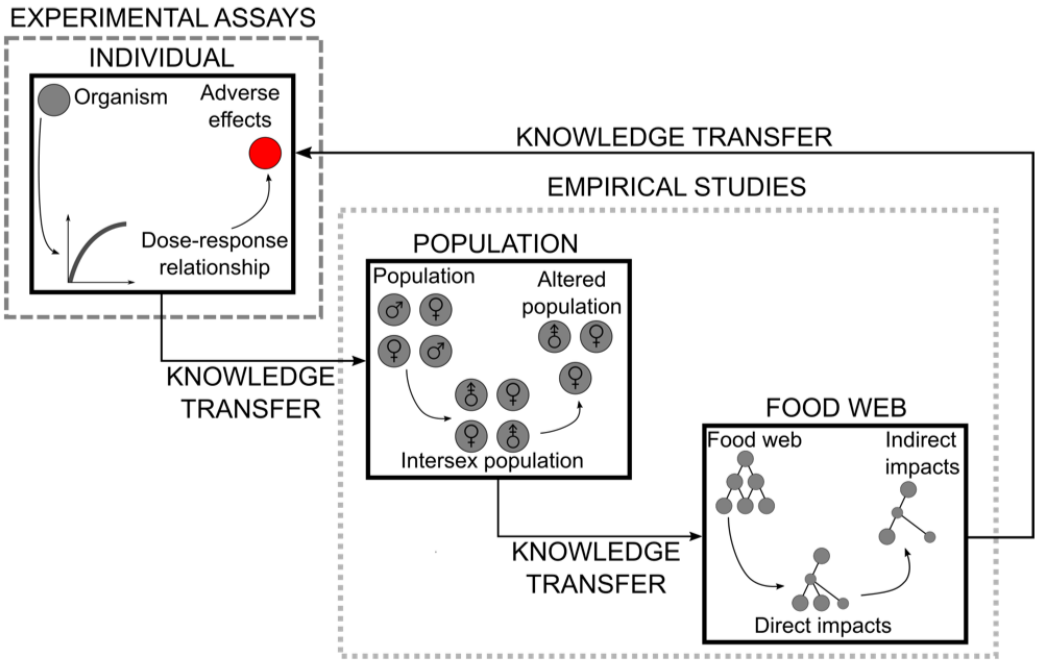
The effects of persistent pollutants on communities and food webs are poorly understood. Data from previous chapters, however, indicate levels of persistent contaminants in the tissues of invertebrates, fish and river birds that suggest potential ecological effects. This field study investigates the ecological effects of xenobiotic pollutant mixtures at 18 sites in three catchments,

presenting evidence of the potential for PCBs and PBDEs to reduce the taxonomic and functional diversity of river food webs. The chapter links the influence of source-dynamics, spatial variation, biological processes and multiple chemical stressors from previous chapters to the potential impacts of xenobiotic pollution at broad spatial and ecological scales.

Chapter 8: General discussion

The final chapter synthesises the study's findings within the wider framework of multiple stressors in global freshwater ecosystems. The results are also used to comment on the potential efficacy of contemporary monitoring schemes and current policy regarding persistent pollutants within freshwater ecosystems. The chapter ends by discussing future avenues of research, including the necessary developments required to understand fully the risk presented by xenobiotic pollutants at higher levels of biological organisation and across broad spatial scales.

Chapter 2: A review of xenobiotic pollution and endocrine disruption in aquatic ecosystems



This chapter is based upon a review published in *Biological Reviews.* The review was written by Fred Windsor (FMW) and subsequent revisions and edits provided by Steve Ormerod (SJO) and Charles Tyler (CRT).

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2.1. Abstract

1. Xenobiotic pollutants can alter biological function in organisms at environmentally relevant concentrations and are a significant threat to aquatic biodiversity, but there is little understanding of exposure consequences for populations, communities and ecosystems. The pervasive nature of xenobiotic endocrine disrupting chemicals (EDCs) within aquatic environments and their multiple sub-lethal effects make assessments of their impact especially important but also highly challenging.
2. Herein, a review of the ecological effects of EDCs in aquatic systems is presented. The review focuses on studies assessing populations and ecosystems, and including how biotic and abiotic processes may affect, and be affected by, responses to EDCs.
3. Recent research indicates a significant influence of behavioural responses (e.g. enhancing feeding rates), transgenerational effects and trophic cascades on the ecological consequences of EDC exposure. In addition, interactions between EDCs and other chemical, physical and biological factors generate uncertainty in our understanding of the ecological effects of EDCs within aquatic ecosystems.
4. Syntheses illustrate how effect thresholds for EDCs generated from individual-based experimental bioassays of the types commonly applied using chemical test guidelines (e.g. Organisation for Economic Co-operation and Development [OECD]) may not necessarily reflect the hazards associated with endocrine disruption.
5. Improved risk assessment for EDCs in aquatic ecosystems urgently requires more ecologically oriented research, as well as field-based assessments at population-, community- and food web-levels.

2.2. Introduction

Xenobiotic endocrine disrupting chemicals (EDCs) remain an active topic in contemporary ecotoxicology due to their proven environmental impacts (Wang & Zhou, 2013; Zhou, Cai, & Zhu, 2010) and postulated health effects (Kabir, Rahman & Rahman 2015). Over the past decade, published work on EDCs has provided a strong mechanistic understanding of exposure effects (Colborn, vom Saal & Soto 1993; Tyler, Jobling & Sumpter 1998; Kloas *et al.* 2009; Söffker & Tyler 2012; Tijani, Fatoba & Petrik 2013; Orton & Tyler 2015). Far less consideration, however, has been given to processes and interactions controlling the effects of EDCs at broader ecological scales, including inter- and intra-specific interactions within populations and food webs (Brodin *et al.* 2014; Schoenfuss *et al.* 2015; Segner 2011). Understanding the effects of EDCs on processes operating at these broader scales is essential, but also challenging, because their effects can be pervasive and sub-lethal. Although EDCs can induce deleterious effects in a wide range of organisms across different trophic levels (Brander 2013), there is insufficient knowledge for environmental regulators to assess the impacts and risks posed by EDCs to populations, communities and ecosystems (Mills & Chichester 2005; Hallgren *et al.* 2012).

Herein, is a critical evaluation of the known and potential effects of EDCs on natural ecological systems. This review highlights a need for research to incorporate processes and effects at broader spatial and temporal scales, illustrating how such studies have helped to advance our understanding of EDC impacts beyond common approaches to testing. Findings also indicate the importance of integrated research strategies for EDCs that develop upon previous frameworks from other pollutants to generate environmentally relevant data. Finally, existing research is synthesised to highlight further research required to understand the effects of EDCs in natural systems.

2.3. The benefits of up-scaling xenobiotic pollutant research

The requirement for information on population effects of EDC exposure to inform risk assessments has led to the extrapolation of individual-based bioassays (Jobling *et al.* 2002a; Miller & Ankley 2004; Gutjahr-Gobell *et al.* 2006; Lange *et al.* 2008; Brander *et al.* 2016). Such extrapolations assume,

however, that effects of EDC exposure in individual-based bioassays generally show simple, direct and invariant relationships, with impacts on populations and communities, even if safety factors are used to account for uncertainties. Assessments involving wild populations, however, indicate discontinuities between the results of individual- and population-level assessments (Jobling *et al.* 2002b; Brown *et al.* 2005; Lange *et al.* 2011; Hamilton *et al.* 2014). Differences in the ecological processes represented in micro-, meso- and macro-scale studies (Fig. 2.1) are potentially responsible for this disparity. Specifically, these differences include the nature of the EDC exposure regime, possible compounding environmental influences (e.g. multiple stressors), and the fact that multiple effect mechanisms may operate through trophic interactions across food webs at the macroscale (Hamilton *et al.* 2016a). There are several inconsistencies in findings about endocrine disruption from different biological, spatial and temporal scales. For example, cause–effect relationships reflect the methods used and the scales at which studies are completed, and this creates a challenge in determining mechanistic relationships and emergent effects at broader spatio-temporal extents. As an example, feminisation at the individual level would suggest significant potential population effects, but studies at broader spatial scales have indicated that population-level effects depend on mating-system dynamics (White *et al.* 2017). On the one hand, the low cost of sperm production relative to eggs means that males are able to fertilise multiple females, thus the feminisation of males may have little effect on population dynamics (White *et al.* 2017). On the other hand, mating systems may prevent male promiscuity, meaning that feminisation and minor alterations in the sex ratio result in negative effects on populations (White *et al.* 2017). Currently, little consideration is generally given to natural complexity in ecological and toxicological processes within experimental research designs (Barton 2003). Models developed for up-scaling from individual-based assessments to population scales are therefore inherently weak, and may even be flawed, as they provide limited appreciations of wider controls on higher levels of biological organisation. Factors such as density-dependence, adaptation, trophic interactions, likelihood of population exposure (habitat preferences), as well as species-specific life-history traits of organisms, are all likely to have a significant impact

on endocrine disruption, yet none of these characteristics are considered in common experimental assessments used to investigate the ecological impacts of EDC exposure.

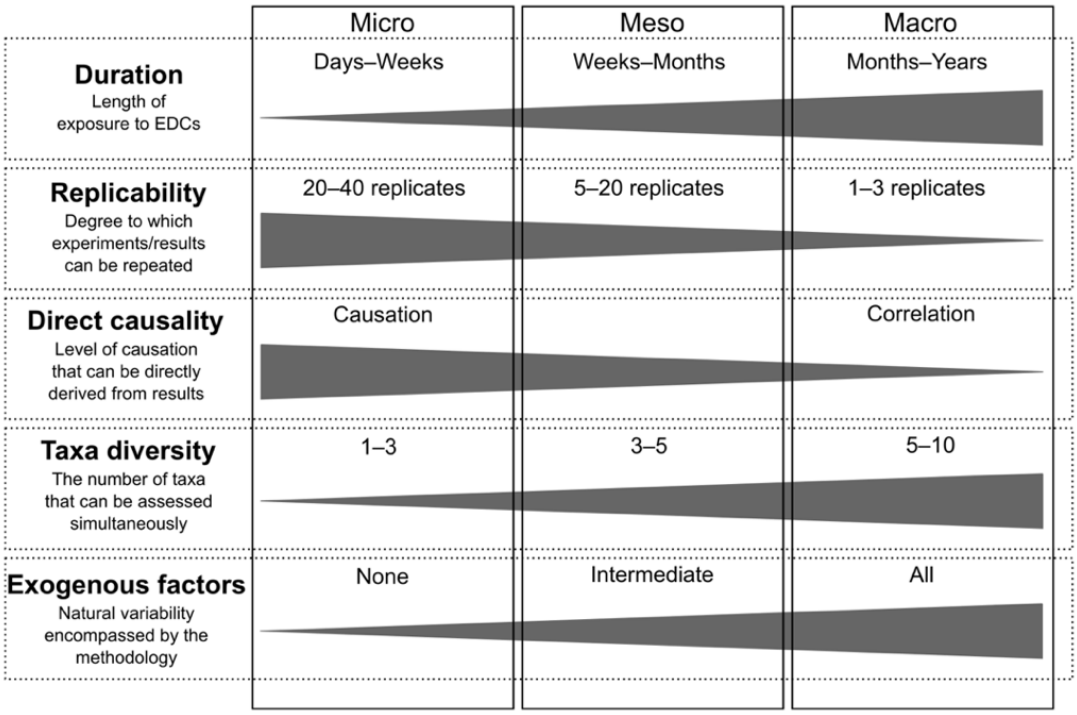


Fig. 2.1. Conceptual differences in framework design and expected outcomes of micro-, meso- and macro-scale assessments.

Research that considers processes over longer periods of time (e.g. entire life cycles) and at higher levels of organisation (e.g. populations and food webs) overcomes several limitations associated with most current experimental ecotoxicology bioassays (Geiszinger *et al.* 2009). The complexity associated with analysis of mesocosm and field scenarios, however, has restricted the uptake of these research designs. Furthermore, many field studies are characterised by correlation and weak inference in comparison to well-established mechanistic knowledge developed under more-controlled experimental conditions. A combination of experimental and field-based studies across a range of ecological scales is thus required for an improved understanding of population- and food-web-level responses to EDC exposure. This approach has, however, had relatively little uptake (Patiño & Carr 2015)

and studies assessing the effects of EDCs at community and food-web scales remain scarce (Boxall *et al.* 2012). Contemporary studies have consequently called for a greater focus on broader scale ecological and toxicological processes (Brodin *et al.* 2014; Kidd *et al.* 2014).

2.4. Advances in broad-scale xenobiotic pollutant research

Here, a critical assessment of recent findings focuses on processes operating at broad spatial and temporal scales, and highlights the limitations associated with using experimental bioassays, conducted without due consideration of natural dynamics. This builds upon previous reviews of the role of theoretical ecology in enhancing ecotoxicology (Relyea & Hoverman 2006).

2.4.1. Biotic interactions and trophic transfer of xenobiotic pollutants

The effects derived from EDC exposure within natural systems are variable and influenced by biological processes including competitive interactions and predation. Only a few examples exist of how biotic factors affect the severity of endocrine disruption, but a suite of processes appear to provide an important influence on the risk associated with EDC exposure within ecosystems. The behaviour of organisms in response to EDC exposure, in particular, can result in important ecological effects and, in some cases, behavioural changes enhance adverse effects of EDC exposure (Melvin & Wilson 2013). As well as providing the potential to exacerbate an effect at higher levels of biological organisation, interactions among individuals can also buffer the observed effects of EDC exposure. An example of this is density–dependent compensatory effects in zebrafish (*Danio rerio*) which alleviate negative individual reproductive effects of octylphenol exposure (Hazlerigg *et al.* 2014). Effects such as those detailed above are rarely considered or captured in laboratory–based studies and the consequences of these alterations could exacerbate the effects of EDCs at higher levels of biological organisation and within natural systems.

Biotic and abiotic processes can influence the trophic transfer of EDCs within aquatic ecosystems. Alkylphenols, pyrethroids, PCBs, PBDEs and diclofenac have been shown to partition, accumulate and magnify within components of aquatic food webs (see Table 2.1), and exhibit different entry and transfer

pathways within the environment (Burreau *et al.* 1997, 2006; Correa-Reyes *et al.* 2007; Corcellas, Eljarrat & Barceló 2015; Muggelberg *et al.* 2017). Many EDCs are hydrophobic in nature and readily partition out of the water column through adsorption to both suspended and benthic sediments (Petrović *et al.* 2001). Consequently, a significant proportion of the total pollutant load entering aquatic food webs is likely to be through benthic taxa interacting with sediments (e.g. sediment ingestors) (Wu *et al.* 2009; Brooks, Gaskell & Maltby 2009). Dietary transfers, however, are not the main route of uptake for many EDCs, and for selected compounds (e.g. carbamazepine and diphenhydramine), direct adsorption from the water column is a major route for their bioaccumulation (Du *et al.* 2014, 2016). This transfer of EDCs directly from the water column into aquatic organisms can occur either through passive adsorption, whereby the skin and respiratory surfaces enable diffusion, or *via* assimilation of EDCs adhering to suspended organics (Zhou *et al.* 2007). In natural systems, it is likely that most EDCs enter organisms by multiple uptake pathways. Thus, EDC exposure in natural systems may be intermittent, as in dietary intake, or possibly continuous *via* the water column.

Upon entry into organisms the transfer of EDCs within aquatic food webs is affected by a series of biological controls, including the organism's physiology, and *via* biotic interactions. The biological traits of organisms, including feeding guilds, influence the bioaccumulation, biomagnification and transfer of EDCs (Muñoz *et al.* 2009; Damásio *et al.* 2011). Bioaccumulation can vary across trophic levels (Ruhí *et al.* 2015), but even within the same trophic level individual biological traits, including size, can influence EDC uptake (Sidney *et al.* 2016). Many organisms exhibit an ability effectively to eliminate selected EDCs from tissues, thereby mitigating their accumulation via diet or water, and subsequent transfer (Norman *et al.* 2007; Al-Ansari *et al.* 2010). These assessments highlight the importance of biological interactions in the trophic transfer of pollutants in natural systems and indicate why responses may deviate from those expected from experimental, laboratory-based exposure on individual organisms. Further research is, however, required to understand the influence of traits on the bioaccumulation and ecological risk of pollutants.

Table 2.1. Bio-concentration and -accumulation factors (BCFs and BAFs) for xenobiotic pollutants in aquatic organisms.

Chemical group	Compound	log K_{OW}	log BCF/BAF	Trophic level	Organism	Source
Organobromines	BDE-100	7.24	7.50	3	*Salvelinus namaycush*	Streets *et al.* (2006)
	BDE-47	6.81	7.30	3	*Salvelinus namaycush*	
	BDE-99	7.32	6.70	3	*Salvelinus namaycush*	
	γ-HBCD	5.48	4.51	3	*Carassius auratus*	Wu *et al.* (2011)
	HBB	6.09	3.48	2	*Cipangopaludina chinensis*	
		6.09	4.47	3	*Carassius auratus*	
	PBDEs	6.27	0.96	2	*Gammarus pulex*	Tlili *et al.* (2012)
		6.27	0.79	2	*Echinogammarus stammers*	Viganò *et al.* (2009)
Organochlorines	DDE	6.51	1.65	3	*Rana* spp.	Albanis *et al.* (1996)
		6.51	2.40	5	*Egretta garzetta*	
	DDT	6.52	4.00	2	*Pomacea* spp.	Siriwong *et al.* (2009)
		6.52	4.40	2	*Macrobranchium lanchesteri*	
		6.52	6.60	2	*Filopaludina mertensi*	
	HCB	5.72	6.20	2	*Tubifex tubifex*	Egeler *et al.* (1997)
		5.72	2.00	2	*Eisenia fetida/andrei*	
	Lindane	3.80	2.20	3	*Rana* spp.	Albanis *et al*. (1996)
		3.80	2.35	5	*Egretta garzetta*	
		3.80	4.40	2	*Tubifex tubifex*	Egeler *et al*. (1997)
		3.80	2.50	2	*Eisenia fetida/andrei*	
	PCBs	6.50	7.63	3	*Perca fluviatalis*	Bremle, Okla & Larsson (1995)
		6.50	6.60	1	*Selenastrum* spp.	Stange & Swackhamer (1994)

Chemical group	Compound	log K_{OW}	log BCF/BAF	Trophic level	Organism	Source
		6.50	6.10	1	*Anabaena* spp.	
Organophosphates	Chlorpyrifos	4.96	5.99	2	*Mytilus galloprovincalis*	Serrano *et al.* (1997)
	Methidathion	2.42	5.26	2	*Mytilus galloprovincalis*	
	TrBT	9.49	3.37	2	*Ancylus fluviatalis*	Ruhi *et al*. (2015)
		9.49	3.61	2	*Hydropsyche* spp.	
		9.49	3.53	3	*Phagocata vitta*	
Pharmaceuticals	Carbamazepine	2.25	3.03	3	*Oreochromis niloticus*	Garcia *et al.* (2012)
	Diclofenac	4.01	0.92	3	*Oncorhynchus mykiss*	Fick *et al.* (2010)
		1.90	6.86	3	*Hemiculter leucisculus*	Liu *et al.* (2015)
	Dilitiazem	2.70	3.18	3	*Oncorhynchus mykiss*	Fick *et al.* (2010)
	Diphenhydramine	3.11	2.77	3	*Gambusia holbrooki*	Wang & Gardinali (2013)
	Erythromycin	3.16	5.67	2	*Planorbidae* spp.	Du *et al.* (2015)
	Gemfibrozil	4.77	4.73	3	*Gambusia holbrooki*	Mimeault *et al.* (2005)
	Ibuprofen	3.79	4.06	3	*Oncorhynchus mykiss*	Fick *et al.* (2010)
	Oxazepam	2.24	0.30	2	*Coenagrion hastulatum*	Brodin *et al*. (2013)
	Propranolol	3.48	8.29	3	*Hemiculter leucisculus*	Liu *et al*. (2015)
	Roxithromycin	2.75	8.87	3	*Hemiculter leucisculus*	
Phenols	BPA	3.40	4.97	2	*Pisidium amnicum*	Heinonen *et al.* (2002)
		3.40	8.48	1	Benthic algae	Yang *et al.* (2014)
	Nonylphenol	4.48	8.85	1	*Isochyrysis galbana*	Correa-Reyes *et al.* (2007)
		4.48	2.64	2	*Lumbriculus variegatus*	Mäenpää & Kukkonen (2006)
	NPEO2	4.20	3.14	1	*Cladophora glomerata*	Ahel, McEvoy & Giger (1993)

Chemical group	Compound	log K_{OW}	log BCF/BAF	Trophic level	Organism	Source
		4.20	–0.22	3	*Oncorhynchus mykiss*	Staples *et al.* (1998)
Pyrethroids	Cypermethrin	5.20	5.74	2	*Chironomus tentans*	Muir *et al.* (1985)
	Deltamethrin	5.20	5.76	2	*Chironomus tentans*	
	Fenvalerate	5.20	4.93	2	*Chironomus tentans*	
	Parathion	3.83	4.62	3	*Gnathopogon caerulescens*	Tsuda *et al.* (1994)
	Permethrin	6.20	5.56	2	*Chironomus tentans*	Muir *et al*. (1985)
	Vamidothion	0.12	6.56	3	*Gnathopogon caerulescens*	Tsuda *et al*. (1994)
Steroidal Androgens and Oestrogens	4-AD	–	5.39	2	*Meretrix lusoria*	Liu *et al*. (2015)
	ADD	–	6.33	2	*Meretrix lusoria*	
	Boldenone	–	8.01	2	*Meretrix lusoria*	
	EE2	4.01	0.80	2	*Chironomus tentans*	Dussault *et al.* (2009)
		4.01	4.23	1	Phytoplankton	Xie *et al.* (2015)
		4.01	4.89	3	*Pelteobagrus fulvidraco*	
	Norgestrel	3.48	6.28	2	*Meretrix lusoria*	Liu *et al*. (2015)
		3.48	6.14	3	*Lutjanus erythopterus*	
	Progesterone	3.87	7.70	2	*Meretrix lusoria*	
	Testosterone	3.32	8.29	2	*Meretrix lusoria*	

4-AD, 4-androstene-3,17-dione; ADD, androsta-1,4-diene-3,17-dione; BDE, Brominated Diphenyl Ether; BPA, bisphenol A; DDT, Dichlorodiphenyltrichloroethane; DDE, Dichlorodiphenyldichloroethylene; EE2, 17α-ethinylestradiol; HBB, Hexabromobenzene; HBCD, Hexabromocylcododecane; HCB, Hexachlorobenzene; NPEO2, nonylphenol ethoxylate 2; TrBT, tris-(2-butoxyethyl)-phosphate; BCF/BAF, Bioconcentration/accumulation; log K_{OW}, octanol/water partition coefficient (https://pubchem.ncbi.nlm.nih.gov/)

Interactions between the direct effects of endocrine disruption and the subsequent transfer of EDCs through ecosystems may also occur, supporting that alterations in individual-level effects may have consequences for wider biological systems (Brooks, Gaskell & Maltby 2009). A specific illustration of this is provided by Brodin *et al.* (2013, 2014) where an increased feeding rate of perch (*Perca fluviatilis*) in a behavioural response to oxazepam exposure resulted in enhanced consumption of its damselfly prey (*Coenagrion hastulatum*), and in turn an increase in the transfer and bioaccumulation of oxazepam. These examples illustrate that ecological risks for some EDCs that affect ecosystem processes (e.g. feeding behaviour and bioaccumulation potential) may be greater than commonly appreciated within aquatic ecosystems.

2.4.2. Adaptation to xenobiotic pollutant exposure

Individuals, populations and food webs have varying levels of resilience to environmental stressors (Harrison 1979), but in most cases organisms in aquatic ecosystems are able to persist at low levels of stress, even in multi-stressor environments (Vinebrooke *et al.* 2004). There is little field-based information, however, on the ecological and evolutionary resilience of individuals and populations to endocrine disruption, although the presence of adaptation is widely displayed within experimental assessments (Wu, Siu & Shin 2005). Many existing studies do not assess adaptations directly, instead indicating the reduction in effect size over the duration of exposure, which occurs more rapidly for individuals, in comparison to populations and communities (Wu, Siu & Shin 2005). Several field studies have identified populations of aquatic organisms resistant to certain EDCs. For example, Weston *et al*. (2013) indicated that point mutations at the pyrethroid target site (voltage-gated Na^+ channel) in amphipod (*Hyalella Azteca*) populations meant that resistant individuals did not experience the neurotoxic effects observed in non-resistant populations, instead exhibiting oxidative stress only at considerably higher pyrethroid concentrations. Varying levels of resistance were found across several populations. Adaptation has also been observed within fish assemblages (Hamilton *et al.* 2016b). Both the Atlantic tomcod (*Microgadus tomcod*) and the Atlantic killifish (*Fundulus heteroclitus*) can

adapt to polycyclic aromatic hydrocarbon (PAH) and PCB exposure in natural systems (Clark *et al.* 2010; Wirgin *et al.* 2011), but through different mechanisms. In *M. tomcod*, a six-base deletion in the aryl hydrocarbon receptor 2 (AHR2) restricted inducible gene expression, and was responsible for the observed resistance to EDC exposure (Wirgin *et al.* 2011). In comparison, resistance in *F. heteroclitus* individuals was generated by single nucleotide polymorphisms in the regulatory regions of the cytochrome P4501A gene (Clark *et al.* 2010; Reid *et al.* 2016).

Resistance, and/or adaptation has significant implications for the potential broad-scale effects of endocrine disruption in aquatic systems. A recent example in *H. azteca*, showed that populations pre-exposed to the pyrethroid pesticide Permethrin were able to persist under higher environmental concentrations (>210 ng L^{-1}) than those populations which were not pre-exposed (Muggelberg *et al.* 2017). This adaptation meant that resistant individuals provided a source of dietary exposure for fathead minnows (*Pimphales promelas*) under conditions within which non-resistant individuals could not survive. Within natural systems, adaptation of individuals or populations leads to an enhanced risk of bioaccumulation with increasing concentrations of EDCs. Adaptation to endocrine disruption indicates that organisms may be able to persist at environmentally relevant concentrations of EDCs, yet it also suggests potential for increased flux of EDCs through food webs. Changes in the bioaccumulation and transfer of EDCs potentially lead to increases in the body burden of higher trophic-level organisms, increasing the likelihood of adverse effects across food webs.

2.4.3. Long-term, life-cycle and transgenerational effects

There have been relatively few assessments of EDCs for long exposure durations, over full organism life cycles and/or over multiple generations, even though many organisms will be exposed for prolonged periods of time. Chronic exposure studies that have been undertaken have provided several significant advances. Firstly, in most cases they have shown that the effects are greater than for short-term exposures (Keiter *et al.* 2012; Tassou & Schulz 2013). Secondly, different health effects have been identified for longer-term exposures in comparison to short-term exposures. For example, for 17α-

ethinyloestradiol (EE2) exposure, effects reported on mating behaviour, growth and survival in *D. rerio* individuals differed between exposure periods of 0–21 and 0–75 days post-fertilisation (Segner *et al.* 2003). Thirdly, unanticipated effects have been identified following chronic exposures to EDCs. Exposure of rainbow trout (*Oncorhynchus mykiss*) eggs to an environmental oestrogen, bisphenol A (BPA), over a range of concentrations including 300 and 3000 ng L^{-1} resulted in lower energy levels allocated to larval feeding, reductions in specific growth and restricted food conversion ratios (Birceanu, Servos & Vijayan 2015). Finally, chronic exposure studies have helped to highlight life-stage-specific susceptibilities to the effects of EDCs. Schäfers *et al*. (2007) illustrated that chronic effects on sexual differentiation in *D. rerio* resulting from lifelong exposure to 10 ng L^{-1} of EE2, were more pervasive than the reversible effects induced by exposure extending over the period of gonadal differentiation only.

It must be emphasised that not all EDC effects are necessarily permanent; some are transient in nature and the organism may recover after the exposure is removed. Examples include the reported partial recovery from the effects of EE2 (5 ng L^{-1}) on gonad differentiation in *D. rerio* after a five-month depuration period post-EE2 exposure (Nash *et al.* 2004). Complete recovery of biological function was observed in a full-life-cycle analysis of *D. rerio* after exposure to EE2 (3 ng L^{-1}) (Fenske *et al.* 2005). Here, exposure to EE2 from the fertilised egg stage for 118 days post-fertilisation inhibited gonad differentiation in males, but a 58-day post-exposure period of depuration resulted in resumption and subsequent completion of testicular differentiation. Reproduction in *D. rerio* has also been shown to recover completely after exposure to zearalenone; exposure to 1,000 ng L^{-1} zearalenone for 140 days induced a female shift in the population sex ratio, but a subsequent period of depuration for 42 days resulted in recovery of relative fecundity (Schwartz *et al.* 2013). The ability to recover will, in part, depend on EDC exposure concentration, and the consequent nature and severity of effect(s). In other studies on *D. rerio,* for example Schäfers *et al.* (2007) and Baumann *et al.* (2014), individuals did not show full recovery following EE2 exposure at 9.3 ng L^{-1} or trenbolone (an androgen used as a growth promotor for cattle in the USA) exposure at

30 ng L^{-1}. The length of both exposure and period for depuration thus appear to be important in weighing up the potential for biological impacts of EDCs in natural systems. The fact that EDCs can act through multiple pathways means that it is especially difficult to identify chronic and life-stage-specific effects (Sohoni & Sumpter 1998). Pinpointing these effects is further hindered by the fact that effect mechanisms for many EDCs are not well defined. As an example, phthalate esters [e.g. di-n-butyl phthalate and di(2-ethylhexyl)phthalate] have been identified as both oestrogen receptor agonists and androgen receptor antagonists (Takeuchi *et al.* 2005). Furthermore, exposure to these compounds maintains a range of individual-level effects, including alterations in cellular proliferation, biosynthesis and apoptosis, as well as several immune responses (Milla, Depiereux & Kestemont 2011; Mankidy *et al.* 2013). Thus, when considering the spatio-temporal dynamics of EDC pollution within aquatic systems, it is important to assess all effects that may manifest. In natural systems, exposure to EDCs through periodic urban run-off may result in different effects compared to continuous emissions from wastewater treatment works (WwTWs).

Transgenerational studies on the effects of EDCs further highlight the importance of considering temporal scale in effect analyses. There is a mounting consensus that EDC exposure effects can span multiple generations, and may induce different impacts in offspring compared with the parental generation (Skinner, Manikkam & Guerrero-Bosagna 2011; Bhandari, vom Saal & Tillitt 2015). Some of the adverse effects observed in subsequent generations have been shown not to be induced through the direct modulation of DNA sequences, but rather through permanent alterations in the epigenome promoting transgenerational phenotypes (Skinner, Manikkam & Guerrero-Bosagna 2011; Head 2014). This mechanism can promote transmission of potentially susceptible phenotypes to the offspring of affected organisms, and may enhance the adverse impacts of EDCs within subsequent generations (Sowers *et al.* 2009). Exposure during early life or at particularly susceptible life stages can also have effects that span the lifetime of the affected organism and potentially lead to adverse effects in subsequent generations (Head 2014). These changes can be through somatic and gametic effect pathways (Faulk &

Dolinoy 2011). Consequently, epigenomic changes resulting from EDC exposure may lead to transgenerational effects, and possibly different population-level impacts within natural systems because of cumulative adverse effects in multiple generations (Bernal & Jirtle 2010).

Contemporary assessments of EDCs in the laboratory are confined to a restricted range of short-lived species suitable for experiments; for fish, notably *D. rerio, P. promelas* and medaka (*Oryzias latipes*). Whilst these taxa are convenient study models, they may not necessarily allow the accurate prediction of effects in populations of longer-lived organisms which may accumulate greater levels of EDCs over longer periods of time and have slower generational turnover, and thus a reduced ability to adapt. Further efforts to understand long-term exposure effects across a wider range of taxa are urgently required.

2.4.4. Interactive mixtures of xenobiotic pollutants

Wastewater effluents and other pollutant sources are often composed of highly complex mixtures, and interactions between EDCs, and of EDCs with other chemicals, could alter their biological effects (Keiter *et al.* 2012; Schoenfuss *et al.* 2016). The potential for additive effects of EDCs and other chemicals is significant. Most experiments on EDCs, however, have assessed only the effects of individual chemicals, with a small number of exceptions (e.g. Thorpe *et al.* 2003; Brian *et al.* 2007). A range of adverse, sub-lethal impacts may occur that are not always predictable from assessments of individual components (Kortenkamp 2007; Viñas, Jeng & Watson 2012) or *via* simple additive-effect modelling (Silva, Rajapakse & Kortenkamp 2002). Compounds with dissimilar modes of action may induce novel effects, operating through multiple mechanisms (Viñas, Jeng & Watson 2012). Sárria *et al.* (2011) demonstrated that exposure to EE2 and tributyltin (TBT) caused alterations in the behavioural responses of juvenile black-striped pipefish (*Syngnathus abaster*). Tributyltin depressed the burst-swimming response known to result from EE2 exposure, whilst EE2 influenced the alterations in the time spent in secluded areas generated by high concentrations of TBT. Consequently, when EDC mixtures combine with processes (e.g. competition and predation), a range of complex and often unpredictable effects can result.

There are also reports of a non-monotonic dose–response relationship resulting from exposure to EDC and their mixtures (Vandenberg *et al.* 2012). Non-monotonic dose–response relationships are not unique to EDCs, but they have been reported more frequently for EDCs than for other toxicants (Vandenberg 2014), in part reflecting the use of more sensitive endpoints or the wider range of concentrations tested (vom Saal *et al.* 2010; Vandenberg *et al.* 2013; Vandenberg 2014). Controversially, it has been proposed that hormesis, where marked beneficial low-dose effects are observed, may be responsible for the non-monotonic dose–response relationships (Calabrese 2005). This conclusion has been disputed, with some arguing that the impacts of oestrogenic EDCs always remain negative irrespective of concentration (Weltje, vom Saal & Oehlmann 2005). Many examples exist of non-monotonic dose–response relationships for EDCs with markedly different physicochemical properties. Pyrethroid pesticides, for example, generally exhibit greater negative effects at lower concentrations (Brander *et al.* 2016), and BPA shows a non-monotonic transcriptional-effect response (Villeneuve *et al.* 2012). There appears to be a wide range of effects that exhibit non-monotonic relationships with several EDCs. The identification of non-linear, non-monotonic, and in some cases hormetic, relationships across many studies has led some authors to suggest that effects observed at high EDC concentrations may not represent those at environmentally relevant concentrations or for mixtures of EDCs (Beausoleil *et al.* 2013; Vandenberg 2014). Thus, the lowest observed effect levels (LOELs) recorded within experimental bioassays may not accurately extrapolate to the lowest concentrations present within natural systems (Vandenberg *et al.* 2012). It has been suggested that alternative relationships (U- or inverted U-shaped) may better reflect effects associated with environmental EDC exposure (Vandenberg *et al.* 2014; Vandenberg & Bowler 2014; Zoeller & Vandenberg 2015). This potentially challenges the concentration-specific understanding of endocrine disruption within natural systems and poses a significant challenge for risk assessment (Futran Fuhrman, Tal & Arnon 2015).

2.4.5. Xenobiotic pollutants within the context of multiple stressors

Accounting for environmental variation is crucial in determining the effects of EDC exposure within natural systems, as multiple covariant environmental variables influence observed effects within natural systems (Daughton 2004; Damásio *et al.* 2011). Previous assessments have used the statistical and environmental control provided by experimental bioassays to eliminate confounding relationships between influential variables present within natural environments. However, interactions between multiple stressors ultimately dictate the relative severity of EDC exposure and subsequent ecological risk within ecosystems (Hooper *et al.* 2013). Recent research has demonstrated the importance of assessments incorporating and accounting for exogenous environmental characteristics, such as water temperature, physicochemical conditions and biotic interactions. These abiotic and biotic stressors may interact with one another as well as with EDCs to affect the outcome in exposed organisms. A modelling study by An *et al.* (2009) assessing wild roach (*Rutilus rutilus*) populations demonstrates the potential for interactive effects of multiple stressors. In this study, the feminisation of individuals generated by endocrine disruption appeared to have negligible effects on population extinction risk, yet the combination of exposure and selective fishing practices resulted in significant increases in local population extinction rates. The feminising effect of oestrogenic EDCs in isolation does not always result in significant population effects (Hamilton *et al.* 2016a) and, in some cases, the population-level threats from masculinisation are greater than from feminisation. The relative threat of both feminisation and masculinisation, however, is dependent on the optimal sex ratio of individual populations (White *et al.* 2017). Fish species exhibiting a non-linear mating function (non-linear response of reproductive capacity to changing sex ratio) did not exhibit reduced reproductive output when few males were present, however, the overall reproductive output of the population was significantly reduced by declines in the relative abundance of females (White *et al.* 2017).

Studies assessing temperature and EDC exposure indicate that stressor–EDC interactions may take multiple forms, with EDC exposure in some cases driving alterations in the effects of temperature increases (Jenssen 2006), while in

other cases temperature determines the severity of ecological effects derived from EDC exposure (Moe *et al.* 2013). The importance of interactions between two stressors has been relatively well demonstrated by contemporary research, yet these studies are still not representative of the true complexity present within natural systems. More recent research has attempted to encapsulate a greater number of stressors. For example, Brown *et al.* (2015) showed that a combination of EDC exposure, temperature increases and inbreeding led to a significantly skewed sex ratio in *D. rerio* populations. Increases in temperature (28–33 °C), clotrimazole exposure (2,000 and 10,000 ng L^{-1}) and inbreeding together had an additive effect, with a marked increase in the male-skew of populations relative to the effects generated by individual stressors. The results of multiple-stressor studies have indicated additive and synergistic interactions between stressors and endocrine disruption, but this depends on the level of biological organisation included (Fischer, Pomati & Eggen, 2013; Sulmon *et al*. 2015). Consequently, such processes are significant in altering the observed effects of EDC exposure whilst also demonstrating the need for analyses to encapsulate the effects of ecological processes on sub-lethal EDC impacts.

2.4.6. Effects of genetics on responses to xenobiotic pollution

Interactions between the wider spatial connectivity of aquatic environments (e.g. isolated and connected populations) and chemical contamination can have marked effects on the genetic diversity present within populations (Bickham *et al.* 2000). Genetics, specifically genetic diversity, can play an important role in determining the effects of EDC exposure, with reductions in genetic diversity derived from inbreeding potentially increasing the adverse ecological effects of EDC exposure (Bickley *et al.* 2013). Söffker *et al.* (2012) reported that despite a generally similar response of genetically divergent *D. rerio* populations to EE2 exposure, differences in their breeding biology and response sensitivity were apparent. Inbreeding within laboratory fish stocks is a major issue for experimental assessments of EDCs, especially when intending to inform further research in systems involving outbred individuals (Brown *et al.* 2009). Although perhaps of limited value for building understanding of the effects of EDCs in outbred populations, experimental

bioassays using inbred individuals may be useful for indicating the increased susceptibility of isolated natural populations to EDC exposure. In the event of habitat reconnection, whereby inbred and outbred populations interact, adverse impacts on fertility within inbred populations can facilitate a reduction in reproductive output of inbred individuals (Bickley *et al.* 2013). Assessments analysing interactions between genetic diversity and endocrine disruption within natural populations, however, remain scarce, and future research is required to test hypotheses relating to genetic diversity and endocrine disruption across aquatic environments.

2.4.7. Trophic cascades and indirect effects of xenobiotic pollutants

Direct effects of endocrine disruption may cause alterations in processes and interactions within aquatic ecosystems, in turn generating indirect effects across wider levels of biological organisation (Relyea & Hoverman 2006; Schulz *et al.* 2015). Such secondary effects may result from changes in competition and predation interactions within food webs, and subsequent release from biotic stressors (Knight *et al.* 2005). Similar trophic cascades have been identified to result from other anthropogenic contaminants, such as petroleum hydrocarbons and heavy metals (Fleeger, Carman & Nisbet 2003). Very few studies, however, have assessed these phenomena for EDCs. These indirect processes could alter the perceived impacts of EDC exposure within natural populations, as well as affect the transfer of EDCs within food webs. Indirect effects may occur through several mechanisms. Knapp *et al.* (2005) demonstrated that changes in nutrient fluxes resulting from invertebrate mortality in response to deltamethrin exposure (2000 ng L^{-1}) increased microbial community biomass. A more commonly observed indirect mechanism is provided by the adverse effects of EDC exposure within predator assemblages and a subsequent top-down cascade through the food web. Alterations in the structure of invertebrate communities have been recorded in response to failed recruitment of secondary-consumer fish species when an entire Canadian lake was dosed with EE2 (5–6 ng L^{-1}) over a period of three summers (Kidd *et al.* 2014). A similar example exists in a differently structured ecosystem, with endocrine disruption in *R. rutilus* populations resulting in a reduction in predation of phytoplankton and increased copepod

abundance (Hallgren *et al.* 2014). The indirect effects of endocrine disruption and their influence over multiple trophic levels further indicates the potential for the observed effects of EDC exposure within natural systems to deviate from those predicted from experimental laboratory bioassays.

2.5. Limitations of xenobiotic pollutant impact assessments

The results of assessments of the impacts of EDCs at broad spatial and temporal scales depart significantly from predictions from laboratory-based experimental studies. These results highlight: (1) the limitations of using individual-based bioassays to predict the effects of EDCs at population- and food-web scales (also see Forbes *et al.* 2010; Hommen *et al.* 2010), and (2) the need for research at a range of spatial and temporal scales to advance knowledge of broad-scale ecological effects and risk assessment. The restricted scope of common experimental assessments has been highlighted previously (Matthiessen 2008; Lecomte *et al.* 2013), with calls for additional data to inform existing protocols and enhanced higher-tier tests to replace unsuitable testing methods (Taenzler *et al.* 2007). Although frameworks such as the OECD guidelines promote an increase in the complexity of assessments (Gourmelon & Ahtiainen 2007), the methodologies used in these assessments inherently simplify the large range of controls on the effects of EDCs present within natural systems. Population-level interactions, including density-dependent relationships such as intra-specific competition, provide inherent controls on the effects of EDC exposure within the environment, yet these controls remain absent from ecological impact and risk assessments (Mills & Chichester, 2005). The low ecological complexity inherent in these protocols therefore appears to provide a major constraint on the accuracy and wider applicability of such tests.

Models developed from standard, individual-based bioassay protocols currently provide limited value for the investigation of the effects of EDCs within natural systems. As identified by Hazlerigg *et al.* (2014), isolation of the effects of chemical-mediation from other sub-lethal effects may be responsible for the underestimation of population-level impacts in model scenarios. Although population-level models are suggested as a method for generating environmentally relevant predictions across natural systems (Forbes, Calow &

Sibly 2008; Forbes *et al.* 2010, 2011), extrapolating from overly simplified experimental data must be done with caution. Furthermore, the availability of limited data at higher levels of biological organisation (e.g. populations) restricts the validation of model simulations (Rose *et al.* 1999; Forbes, Calow & Sibly 2008; Raimondo *et al.* 2009). The application of these models to the prediction of EDC effects thus remains prone to inaccuracies (Munns *et al.* 2008).

2.6. Integrated research for studies on xenobiotic pollutants

Low environmental concentrations of EDCs, coupled with their high propensity for sub-lethal impacts, means that assessments at broader scales are essential for understanding the true implications of EDC exposure. Nonetheless, the complex mechanisms through which endocrine disruption can occur requires a detailed causal understanding which is difficult to derive from large-scale studies (e.g. mesocosm or field assessment) (Schindler 1998; Forbes *et al.* 2010). The requirement for a multi-tiered research strategy may apply to all chemicals but is arguably most relevant to EDCs due to their wide range of sub-lethal effects that operate at different ecological scales, together with their potential for multiple biotic and abiotic interactions within and among spatial and temporal scales. The need to develop a cohesive, broad-scale biomonitoring strategy is frequently identified in reviews of ecological risk assessments (Besse, Geffard & Coquery 2012; Gavrilescu *et al.* 2015).

Knowledge acquired at multiple spatial and temporal scales provides a suitable framework to mitigate previous limitations and to increase our understanding of EDC effects over wider ecological scales. Similar integrated research has proved effective when assessing the complex effects of stressors within a range of ecosystems, including multiple stressors in freshwater systems (Altshuler *et al.* 2011) and heavy metals in coastal areas (Vlahogianni *et al.* 2007). In the case of endocrine disruption, such a focus will enable an increase in mechanistic knowledge at broad scales and the development of environmentally relevant experimental bioassays. The product of this framework is environmentally relevant knowledge at a range of scales, enabling the provision of suitable information (and uncertainties) to

practitioners and managers, potentially facilitating a reduction in adverse EDC effects across aquatic environments.

As in other research fields (see Culp *et al*. 2000), experiments on individuals can initially be used to understand the direct impacts of stressors at the organism level, and these can then be translated to research designs operating at broader scales. The multi-tiered research strategy that is proposed here, unlike other more-specific ecosystem-based strategies, is applicable to a wide range of ecosystems and a suite of EDCs. Furthermore, it surpasses previous methodological designs which focus more on the identification of ecological risk (using experimental bioassays) and subsequent biomonitoring programs (e.g. Maruya *et al.* 2014), rather than providing a framework for understanding the risks within all levels of biological organisation across ecosystems. Microcosm assessments within this research strategy allow for an assessment of EDC exposure on reproductive morphology, physiology and behaviour, in turn allowing for mechanistic knowledge at the organism and sub-organism scales. Similarities and discrepancies identified between individual- and population-level assessments can, in turn, indicate the population-level processes and controls (e.g. density dependence and habitat-mediated exposure) influencing the effects of EDCs within populations of aquatic organisms. Significant effects identified at the population level can be used to pinpoint areas of research suitable for further individual-based studies. In terms of food-web assessments, the initial direct effects identified within individual-based assessments can indicate the potential for indirect effects and trophic cascades, allowing for the derivation of a suitable research design to identify these processes within natural systems. Furthermore, the high replicability and mechanistic understanding developed within individual-based studies provides a valuable tool for broad-scale assessment, enabling causal relationships to be derived for processes observed within aquatic food webs. The combination of individual-, population- and food-web-level analyses can therefore enable improved realism of investigations and facilitate up-scaling of results to suitable levels for utilisation by practitioners.

2.7. Future directions

2.7.1. Spatial variation in xenobiotic pollution across aquatic systems

Contemporary research focuses on up-scaling EDC exposure to populations and food webs within aquatic environments. The spatial coverage of these assessments, however, is restricted when using individual systems to exemplify the wider conditions present across the landscape. An example of this is the focus on WwTWs and their downstream impacts across aquatic systems. A focus on wild populations and the effects of regulated effluent discharges (containing EDCs) has made significant contributions to establishing the effects of effluent discharges on aquatic organisms across aquatic environments. A focus on WwTWs discharges has, however, also led to limitations in our understanding of the spatial variation in EDC occurrence and their impacts within and between different types of aquatic systems. Up-scaling research strategies to landscape scales to understand these spatial variations is much needed to extend our knowledge of the effects of EDCs within natural systems. This will enable improved impact and risk assessment, with practitioners able to assess more accurately the degree to which potential concerns vary across the aquatic environment. Water-quality data regarding WwTWs discharges are available in many countries, consequently high-risk WwTWs can be targeted for regulation and remediation. A range of techniques are available to achieve this objective, including spatial and statistical modelling. Modelling at extremely broad scales has identified variations in emission of steroidal oestrogens between catchments, highlighting spatial variation in effects (Zhang *et al.* 2014). Furthermore, a significant role of mixing zones in determining the distribution of EDCs has been identified at high resolutions (~500 m) (Pagsuyoin, Lung & Colosi 2012). Assessments investigating intra-catchment variation, along aquatic continuums and among systems, however, are scarce. Understanding how EDC concentrations and subsequent exposure varies at this scale is extremely important for River Basin Management strategies currently employed by water managers.

2.7.2. Xenobiotic pollutant transfers across food webs

A detailed understanding of the transfer of EDCs across entire aquatic food webs is not yet available, with studies predominantly focusing on

bioaccumulation and biomagnification of EDCs within upper trophic levels (Berglund, Nyström & Larsson 2005). Assessments aiming to evaluate entire food webs are generally restricted to a small range of organisms representing several trophic levels. Controls on food-web organisation, such as environmental conditions, may significantly influence EDC bioaccumulation, biomagnification and effects, whilst a range of other biological factors also provide important regulatory impacts. The extent to which these factors enhance (or mitigate) the transfer of toxicants through food webs, however, remains relatively unknown. Moreover, although existing studies document relatively variable relationships between biological controls and bioaccumulation of different EDCs across aquatic food webs, explanations for such variability are absent. Future work is required to detail the specific pathways of accumulation and magnification throughout the lower trophic levels to understand the routes of dietary EDC exposure and biomagnification within higher trophic-level organisms. The first stage will be identifying the role of biotic and EDC-specific processes in controlling trophic transfers. Comprehensive biological-trait databases for aquatic organisms, such as Tachet *et al.* (2002), provide a valuable resource for such work.

2.7.3. Biomarkers for quantifying xenobiotic pollutant effects

Biomarkers, used to identify endocrine disruption within individuals, are well established for a small number of taxa (e.g. fish; Ankley *et al.* (2009)). Methods for other taxa have received less attention, and their utilisation and validation is relatively poorly developed (see Matozzo *et al.* 2008). A recent review identified a wide range of established and novel techniques for identifying endocrine disruption across environmental samples, yet there is an absence of suitable data for their validation (Kudłak *et al.* 2015). Furthermore, the relative accuracy of biomarker assessments is widely debated, with inconclusive results for some novel biomarker techniques. For example, the use of vitellogenin as a biomarker of endocrine disruption in an amphipod (*Gammarus fossarum*) proved inconclusive as vitellogenin expression was shown to vary with unexplained environmental conditions (Jubeaux *et al.* 2012). The unknown, potentially pleiotropic, function of the vitellogenin gene within male invertebrates also may limit the application of this biomarker in the

assessment of endocrine disruption (Jubeaux *et al.* 2012). Further development and validation of biomarkers specific to EDCs remains an important challenge (Kudłak *et al.* 2015). Relating the severity of endocrine disruption (*via* biomarker assessments) to analytical quantification of environmental EDC concentrations (e.g. *via* gas chromatography mass spectrometry) is essential for advancing our understanding of endocrine disruption in natural systems. Such comparisons will allow evaluation of the robustness of biomarkers in assessing ecological risk from EDCs and stimulate the refinement of *in vivo* methods. The currently restricted focus on a few chemicals and organisms limits the ability of practitioners to utilise biomarkers for ecological risk assessment and environmental decision-making (Hutchinson *et al.* 2006). Establishing a wider database of biomarkers for multiple species and EDCs is therefore an important future goal.

2.7.4. Applying genetics and modelling to broad-scale analysis

A significant concern surrounding EDCs is the potential for impacts on the genetic structure of populations and thus on the integrity of wild populations (Coe *et al.* 2010). Genetic assessments within natural systems, including DNA microsatellite and single nucleotide polymorphism (SNP) analysis, and other sequencing methods, provide the potential to assess whether EDCs affect population structure *via* genomic pathways (e.g. Harris *et al.* 2011). Olmstead *et al.* (2010) reported with EDC-induced sex reversal identifiable from genetic polymorphisms within the western clawed frog (*Xenopus tropicalis*). As well as allowing for broad-scale analyses, these techniques enable a reduction in the previously large number of samples required for field-based assessments to detect reproductive impacts and sex reversal at low EDC concentrations.

Up-scaling research into the effects of EDCs also requires improved models for populations and food webs. One major constraint in currently available population models is the absence of suitable parameterisation and validation data at the population level collected using field assessments (Rose *et al.* 1999; Raimondo *et al.* 2009). Future models must also aim at an improved representation of the biotic and abiotic controls present within natural systems (Borgå *et al.* 2004). Complexity, nonetheless, does not always facilitate accuracy, and highly site-specific, overly complex models may lack wider

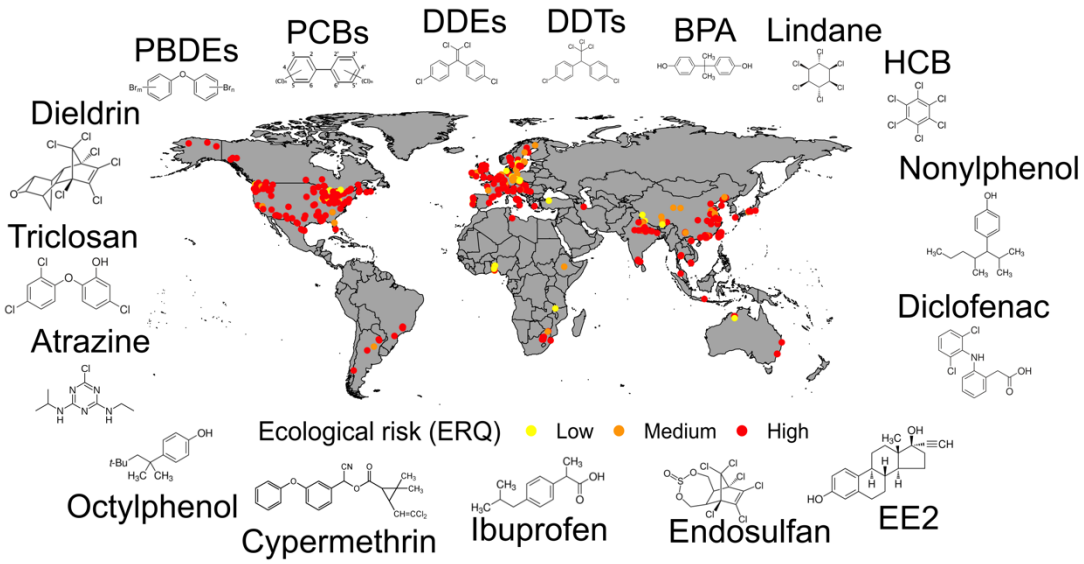
applicability (Miller *et al.* 2007). New model strategies, such as developed by Rose *et al.* (2003), provide the way forward for future models, with a nested structure allowing incorporation of a range of multi-scalar data, and in turn generating model simulations which replicate well the natural conditions found within ecological systems. Such work will enable an amalgamation of laboratory and field-based data, facilitating an understanding of causality and environmental relevance within future research.

2.8. Conclusions

The review highlights the benefits of applying previously derived mechanistic knowledge at broader spatial and temporal scales to assess the ecological impacts in natural systems. A range of abiotic and biotic characteristics, and processes can alter the effects and transfer of EDCs within aquatic food webs and cause deviations of observed effects from those identified in experimental assessments. Indirect effects also occur within natural systems, thus accurate assessment of endocrine disruption risk in aquatic ecosystems requires an appreciation of ecological processes at a range of spatial and temporal scales. Several limitations of experimental bioassay designs are highlighted by recent research. Consequently, the results of experimental bioassays should be interpreted with caution as such investigations often poorly represent the processes present in natural systems.

A complementary suite of assessments at a range of scales should be adopted within a multi-tier integrated research strategy to promote the development of environmentally relevant knowledge suitable for use by practitioners. Understanding the various direct and indirect impacts of EDCs, across a range of different spatial and temporal scales, should allow us to determine more effectively the transfer and ecological effects of EDCs within natural systems. Increasing the effectiveness of empirical and experimental research through methods such as integrated frameworks is therefore an important development. Future research should focus on expanding field-based research across a range of different aquatic environments. To achieve this objective, however, methodological and theoretical advances are required to provide data applicable to natural systems and develop more comprehensive methods of risk assessment for EDCs.

Chapter 3: A global assessment of the ecological risks from xenobiotic pollutant bioaccumulation in freshwater ecosystems



This chapter forms the basis for an article currently in preparation. The data collected, analysed and paper written by FMW, with revisions and edits provided by CRT and SJO.

3.1. Abstract

1. Bioaccumulative xenobiotic pollutants are considered a major threat to freshwater ecosystems, but spatial and temporal variation in the associated ecological risks are not well understood.

2. Presented here is an analysis of the spatial distribution of 26 xenobiotic pollutants, selected based on their persistence, bioaccumulative nature and toxicity, as reported in ecotoxicological databases and also identified in priority substance lists. This list included industrial chemicals (e.g. PCBs, PBDEs), pesticides (e.g. dieldrin, chlorpyrifos), pharmaceuticals (e.g. ibuprofen, diclofenac) and personal care products (e.g. triclosan). Concentrations of pollutants, measured in the tissues of freshwater organisms, were used to understand their potential ecological risks. The dataset consisted of 4603 observations from 192 peer-reviewed studies, for >1,000 sites across 45 countries.

3. The distribution of the chemicals studied varied spatially but data available were highly skewed, with most data from regions such as North America and comparably few observations for other regions such as Australasia. Across freshwaters globally, tissue burdens of studied chemicals were related to localised environmental concentrations, chemical lipophilicity and organism trophic level, as expected.

4. Using laboratory-derived toxicity thresholds, tissue concentrations indicated strong potential for individual- and population-level effects in 75.7% of observations from freshwaters. Chemicals identified as highest risk using experimental ecotoxicological data (e.g. atrazine, ß-estradiol, methoxychlor, gemfibrozil), however, were not exclusively associated with high ecological risk in field data. Instead, a range of legacy pollutants (PCBs, PBDEs and OCs) were responsible for a large proportion of observed ecological risk in global freshwater environments.

5. The synthesis herein, illustrates that the bioaccumulation of a series of selected chemicals of environmental concern presents a significant potential ecological risk across global freshwater ecosystems.

3.2. Introduction

Anthropogenic activities are a significant global threat to freshwater biodiversity (Strayer & Dudgeon 2010), with pressures on them arising through impaired water quality, habitat modification, fragmentation, invasive non-native species, pollution, and climate change (Vörösmarty *et al.* 2000; Dudgeon *et al.* 2006; Reid *et al.* 2018). Pollution is widely recognised as one of the greatest threats (Stendera *et al.* 2012). More than 30,000 chemicals are discharged regularly into freshwater systems via wastewater treatment, industrial effluent, surface run-off and leachates from buried wastes (Schwarzenbach *et al.* 2010), in many cases causing adverse ecological effects (Carpenter, Stanley & Vander Zanden 2011). Xenobiotic pollutants – those that would not occur naturally – of particular ecotoxicological importance in freshwater ecosystems, are those that are persistent, bioaccumulative and/or toxicologically potent (PBT) (Zhou, Cai & Zhu 2010; Malaj *et al.* 2014; US EPA 2018a). The extent of ecological risk posed by these pollutants will depend on a combination of their spatial and temporal distribution, concentration and mixture (Diamanti-Kandarakis *et al.* 2009; Ankley *et al.* 2009).

The effects of endocrine-disrupting xenobiotic chemicals tend to cascade up food chains and are more likely to cause effects across different levels of biological organisation (Chapter 2). As an example, exposure to steroidal estrogens has been shown to induce ecological effects at both population and community levels, with the loss of species, alterations in population dynamics and food web restructuring observed in response to field-scale exposure of lentic food webs (Kidd *et al.* 2007, 2014). Persistent organic pollutants (POPs), including PCBs and PBDEs, have also been shown to have food web level effects, with clearly identified associations between high tissue concentrations, and both reproductive and developmental impairment in some Arctic systems (Letcher *et al.* 2010). These food-web scale effects, in turn, maintain significant implications for the structure and function of entire ecosystems (Nienstedt *et al.* 2012). Furthermore, these effects may interact with other global scale drivers, for example climate change, eutrophication and habitat fragmentation, to generate significant reductions in biodiversity.

The ecological risks posed by xenobiotic pollutants is dictated clearly by their distribution and concentration within the environment. The relationships between pollutant concentrations (both environmental and tissue) and ecological risks are relatively well founded, yet linkages in the environment are not necessarily linear and are complex (DeForest, Brix & Adams 2007). For example, concentrations of bioaccumulative compounds may be below analytical limits of detection in soil and water, yet they can accumulate to significant concentrations in organisms (Chapter 4). For this reason, environmental monitoring schemes operated by national regulatory authorities (e.g. US Environmental Protection Agency, Environment Canada, Environment Agency, Natural Resources Wales, Scottish Environmental Protection Agency) are used in combination with laboratory data and standardised ecotoxicity assessments to appraise the ecological risks derived from environmental or tissue concentrations of xenobiotic substances (Van der Oost, Beyer & Vermeulen 2003).

The combination of measures and techniques, however, makes ecological risk assessments for xenobiotic pollutants challenging, particularly at scale and across contrasting regions of the planet. Several studies have assessed the global spatial distribution of selected compounds, or groups of chemicals (Stehle & Schulz 2015; Corrales *et al.* 2015), yet few have investigated the global distribution of ecological risk that may arise from the occurrence of multiple xenobiotic pollutants with the capacity to interact. Knowledge is also lacking on the varying interactions between physicochemical, environmental and biological factors that influence the bioavailability, bioaccumulation, tissue concentrations and ecological risks of xenobiotic chemicals across natural systems (Windsor, Ormerod & Tyler 2018).

Here a global, peer-reviewed dataset spanning 45 countries and in excess of 1,000 sample sites, is used to investigate spatial variability in the tissue concentrations of xenobiotic pollutants in freshwater organisms, and the potential ecological risks that result. A meta-analysis compares observed tissue concentrations of several classes of chemicals with ecological risks identified from extrapolating from toxicity tests. The analysis aims to generate an improved understanding relating to the ecological risk of bioaccumulative

xenobiotic pollutants across global freshwater ecosystems. The overarching objectives of the study were to:

1. Identify global hotspots of xenobiotic pollution bioaccumulation within freshwaters
2. Assess critically the potential ecological risk resulting from the bioaccumulation of selected xenobiotic pollutants
3. Highlight knowledge gaps affecting ecological risk assessment for xenobiotic pollution

3.3. Material and methods

3.3.1. Selection of high-risk xenobiotic pollutants

The diversity of xenobiotic pollutants practically precludes an assessment of all chemicals; thus, a subset of xenobiotic pollutants was selected for this global analysis of risk. Pollutants were selected for this analysis based upon their potential ecological risk to freshwater ecosystems, as appraised from experimental studies assessing their toxicity and toxicodynamic behaviour. A systematic approach was applied to rank chemicals based upon their toxicity to freshwater organisms, their octanol/water partitioning coefficient (log K_{OW}) values and their annual global production volume.

Data for the risk categorisation were collated from several secondary sources as follows. Toxicity data for xenobiotic pollutants were collated from the US EPA ECOTOX database (US EPA 2018b). These data were used to provide 'No Observed Effect Concentrations' (NOECs) for all recorded compounds, across a range of endpoints that related to potential population-level effects within natural systems (e.g. growth, survival and reproduction; see Appendix S1). Although previously criticised for not accurately representing dose-response relationships (Landis & Chapman 2011), NOEC measurements allowed for the greatest taxonomic and physicochemical coverage within the database. Toxicity data for compounds across freshwater taxa were incorporated into the final dataset to allow for an environmentally relevant assessment of toxicity. Only endpoints quantified (e.g. mean values in $\mu g\ L^{-1}$) were used within the final dataset (n = 47,755).

Table 3.1. Summary of the ecological risk (mean P_{ER}) of xenobiotic pollutants identified as 'high risk'.

Group	Chemical	log K_{ow}	HPV	NOEC (μg L^{-1})*					P_{ER}
				Algae (Population)	Mollusca (Mortality)	Crustacea (Growth)	Amphibia (Growth)	Fish (Growth)	
Pesticides	Endosulfan (END)	3.83	No	29.25	52.75	49.00	15.88	695.37	0.12
	Cypermethrin (CYP)	6.60	Yes	47556.36	-	0.03	-	0.39	0.09
	Deltamethrin (DEL)	6.20	Yes	256.51	-	0.02	-	0.60	0.10
	Chlorpyrifos (CLP)	4.96	Yes	291.07	240.27	0.78	148.11	27.67	0.05
	Esfenvalerate (EFV)	6.22	Yes	3.98	-	-	-	0.08	0.17
	∑ DDTs (DDT)	6.79	No	-	-	-	-	69.40	0.05
	∑ DDEs (DDE)	6.51	Yes	-	-	-	-	37.50	0.11
	Lindane (LI)	3.72	No	28706.15	-	76.34	-	635.50	0.10
	Dieldrin (DI)	5.40	No	-	-	-	8.63	0.55	0.10
	Hexachlorobenzene (HCB)	5.73	Yes	-	-	-	-	4.78	0.10
	Atrazine (AT)	2.61	Yes	941.29	3748.87	6733.33	473.78	2741.54	0.01
	Methoxychlor (MO)	5.08	Yes	-	-	-	-	2850.00	0.07
Industrial chemicals	Bisphenol a (BPA)	3.32	No	21952.38	370.00	1941.82	6000.00	602.81	0.13
	Nonylphenol (NP)	5.92	Yes	-	258.00	43.56	100.00	58.93	0.15
	Octylphenol (OP)	5.28	Yes	-	100.00	-	10.00	47.00	0.50
	∑ PCBs (PCB)	7.10	Yes	-	-	-	100.00	39.19	0.40
	∑ PBDEs (PBDE)	7.66	Yes	12.50	-	-	-	128.08	0.35

Group	Chemical	log K_{ow}	HPV	NOEC ($\mu g\ L^{-1}$)*					P_{ER}
				Algae (Population)	Mollusca (Mortality)	Crustacea (Growth)	Amphibia (Growth)	Fish (Growth)	
Pharmaceuticals and personal care products	Diclofenac (DIC)	4.51	Yes	63.00	-	0.36	-	4566.60	1.21
	Gemfibrozil (GEM)	4.77	Yes	-	-	-	-	1520.00	0.09
	Estrone (E1)	3.13	Yes	-	-	-	-	0.28	0.19
	Progesterone (PR)	3.87	Yes	-	-	100.00	-	-	0.88
	17α-Ethinylestradiol (EE2)	3.67	Yes	-	-	12.72	14.15	6.07	1.45
	β-Estradiol (E2)	4.01	Yes	35934.29	-	-	56.46	598.78	0.03
	Ibuprofen (IB)	3.97	Yes	10.00	4627.50	-	2000.00	724.97	1.48
	Testosterone (TES)	3.32	Yes	-	-	100.00	-	500.00	1.63
	Triclosan (TR)	4.76	Yes	98.33	-	-	230.00	71.19	0.10

* Data reported here provide examples of NOEC values for a subset of taxonomic groups and biological endpoints collated from ecotoxicological databases (Section 3.3.1).

Global annual production volumes for a variety of xenobiotic pollutants were not readily available, consequently, the OECD High Production Volume (HPV) listing (OECD 2004) was categorised into an ordinal variable to separate chemicals produced in large quantities across the globe (production of >1000 metric tons year^{-1} within at least one OECD member country) from those either no longer produced, or produced in lower quantities. The variable was used as a coefficient in the calculation on potential ecological risk; generating increased ecological risk for compounds produced in large quantities. Finally, the log K_{OW} values for pollutants were collated from the PHYSPROP© database (Syracuse Research Corporation 2013). The product of global compound production volumes and the natural logarithm of toxicity estimates (NOEC values) was divided by the log K_{OW} values for all combinations of compounds, taxa and biological endpoints to provide a composite metric indicating potential ecological risk, see Equation 1:

$$P_{ER} = \sum Pv(ln\ TR) / \log K_{OW} \quad (3.1.)$$

where P_{ER} is potential ecological risk, TR is the toxicity rating (NOEC [$\mu g\ L^{-1}$]), P_v is HPV classification (low production = 0.25, high production = 1) and log K_{OW} is the octanol-water partitioning coefficient. Lower values of P_{ER} are associated with increased ecological risk. Mean P_{ER} values were calculated for each combination of xenobiotic pollutant, species, effect and biological endpoint. Values below zero were removed as they indicated a low bioaccumulative potential. The minimum value for P_{ER} was then derived for each pollutant and endpoint. Compounds with minimum P_{ER} value in the lowest 10% of records were selected. This process generated a list of 113 compounds with physical properties, toxicity and production volumes that mean that these chemicals are likely to present an ecological risk to freshwater systems.

Of the chemicals on this list (Table 3.1), almost one third (31%) were EU priority substances (Directive 2000/60/EC, Directive 2009/90/EC and Decision 2013/480/EU), 18% were on the US Environmental Protection Agency priority pollutants list (Clean Water Act USC 1361), 25% on the Agency for Toxic Substances and Disease Registry (ATSDR) substance priorities, and 20% were on the OSPAR Commission substances of possible concern list. In total, 42% of compounds identified as high risk within this study are also identified

as of significant concern in the above reports. For the list of 113 pollutants identified as potentially high risk, measured concentrations within the tissues of freshwater organisms were available for 26 chemicals (Table 3.1). This list of potentially 'high-risk' compounds was subsequently used to assess the potential vulnerability and ecological risk to freshwater ecosystems posed by this suite of xenobiotic chemicals.

3.3.2. Bioaccumulation of selected 'high-risk' xenobiotic pollutants

A systematic analysis of the available published literature was conducted to identify datasets on the bioaccumulation and/or biomagnification of the selected 'high-risk' xenobiotic chemicals. Data were collected using a number of academic search engines (e.g. Scopus, ISI Web of Science and Google Scholar), to provide a comprehensive analysis of all available peer reviewed research between the period between 1990 to 2017 (to represent contemporary concentrations of pollutants and account for temporal heterogeneity in data collection) (Koricheva, Gurevitch & Mengersen 2013). The search terms used primarily consisted of the following nested query fields and operators: (bioaccumulation OR biomagnification OR bioconcentration OR concentration) AND (freshwater OR river OR stream OR lake OR pond OR wetland) AND ("*compound name*"), an exhaustive list of search terms used to collate data for each compound is included in Appendix S2. Additional sources of data were collated from the bibliographies of studies. A complete description of the systematic protocol is provided in Appendix S2.

Several criteria were applied to collate studies appropriate for this global distributional analysis and studies were selected for analysis, based on the following criteria: (1) they provided data on the bioaccumulation of xenobiotic pollutants in organisms inhabiting freshwater environments (e.g. rivers, lakes and ponds); (2) tissue concentrations of xenobiotic pollutants were reported in wet weight or lipid weight, with appropriate lipid % value for tissues if the latter was reported; and (3) the studies included replication in the study design.

The original field-based studies collected report xenobiotic pollutant bioaccumulation within different tissues and organs, including; muscle, liver and stomach and across a range of taxa, spanning several trophic levels; ranging from primary producers at the lowest trophic levels (e.g. algae and

macrophytes) to quaternary consumers (e.g. predatory fish and reptiles). Bioaccumulation values were standardised, by converting data to ng g^{-1} wet weight, to allow for suitable comparison (see Section 3.3.6) and risk analysis. All data were collected from the original paper and associated supplementary materials. The final database comprised of 192 studies with 4603 concentrations of xenobiotic pollutants within the tissues of freshwater organisms. Further information on the distribution and spatial coverage of the data is provided in Appendix S2 of the Supplementary Information.

3.3.3. Quality scoring criteria

Data were assigned a score relative to the methodological quality of the study from which they were derived. A quality scoring system was required to account for variability between studies as a result of their different methodologies, for example the limits of detection used for analysis. The scoring system was based upon the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) system (Moermond *et al.* 2016). The quality score values in this study were determined by the study design, and the data collection and processing methodologies applied (see Hobbs *et al.* 2005). Appendix S3 outlines the questions and relative score used to construct a quality score for each study, QS [(Quality score ÷ Total possible score) ÷ 100]. Briefly, the quality score gives a measure on the precision and accuracy of the study methodology, with higher scores indicating a greater reliability, relevance and validity/veracity of results. To be included within the following analysis studies were required to meet the minimum requirements – above 50% score for all criteria.

3.3.4. Ecological risk evaluation

The freshwater risk assessment for the selected xenobiotic pollutants was estimated from tissue and environmental (sediment and water) concentration data. The basis of the risk assessment was an ecological risk quotient (ER_Q); the ratio between measured environmental concentrations (MEC) and NOEC (both in $\mu g\ L^{-1}$) for biological endpoints (Appendix S1):

(3.2.) $$ER_Q = MEC / NOEC$$

NOEC values were derived from the toxicity dataset (see Section 3.3.1) and mean NOEC values for different classes of freshwater organisms (e.g. Actinopterygii, Gastropoda and Oligochaeta) were used to evaluate risk relating to the measured environmental and tissue concentrations from field data. Values for ER_Q were calculated for environmental (eER_Q) and tissue concentrations (tER_Q). The resulting ER_Q values were then categorised into a multinomial classification based upon Hernando *et al.* (2006), with ER_Q values of 0–0.1 indicating low risk, 0.1–1 medium risk and >1 high risk.

3.3.5. Independent predictor variables

The majority of explanatory/independent variables for models were derived from information provided within study methodologies (continent, aquatic habitat, chemical and source) or identifiable from data provided therein, for example the location of sample sites. Several other variables were derived from latitude and longitude data. For example, the climate zone for each sample location was defined based on the Köppen-Geiger classification (Peel, Finlayson & Mcmahon 2007). Data for these spatial variables were extracted using ArcGIS (version 10.4.1).

3.3.6. Data preparation, grouping and analysis

Data preparation and analysis was conducted using the 'R' statistical software package (version 3.2.2) (R Core Team 2015). Raw tissue concentration data collated from studies were first converted and standardised to ng g^{-1} wet weight (ng g^{-1} ww) using the "OrgMassSpecR" package (Dodder 2014). Wet weight standardisation was completed to provide a better understanding of potential risk to the given organism, as lipid corrections can bias model results and affect interpretations regarding environmental health (Schisterman *et al.* 2005; Gaskins & Schisterman 2009). Standardised tissue concentration of the study chemicals data ranged between 0.01 to 28,226.21 (ng g^{-1} ww) and environmental concentration data exhibited a range of between 0.001 and 4,000 (water = ng ml^{-1}; and sediment = ng g^{-1}). Data were initially analysed, using a series of exploratory steps, to prevent statistical errors using the framework in Zuur *et al.* (2010).

Due to the non-linear distribution of both environmental and tissue concentration data, natural logarithmic transformations (log^e) were used to normalise data. Generalised Linear Mixed Models (GLMMs) were then applied to understand the dominant spatial, chemical and biological influences on pollutant concentrations. Model structures used for analysis are provided in Table 3.2. Multicollinear or nested variables (VIF > 10) (Dormann *et al.* 2013), were removed and replaced by representative variables at appropriate ecological scales. From broad model structures, model selection was completed using both shrinkage and backward selection methods (Marra & Wood 2011). Further to this, model validation procedures, following Zuur *et al.* (2007) and Thomas *et al.* (2015), were used to assess model validity and accuracy. Residual normality was assessed using QQ plots, homogeneity of variance was determined by plotting residuals against fitted values, and influential observations were investigated using Cook's leverage distances.

Table 3.2. Model structures for GLMM analyses of high-risk xenobiotic pollution concentrations in freshwater ecosystems.

Dependent variable	Model, family and link function	Independent variables
log(Environmental concentration)	GLMM Gaussian Identity **(QS, Date)**	Continent Climate zone Aquatic habitat Chemical Source
log(Tissue concentration)	GLMM Gaussian Identity **(QS, Date)**	log(Environmental concentration) Continent Aquatic habitat Chemical Source Trophic level
Ecological risk	GLMM Binomial Probit **(QS, Date)**	Continent Climate zone Aquatic habitat Source

Explanatory variables within the table are utilised within the final model, post-model refinement. **(Bold)** indicates random effects.

3.4. Results

3.4.1. Spatial distribution of available chemical data

Most data on chemicals in the environment and in biota came from Asia (n = 1,676), Europe (n = 1,264), and North America (n = 1,104), and were more limited for Africa (n = 325), South America (n = 147) and Australasia (n = 87; Fig. 3.1). The majority of records were derived from within temperate climates (n = 3,438), with significantly fewer in equatorial (n = 122), polar (n = 143), Mediterranean (n = 56), tropical (n = 243) and arid climatic systems (n = 247). Local variation within countries and continents was also apparent, for example there were a greater number of samples collected across several study catchments (e.g. St. Lawrence catchment, Canada).

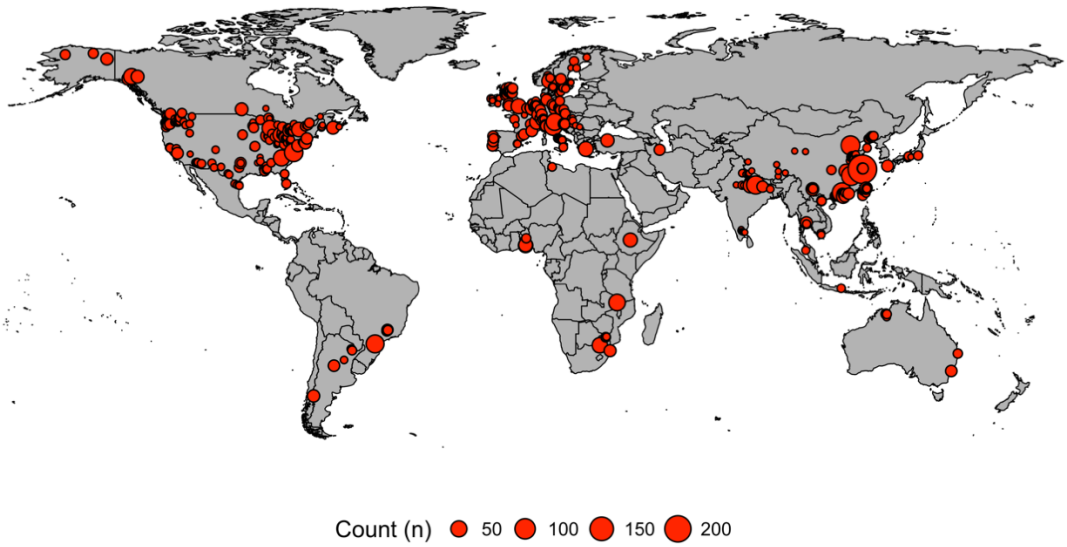


Fig. 3.1. Global distribution of tissue concentration data (n = 4603) summarised across 1° grid squares. The density and abundance of observations were far greater in Europe, North America and Asia than other broad geographic regions (see main text).

Omnivorous vertebrate taxa dominated the biological data, primarily fish species (73.3%). Mammals, birds, algae and invertebrates were all under-represented (2.7%, 5.5%, 3.1% and 14.1% of records, respectively). Observations were more evenly distributed between chemical groups, yet several industrial (e.g. PCBs, PBDEs) and agricultural compounds (e.g. DDE,

LI, DDT, HCB) more prevalent across freshwater ecosystems (see Appendix S2 for full listing). This distribution of observations (unbalanced) between groups was accounted for in generalised linear modelling frameworks.

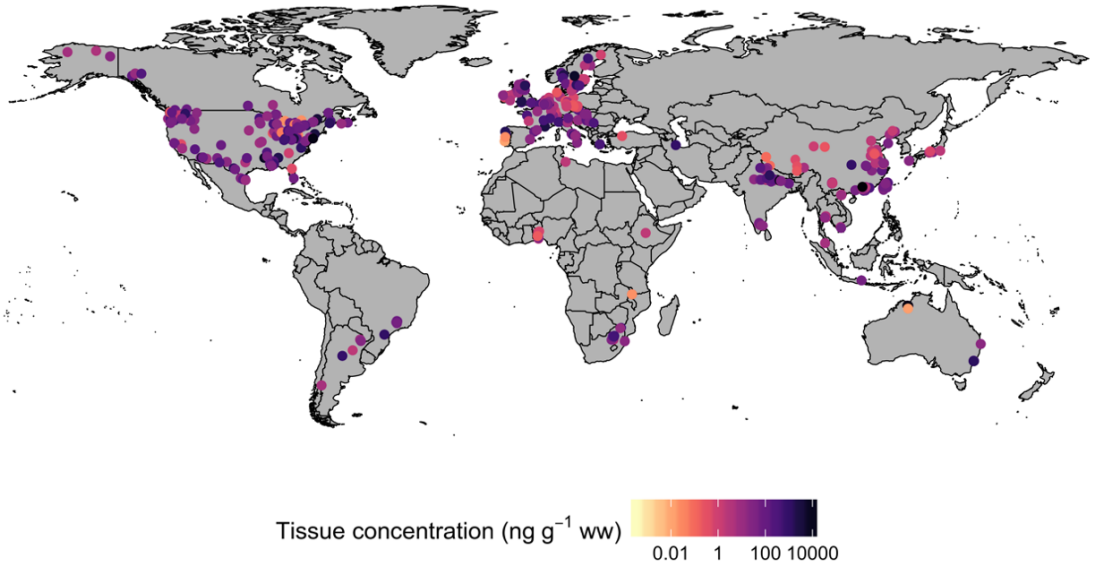


Fig. 3.2. The global distribution of xenobiotic pollutant concentrations in the tissues of freshwater organisms (n = 4,603). Concentrations are reported on a log10 scale. Tissue concentrations were variable across the globe, at a range of spatial scales, including across continents and between different freshwater habitats ($R^2c = 0.64$, $F_{44,508} = 834.13$, $p < 0.001$).

The QS for samples did not explain a large amount of variation in the tissue concentrations of xenobiotic pollutants across samples, yet, the model explained significant variation ($R^2c = 0.01$, $F_{1,4601} = 59.51$, $p < 0.001$). The QS was subsequently included as a random factor within mixed effect analyses to account for any residual effects of study and methodological quality.

3.4.2. Xenobiotic pollutant concentrations in freshwater systems

Environmental concentrations of all xenobiotic pollutants were highly variable across freshwater environments and explained by a series of spatial variables ($R^2c = 0.85$, $F_{33,394} = 1059.50$, $p < 0.001$). At broad spatial scales, continental variation in the environmental concentration of pollutants was apparent, with Asia, Europe and North America exhibiting greater levels of pollution within

freshwater ecosystems compared to other regions of the globe ($F_{4,1342} = 33.12$, $p < 0.001$). At lower spatial scales, different freshwater habitat types (ponds, rivers, lakes, wetlands) were associated with different concentrations of xenobiotic pollutants in sediment and water ($F_{3,1482} = 6.19$, $p = 0.004$), with wetlands and ponds exhibiting the highest concentrations. This variation between different freshwater habitats was, in part, related to the greater contributions from pollution sources. For example, freshwater systems contaminated by WwTW discharges and urban runoff were associated with greater environmental concentrations of pollutants ($F_{2,1471} = 4.17$, $p = 0.04$), due to high concentrations of domestic and industrial chemicals within the dataset (e.g. PCBs, PBDEs, EE2). In spite of spatial variation, the environmental concentrations of each chemical were different ($F_{21,1394} = 32.42$, $p < 0.001$), and some chemicals exhibiting consistently high concentrations (e.g. PCBs, PBDEs and organochlorine pesticides).

Concentrations of xenobiotic pollutants in the tissues of organisms were related to their environmental concentration ($F_{1,1400} = 156.64$, $p < 0.001$) and explained by a series of spatial, physicochemical and biological variables ($R^2c = 0.64$, $F_{44,508} = 834.13$, $p < 0.001$; Fig. 3.3). Examples of spatial variation includes, differences in concentrations of pollutants between continents ($F_{4,508} = 15.93$, $p < 0.001$; Asia > South America > Europe = North America = Australasia) and between the sources of pollution present within freshwater environments ($F_{1,1474} = 33.73$, $p < 0.001$; Urban = WwTW > Rural).

Field and experimental log BAF values calculated from available field and experimental data (Appendix S4) were significantly different ($W = 153.00$, $p < 0.001$; Paired Wilcoxon signed rank test), with field log BAF values in general lower than those derived from experimental manipulations (Table 3.3). For example, although the maximum field log BAF values for PBDEs were 2.40 higher, on average these values were lower than those calculated from experimental assessments. Other chemicals with maximum field log BAF values greater than those derived from experiments are PCBs, HCB, endosulfan, chlorpyrifos and β-estradiol (Table 3.3). Actual differences between field and experimental log BAF values were relatively small and large variation was observed in both field and laboratory data. Thus, although

several chemicals maintained statistically different experimental and field BAFs, biologically meaningful differences appeared to be small.

Table 3.3. Mean values for risk quotients (and summary statistics) and log BAFs for bioaccumulative xenobiotic pollutants.

	eER_Q	tER_Q	% high-risk *		log BAF (min, max)	
			eER_Q	tER_Q	Field	Lab*
AT	0.002	207.44	0.00	100.00	5.86 (4.97, 6.88)	-
BPA	0.019	12.28	0.00	61.54	-1.63 (-9.15, 2.85)	
CLP	0.001	25.66	0.00	75.56	1.89 (-1.49, 4.38)	1.35 (0.27, 1.76)
CYP	-	18.03	-	42.85	-	-
DDE	0.001	118.60	0.00	76.69	2.79 (-7.44, 11.17)	6.02 (4.48, 7.05)
DDT	0.001	8.17	0.00	38.23	1.99 (-8.48, 8.84)	4.47 (3.45, 4.96)
DEL	-	0.90	-	33.34	-	-
DI	0.001	24.47	0.00	56.39	1.87 (-6.15, 10.31)	3.96 (3.95, 3.98)
DIC	0.029	8.28	0.00	87.22	0.11 (-6.00, 3.24)	2.37 (0.69, 3.43)
E1	0.021	24.56	0.00	95.74	-0.49 (-3.56,1.89)	2.35 (2.22, 2.44)
E2	0.011	22.87	0.00	84.44	0.59 (-2.52, 3.45)	0.49 (0.18, 0.81)
EE2	0.013	17.45	0.00	79.69	0.88 (-2.51, 9.75)	-
EFV	0.001	12.10	0.00	100.00	7.61 (6.83, 8.38)	-
END	0.001	6.15	0.00	42.61	2.20 (-1.87, 5.37)	2.39 (1.47, 4.22)
GEM	0.022	27.78	0.00	72.73	-0.41 (-3.69,5.29)	2.33 (1.96, 2.70)
HCB	0.001	12.00	0.00	45.91	3.61 (-0.11, 6.98)	4.42 (3.08, 5.74)
IB	0.025	15.69	0.99	94.12	-0.07 (-3.87, 7.53)	-0.15 (-1.09, 0.16)
LI	0.001	11.76	0.00	29.62	0.34 (-2.34, 7.17)	2.61 (1.40, 4.04)
MO	0.023	19.13	0.00	44.07	-2.60 (-9.74, 0.84)	3.12 (0.21, 4.08)
NP	0.131	183.86	13.14	94.37	1.30 (-6.21, 7.27)	1.47 (-0.09, 3.53)
OP	0.001	22.35	0.00	52.74	2.44 (-5.01, 9.54)	-
PBDE	0.013	71.31	0.00	83.51	2.21 (-4.71, 7.70)	4.10 (2.90, 5.30)
PCB	0.021	224.42	0.00	93.32	2.31 (-3.08, 9.01)	5.21 (4.69, 5.87)
PR	0.238	463.61	0.00	100.00	-	-
TES	-	2.42	-	62.96	-	-
TR	0.007	15.11	0.00	80.00	3.68 (0.51, 7.63)	-

* Percentage of records categorised as high ecological risk (see Section 3.3.4).

3.4.3. Ecological risk from xenobiotic pollution

The ecological risk (ER_Q) posed by the suite of bioaccumulative xenobiotic pollutants was identified to be high for several regions across the globe. There were, nevertheless, significant variations in ER_Q values for tissue concentrations among geographical areas (R^2c = 0.36, $F_{12,4591}$ = 293.00,

$p < 0.001$; Fig. 3.3). The ecological risk was different between continents ($F_{5,4598} = 60.77$, $p < 0.001$: North America = South America > Europe = Asia = Australasia = Africa), pollution sources ($F_{3,4600} = 65.41$, $p < 0.001$: WwTW > Urban > Rural) and aquatic habitat types ($F_{2,4601} = 153.48$, $p < 0.001$: Pond = Wetland > Lake > River) (see Appendix S5). Thus, organisms within standing freshwater habitats in urban areas of North America appear at greatest risk.

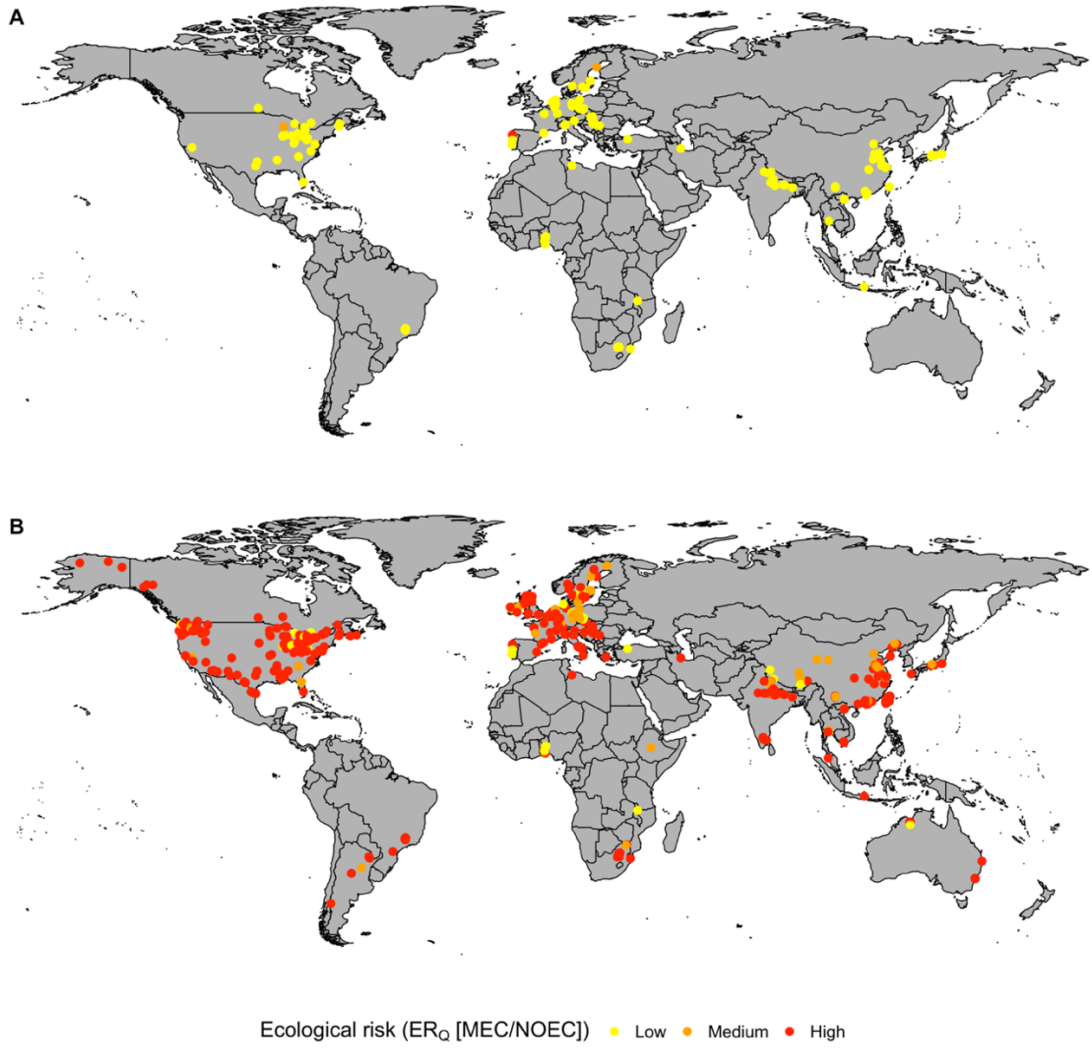


Fig. 3.3. Global ecological risk (ER_Q) associated with xenobiotic pollutant concentrations within the tissues of freshwater organisms. (A) eER_Q values calculated from environmental (sediment and water) concentrations (n = 1,552). **(B)** tER_Q values calculated from the concentrations of pollutants in the tissues of freshwater organisms (n = 4,603). Although risks were widespread at broad scales (catchment, region and continent), the risk at specific locations was highly variable ($R^2c = 0.36$, $F_{12,4591} = 293.00$, $p < 0.001$).

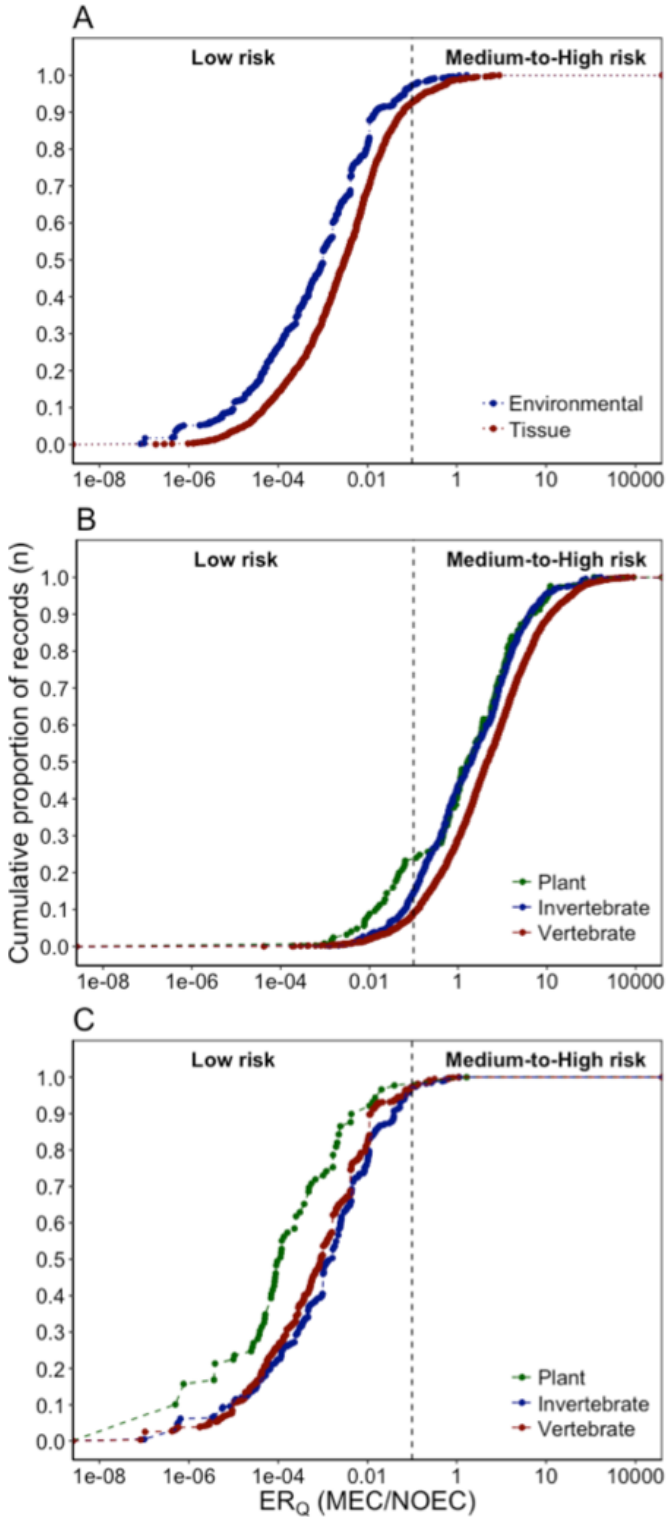


Fig. 3.4. Distribution of ecological risk (ER_Q) from data on global xenobiotic pollutant concentrations. Dashed lines indicate a threshold for ER_Q over which medium-high ecological risks are predicted. **(A)** ER_Q values calculated from tissue and environmental concentrations (n = 4603 and n = 1522, respectively) are compared. **(B)** tER_Q values for different taxa (n = 4603). **(C)** eER_Q values for different taxa (n = 1522).

There was large variability in the ecological risk, with several xenobiotic pollutants posing a greater risk to freshwater ecosystems than others (Table 3.3). EE2, PCBs and PBDEs had particularly high tER_Q values and were responsible for the ubiquitous, high ecological risk identified across global freshwater ecosystems for these specific chemicals (483.26 ± 262.35, 397.09 ± 93.15 and 87.74 ± 14.42, respectively). Other compounds were observed posing relatively low potential ecological risks, including Lindane and Chlorpyrifos (0.22 ± 0.05 and 0.04 ± 0.02, respectively).

Differences in the levels of ecological risk were also observed between the broad taxonomic groups encapsulated within the dataset; plants, invertebrates and vertebrates (X^2 = 29.97, df = 2, $p < 0.001$; Kruskal-Wallis rank sum test; Fig. 3.4). Plants appeared at lowest risk, with a greater cumulative proportion of recorded concentrations falling into the low risk category for eER_Q and tER_Q values. The ecological risk posed to vertebrates and invertebrates remains relatively similar yet based on tissue concentrations a greater cumulative proportion of records for vertebrates fall within the high-risk category (Fig. 3.4), with the inverse true for environmental ER_Q values, where invertebrates have a greater proportion of recorded concentrations falling into the high-risk category.

3.5. Discussion

High concentrations of several xenobiotic pollutants occur in the tissues of freshwater organisms across the globe. This systematic review and meta-analysis identified spatial hotspots of pollution, with lentic freshwater ecosystems appearing at highest risk, particularly across regions of Europe, North America and Asia. Bioaccumulation factors calculated under laboratory conditions were statistically different to those observed within field studies, yet the actual differences between the values were relatively small (less than an order of magnitude). Ecological risk values calculated from concentrations of xenobiotic pollutants in sediments and water (eER_Q) were relatively low across the globe, indicating restricted potential for ecological risk. However, values for ecological risk calculated from tissue concentrations (tER_Q) frequently exceeded the “high-risk” threshold as defined by ER_Q values. The combination of tissue concentration data, tER_Q values, field BAFs and the observed

relationship between environmental and tissue concentrations, thus indicates potential for effects at multiple levels of biological organisation.

The results of this study are based on several fundamental assumptions. Firstly, it is assumed that the data collated are representative of wider trends across regions and habitats without representation. Localised patterns in tissue concentrations and ecological risk, nevertheless, are not presumed to be representative of un-sampled categories or locations. Secondly, understanding ecological risk from tissue concentration data is uncertain, and links between bioaccumulation and the effects of xenobiotic pollutants are infrequent across the literature (Escher & Hermens 2004; Escher *et al.* 2011). Risk assessments based on tissue concentrations presented herein, thus assume that both internal and external exposure, to a given concentration of pollutant, result in similar ecological effects. This poorly tested assumption highlights the need for better mechanistic understanding of internal exposures and presents a significant opportunity for future research.

High profile, hydrophobic compounds, including DDT, DDE and PCBs, dominated the database, as a result of the perceived risk for this group (Colborn, vom Saal & Soto 1993; Vos *et al.* 2000). Although bioaccumulation was markedly higher for the hydrophobic xenobiotic compounds, other chemicals may pose a significant threat to freshwater organisms also, for example EE2 (Al-Ansari *et al.* 2010). The skewed distribution of data, with a large proportion of data pertaining to a handful of chemicals and a small number of species, may influence understanding of risk within freshwaters and potentially affect assessment of ecological effects. The dominance of specific phyla (e.g. Chordata = 90.5%) indicates the overreliance of current studies on specific taxa. Within Chordata, records were dominated by several omnivorous fish species, also causing a bias towards individuals occupying the mid-trophic levels of freshwater food webs. These biases within bioaccumulation studies poses problems for a broader understanding of the ecological risk of xenobiotic pollutants, as our knowledge regarding the transfer and effects across lower trophic levels remains poor. The risk associated with bioaccumulative xenobiotic pollutants at the food web and ecosystem scale is therefore poorly understood and potentially underestimated.

The global analysis provided here enables an improved understanding of the factors influencing both tissue concentration and ecological risk resulting from exposure to xenobiotic pollutants across natural systems. Factors associated with variation in tissue concentrations were classified into three broad categories; spatial, physicochemical and biological. The relationships identified across global data conform to previous research, with common relationships (e.g. trophic magnification of hydrophobic compounds) observed, even across large spatial scales and despite significant variation in other influential factors. In particular, trophic level and habitat were significant influences on the concentrations of xenobiotic pollutants in the tissues of freshwater biota. These factors are nested and produce the heterogeneous distribution of tissue concentrations observed across the globe.

The discrepancies in both bioaccumulation and the associated ecological risk observed between field and experimental data indicate that ecological processes in natural systems are responsible for generating differences in bioavailability, exposure and associated ecological risk. This is particularly important for biomonitoring schemes which either; use environmental concentrations to estimate risk or derive tissue concentration data and ecological risk from concentrations measured within water and sediments. Data here highlight the fact that contemporary risk identification and prioritisation based on methods that infer ecological risk from environmental concentrations (sediment and water), even when used in conjunction with innate toxicity and safety factors, may not be suitable for all chemicals. This is principally the case for chemicals which accumulate and magnify within the tissues of organisms, especially when these chemicals are also present in low concentrations across abiotic compartments (e.g. sediment and water) of freshwater environments.

Ecological risk quotients calculated from environmental and tissue concentration data for persistent pollutants across the globe indicates the widespread ecological threat posed by these chemicals. The risk presented by persistent and bioaccumulative pollutants is generated through both internal and external exposure (Escher & Hermens 2002). tER_Q values, calculated from tissue concentrations (internal exposure), suggested higher risk of

xenobiotic pollution exposure in freshwater organisms, compared to eER_Q values calculated from concentrations measured in sediment and water samples (external exposure). The disparity between risk quotients calculated from these measures highlights a significant knowledge gap in our understanding of the contemporary risks posed by persistent xenobiotic pollutants in aquatic systems. Although the risk from internal exposure is likely overestimated using NOECs, which are developed for external exposure thresholds. This highlights the importance of considering multiple exposure pathways when assessing the risk of chemicals that may be in low environmental concentrations.

Freshwater organisms are differentially at risk from bioaccumulative xenobiotic pollutants. Environmental exposures generated lowest ecological risk for plants, followed by vertebrates, with NOEC values for invertebrates most frequently exceeded across the globe. Internal exposures, however, indicated that vertebrates were at greatest risk from internal exposure, followed by invertebrates and plants. The differential risk profiles between internal and external exposures are likely due to the higher trophic position of vertebrates in freshwater systems and subsequent magnification of bioaccumulative xenobiotic pollutants. In spite of differences in taxonomic variation between tER_Q and eER_Q, the ecological risk provided by bioaccumulative xenobiotic pollutants, through both internal and external exposure pathways, appears significant across the globe.

The analyses conducted here using a global dataset on the presence of chemicals within freshwater organisms illustrates several knowledge gaps and potential issues with contemporary monitoring and regulatory frameworks in chemical risk assessment. Firstly, although a significant log-linear relationship was identified between the environmental and tissue concentrations of several xenobiotic pollutants, the high variability in the overall relationship for all pollutants, means that estimating potential ecological effects based on environmental concentrations is challenged by variable relationships for individual chemicals, as well as relatively poor prediction accuracy. These estimation methods, however, are frequently used as a tool for understanding potential ecological risk and bioaccumulation within national monitoring

programmes (e.g. EA 2007). Secondly, the data analysed here have demonstrated significant sub-continental variation in the prevalence of xenobiotic pollutants, meaning that priority substances should perhaps be identified at finer geographical scales, e.g. nationally. Thus, national regulation of xenobiotic pollutants through continental priority substance lists (e.g. European Union list of priority endocrine disruptors: Vorkamp *et al.* 2014) may not be appropriate. Finally, the significant variation in spatial, biological and physicochemical factors influencing observed tissue concentrations indicates that caution is required when extrapolating results of bioaccumulation and risk assessments from site-specific studies. Modelling studies have previously highlighted this fact (e.g. Miller *et al.* 2007), and the data here reinforces the need for careful interpretation of site-specific data.

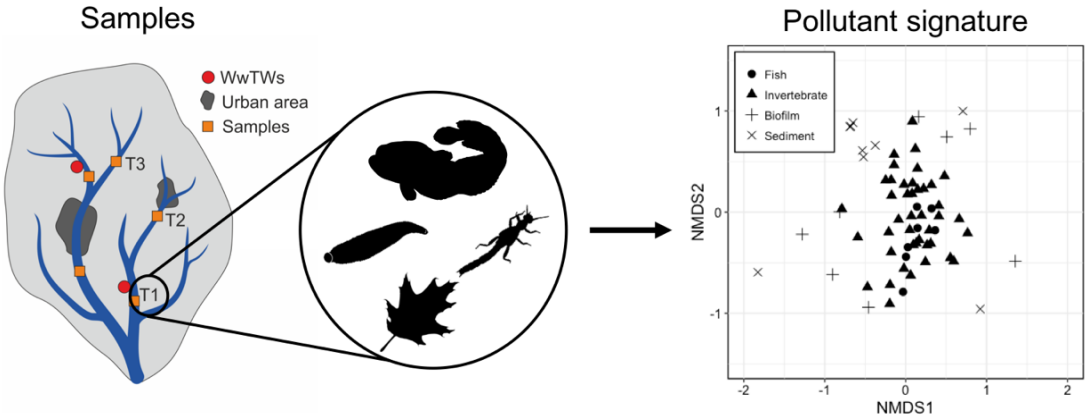
The heterogeneous spatial and biological distribution of data observed in the study requires attention. Research assessing the bioaccumulation of xenobiotic pollutants is necessary within lower trophic levels of freshwater food webs to understand the mechanisms and pathways of bioaccumulation and biomagnification. More emphasis needs to be placed on expanding research to regions of the globe where assessments are currently scarce, such as Africa and South America. These areas, as well as others poorly represented by data, are locations where water resources are most at threat from increasing population density and associated municipal, industrial and agricultural pressures. Developing a broader knowledge of xenobiotic pollutants across different landscapes will not only provide suitable ecological risk assessments and bases for management, but also help to inform our understanding of interactions between environmental conditions, multiple stressors and xenobiotic pollutants within freshwater ecosystems.

3.6. Conclusions

Global analyses of bioaccumulation in freshwater organisms are infrequent within the literature. This assessment demonstrates the severity of potential ecological risks derived from long-term exposure to xenobiotic pollutants. The suite of bioaccumulative compounds analysed herein provides an indication of the total risk presented within these systems. In this case, ecological risk was calculated from exposure to individual compounds, nevertheless, cumulative

and synergistic effects are likely generated from simultaneous exposure to multiple xenobiotic pollutants. The ecological risk posed by bioaccumulative xenobiotic pollutants therefore appears widespread across the globe.

Chapter 4: Biomonitoring and spatial variation of persistent organic pollutants across river ecosystems in South Wales



This chapter provides the data and analysis contributing to an article in revision at *Environmental Pollution*. FMW collected, extracted and analysed (GC-MS) the samples under supervision by Glória Pereira (MGP), analysed data and wrote the manuscript. MGP, CRT and SJO provided comments and revisions on subsequent iterations.

4.1. Abstract

1. Persistent organic pollutants (POPs) continue to threaten aquatic organisms, but risk assessments are restricted by poor knowledge of POP distribution and quantity in biota.
2. Here, variation in PBDEs, PCBs and OCs across benthic sediments, biofilms, macroinvertebrates and fish across river systems in South Wales (UK) is investigated.
3. Persistent PCB (118, 153 and 180) and PBDE (47, 99 and 100) congeners, and OCs (*p,p'*-dichlorodiphenyldichloroethylene: *p,p'*-DDE; and dieldrin: HEOD), dominated the POPs detected, indicating links to historical emissions. Low concentrations of less persistent PBDEs, PCBs and OCs, however, suggest more contemporary sources.
4. Concentrations of POPs were 2 to 22-fold greater in fish than invertebrates, but their detection frequency and concentration were greater in these organisms (>90%; 0–304.2 ng g^{-1} wet weight) than in sediments or biofilms (<10%; 0–11.8 ng g^{-1} wet weight). Invertebrates and fish also revealed the presence of several PCB congeners (28, 52, 77 and 105) and *p,p'*-dichlorodiphenyltrichloroethane (*p,p'*-DDT) that were not detected in the environment. Concentrations of PBDEs, PCBs and OCs differed among invertebrate taxa and feeding guilds.
5. After controlling for variation among sample types and taxa, PBDEs increased with urban land cover, while PCBs increased with urban land cover and wastewater discharge.
6. These data illustrate how POP body burdens from invertebrates and fish provide valuable information on the spatial variation and likely sources of persistent pollutants in freshwater ecosystems. More work is required to resolve differences in POP contamination between taxonomic groups.

4.2. Introduction

A range of xenobiotic or anthropogenic chemicals from both legacy and contemporaneous sources occur in most aquatic environments (Sumpter 2009; Gavrilescu *et al.* 2015; Sun *et al.* 2015). Such pollutants alone or in mixtures can have harmful effects on aquatic organisms (Wasi, Tabrez & Ahmad 2013; Malaj *et al.* 2014; Arnold *et al.* 2014), in some cases altering species communities or food webs (Chapter 2). While recent studies in freshwater systems have focussed more on emerging pollutants, the 'legacy' or persistent organic pollutants (POPs) continue to present an ecological risk to organisms because of their toxicity and persistence (Rasmussen *et al.* 2015; McKnight *et al.* 2015).

Previous modelling studies have linked the distribution and quantity of POPs in river systems to activities and land uses historically associated with pollutant sources (Nizzetto *et al.* 2010). In reality, however, the distribution of POPs in river catchments is more complicated, and includes remobilisation from contaminated soils or sediments (Zoumis *et al.* 2001) as well as release from landfill or discarded equipment (Diamond *et al.* 2010; Iqbal *et al.* 2017). POPs can also be emitted from wastewater treatment works (WwTWs) when historical wastes are discharged inadvertently or illegally via sewers (Jones, Gardner & Ellor 2014). In general, however, contemporary and legacy sources of POPs are poorly understood (Lohmann *et al.* 2007) and there is a need for field-based assessments to validate models, appraise sources, and determine the quantity, composition and transfer of POPs in environmental circulation.

Assessments of the dynamics, sources and distribution of POPs have tended to focus on measurements in water or sediment samples, but concentrations here are low or non-detectable (Schwarzenbach *et al.* 2006; Loos *et al.* 2009). In contrast, the hydrophobic nature of many POPs means that they accumulate in organic matter, lipid-rich sediments or biota (Geyer *et al.* 2000). For this reason, aquatic organisms offer potential advantages in detecting the concentration and composition of POPs through space and time (Van der Oost, Beyer & Vermeulen 2003; Schäfer *et al.* 2015). At one extreme, pollutant concentrations in organisms with small home ranges can reveal specific sources of pollution, while those with larger territories or those abundant

enough to be sampled at multiple locations can integrate pollution signals across whole regions (Ormerod, Tyler & Jüttner 2000; Van der Oost, Beyer & Vermeulen 2003; Morrissey, Elliott & Ormerod 2010).

Although the benefits of sampling POPs in organisms have long been recognised, particularly in apex predators (see Crosse *et al.* 2012), some taxonomic groups have been largely overlooked. Community composition in freshwater organisms such as aquatic invertebrates, for example, is used widely to indicate physicochemical conditions or abiotic stressors on ecosystems, but the prevalence and concentrations of legacy pollution in these taxa is poorly known (Bonada *et al.* 2006; Buss *et al.* 2015). Moreover, invertebrates have a range of contrasting ecological functions, traits and trophic levels in food webs that could reveal different exposure pathways, transformations and cascading effects for xenobiotic substances (Chapter 2). Improved information on the interactions between biota, xenobiotic contaminants and accumulation of pollutants could provide fuller understanding of such processes in natural systems, while also augmenting the general indicator of freshwater organisms.

In this paper, variations among PCBs, PBDEs and a suite of OCs across compartments of river systems in South Wales are investigated by measuring the concentration of POPs in environmental (sediments and biofilms) and biological samples from different invertebrate taxa and a benthic fish. The study compares contrasting sample types, assessing their value for monitoring persistent contaminants in the environment, whilst also assessing the distribution, quantity and potential sources of POPs across a samples of river systems. Specific hypotheses were that:

1. POP composition and concentrations differ between sample media commonly used in monitoring (sediments, invertebrates and fish)
2. Variation in POP concentrations in macroinvertebrates are positively related to concentrations in other samples (sediments, biofilm and fish)
3. Variations in POP concentrations and composition among different invertebrate taxa are similar across river systems
4. Environmental and biological samples reveal local and regional sources of POP contamination

4.3. Material and Methods

4.3.1. Sample sites and source identification

The study was carried out across nine river reaches (Fig. 4.1) distributed evenly across three catchments in South Wales (Taff, Usk and Wye). Land use varied (Table 4.1) such that the Taff catchment has a large percentage of urban land, as well as a legacy of large-scale industrial activity associated with coal mining, coal gasification and metal smelting (Learner *et al.* 1971). In comparison, both the Usk and Wye are dominantly agricultural catchments, with land uses ranging over arable farming, horticulture, fertilised grassland and rough pasture (HMSO 1978).

As well as land use, sample sites varied in stream discharge, physicochemical conditions (e.g. conductivity, pH, total dissolved solids) and consented effluent emission from WwTWs (Table 4.1). The combination of land use and industrial history, with local variations in effluent contributions and other point sources, covers a range of potential point and diffuse POP sources across sites and between catchments (Table 4.1; Appendix S6).

To reveal potential point and diffuse sources of POPs across catchments, land use was determined using ESRI ArcMap (version 10.2). The contributing upstream land cover (urban, agricultural and improved grassland) was determined for each sample site using JNCC phase 1 habitat classification data (JNNC 2010), in conjunction with the Spatial Tools for the Analysis of River Systems (STARS) and Spatial Stream Network (SSN) tools (Ver Hoef *et al.* 2014). Additionally, specific information on point sources of pollution, such as WwTW discharges, were collated from Natural Resources Wales (NRW) routine monitoring. At sampling sites downstream of WwTW discharges, the ratio of wastewater effluent to river discharge (both in $m^3\ s^{-1}$) was calculated.

For an additional description of site attributes, data available from routine monitoring by Natural Resources Wales (NRW) and the Environment Agency (EA) collected during the period 2010–2015 were used (Table 4.1). From these data, two macroinvertebrate indices were derived to assess *in situ* benthic environments: (1) the British Monitoring Working Party (BMWP), a monitoring metric scoring taxa based on invertebrate tolerance to pollution (high scores

indicate the presence of pollution sensitive taxa); and (2) Average Score Per Taxon (ASPT), which is the average BMWP score across the taxa sampled and accounts for sampling bias induced by differences in the number of invertebrate families sampled (Armitage *et al.* 1983; Hawkes 1998).

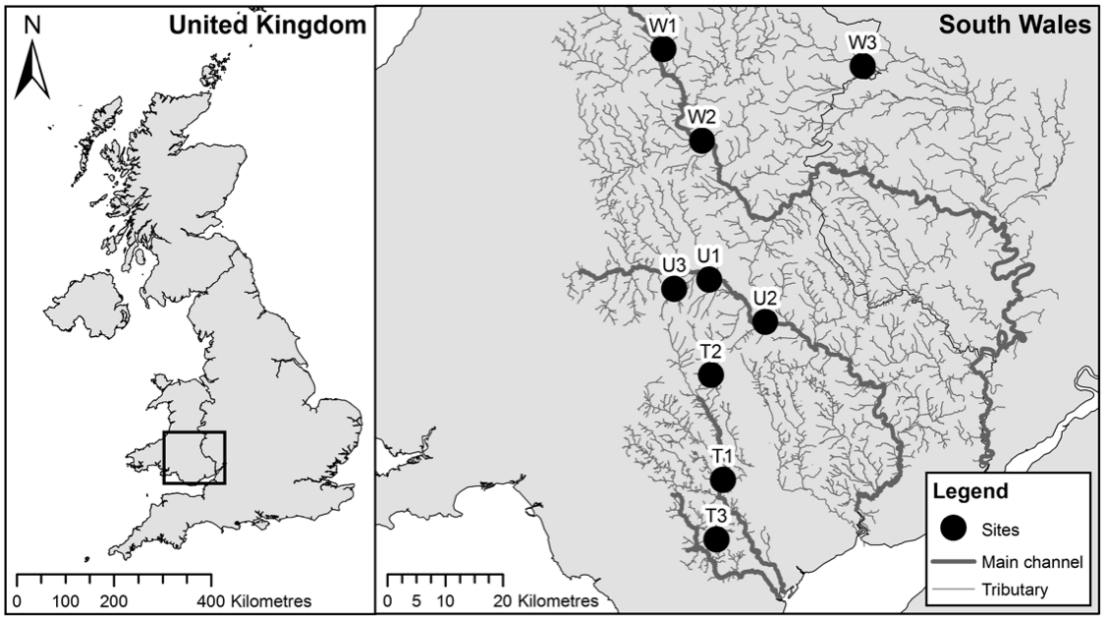


Fig. 4.1. The location of sample sites across South Wales. Sample sites were located across the Taff (T), Usk (U) and Wye (W) catchments.

4.3.2. Sample collection

At each of the nine sites, environmental and biological samples for POP analysis were collected along a 20 m river reach downstream of urban areas and point source discharges (e.g. WwTWs). Samples of sediments, biofilms (from the surface of cobbles), invertebrates (Heptageniidae, Baetidae, Rhyacophilidae, Gammaridae, Hydropsychidae, Leuctridae) and European bullhead (*Cottus gobio*) were collected, under consultation and licencing from NRW, during June–August 2016. Each sample comprised of either multiple organisms (n = 5–200), or composite samples amalgamated from samples collected across multiple locations (n = 5) for sediments and biofilms, hereafter referred to as environmental samples. Samples were kept at –80 °C until analyses. Specific protocols for all sample matrices are detailed in Appendix S7.

Table 4.1. Environmental characteristics at sample sites across South Wales, UK.

Site	Mean annual river discharge ($m^3\ s^{-1}$)	Ratio (E:R)*	Urban (km^2)	Arable (km^2)	Total (km^2)	BMWP	ASPT
T1	20.76	0.0109	33.50	4.43	304.44	78.61	5.88
T2	0.78	0.0022	0.11	0.02	32.20	72.11	6.36
T3	0.89	0.0032	4.29	0.97	20.42	25.56	4.25
U1	18.06	0.0043	5.01	8.81	440.49	65.66	6.06
U2	18.06	0.0002	6.32	23.54	582.30	60.83	5.85
U3	1.03	0.0042	0.16	0.12	16.49	70.72	6.26
W1	6.65	0.0045	0.80	7.53	169.53	74.86	6.49
W2	37.24	0.0005	7.39	77.07	1117.66	81.54	6.24
W3	3.93	0.0034	2.47	5.42	108.27	72.69	6.21

* Ratio of consented effluent discharges to stream discharge and land use conditions in the contributing area upstream of each stream reach across South Wales. Consented effluent discharges used in the calculation of effluent ratio is from NRW and EA licencing data.

4.3.3. Chemical analyses

Environmental and biological samples were analysed at the Centre for Ecology and Hydrology (CEH, Lancaster) for a range of chemical contaminants (OCs: *p,p*'-DDT, *p,p*'-DDE and *p,p*'-DDD [TDE], dieldrin [HEOD], α- and γ-hexachlorocyclohexane [HCH], hexachlorobenzene [HCB]; 36 PCB congeners and 23 PBDE congeners; see Appendix S8). Samples (0.5–2 g) were thawed, accurately weighed, ground with sand, dried with anhydrous sodium sulphate, spiked with internal recovery standards (^{13}C OCs, ^{13}C PCBs and ^{13}C PBDEs), and Soxhlet-extracted with dichloromethane for 16 hours. A small proportion of the extract was subsampled and evaporated to zero volume under N, the lipid content was then determined gravimetrically. The remaining extract was subsequently cleaned using automated size exclusion chromatography followed by filtering through an alumina glass column packed with pre-treated alumina (12 hours at 550 °C) that was deactivated using deionised water 5% (w/w). The extract was divided into two: one fraction was spiked with labelled internal standards OCs and PCBs, and the other was spiked with PBDEs.

An aliquot of extract was injected into the gas chromatograph – mass spectrometry (Agilent, Wokingham, UK) using a 50 m (OCs and PCBs) or 25 m (PBDEs) HT8 column (0.22 mm internal diameter and 0.25 μm film thickness; SGE, Milton Keynes, UK), and programmable temperature vaporization (PTV) inlet using two different methods for OC/PCBs and PBDEs. The injector temperature was set to 250 °C and helium was used as the gas carrier (2.0 mL min^{-1}). An isothermal temperature regime was programmed at 50 °C for 2 min, then ramped at 45 °C min^{-1} to 200 °C, 1.5 °C min^{-1} to 240 °C, 2 °C min^{-1} to 285 °C, 50 °C min^{-1} to 325 °C and 350 °C for 10 minutes. Compounds were detected in electron ionisation (EI) mode.

The internal standard method was used to quantify residues as well as calibration curves of commercially available standards for PCBs and OCs (Greyhound Ltd, Birkenhead, UK), and PBDEs (LGC Ltd., Teddington, UK). A series of procedural blanks were concurrently run, and samples were recovery corrected based on values from recovery spikes. Recovery values were relatively consistent across all sample media and all compounds/congeners (85.8–103.9%). The detection limits for all compounds averaged 0.04–0.11 ng g^{-1} ww for all congeners and compounds analysed (Appendix S8). Octanol-water partitioning coefficients (log K_{OW}) were collated from a range of sources: PCBs (IARC 2016), PBDEs and OCs (ChemSpider 2018).

4.3.4. Statistical analyses

All statistical analyses were conducted using R Statistical Software (version 3.4.3) (R Core Team 2015). Individual pollutants and congeners were recorded on a wet weight basis (ww) due to the low variation in lipid concentrations across all samples (0–1.62% lipid). Values for PCBs, PBDEs and OCs below the detection limits are noted throughout as not detected (ND) and given a value equal to the minimum detection limit (0.04 ng g^{-1} ww) for statistical analysis. Spatial variables and environmental covariates (land cover, catchment area and effluent contribution), were transformed logarithmically (log10) to normalise variances and aid analyses.

To address the first hypothesis, Multivariate Generalised Linear Models (M-GLMs) were fitted using the ‘mvabund’ package (Wang *et al.* 2012) and used

to analyse variations in the composition of POP compounds and congeners among sample media, as well in relation to land use, sites and catchments with environmental variables treated as covariates. All models were fitted with a negative binomial structure to account for the distribution of data. To further interrogate multivariate relationships in POP composition across samples and sites, non-metric multidimensional scaling (NMDS) (Kenkel & Orloci 1986), calculated using Jaccard similarity indices with a double-Wisconsin square root standardisation, was used. Differences in the concentration of POPs across sample media were assessed using a series of Generalised Linear Models (GLMs). Regression-based analyses were used to assess how the concentrations of POPs covaried across sample media (sediments, biofilms, invertebrates and fish).

To test the second hypothesis, POP composition and concentration measured in different invertebrate taxa were analysed separately. Generalised Linear Models (GLMs) were used to investigate differences in POP concentrations between invertebrate taxa and feeding guilds (filterers, grazers, shredders and predators; Cummins 1973), with sample site included as a variable to account for variation in POP concentrations across river systems.

The third hypothesis was tested using Generalised Linear Mixed Models (GLMMs) (Bolker *et al.* 2009), implemented using the 'lme4' package (Bates *et al.* 2015). These models were used to assess relationships between POP concentrations and environmental variables. Concentrations of POPs were summed for groups (e.g. ∑PCBs, ∑PBDEs and ∑OCs) due to the low detection frequency of congeners and chemicals, while the sample media (sediments, microbial biofilms, invertebrates and fishes) was included as a random effect. Linear regression with log transformed POP concentrations were used to assess covariation in the levels of frequently detected PBDEs, PCBs and OCs to appraise whether patterns in sources and dynamics were similar.

The validity and accuracy of statistical tests and models was assessed following Zuur, Leno and Smith (2007) and Thomas *et al.* (2015). Briefly, residual normality was assessed using QQ plots, homogeneity of variance was determined by plotting residuals against fitted values and influential

observations were investigated by calculating Cook's leverage distances. Only valid and accurate models are reported in the subsequent sections.

4.4. Results

4.4.1. POP contamination in environmental and biological samples

Most samples contained PBDEs, PCBs and OCs (86.6%, 61.1% and 98.51%, respectively), but their concentrations and composition varied across sample media, sites and river catchments (Table 4.2; Fig. 4.2A). Samples were dominated by several congeners for PBDEs (47, 99 and 100) and PCBs (81, 118, 153, 138, 169, 170 and 180), whereas the composition of OCs was relatively uniform, and neither γ-HCH nor α-HCH was detected. Some of the scarcer PBDEs, PCBs and OCs were detected, but only in a small proportion of environmental and biological samples ($n \leq 25\%$) or infrequently across sample sites (Appendix S8).

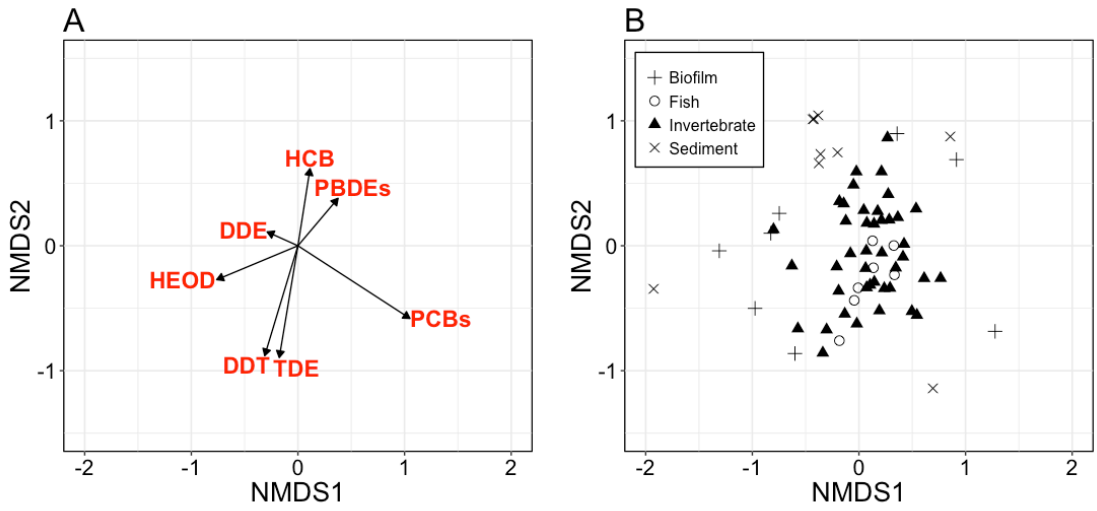


Fig. 4.2. Concentration and composition of POPs across sites and sample media (sediments, biofilms, invertebrates and fish). (A) NMDS of POP congeners and chemicals across sites (n = 9) and catchments (n = 3). **(B)** Variation in POP composition between sample matrices.

Multivariate analysis of POP composition indicated relatively similar distribution and concentration of PCBs, PBDEs and OCs across sites, but significant variation among sample media (Fig. 4.2). In general, differences

between sites explained a greater amount of variation in the concentration of PBDEs, PCBs and OCs than catchment, but in neither case were effects statistically significant (Sites: $F_{8,56} = 21.91$, $p = 0.81$; Catchments: $F_{2,64} = 7.66$, $p = 0.61$). Differences between sites were likely confounded by large variation between sample media ($F_{11,48} = 195.68$, $p < 0.001$) and differences in detection frequencies of POP compounds across sites.

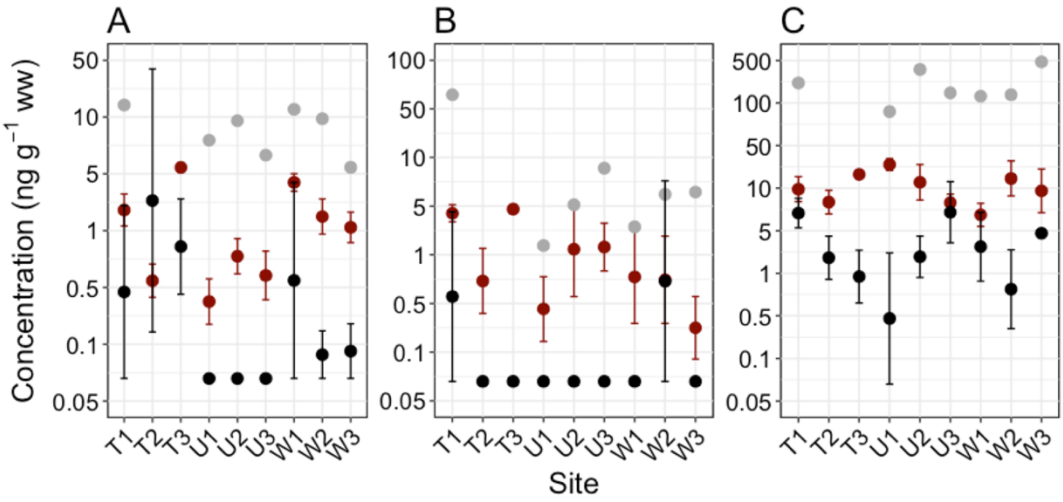


Fig. 4.3. Concentrations of POPs in environmental (sediment and biofilm), invertebrate and fish samples across river systems. (A) PBDEs. **(B)** PCBs. **(C)** OCs. Black symbols are mean values for environmental samples, grey symbols are *Cottus gobio* samples and red samples are mean values for invertebrate taxa. Errorbars for invertebrate and environmental samples indicate ± 1 standard error.

The concentrations of PCBs, PBDEs and OCs were an order of magnitude lower in environmental samples (sediments and biofilms) than invertebrate or fish samples ($R^2 = 0.26$, $F_{1,65} = 23.04$, $p < 0.001$; $R^2 = 28$, $F_{1,65} = 25.44$, $p < 0.001$; $R^2 = 0.38$, $F_{1,65} = 40.07$, $p < 0.001$; for PBDEs, PCBs and OCs respectively), while POP concentrations in biological and environmental samples from the same sites were not correlated ($R^2 = 0.01$, $F_{1,47} = 0.31$, $p = 0.584$). There were also differences in the detection frequency of POPs, with at least one PCB, PBDE congener or OC chemical detected in 63.8% of biological samples but only 27.5% of environmental samples. Furthermore, a

range of PBDEs, PCBs and OCs not in sediments were detected in biological samples: PBDEs (119, 85), PCBs (28, 52, 77, 81, 101, 114, 118, 149, 153, 209, 214) and OCs (TDE, *p,p*'-DDT). Only BDE-66, was detected in sediments yet not in other sample media (biofilms, invertebrates and fish).

4.4.2. Variation in POP contamination across taxonomic groups

Large differences were observed in the composition, concentrations and spatial variation of POPs measured across different taxonomic groups (Figs. 4.2 and 4.3). The concentrations of PBDEs, PCBs and OCs in the tissues of invertebrates were not significantly related to the concentrations measured in the benthic fish *Cottus gobio* ($F_{1,7}$ = 0.28, p = 0.790; $F_{1,7}$ = 0.19, p = 0.853; $F_{1,7}$ = 0.51, p = 0.631; for PBDEs, PCBs and OCs, respectively). Furthermore, the concentrations of POPs in sediments, biofilms, invertebrates and fish were generally not significantly related to one another. Dissimilarities were observed in the concentrations measured across organisms in the same broad taxonomic groups (Appendix S9).

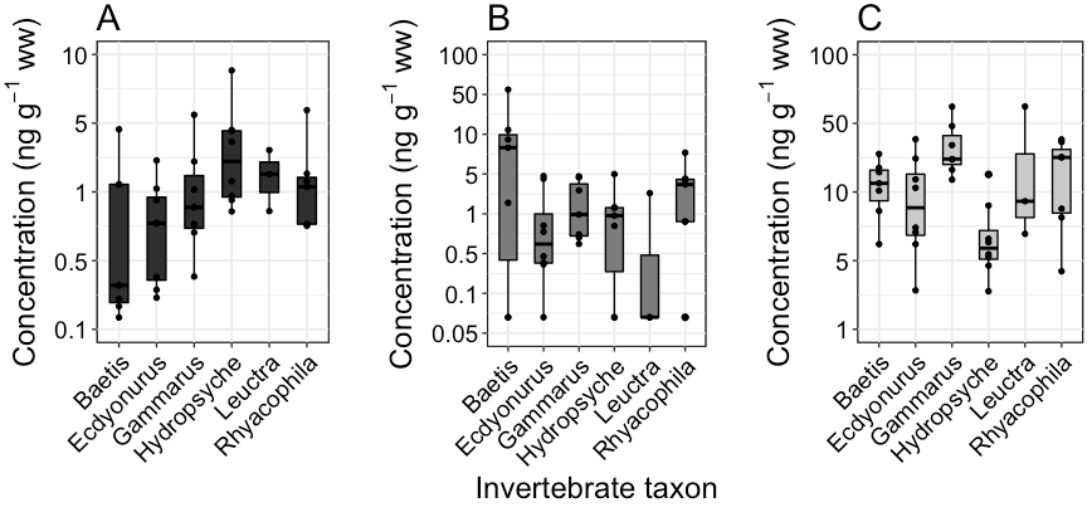


Fig. 4.4. Concentrations of POPs in the tissues of invertebrate taxa across sample sites. (A) PBDEs. **(B)** PCBs. **(C)** OCs. The results of statistical analyses are reported in the main text.

Table 4.2. Concentrations of persistent xenobiotic pollutants within riverine food webs.

Location	Site	Sample	∑PCBs	∑PBDEs	∑OCs
Taff	T1	Sediment	2.73	ND	7.54
		Biofilm	ND	1.62	3.37
		Invertebrates	2.92 (1.32–5.82)	2.19 (0.99–7.60)	12.48 (3.31–24.09)
		Fish	44.09	12.74	172.01
	T2	Sediment	ND	ND	0.81
		Biofilm	ND	0.08	2.67
		Invertebrates	1.29 (ND–2.94)	0.46 (0.12–0.82)	8.51 (1.84–17.23)
	T3	Sediment	ND	0.23	1.82
		Biofilm	ND	1.85	0.40
		Invertebrates	2.91	3.59	14.49
Usk	U1	Sediment	ND	ND	1.68
		Biofilm	ND	ND	ND
		Invertebrates	0.71 (ND–2.90)	0.36 (0.07–0.88)	19.88 (13.39–30.14)
		Fish	1.24	6.25	79.34
	U2	Sediment	ND	ND	0.85
		Biofilm	ND	ND	2.67
		Invertebrates	8.13 (ND–36.28)	0.72 (0.16–1.15)	17.37 (2.86–41.77)
		Fish	3.27	9.27	247.41
	U3	Sediment	ND	ND	2.23
		Biofilm	ND	ND	11.88
		Invertebrates	4.94 (0.50–15.85)	0.54 (0.10–1.61)	8.43 (3.48–13.21)
		Fish	7.76	4.61	131.39
Wye	W1	Sediment	ND	ND	5.15
		Biofilm	ND	2.61	0.76
		Invertebrates	2.46 (ND–8.51)	2.77 (1.64–3.88)	5.69 (2.60–11.53)
		Fish	1.94	11.68	120.06
	W2	Sediment	ND	ND	1.84
		Biofilm	5.7	0.08	0.17
		Invertebrates	1.57 (ND–6.72)	1.40 (0.54–2.72)	16.16 (4.26–41.91)
		Fish	4.18	9.66	124.94
	W3	Sediment	ND	ND	3.24
		Biofilm	ND	0.10	2.60
		Invertebrates	0.30 (ND–0.75)	1.23 (0.53 – 2.27)	13.70 (1.87–23.12)
		Fish	4.42	3.60	304.24

Mean (min–max) in ng g^{-1} ww. ND = Not detected (i.e. below the limits of detection).

4.4.3. Comparisons of POP contamination between invertebrate taxa

There was marked variation in the concentrations of PBDEs PCBs and OCs among invertebrates across sites (Fig. 4.4). For PBDEs, concentrations varied across sites as well as invertebrate taxa (R^2 = 0.75, $F_{13,26}$ = 9.95, p < 0.001); mayflies had the lowest concentrations (*Baetis* spp. and *Ecdyonurus* spp.), with intermediate concentrations in *Gammarus* spp. (crustacean) and *Leuctra* spp. (stonefly), and the highest concentrations in caddisflies (*Rhyacophila* spp. and *Hydropsyche* spp.). Conversely, concentrations of PCBs and OCs varied less among invertebrates, with the majority of taxa containing similar concentrations. The exceptions to this were lower concentrations of PCBs in *Leuctra* spp. and lower concentrations of OCs in *Hydropsyche* spp. (R^2 = 0.40, $F_{13,26}$ = 1.34, p = 0.253; R^2 = 0.59, $F_{13,26}$ = 2.88, p = 0.010; for PCBs and OCs, respectively). PCBs and OCs also varied less across sites ($F_{8,26}$ = 1.77, p = 0.129; $F_{8,26}$ = 1.29, p = 0.290; for PCBs and OCs, respectively).

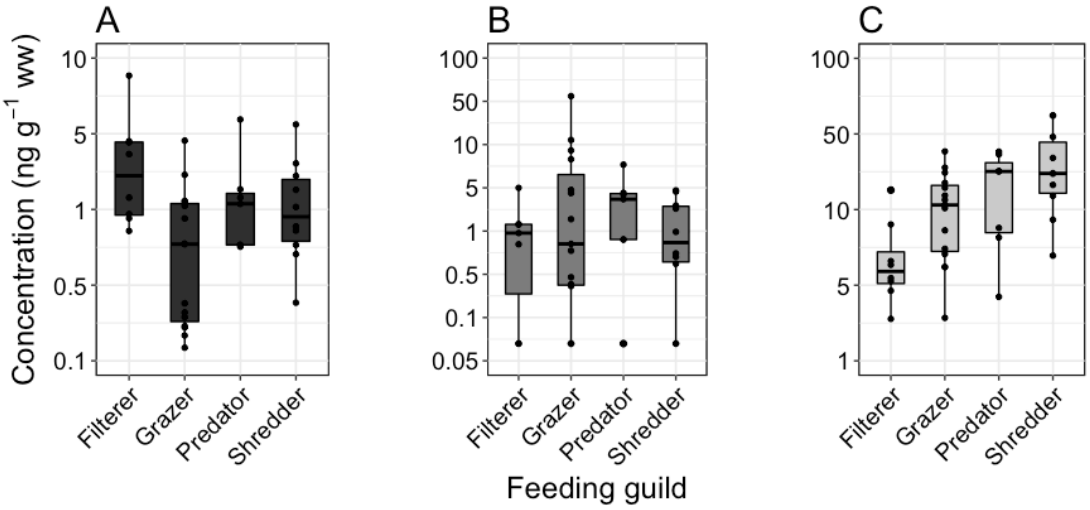


Fig. 4.5. Differences in (A) PBDE, (B) PCB and (C) OC concentrations between invertebrate feeding guilds. Not all taxa or feeding guilds were represented across all sample sites.

Concentrations of POPs in some taxa were consistently higher or lower across all sites (Fig. 4.4). For example, the mayfly genus *Ecdyonurus* had consistently lower POP concentrations than the majority of other invertebrate taxa, while *Baetis* spp. had significantly higher concentrations of PCBs in the Usk and

Wye catchments (Fig. 4.4). In general, however, there was large variability in the concentrations of POPs for different taxonomic groups at the same sites. Only taxa with similar feeding behaviours had concentrations of POPs that varied in similar ways across multiple sites, for example; the grazing mayfly genera *Baetis* and *Ecdyonurus* (Appendix S9).

Persistent organic pollutants varied in different ways among invertebrate feeding guilds and were also complicated by significant inter-site variation (Fig. 4.5). In combination, however, site and feeding guild explained a significant proportion of the variation in PBDEs ($R^2 = 0.76$, $F_{11,28} = 12.31$, $p < 0.001$) and OCs ($R^2 = 0.55$, $F_{11,28} = 3.15$, $p = 0.007$), but not PCBs ($R^2 = 0.28$, $F_{11,28} = 0.99$, $p = 0.471$). After controlling for variation among sample sites, PBDEs differed among feeding guilds in the order Filterer = Predator > Shredder > Grazer ($F_{3,36} = 14.77$, $p< 0.001$) while OCs varied in the order Shredder > Predator = Grazer > Filterer ($F_{3,36} = 6.93$, $p = 0.001$); in the latter case (OCs) there was less variation among sites compared to PCBs and PBDEs ($F_{8,28} = 1.74$, $p = 0.134$).

4.4.4. Distribution of POPs in relation to environmental covariates

After controlling for the large variation in POP concentrations among sample media using random effects, total concentrations of PCB, PBDE and OCs were related to land-use and other environmental factors. PBDEs increased with urban land-cover ($R^2c = 0.53$, $F_{7,54} = 9.21$, $p = 0.03$) while PCBs reached their highest concentrations with urban land cover and low wastewater dilution ($R^2c = 0.55$, $F_{1,53} = 5.19$, $p = 0.027$). Concentrations of OCs were highest in the Usk and Wye (Table 4.2), but none of urban land use, agricultural activity, or point-sources, explained significant variation.

The widespread PBDE and PCB congeners occurred at similar concentrations in samples (Fig. 4.6) and were significantly intercorrelated across the sites (Appendix S10). The concentrations of different OCs were also related to one another, although less clearly (Fig. 4.6; Appendix S10). There was some evidence that structural properties affected POP occurrence as the concentrations between congeners or chemicals with structural similarities were significantly correlated. Water-lipid solubility, however, had no detectable effect: the log K_{OW} of compounds was not significantly related to the observed

concentrations of individual PCBs, PBDEs or OCs across sites (R^2 = 0.06, $F_{1,41}$ = 2.47, p = 0.12). Instead, concentrations were highest in a number of compounds with relatively low (~5.0 log K_{OW}) and intermediate (~7.0 log K_{OW}) values for example HEOD, *p,p*'-DDE, BDE-47, BDE-99 and PCB-153.

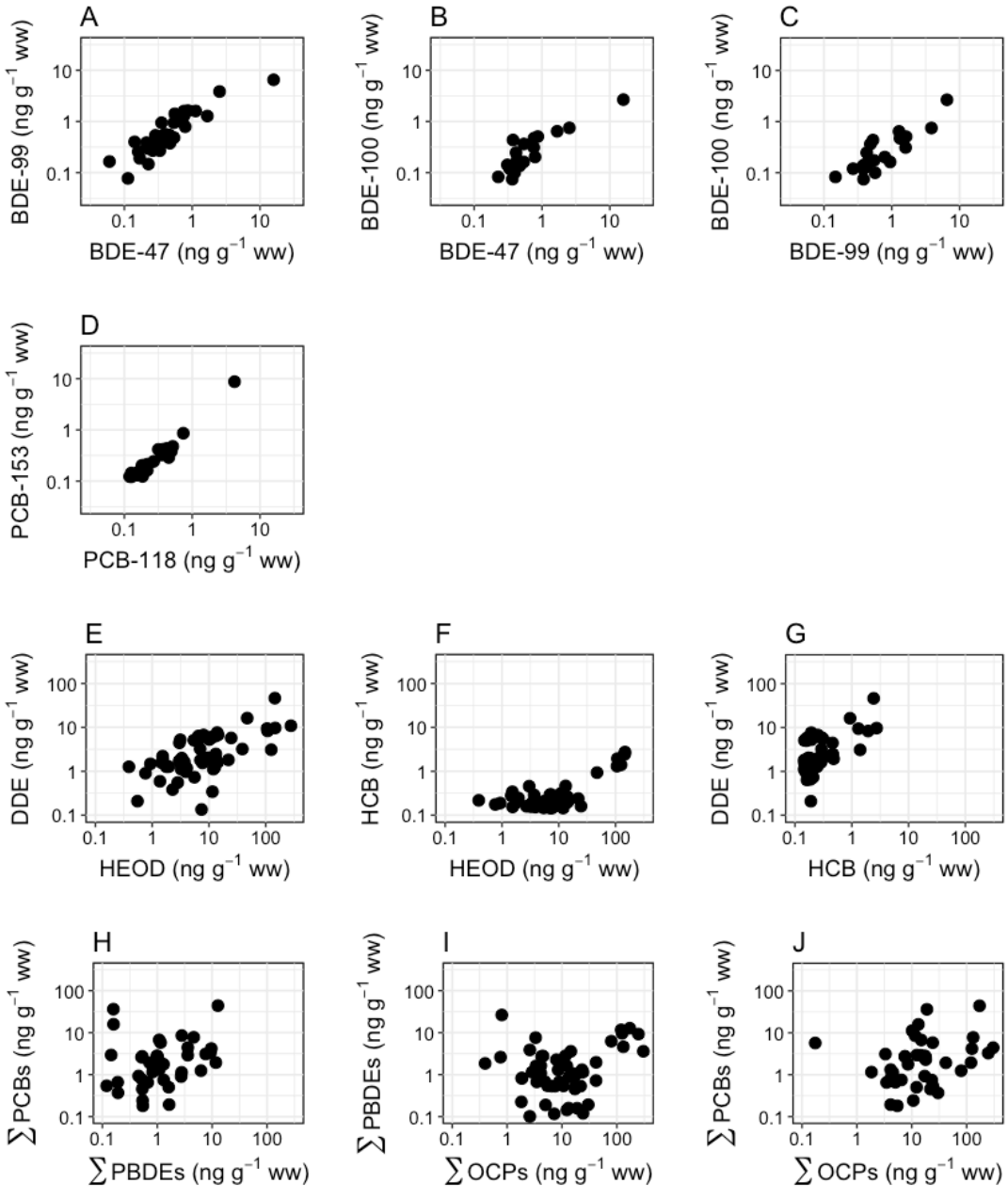


Fig. 4.6. Relationships between the concentrations of frequently detected (≥30% of samples) PBDE and PCB congeners, as well as OCP compounds. The results of statistical analyses are in Appendix S10.

4.5. Discussion

Persistent organic pollutants were detected in both environmental and biological samples across rivers in South Wales. The concentration and composition of POPs, however, was different between sample media, with several PBDEs, PCBs and OCs below detectable concentrations in sediments and biofilms, but present in high concentrations in invertebrates and fish. The question of whether invertebrates provide a suitable taxonomic group for monitoring POPs was assessed, showing that inconsistencies in the composition and concentrations of POPs across invertebrate taxa provided novel information on the distribution of pollutants in river systems (e.g. across basal resources and mesohabitats). This same variation, however, limited the use of invertebrates as representatives for POP levels in both the environment and other aquatic taxa. In general, there were large differences in POP concentration and composition among biota, and no single group of organisms consistently represents the distribution and variation of POPs in river environments. Once variation between sample media was accounted for, body burden data provided important information about POP distribution and potential sources, but only a modest amount of spatial variation in PCBs, PBDEs and OCs was explained by surrounding land use. The relatively high concentrations of POPs indicated their ubiquity at both local and regional scales, whilst demonstrating the persistence of several POPs but also potential for continued low-level releases of others. In combination, these data indicate the widespread distribution of POPs in the study area, but also the value of biological samples to understanding the distribution, quantity and potential ecological risk of POPs in river systems.

Differences in the composition and concentration of PBDEs, PCBs and OCs between environmental and biological samples indicates the importance of body burden data to monitoring POPs in aquatic systems (Van der Oost, Beyer & Vermeulen 2003). In general, the low detection frequencies across environmental samples restricted spatial analysis, thus limiting understanding of potential sources of pollution across the landscape. For biota this was not the case, with lower chlorinated PCB congeners and a range of other less stable PBDEs and OCs, as well as higher detection frequencies enabling the

identification of POP sources across the contrasting river systems. Discrepancies between environmental and biological samples also have implications for understanding exposure and potential ecological risk in natural systems – with the use of POP composition data from different sample media potentially misrepresenting the toxicity of POP mixtures (e.g. sediments not representing the non-dioxin like PCBs observed in invertebrates and fish). The present study demonstrates that monitoring both environmental and biological media allows for an improved accuracy in determining the risks posed by a range of chemicals that can translocate in the environment multiple exposure pathways.

Among existing biomonitoring schemes that analyse the body burden of pollutants in biota, the vast majority have used fishes and mammals (Van der Oost, Beyer & Vermeulen 2003; Bettinetti *et al.* 2011; Pountney *et al.* 2015), with invertebrates used infrequently (Ravera 2001). This contrasts with their more widespread incorporation into metrics to appraise, and sometimes diagnose, the effects of acidification, gross pollution, sediments, pesticides and other water quality alterations in freshwater ecosystems (Armitage *et al.* 1983; Liess & Von Der Ohe 2005; Davy-Bowker *et al.* 2005). In the data, the composition and concentration of POPs in invertebrates varied among taxa and feeding guilds in ways that differed among compounds in ways that require more investigation. Once these variations were accounted for in statistical analysis, however, invertebrate data provided information that extended that available from environmental samples on both local (meso-habitat) and regional distribution of pollutants across the study area, while also revealing contrasting patterns in the accumulation of different pollutants in organisms at the lower trophic levels of food webs. This suggests an optimum sampling strategy for POPs in which different taxa are sampled in ways that allow data from different taxa to be combined to provide an overall indication of pollutant distribution, but also specific details on food-web transfers and contrasting sources. Further studies in other freshwater systems should be completed to evaluate these possibilities as well as assessments of more extensive relationships between POP concentrations in invertebrates and more conventional bioassessment metrics.

With differences between sample media and taxonomic groups accounted for as indicated above, spatial variation among POPs became clearer – at least for PCBs and PBDEs. The concentrations of these two groups of compounds increased in urbanised regions or where wastewater effluents contributed most to river discharge. As well as the persistence and local remobilisation of these compounds from secondary sources (e.g. temporary sediment stores; Weber *et al.* 2008; Kallenborn *et al.* 2012), the extremely persistent, dioxin like PCB congeners (81, 118, 153 and 180), and less stable, non-dioxin like PCBs (66 and 105) appear to remain in the urban rivers environments of South Wales. Similarly, stable PBDE congeners (47, 99 and 100) were dominant in urban regions. This is consistent with the detection of elevated concentrations of PCBs and, particularly, PBDEs in the eggs of a river bird, the Eurasian dipper (*Cinclus cinclus*) along the urban rivers of South Wales (Morrissey *et al.* 2013b). Although the precise sources are uncertain, links to the widespread and intense industrial activity in this region are likely to be implicated. At its industrial peak, South Wales supported around 600 collieries, together with associated coking, gasification, smelting and manufacturing industries, with smaller hundreds of more mechanised mines still operating up to their progressive closure from the late 1970s and early 1980s onwards (see Bateson *et al.* 2015).

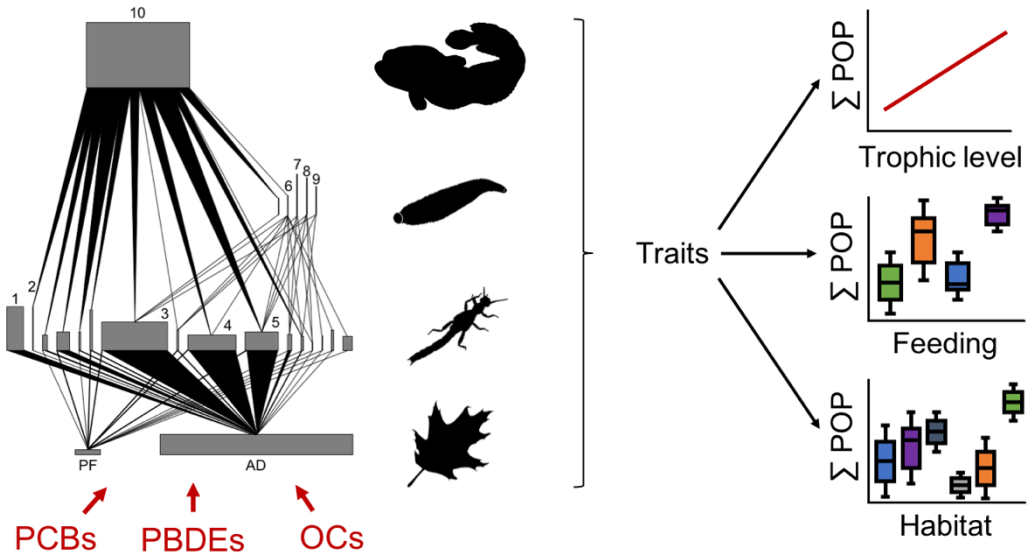
In contrast, OCs reached their highest concentration in the more rural Usk and Wye catchments, although statistically significant patterns were not detected. Although OCs were widely utilised in agricultural, domestic and industrial activities across the landscape (Barber, Sweetman & Jones 2005), the concentrations of several compounds (dieldrin, DDE, HCB) in the samples exceeded levels typically associated with persistence and remobilisation. The concentrations observed were high compared to freshwater organisms from other regions where the use of such chemicals has been restricted, for example Europe (Chapter 3). Elevated concentrations of these chemicals were detected in the eggs of Eurasian dippers during the 1980s along tributaries of the Usk and Wye where previous agricultural uses, such as sheep dipping using dieldrin (HEOD), had once been more widespread (Ormerod & Tyler 1990, 1993; Morrissey *et al.* 2013b), yet, some of the high OC

concentrations detected could be more recent. For example, hexachlorobenzene (HCB) was relatively absent in the eggs of the river bird *Cinclus cinclus* the 1980s (Ormerod & Tyler 1990), yet detected at significant concentrations over 2008–2010 (Morrissey *et al.* 2013b), as well as in biota within this study. Though now banned for direct use, recent sources include fungicidal use in agriculture and emission from metal production, while lower level emissions might arise from the domestic waste combustion (Barber, Sweetman & Jones 2005; NAEI 2016).

4.6. Conclusions

Overall, using environmental and biological samples, data illustrate how significant concentrations of POPs continue to pervade river systems across South Wales (UK). Although discrepancies in the composition and concentration of pollutants across different taxonomic groups were identified, the study indicates that a combination of samples from different media and different organisms could optimise the detection of different legacy pollutants in river systems. By accounting for variation in this way, the contaminant profiles for POPs were shown to relate to potential sources including the remobilisation or circulation of legacy contaminants, as well as the continued or recent emission of some POP compounds. The widespread contamination of river ecosystems by persistent, legacy contaminants highlights a need to consider these pollutants, alongside current-use and emerging compounds, in contemporary risk assessments.

Chapter 5: Transfer pathways of persistent organic pollutants through a freshwater food web



This chapter forms the basis of a paper currently in review at *Environmental Science & Technology*. FMW collected, processed and analysed samples for POPs (GC-MS) under supervision from MGP, analysed data and wrote the manuscript. MGP, SJO and CRT provided comments and revisions.

5.1. Abstract

1. Freshwater organisms are still at risk from the bioaccumulation and biomagnification of persistent organic pollutants (POPs), but factors affecting their transfer through food webs are poorly understood.

2. Here the transfer pathways of PCBs, PBDEs and OCs through a complex river food web were investigated, assessing the distribution and flux between basal resources (n = 3), macroinvertebrate taxa (n = 22) and fish (n = 1). Furthermore, the effects of biological traits on the observed patterns and use trait-based models to predict POP bioaccumulation were assessed.

3. Transfer pathways differed among chemical groups, with PCBs apparently associated with detrital pathways, PBDEs with autochthony and herbivory (e.g. microbial biofilms), and OCs associated equally with both detrital and herbivorous transfer pathways. Biological traits such as habitat affinity, feeding behaviour and body size also explained some of the variation in pollutant burdens between organisms occupying similar trophic levels.

4. Trait-based models indicated that relationships between contaminants, trophic transfers and traits were relatively well conserved across a wider array of river food webs. Although providing more consistent predictions of POP bioaccumulation than steady-state models, variability in POP bioaccumulation across food webs limited the accuracy of trait-based predictions.

5. As some of the first data to illustrate how ecological processes alter the fluxes of persistent pollutants through river food webs, analyses reveal potentially important links between POPs and contrasting energetic pathways. The data also show the potential utility of models and trait-based methods to predict the ecological risk posed by persistent contaminants, but further field validations are required.

5.2. Introduction

Xenobiotic pollutants – chemicals which do not occur naturally in the environment – are distributed widely across the Earth's freshwater ecosystems (Malaj *et al.* 2014; Chapter 3). These pollutants are particularly hazardous to individual organisms and have impacts through a diverse array of pathways, including endocrine disruption (Chapter 2). Multiple taxonomic groups are at risk, including microbes (López-Doval *et al.* 2010), benthic invertebrates (Soin & Smagghe 2007), fish (Kloas *et al.* 2009) and aquatic birds (Morrissey *et al.* 2014). In natural systems, however, negative ecological effects can transcend levels of biological organisation to affect populations, communities and ecosystems, with consequences for the provision of ecosystem services and the functioning of socio-biological systems (Chagnon *et al.* 2015; Chapter 2).

Legacy organic chemicals are a group of xenobiotic pollutants that have largely been withdrawn from legal use, but remain widespread across freshwater ecosystems either because of their persistence, or because of low-level recirculation from discarded materials, land-fill or local illicit use (McKnight *et al.* 2015). Generally referred to as persistent organic pollutants (POPs), these chemicals often occur at relatively low environmental concentrations (Rasmussen *et al.* 2015), but their hydrophobic and lipophilic nature enables significant accumulation in the tissues of freshwater organisms (invertebrates, fish, mammals) as well as significant magnification across the trophic levels of aquatic food webs (Streets *et al.* 2006; Kelly *et al.* 2007; Walters *et al.* 2008, 2011, 2016; Wu *et al.* 2009). Thus, although the environmental concentrations of these chemicals might suggest a relatively low potential for ecological risks, long-term exposure, assimilation and subsequent accumulation and/or magnification of persistent contaminants could represent a continued threat to individual organisms, population and communities across the wider freshwater ecosystem (Chapter 2).

Although the bioaccumulation and biomagnification of POPs are key to understanding ecological risks from persistent chemicals, factors affecting transfer processes and cascading ecological effects across trophic levels are poorly understood (Escher *et al.* 2011; Meador *et al.* 2011). Existing research suggests that compound chemistry, biology of exposed organisms and

environmental characteristics might interact to influence the distribution and concentrations of POPs in aquatic and terrestrial food webs (Kelly *et al.* 2007, 2008; Walters *et al.* 2016), with chemical structure and concentration particularly important (Fisk, Hobson & Norstrom 2001; Walters *et al.* 2008). Food web structure might also affect POP behaviour, but much of the existing work has involved relatively simple ecosystems with restricted taxonomic or functional diversity and limited trophic interactions. Thus, although the general principle of bioaccumulation and trophic magnification of persistent pollutants is well established globally (Walters *et al.* 2016), there is limited knowledge of how complex, potentially heterogeneous and multi-layer trophic interactions affect the partitioning and transfer of POPs through freshwater food webs. This includes significant gaps in understanding how persistent organic contaminants might enter and pervade river food webs linked to basal energetic resources arising from autochthony (i.e. within-river primary production) or allochthony (i.e. matter such as leaf-litter originating from the riparian zone or wider catchment). These two resources contribute significantly to carbon and energy fluxes throughout freshwater ecosystems and are fundamental to their functioning (Brett *et al.* 2017). Thus, any influence of the transfer of energy from basal results on fluxes of pollutants has the potential to affect organism exposure at a range of trophic levels.

As well as insights from food-webs, biological trait analysis might also improve understanding of POP fluxes through ecosystems. Trait analysis has expanded generally in freshwater ecology, and has been applied to an increasing array of problems (Menezes, Baird & Soares 2010). Although experimental studies have assessed the influence of biological traits on the accumulation of xenobiotic pollutants within the tissues of aquatic organisms (Gaskell, Brooks & Maltby 2007; Rubach *et al.* 2011; Diepens, Van den Heuvel-Greve & Koelmans 2015; Sidney *et al.* 2016), the primary focus has been on physiological traits (e.g. size, growth) in just a small number of organisms from individual taxonomic groups. In natural systems, however, trait diversity is large (Schmera *et al.* 2015) and influences several processes through variations in: (1) morphological and physiological traits, including biomass, mouthpart morphology and life-cycle duration; (2) ecological traits,

regulating events or processes, including factors such as time of emergence, growth rate and dispersal mechanisms; and (3) behavioural traits, relating to the specific activities of the organism, for example feeding and habitat preferences. All might affect the transfer and effects of POPs in freshwater communities (see Chapter 2).

In this study, the aim was to assess transfer pathways of persistent pollutants (PCBs, PBDEs and OCs) through river food webs while also investigating the relative influence of biological traits on POP bioaccumulation across different aquatic taxa. POP body burdens and trophic interactions were quantified across a complex river food web in South Wales (United Kingdom), and relationships among trophic transfers, biological traits and contaminant data were used to develop trait-based models to estimate POP bioaccumulation across eight further aquatic food webs sampled from UK river systems. By comparing trait-based model predictions, estimates from a steady state equation and direct measurements of bioaccumulation for the wider suite of invertebrate taxa, the applicability of trait-based methods for broad-scale assessments of environmental pollution was tested. It was hypothesised that:

1. The transfer of POPs through food webs occurs alongside the flux of energy associated with different aquatic resources (allochthony versus autochthony)
2. Variation in bioaccumulation and biomagnification of POPs in food webs are related to biological traits
3. Trait-based models suitably predict the bioaccumulation of persistent organic pollutants across multiple food webs

5.3. Material and methods

5.3.1. Sample site and the T1 food web

The analysis of bioaccumulation, biomagnification trophic transfer of persistent pollutants through a river food web focussed on a 20 m stretch downstream of the Cynon Valley Wastewater Treatment (WwTWs) discharge into the River Taff, South Wales (51°37’41.8” N, 3°19’45.4” W) (T1; Fig. 5.1A). This facility receives wastewater from the Rhondda-Cynon Valleys (approx. 63,000 people), and involves primary and secondary treatment, consisting of

settlement, mechanical filtering and biological processing using percolating filter-beds. The surrounding catchment is highly urbanised (~20%), and contains a range of pollution sources, including combined sewer overflows, storm drains and road runoff drains, all of which have previously been shown to contribute to anthropogenic pollution loads in the Taff river system (Williams & Simmons 1999). The diversity of discharges and presence of legacy pollutants in benthic sediments and biota at this location make it suitable to assess pollutant transfer through the food web – which consist of a range of invertebrate, and fish taxa that are representative of other streams recovering from past insanitary pollution (Vaughan & Ormerod 2012; Chapter 7).

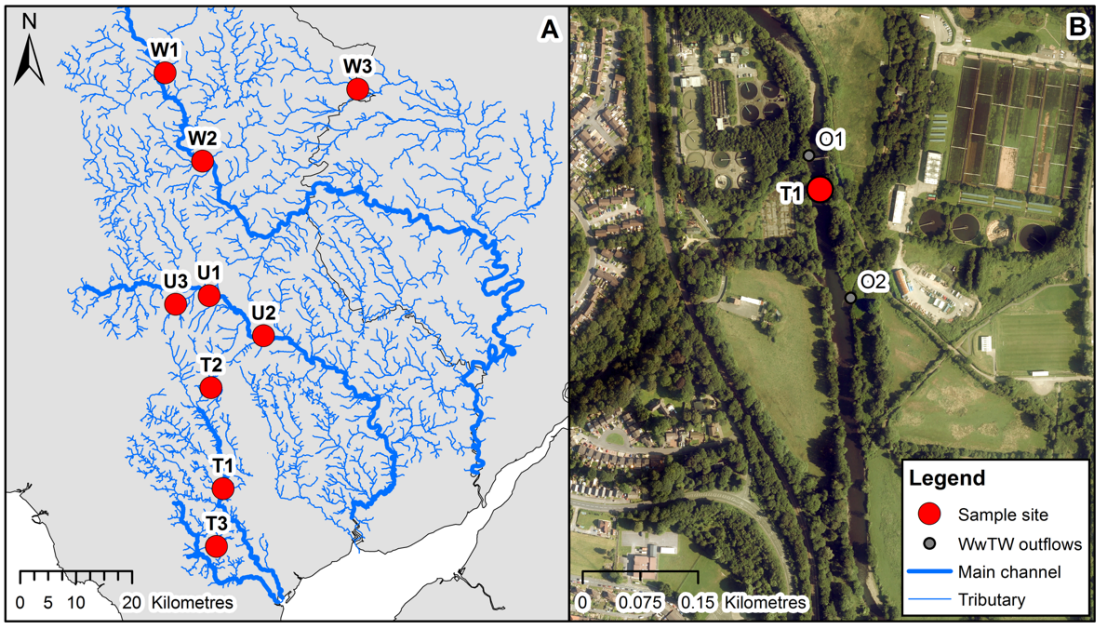


Fig 5.1. Location of the T1 sample site in relation to the Cynon Valley WwTWs. The T1 site is part of a wider network of sites shown in **(A)**. In **(B)** O1 and O2 represents the outflow from Cynon Valley and Cilfynydd WwTWs, respectively. Aerial photography was derived from ©Getmapping Plc.

Samples were collected from 26 components of the river food web at T1 (May–August 2017) including: basal resources (n = 3; microbial biofilm, macrophytes and allochthonous detritus), invertebrate taxa (n = 21), benthic fish (n = 1). All samples were collected under consultation and licence from Natural Resources Wales (see Table 5.1 for taxonomic detail). Methods varied with

sample types, with area-based sampling used for basal resources, composite samples of whole organisms for invertebrate taxa (5–200 individuals per sample), liver samples from fish (n = 5 individuals). Approximately 1–2 g of each sample was collected and stored at -80°C in a glass vial previously rinsed in hexane/acetone (1:1, v/v) until analysis (see Appendix S7 for methods).

5.3.2. Chemical analyses

See Chapter 4, Section 4.3.3.

5.3.3. Quantitative food web construction

To construct a quantitative network for the food web at T1, so as to act as a basis for understanding pollutant flux, the trophic basis for production method was used (Benke & Wallace 1997). In outline, food web links are expressed as the flow of biomass from resources to consumers based on dietary information and estimates of secondary production that form the basis for energy flux (see Appendix S11).

For macroinvertebrates, secondary production (mg m^{-2} yr^{-1}) estimates were derived from monthly samples over 2016–2017 (n = 36) in which individuals were identified to the lowest practical taxonomic unit (usually species or genus), counted and biomass derived from head width or body length measurement to the nearest 0.1 mm (n = 9,921) using a Nikon SMZ800N stereomicroscope (Nikon, Tokyo, Japan), with a Lumenera Infinity 1-1M camera (Lumenera, Ontario, Canada) and visual analysis software (Infinity Analyse, version 6.5.4). Individual biomass (mg dry weight) was then calculated following published length-mass relationships (Benke *et al.* 1999; Baumgärtner & Rothhaupt 2003), and secondary production calculated using the size-frequency method (Waters 1977). For rare taxa (n < 5) production was estimated using the Production/Biomass (P/B) value for the most closely associated taxa. Fish secondary production could not be directly estimated, and instead a P/B ratio (2.0) derived from existing literature on *Cottus gobio* (Mills & Mann 1983) was coupled with an estimate of biomass (g m^{-2}) generated from an electrofishing survey during July 2017, under consultation and licence from Natural Resources Wales.

Trophic links between organisms were derived from gut dissection of individual macroinvertebrates (n = 545) and fish (n = 15) in which the digestive tract of each individual was removed, and relative proportions of prey items enumerated using a gridded graticule. A mean value was calculated for each taxon to provide an estimate of the proportion of resources utilised, including both basal resources and other invertebrates. These proportions were then used in conjunction with the trophic basis of production method, to quantify food webs (Benke & Wallace 1997). Based on data, the flux of biomass to consumers (consumption) for each taxon was separated among the two resources, microbial biofilms (autochthonous), or sediments, detritus, organic matter and plant fragments (allochthonous).

The trophic level of each component of the quantitative food web (n = 26), was calculated based on invertebrate community data, and modelled links across the wider food web, which comprised of 71 taxa in total (see Chapter 6, Section 6.3.3). In this study, trophic level was chain-averaged (mean chain length of paths from the organism to the basal resources; Martinez, 1991) to allow for extrapolation across river food webs in South Wales.

5.3.4. Statistical analyses

All data analysis was completed using 'R' statistical software (version 3.4.0) (R Core Team 2015). Values for PCBs, PBDEs and OCs below the detection limits were noted throughout as not detected (ND), and for statistical analyses a value equal to the minimum detection limit (0.04 ng g^{-1} ww) was applied. Prior to further analyses a series of exploratory steps, following Zuur, Leno and Elphick (2010), were completed to understand the structure of POP concentration data (heteroscedasticity, normality, outliers), and to inform the selection of further statistical tests and models.

To address the first hypothesis covariation in POP and transfer pathway data were assessed directly using Generalised Linear Models (GLMs) (Nelder & Baker 2006). These data were also used to calculate metrics describing the accumulation, magnification and transfer of POPs between organisms sampled from the T1 food web. Sediment bioaccumulation factors (BSAFs) for each taxon were calculated using tissue concentration data in conjunction with concentrations measured in sediments, following Equation 5.1:

(5.1) $$BSAF = \frac{C_B}{C_{STO}}$$

where C_B is the concentration of POP groups (PBDEs, PCBs and OCs) measured in the tissues of the target organism (ng g^{-1} ww), and C_{STO} is the concentration measured in organic matter and sediments (ng g^{-1} ww). Biomagnification factors (BMFs) were also calculated for taxa to assess the organism-specific levels of biomagnification, following Equation 5.2:

(5.2) $$BMF = \frac{C_B}{\sum(P_i\, C_{Di})}$$

where C_B is the concentration of xenobiotic pollutants measured in the tissues of the target organism (ng g^{-1} ww), P_i is the proportion of prey organism or basal resource (sediment, detritus, plant material or biofilm) observed in the diet of the target organism *i* (0–1) and CD_i is the concentration of persistent pollutants measured in the tissues of the prey organism *i* (ng g^{-1} ww).

For the second hypothesis, trait data for the macroinvertebrates sampled at the T1 food web, collated from a European fuzzy-coded trait database (Tachet, Bournaud & Usseglio-Polatera 2002), was used to investigate inter-taxon variation within communities (Chevene, Doléadec & Chessel 1994; Statzner, Resh & Roux 1994) and to understand the consequences of such structure for the transfer of pollutants. These trait data were supplemented by non-fuzzy, categorised feeding guild data for macroinvertebrate taxa of South Wales (Durance I. & Ormerod S.J., unpublished data). In the following analyses, disaggregated trait data were utilised to assess the relationships between biological traits, bioaccumulation and biomagnification of POPs. Prior to trait-based modelling, trait affinity data were standardised across grouping features (overarching trait groups, for example feeding preference) to allow for improved comparisons between different traits and organisms. The exact methods used in the preparation and standardisation of fuzzy-coded trait data are described in more detail by Gutiérrez-Cánovas *et al.* (2015). Traits were selected initially based on their correlation with the BSAF across the sampled taxa, for each chemical group (PBDEs, PCBs and OCs). BSAF values were used in models to minimise the effect of concentration-dependence which influences bioaccumulation of POPs and restricts the applicability of these models to other systems where environmental concentrations may differ to

those observed at the T1 food web. This allows for predictions across multiple sites, used to test the third hypothesis. Traits with an average coefficient of $R \geq |0.40|$ were selected for further analysis. For this subset of selected traits the relationships between trait affinity and BSAF values for PBDEs, PCBs and OCs across taxa, were assessed using GLMs. Global models were constructed for each chemical using the corrected Akaike Information Criterion (AICc) and the *dredge* function in 'MuMin' (Bartoń 2009). GLMs were then validated following the procedures detailed in Zuur *et al.* (2007) and Thomas *et al.* (2015). Residual normality was assessed using QQ plots, homogeneity of variance was determined by plotting residuals against fitted values and influential observations were investigated using Cook's leverage distances.

The third hypothesis was tested using the BSAF GLMs (detailed above), a steady-state equation for estimating chemical bioaccumulation (AQUAWEB 1.2; Arnot & Gobas, 2004), and a wider dataset of BSAFs calculated for invertebrate taxa from eight other river food webs sampled across South Wales, United Kingdom (Chapter 4; Fig. 5.1A). Through comparisons between the predictions from these two models and measured BSAFs, the relative accuracy of both trophic- and trait-based models was assessed. Here Mean Absolute Error (MAE; Willmott & Matsuura, 2005) calculated for relationships between the observed and predicted BSAFs was used to assess the performance and accuracy of models.

5.4. Results

5.4.1. Concentrations of PCBs, PBDEs and OCs in the T1 food web

Persistent organic pollutants occurred widely in organisms at T1, but concentrations were variable (Table 5.1) and over 55% of compounds analysed were detected in <10% of samples. This was particularly true for some of the scarcer congeners, and for example PCBs 31, 126 and 157 were only observed in sediments and microbial biofilms. Conversely, PBDE congeners 28, 49, 99, 100, 153 and 154, PCB 52 and *p,p'*-DDT were only observed in tissue samples from invertebrates and fish. Rather than treatment at congener level concentration, data were aggregated for different chemical groups in further assessments of bioaccumulation and trophic magnification.

Table 5.1. Concentrations of POPs in basal resources and organisms in the T1 river food web. Chain averaged trophic level used allow for modelling across multiple food webs across other river systems.

Sample	Trophic level	Functional feeding group	Concentration (ng g^{-1} ww)		
			∑PBDEs	∑PCBs	∑OCs
Sediment	1.00	Basal resource	0.27	2.73	7.53
Fontinalis spp.	1.00	Basal resource	ND	18.30	1.95
Microbial biofilm	1.00	Basal resource	1.97	2.66	0.96
Asellus spp.	2.00	Gatherer	0.54	ND	1.56
Leuctra spp.	2.00	Shredder	1.10	12.24	7.68
Rhithrogena spp.	2.00	Grazer	2.03	ND	6.70
Caenis spp.	2.00	Gatherer	1.22	ND	6.92
Naididae	2.00	Gatherer	1.34	ND	2.87
Ecdyonurus spp.	2.00	Grazer	1.19	1.70	2.54
Baetis spp.	2.00	Grazer	3.64	0.56	4.86
Radix spp.	2.00	Grazer	1.35	4.40	6.30
Heptagenia spp.	2.00	Grazer	1.65	3.81	2.40
Eiseniella tetraedra	2.00	Gatherer	2.00	11.87	6.09
Lepidostoma hirtum	2.00	Gatherer	1.36	ND	24.28
Simuliidae	5.20	Filterer	1.12	0.66	0.36
Serratella ignita	5.24	Grazer	1.78	1.18	7.09
Sericostoma spp.	7.60	Gatherer	9.82	1.30	90.98
Gammarus pulex	8.06	Shredder	1.60	0.36	5.36
Hydropsyche spp.	8.69	Filterer	7.60	1.39	55.93
Polycelis spp.	8.77	Predator	8.29	1.65	127.94
Platambus maculatus	9.44	Predator	6.18	11.24	11.58
Rhyacophila dorsalis	9.98	Predator	1.67	ND	22.09
Polycentropus spp.	10.14	Predator	1.89	ND	27.19
Erpobdella octoculata	10.80	Predator	3.68	8.15	5.90
Cottus gobio	11.09	Predator	32.70	78.60	45.84

Total concentrations of PBDEs (Coefficient of variation [CV] = 0.61), PCBs (CV = 0.51) and OCs (CV = 0.64) remained highly variable across the T1 food web even after aggregation (Table 5.1). This, in part, reflected the magnification of POPs across trophic levels (Table 5.2), with predators such as *C. gobio*, *E. octoculata* and *Polycelis* spp. with the highest concentrations of PBDEs, PCBs and OCs. Trophic level did not explain all variation (Table 5.3), however, and organisms feeding on the same resources and occupying the same trophic level had differing pollutant concentrations (Table 5.1).

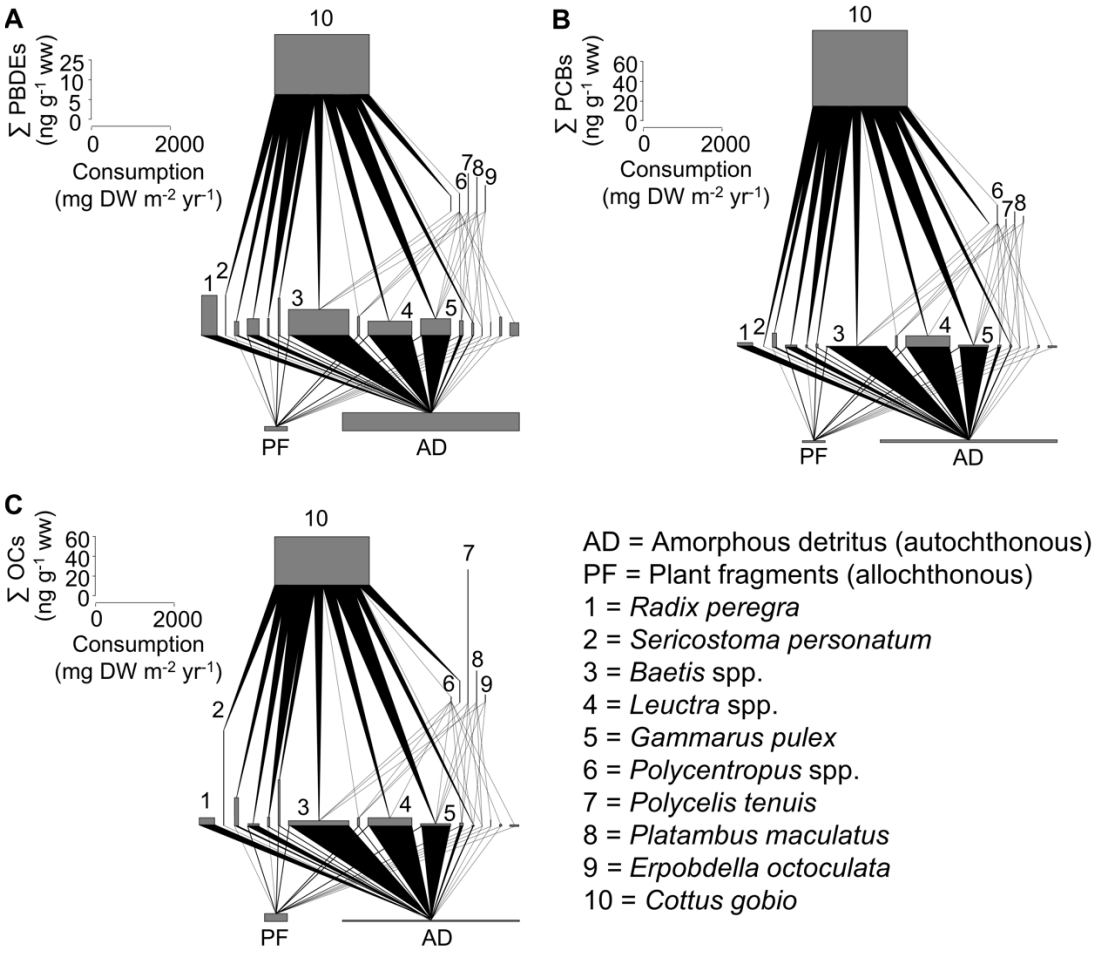


Fig. 5.2. Quantitative food webs representing the flux of contaminants through the T1 food web. Data used to construct food webs were collected from monthly samples over an annual cycle (2016–2017). Food webs were summarised into four trophic levels: the lowest bars are basal resources, the middle bars are primary and secondary macroinvertebrate consumers respectively, and the top bar is a predatory fish (*Cottus gobio*). The relative height and width of bars correspond to the concentration of POP groups (PBDEs, PCBs and OCs) and total consumption (total biomass flux from resources to consumers) for each taxon, respectively (see inset scales). The width of bars for basal resources relates to the total consumption of the resource by invertebrate consumers (total flux from resource to consumers). Black triangles linking the trophic levels are the contributions of resource fluxes to production in each consumer, aggregating to total inflow.

Table 5.2. BSAFs and BMFs for total PCBs, PBDEs and OCs across a riverine food web.

Sample	BSAF			BMF		
	∑PBDEs	∑PCBs	∑OCs	∑PBDEs	∑PCBs	∑OCs
Asellus aquaticus	2.04	-	0.21	0.49	-	0.37
Leuctra spp.	4.15	4.48	1.02	0.99	4.55	2.73
Rhithrogena spp.	7.64	-	0.89	1.03	-	7.99
Caenis spp.	4.59	-	0.92	1.09	-	1.58
Naididae	5.06	-	0.38	1.20	-	1.63
Ecdyonurus spp.	4.48	0.62	0.34	0.61	0.64	0.37
Baetis spp.	13.68	0.20	0.65	3.25	0.21	0.68
Radix spp.	5.07	1.61	0.84	1.21	1.64	0.60
Heptagenia spp.	6.21	1.40	0.32	1.48	1.42	1.15
Eiseniella tetraedra	7.51	4.35	0.81	1.79	4.41	1.67
Lepidostoma hirtum	5.12	-	3.22	1.22	-	1.26
Simuliidae	4.19	0.24	0.05	1.00	0.33	1.28
Serratella ignita	6.69	0.43	0.94	0.90	0.44	2.49
Sericostoma spp.	36.95	0.47	12.08	7.13	0.72	0.51
Gammarus pulex	6.02	0.13	0.71	0.99	0.09	1.01
Hydropsyche spp.	28.60	0.51	7.42	5.64	0.43	3.77
Polycelis spp.	31.19	0.60	16.98	7.55	0.50	26.63
Platambus spp.	23.24	4.12	1.54	23.24	5.42	6.65
Rhyacophila dorsalis	6.28	-	2.93	0.85	-	6.41
Polycentropus spp.	7.13	-	3.61	0.94	-	1.97
Erpobdella spp.	13.84	2.99	0.78	96.91	20.90	51.96
Cottus gobio	123.03	28.79	6.08	9.62	22.73	2.89

*Factors were calculated using environmental (sediment) and organism samples (ng g^{-1} ww).

5.4.2. Transfer pathways of POPs through a food web

Trophic transfer pathways at T1 appeared to differ among PBDEs, PCBs and OCs (Fig. 5.2). Chlorinated compounds, particularly PCBs, were associated with the flux of allochthonous carbon from benthic detritus and organic matter (plant fragments) in samples from sediments (Fig. 5.2). Taxa consuming a greater mass of allochthonous resources, as well as those consuming secondary production derived from allochthonous resources, had higher PCB concentrations ($R^2 = 0.22$, $F_{1,20} = 5.68$, $p = 0.027$). In comparison, higher concentrations of PBDEs were associated with the flux of autochthonous carbon, increasing in taxa consuming primary and secondary production derived from microbial biofilms ($R^2 = 0.48$, $F_{1,20} = 10.79$, $p < 0.001$). Despite significant relationships, the low R^2 values for models indicate unexplained

variation in the POP concentration data, and for the concentrations and flux of OCs, there was no relationship with either autochthonous or allochthonous carbon consumption ($R^2 = 0.02$, $F_{1,20} = 0.33$, $p = 0.574$). Some residual variation related instead to variability in biological traits of different invertebrates, and this is considered below (Table 5.3).

5.4.3. Influence of biological traits on bioaccumulation

Concentrations of POPs across the invertebrate component of the food web were variable and related to biological traits, such as habitat affinity, substrate utilisation, size and voltinism (Table 5.3). These traits explained a significant amount of the variation in PBDEs, PCBs and OCs across the food web (Table 5.3), yet relationships between specific traits and concentrations of chemicals measured in invertebrates were not consistent, with different traits related to variation in the concentrations of PCBs, PBDEs and OCs (Table 5.3). Several traits relating to habitat affinity, feeding behaviour and trophic level, however, were present in multiple models.

Models constructed from biological traits in the T1 food web explained a significant amount of variation in the concentrations of POPs (Table 5.3). As a result, these models were able to predict accurately BSAFs within the T1 system, with strong relationships between the observed and predicted BSAFs values for PBDEs, PCBs and OCs ($R^2 = 0.92$, $F_{1,61} = 712.60$, $p < 0.001$; Fig. 5.3A). The MAE for relationships constructed for the same food web was also relatively low, with models able to predict the concentrations of taxa with a relatively low level of error (MAE = 0.14). The prediction of BSAF values for PCBs were hindered by the lower detection frequency of this group of POPs within the T1 food web (Table 5.1), which resulted in a relatively poor R^2 in the final model. As a result, BSAFs for PCBs were not predicted across the validation sites.

5.4.4. Multi-model comparisons

The ability of trait-based models to predict BSAFs for invertebrates across wider river ecosystems was assessed using a validation dataset from South Wales. The low explained variation in parametrised PCB model ($R^2 = 0.47$) did not enable suitable predictions across the parameterisation data (Fig. 5.3B).

Although predictions from the PCB models were not included in further statistical analyses, the results of these models are displayed in subsequent figures to allow for interpretation and discussion regarding the utility and efficacy of trait-based modelling.

Table 5.3. Results of predictive trait-based models relating biological traits to POP BSAFs in the T1 food web.

Pollutant	AICc	R^2	Grouping feature (trait)	Effect (± SE)	t	p
Σ PBDEs	38.40	0.69	Trophic level (Chain averaged)	0.11 (0.03)	3.46	0.003
			Feeding behaviour (Functional feeding guild)	1.93 (0.71)	2.78	0.081
			Longitudinal distribution (Estuary)	-3.27 (1.23)	-2.65	0.017
			Substrate (Microphytes)	-2.36 (3.01)	-0.79	0.445
			Reproduction (Isolated cemented eggs)	0.64 (0.25)	2.56	0.021
Σ PCBs	76.81	0.47	Trophic level (Chain averaged)	0.17 (0.19)	0.87	0.235
			Feeding behaviour (Functional feeding guild)	1.71 (1.36)	1.25	0.027
			Longitudinal distribution (Estuary)	-5.30 (4.27)	-1.81	0.201
			Transversal distribution (Banks and side pools)	2.57 (2.73)	0.95	0.113
			Respiration (Gills)	-1.91 (1.23)	-1.55	0.042
Σ OCs	54.10	0.84	Trophic level (Chain averaged)	0.14 (0.06)	2.77	0.019
			Feeding behaviour (Functional feeding guild)	1.77 (0.55)	3.23	0.009
			Dispersal mode (Active aquatic)	3.21 (0.82)	3.91	0.003
			Reproduction (Clutch cemented eggs)	0.40 (0.22)	1.83	0.098
			Substrate (Twigs and detritus)	-0.55 (1.51)	-0.55	0.593
			Saprobity (Oligosaprobic)	4.57 (0.83)	5.49	<0.001

Relationships are presented as trait affinities within grouping features (see *Methods and materials*). Data were derived from both fuzzy-coded trait databases and food web data.

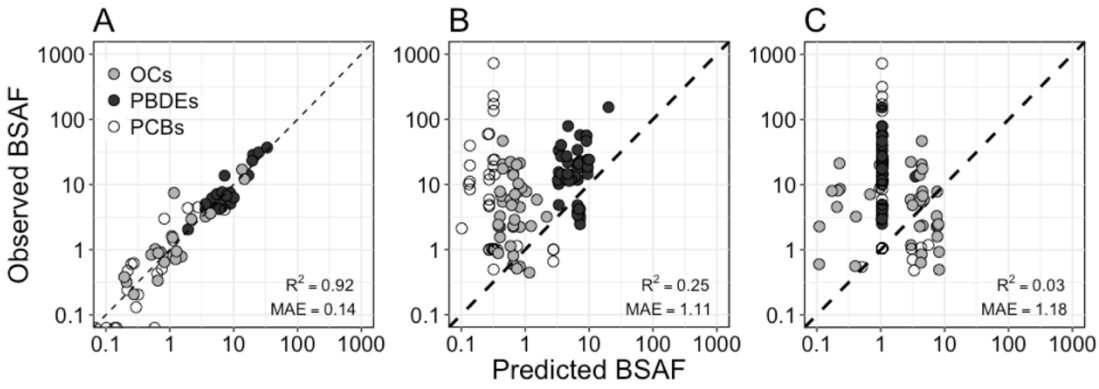


Fig. 5.3. Predicted versus observed POP BSAFs for trait-based and steady-state models data across river food webs in South Wales (UK). (A) Data from the T1 food web. **(B)** Trait-based model for validation data. **(C)** AQUAWEB 2.0 for validation data. Solid black lines indicate a 1:1 relationship between observed and predicted BSAF values. PCBs were excluded from statistical analyses due to the low detection frequency in chemical analyses, and thus the restricted proportion of explained variance in models which reduced the predictive ability of the trait-based model (presented here as hollow points).

For both OCs and PBDEs, trait-based models were shown to perform better than the AQUAWEB model for predicting BSAFs across the catchments (Fig. 5.3), with trait-based models exhibiting stronger linear relationships between observed and predicted values (R^2 = 0.22, $F_{1,82}$ = 28.55, $p < 0.001$), as well as lower MAE for PBDEs and OCs, in comparison to AQAUWEB (R^2 = 0.03, $F_{1,82}$ = 2.51, p = 0.117). The inclusion of biological traits including habitat affinities and physiological characteristics, as well as trophic factors, thus enables an improvement in the accuracy and precision of predictions, although significant unexplained variation remains (R^2 = 0.22). In general, predictions from the AQUAWEB model for PBDEs and PCBs were hindered by the infrequent detection of POPs in sediment samples, which often maintained concentrations below the mean limits of detection (~0.04 ng g^{-1} ww). As trait-based models were not reliant on environmental concentration data once parametrised for a specific group of taxa, these models were less affected by this problem.

5.5. Discussion

The flux of POPs through the T1 river food web appeared to occur through a variety of transfer pathways linked to the flux of primary and secondary production arising from both allochthonous and autochthonous resources. These trophic transfers, however, did not explain all variation in the POP concentrations measured in organisms, and the observed patterns of POP bioaccumulation was related to the physiological, ecological and behavioural biological traits of organisms. Trait-based models constructed from the ecological data of 20 invertebrate taxa were able to capture a significant amount of variation in the BSAFs for POP compounds across a wider suite of river food webs across South Wales. Despite performing better than steady-state equations, however, trait-based models were only able to estimate BSAFs to within one order of magnitude. Both prediction methods were hindered by the low detection frequencies of POPs across environmental matrices (sediments and biofilms), as well as the significant variation in bioaccumulation not related to the combination of trophic interactions, biological traits or environmental concentrations of POPs. In total, these findings show how physiological, phenological and behavioural traits, as well as trophic characteristics of organisms (e.g. proportion of prey ingested and feeding habit), affect the flux and accumulation of POPs, but also highlight the variable nature of pollutant transfers across aquatic food webs.

There are several caveats over field-based assessments of food web pollutant transfers, and several assumptions need to be considered when interpreting findings. Firstly, although trait diversity was relatively high within the T1 food web, only a single food web was analysed in depth. Focusing on a single food web, although increasing the potential influence of site-specific characteristics, limits the potentially confounding variation associated with the structure of food webs and environmental conditions, and facilitates the incorporation of a greater taxonomic and functional diversity than previous assessments. This, in turn, allowed for the construction of trait-based models, and enabled predictions across sites thus filling a gap in the literature. Secondly, there remain challenges associated with the use of fuzzy-coded trait data (Menezes, Baird & Soares 2010): despite representing noisy data (e.g. size, feeding

behaviour and substrate preferences), the multivariate nature of fuzzy coding makes the statistical assessment of relationships between groups of traits (e.g. feeding behaviour), trait affinities and other variables difficult. This study assessed relationships between trait affinities and BSAF values, yet a more parsimonious approach would be to summarise the multivariate trait characteristics of individual taxa prior to modelling. Nevertheless, although trait-space methods exist for summarising and understanding the diversity of traits across taxa or communities (e.g. Gutiérrez-Cánovas *et al.* 2015), there remains a lack of suitable methods for consolidating fuzzy-coded trait data regarding individual taxa for the purposes of predictive model-based analyses. Within these caveats, findings provide novel information about transfer pathways and the influence of biological traits on pollutant dynamics in natural systems.

Contaminant data coupled with data on the flux of energy and material between trophic linkages indicated multiple transfer pathways of PBDEs, PCBs and OCs through the T1 food web. Although widely detected, groups of POPs (PBDEs, PCBs and OCs) appeared to be differentially distributed across food web compartments, with initial partitioning occurring in the basal resources with a subsequent proliferation through the network alongside the transfer of different resources. The partitioning of POPs across the basal resources of the food web may result from several factors. Firstly, the chemical characteristics of the pollutants (e.g. partitioning coefficients and structural stability) may alter the accumulation of POPs and generate differential accumulation in the food web. For example, it has been previously shown that OCs have a high affinity for fine sediments (Sarkar *et al.* 2008) as well as plant detritus (Odum & Drifmeyer 1978), yet PBDEs are often been observed in high concentrations in microbial biofilms (Wang *et al.* 2017a). Thus, it may be that the affinity of different pollutant groups varies across these resources, and the interaction between the composition and structure of the pollutants and basal resources (e.g. organic matter content, polarity, hydrophobicity) may explain variable distributions across these compartments. A second potential explanation is that pollutants may be partitioned as a result of their sources across the environment. For example, OCs and PCBs may be more prevalent

in sediment and benthic organic matter as a result of their remobilisation in benthic sediments and allochthonous carbon inputs (e.g. de Perre *et al.* 2014). This conforms to previous research that indicates that PCBs remain in greater concentrations in allochthonous material due to the enhanced atmospheric deposition of these compounds onto riparian vegetation (Dang, Walters & Lee 2016). Similar findings in stream systems have been observed previously, with increasing levels of terrestrial carbon inputs resulting in increasing levels of PCBs (Berglund, Nyström & Larsson 2005). Further to this, the persistence of OCs in soils and atmospheric deposition, and subsequent uptake by plants (Bartrons, Catalan & Penuelas 2016), may allow for the flux of OCs alongside plant material and detritus entering rivers. In comparison, PBDEs may be present in microbial biofilms as a result of their more contemporary emissions, greater aqueous concentrations, and thus greater potential for storage in surficial biofilms (e.g. Bartrons *et al.* 2012). Certainly, the presence of highly brominated congeners across the food web indicates the potential for recent emissions, as these congeners are liable to degrade in to less brominated congeners in the environment (Siddiqi, Laessig & Reed 2003). Although all plausible explanations, the exact mechanism responsible for the observed partitioning of different chemical groups across the food web remains uncertain. Further research, across multiple food webs, is also required to understand whether these patterns are present across multiple systems, or whether this is an artefact of environmental conditions present at the T1 sample reach.

Concentrations and the levels of bioaccumulation of all POPs in the T1 food web were related to variation in the biological traits of organisms. Some traits consistently influenced the concentrations of POP compounds, for example, affinity of organisms with different habitats in river systems (e.g. side pools, slow flowing regions of the channel and lowland systems), feeding behaviour (e.g. predator, filterer and grazer) and organism trophic level. Such patterns, in particular associations with feeding habitat, have been observed widely across aquatic food webs (Fisk, Hobson & Norstrom 2001; Yu *et al.* 2009; Zhang *et al.* 2010; Sidney *et al.* 2016; Liu *et al.* 2018). The other statistical relationships, between ecological and behavioural traits, further point towards

the potential for the differential distribution of persistent compounds across the longitudinal and transversal profile of river systems. As a specific example, in the T1 food web organisms associated with side pools, twigs and detritus, as well as lowland stream systems exhibited higher OC, PCB and PBDE body burdens. Similar findings have been observed in tropical food webs for OCs, with slow flowing regions of the stream supporting greater volumes of fine sediment and detritus, facilitating an enhanced bioavailability and bioaccumulation of chemicals (Coat *et al.* 2011). Yet in general relationships such as this remain poorly understood. Other trait-pollutant relationships were specific to individual compounds. For example, OC bioaccumulation was greater in smaller organisms, potentially as a result of the biotransformation of compounds within larger invertebrates at higher trophic levels in the food web, as is shown for other organic pollutants (Verrengia Guerrero *et al.* 2002). The absence of this allometric relationship for PCBs and PBDEs may result from the fact that these chemicals are not rapidly transformed (e.g. the absence of PCB congener biotransformation in the tissues of mysids (Wong *et al.* 2004)). Again, however, uncertainty surrounds the basis for these relationships, especially as many processes related to the transformation of persistent chemicals are particularly difficult to assess in natural systems without further information on chiral congeners (see Wong *et al.* 2004).

The enhanced accuracy of predictions from trait-based models, in comparison to steady-state equations, complements previous research that suggests that the biological and ecological characteristics of food webs strongly influences the levels of accumulation and magnification (Walters *et al.* 2008). Furthermore, this supports findings in previous studies which have also shown the relatively limited effectiveness of steady-state models for predicting BSAFs within river food webs (van Beusekom *et al.* 2006). Trait-models, however, were only able to predict BSAFs with approximately an order of magnitude accuracy, and large variation in BSAFs were observed across the wider suite of river food webs. This points towards exogenous drivers of variation in the bioaccumulation of POPs across these food webs. The unexplained variation is likely to result from differences in the bioavailability of POPs across sites or significant differences in the structure of the food webs. Thus, it is suggested

that the trait-based models presented here explained existing variation in bioaccumulation associated with biological traits, yet environmental variation and remaining broad-scale biological variation, relating to food web structure, perturbed accurate predictions.

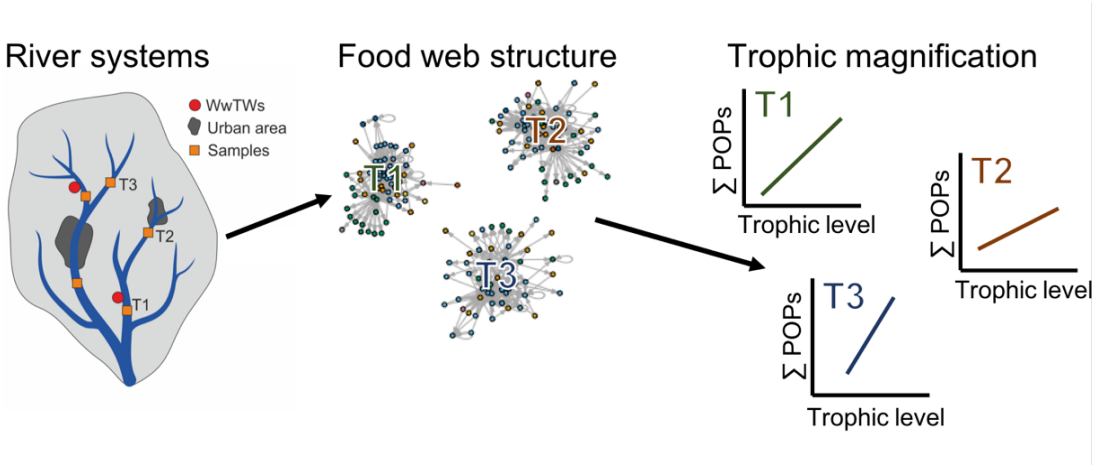
Although proving to be only marginally better than existing methods in this study, the development of trait-based analyses is important, with significant benefits derived from the implementation of such analytical frameworks. Firstly, modelling with invertebrate traits, that are conserved across continental scales (Statzner & Bêche 2010), provides a potential technique for large scale monitoring of the ecological risk from contaminants. Based on the assumption that taxa with similar biological traits respond similarly to pollutant exposure, the problems associated with highly variable regional taxonomy, and thus challenges in estimating risk for individual species, could be avoided. Secondly, such methods present an opportunity to develop pre-emptive monitoring tools to indicate the potential risk of bioaccumulation or ecological effects for organisms with specific combinations of biological traits. The development of such tools may contribute to important next steps in improving assessments of risk relating to chemicals in advance of their introduction to the environment (see Godfray *et al.* 2019). More research, nevertheless, is required to understand just how widely applicable trait-based methods are for the prediction of bioaccumulation and/or ecological risk across different systems.

5.6. Conclusions

In summary, findings from this study demonstrate the importance of transfer pathways and biological traits in influencing the bioaccumulation and trophic magnification of pollutants across a riverine food web. Specific groups of chemicals were shown to accumulate differentially, in response to a variety of resources and transfer pathways within the food web. Magnification was observed for all compounds, yet biological traits influenced the relationship between trophic level and observed bioaccumulation – indicating that organisms occupying the same trophic level may be differential exposed to POPs. Trait-based models constructed with data from a single river food web, were shown to predict relatively accurately the bioaccumulation of POPs

across multiple samples sites in South Wales. The trait-based model required less prior knowledge compared to other bioaccumulation models, such as AQUAWEB, and was based on publicly available trait data for freshwater invertebrates. This study demonstrates the importance of biological traits for the trophic transfer of pollutants and indicates the potential power of trait-based analyses for the prediction of food web scale ecotoxicological processes.

Chapter 6: Trophic magnification of persistent organic pollution across river ecosystems in South Wales



Data collected and analysed in this chapter is the foundation for a manuscript in preparation. FMW collected the majority of data, however, data from Eurasian dipper (*Cinclus cinclus*) eggs were provided by Christy Morrissey (CAM). Chemical analyses were supervised by MGP. FMW wrote the manuscript and revisions and comments on subsequent iterations were provided by MGP, SJO and CRT.

6.1. Abstract

1. The bioaccumulation and biomagnification of legacy persistent organic pollutants (POPs) continues to affect freshwater ecosystems yet understanding across ecosystems with different environmental conditions and biological communities, remains limited.
2. In this study, the bioaccumulation and trophic magnification of PBDEs, PCBs and a suite of OCs, were assessed across nine river food webs in contrasting hydrological catchments in South Wales (United Kingdom).
3. POPs magnified through all food webs to reach hazardous concentrations in the tissues of invertebrates, fish and river birds. Different pollutants accumulated and magnified to different levels, depending on their characteristics (e.g. octanol-water partitioning coefficient), their partitioning across different basal resources (e.g. detritus versus biofilms), the biological traits of organisms and site-specific environmental conditions.
4. The magnification of PBDEs, PCBs and OCs also reflected food-web structure, with greater connectance associated with high levels of trophic magnification. This effect may result from the dominance of generalist taxa in highly connected food webs. These taxa use a wider range of primary and secondary resources and subsequently increase the transfer of contaminants.
5. Data highlight the role of interactions between pollutant characteristics, environmental conditions and biological network structure in altering the transfer and magnification of POPs in river ecosystems. Further investigations of system-specific transfers of contaminants through aquatic food webs are now required as these factors appear to have important implications for the potential risk presented by persistent and bioaccumulative pollutants, such as POPs.

6.2. Introduction

Xenobiotic pollutants are a diverse, widely distributed range of synthetic chemicals associated with significant environmental risks for aquatic ecosystems (Petrović *et al.* 2004; Malaj *et al.* 2014). These pollutants exert harmful effects through a wide range of biological mechanisms, with many associated with the disruption of the endocrine system in exposed organisms (Tyler, Jobling & Sumpter 1998; Solecki *et al.* 2017). The effects of endocrine disruption in aquatic systems, however, are not confined to individual organisms, and there is strong evidence for significant negative effects across populations, communities and ecosystems (Chapter 2). Persistent organic pollutants (POPs), in particular, generate effects at higher levels of biological organisation (Hutchinson *et al.* 2013), and these hydrophobic pollutants accumulate and magnify to hazardous internal concentrations in aquatic organisms through a combination of trophic and non-trophic interactions (Chapter 2).

Although the production and use of many POPs of high concern has discontinued, including PCBs, PBDEs and OCs, their release into global environments continues through the recirculation of historical pollution and in some cases a continued low-level release of chemicals (e.g. DDTs, endosulfan, aldrin, hexachlorocyclohexanes) (Isogai *et al.* 2018). As a consequence, the concentrations of POPs in some aquatic environments are still sufficient to pose an ecological risk through toxic biological effects (Konwick *et al.* 2006; Koenig, Huertas & Fernández 2013; Sun *et al.* 2015; Kim *et al.* 2015). Although POPs are present at relatively low levels in the freshwater environment compared to current-use and emergent chemicals (e.g. pyrethroid and systemic pesticides, pharmaceuticals, personal care products), recent assessments indicate that these chemicals remain an important constituent of the total pollutant load in rivers and lakes (Rasmussen *et al.* 2015; McKnight *et al.* 2015).

The persistent and hydrophobic nature of many of legacy POPs means that even when present in low concentrations in sediments, soils, water and the atmosphere, there is still a significant potential for them to bioaccumulate and biomagnify to cause ecological effects (Hutchinson *et al.* 2013). A range of

biological factors can influence the accumulation of persistent pollutants in aquatic organisms. Firstly, biological traits influence the exposure of organisms to pollutants (Sidney *et al.* 2016; Chapter 5). As an illustration, organisms occupying depositional habitats are more likely to be exposed to hydrophobic chemicals as a result of their close contact with organic matter and fine sediments which accumulate POPs (Leppänen, 1995; Chapter 5). Secondly, the feeding strategies of organisms at lower trophic levels can affect the assimilation of these compounds through the partitioning of pollutants among different basal resources that alter the dietary transfer of POPs to primary consumers (Kukkonen & Landrumb, 1995; Chapter 5). Third, the trophic level of an organism influences its dietary exposure to POPs, with those at higher trophic levels generally at greater risk, due to the magnification of hydrophobic pollutants within food webs (Russell, Gobas & Haffner 1999; Ross & Birnbaum 2003). Finally, the biological or ecological characteristics of individual organisms that affect internal POP exposure might also be influenced by the structure of the wider ecological network – such as the nature and strength of interactions within a food web. Examples include trophic (predator-prey) or non-trophic (e.g. competition or facilitation) interactions which could potentially alter the flux of persistent pollutants through ecological networks (Berlow *et al.* 2004).

Evidence that the general structure of biological communities might influence POP transfers and distribution comes from the variable biomagnification of POPs across different food webs (Kelly *et al.* 2007). An extreme example is where different chemicals bioaccumulate and magnify because the presence of air-breathing taxa interacts with pollutant solubility (air and water octanol partitioning coefficients, K_{OA} and K_{OW}, respectively) to reduce respiratory elimination thus altering the relative magnification of chemicals such as PCB-153 and β-HCH in terrestrial and aquatic food webs (Kelly *et al.* 2007). A further example, across more similar ecosystems, is observed in marine food webs with significant relationships between food-chain length, tissue lipid content, and organism-specific feeding ecology and the levels of trophic magnification (Borgå *et al.* 2004). Existing studies in freshwater systems have also demonstrated the importance of ecological constraints on the distribution of

PCBs within aquatic organisms (Walters *et al.* 2008). The broad structure of ecological networks (e.g. connectance, link density, chain length, generality, vulnerability) and their constituent characteristics (e.g. species composition and ecology), are therefore likely to influence the magnification of bioaccumulative pollutants in freshwater environments. There is, however, only limited research comparing the accumulation and magnification of POPs across different aquatic food webs, despite the potential importance of such information to risk assessment (Chapter 2).

In this chapter, the levels of bioaccumulation and trophic magnification of POPs are investigated across complex aquatic food webs in contrasting river catchments across South Wales (UK). For nine sites with different environmental conditions, samples from sediments, microbial biofilms, macroinvertebrates, benthic fish and river birds were collected to investigate the trophic transfer of PCBs, PBDEs and OCs. To understand the relative importance of food web structure, trophic dynamics, pollutant characteristics and environmental conditions in modifying the accumulation, trophic magnification and ecological risk of POPs, three hypotheses were tested:

1. POP bioaccumulation and biomagnification are different between organisms in river food webs
2. The levels of bioaccumulation and biomagnification are different across sites in contrasting catchments
3. The trophic magnification of POPs is related to the structure of river food webs, pollutant characteristics and site-specific environmental conditions

6.3. Material and methods

6.3.1. Sample sites

The study was conducted across river systems in South Wales (see Fig. 6.1) at sites distributed across the catchments of the Rivers Taff, Usk and Wye. Among these three, the Taff is dominated by urban and industrial land cover (~20%), whereas the Wye and Usk catchments have more pastoral agriculture (1.6–5.1%). At smaller scales, land-use heterogeneity gives rise to potential variations in pollutant sources among sites, and between groups of sites located in different catchments (Appendix S11). Using multiple sites across

catchments provides different environmental contexts (Burdon *et al.* 2016) which may alter pollutant accumulation and magnification.

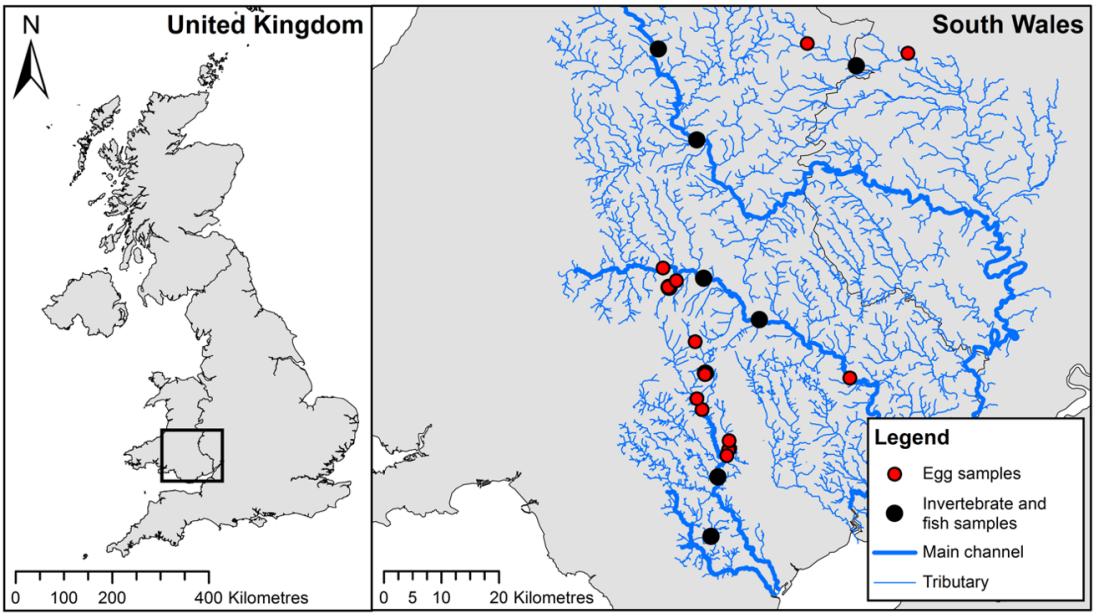


Fig 6.1. The location of sample sites across South Wales. Sample sites for invertebrates and fish samples (black; n = 9), as well as dipper egg samples (red; n = 12) located across the Taff, Usk and Wye catchments.

Sediments, biofilms, invertebrates (Heptageniidae, Baetidae, Rhyacophilidae, Gammaridae, Perlodidae, Leuctridae and Ephemerellidae) and European bullheads (*Cottus gobio*) were collected, when present, from the nine sample sites (2015–2016) across the three catchments to represent a gradient of agricultural and urban land use, as well as differences in effluent contribution from wastewater treatment works (WwTWs). Because of their more dispersed breeding distribution, egg samples from Eurasian dipper (*Cinclus cinclus*) were collected more opportunistically (under licence from NRW) from adjacent breeding sites across the three catchments in 2008–2010 (see Morrissey et al. 2013). Freshly collected samples were placed in acetone-hexane (1:1 v/v) rinsed glass vials and stored at –80 °C until chemical analyses. Specific details of the collection protocols for each sample (sediment, biofilm, invertebrate, fish and bird egg) are in Appendix S7.

6.3.2. Chemical analyses

See Chapter 4, Section 4.3.3.

6.3.3. Food web construction

Aquatic food webs were constructed from community data at each sample location collected monthly over the years 2016–2017 based on 97,308 individuals from 139 invertebrate taxa (Chapter 7). An empirical method, following Gray *et al.* (2015), was used to assemble food web structures based on a database of field data from other observed trophic interactions (isotope, observational and dietary analyses) across the United Kingdom. This method has been shown to generate accurate food webs for rivers across the UK, including those in Wales (Gray *et al.* 2015). Once food webs had been constructed, the trophic level of organisms within this study were extracted for further analysis. Food web metrics were then quantified from these data to assess the influence of food web structure on bioaccumulation and biomagnification. Quantitative food web metrics were calculated for individual food webs (n = 9) using the density of organisms in sample sites (n individuals m^{-2}), collated from monthly benthic community samples collected over 2016–2017 (Chapter 7). The key metrics used in this study were connectance (ratio of observed links to potential links), link density (the number of links per node), maximum chain length (the number of links in the longest chain) and mean chain length (the average number of links in chains across the food web) (Warren 1994; Bersier, Banašek-Richter & Cattin 2002).

6.3.4. Statistical analyses

All statistical analyses were completed using R Statistical Software (version 3.4.0) (R Core Team 2015). Concentrations of individual POPs and their congeners were calculated on a wet weight (ww) and lipid weight (lw) basis. Concentrations are reported as wet weight due to the relatively low variation in lipid concentrations in the organisms studied (0–1.62% lipid), with the exception of *Cinclus cinclus* (3.73–14.17% lipid). Wet weight concentrations were also used to assess food web transfers and body burdens directly following Ross and Birnbaum (2003). All data were initially explored, using the framework by Zuur, Ieno and Elphick (2010), to understand the structure of

POP concentration data (heteroscedasticity, normality, outliers) and to inform the selection of further statistical tests and models.

The bioaccumulation of different classes of POPs (PBDEs, PCBs and OCs) in the tissues of organisms from food webs were estimated using the Biota to Sediment Accumulation Factor (BSAF; Ankley *et al.* 1992), calculated following Equation 5.1.

The trophic transfer and magnification of persistent pollutants within river food webs were analysed using a combination of organism and food web level biomagnification factors. To identify the extent of biomagnification within individual riverine taxa across different food webs, biomagnification factors (BMFs) were calculated for taxa. BMFs were calculated following Equation 5.2.

To assess the levels of trophic magnification across the entire food web, trophic magnification factors (TMFs) were calculated following Fisk et al. (2001) and Walters et al. (2011), see Equation 6.1 and 6.2:

(6.1.) $$\log10\ POP_{ww} = b + (m \times TL)$$

(6.2.) $$TMF = 10^{m}$$

The slope (m) of the relationship between the log10 transformed xenobiotic pollution concentration (ng g^{-1} ww) and trophic level is used to calculate TMF. This analysis was completed for all congeners and compounds detected in over 50% of all biotic samples, as well as total PBDEs, PCBs and OCs (Appendix S8).

Generalised Linear Mixed Models (GLMMs) (Bolker *et al.* 2009), were used to assess the potential trophic influences on the bioaccumulation and biomagnification of xenobiotic pollutants across sampled food webs. All models were fitted using the 'lme4' package (Bates *et al.* 2015). Random effects, where necessary, were used to account for confounding variation associated with variables not of interest within this study, e.g. site-specific environmental conditions. Model validation, used to assess model validity and accuracy, followed the procedures detailed within Zuur *et al.* (2007) and Thomas *et al.* (2015). Residual normality was assessed using QQ plots, homogeneity of variance was determined by plotting residuals against fitted values and influential observations were investigated using Cook's leverage

distances. Only models satisfying the above criteria are presented in the following results section.

6.4. Results

6.4.1. Taxonomic differences in bioaccumulation and magnification

Detection frequency for many congeners was low (<20%; Appendix S8) and as a consequence, the concentrations for all congeners were pooled within classes (PCBs, PBDEs, OCs) for several of the following analyses, with the exception of TMF analyses which were completed for frequently detected compounds (>50% samples).

The total concentrations of persistent organic chemicals (PBDEs, PCBs and OCs) varied across sampled organisms (Table 6.1), largely reflecting significant differences between sample media (sediments, biofilms, invertebrates, fish and birds), as well as between broad taxonomic groups ($R^2 = 0.92$, $F_{11,66} = 85.77$, $p < 0.001$). In particular, sediment/detritus and microbial biofilms (basal resources) had significantly lower concentrations of persistent pollutants than invertebrates and fish across all of the river food webs ($F_{11,66} = 5.14$, $p < 0.001$; Table 6.1).

The levels of bioaccumulation, as described by the BSAF values, were also highly variable across organisms, but differed also across the sample sites and broad classes of POP chemicals ($R^2 = 0.63$, $F_{30,151} = 11.16$, $p < 0.001$). Variation in BSAFs between organisms was related to the feeding guild of the organism (predator, shredder, grazer, filterer) as well as their overall trophic position (chain-averaged trophic level) within the food web. Predatory organisms, in general, had higher BSAF values, with a significant positive relationship observed between trophic level and BSAFs ($F_{1,167} = 74.92$, $p < 0.001$). Further to this, differences in BSAF values were observed between other broad feeding guilds; biofilm grazers, detritus shredders and filterers ($F_{3,168} = 49.09$, $p < 0.001$).

Table 6.1. Concentrations of persistent organic pollutants within compartments of riverine food webs [Mean (min-max)].

Sample	n	% Lipid	Concentration (ng g^{-1} ww)						
			∑PCBs	∑PBDEs	p,p'-DDT	p,p'-DDE	TDE	HCB	HEOD
Sediment	9	<0.01 (0.00-0.91)	0.31 (ND-2.73)	0.03 (ND-0.23)	ND	1.09 (ND-1.58)	ND	0.20 (0.15-0.25)	1.68 (ND-5.15)
Microbial biofilm	9	<0.01 (0.00-0.04)	0.63 (ND-5.70)	0.64 (ND-1.85)	1.05 (ND-1.56)	0.44 (ND-1.04)	0.02 (ND-0.18)	0.02 (ND-0.19)	2.06 (ND-11.53)
Baetis spp.	7	0.05 (0.09-0.15)	9.16 (ND-36.28)	0.77 (0.07-2.80)	1.06 (ND-1.67)	3.74 (1.55-6.39)	0.67 (ND-0.93)	0.27 (0.15-0.48)	6.91 (ND-10.72)
Serratella ignita	1	<0.01	ND	0.68	ND	1.80	ND	0.15	7.07
Ecdyonurus spp.	8	0.12 (0.03-0.22)	0.93 (ND-2.94)	0.63 (0.12-1.65)	1.35 (ND-2.79)	3.08 (0.97-7.74)	0.02 (ND-0.16)	0.21 (0.15-0.46)	6.16 (0.39-14.44)
Leuctra spp.	3	0.16 (0.12-0.19)	0.59 (ND-1.77)	1.31 (0.68-1.96)	ND	3.39 (1.72-5.22)	0.07 (ND-0.19)	0.05 (ND-0.17)	14.94 (3.00-38.69)
Gammarus pulex	8	0.07 (0.00-0.27)	1.41 (0.37-2.90)	1.16 (0.19-3.59)	3.96 (0.56-8.72)	3.55 (1.23-6.31)	1.31 (0.45-2.53)	0.20 (0.16-0.26)	13.45 (9.03-24.62)
Hydropsyche spp.	8	0.75 (0.12 - 4.44)	0.99 (ND - 3.11)	2.36 (0.67-7.60)	ND	2.58 (ND-5.81)	0.02 (ND-0.18)	0.27 (0.16-0.46)	2.65 (ND-7.29)
Rhyacophila dorsalis	8	0.13 (0.06 - 0.25)	2.13 (ND - 5.82)	1.28 (0.52-3.88)	1.73 (ND-2.38)	3.62 (0.73-7.63)	0.49 (ND-0.61)	0.22 (0.19-0.30)	9.18 (0.93-22.03)
Isoperla grammatica	1	0.13	15.85	0.16	ND	1.12	ND	0.15	11.94
Cottus gobio	7	0.95 (0.57 - 1.62)	9.56 (1.24 - 44.10)	3.60 (8.26-12.74)	13.26 (1.45-42.62)	14.87 (3.10-46.34)	3.37 (ND-0.53)	1.79 (ND-2.74)	136.41 (47.16-277.87)
Cinclus cinclus	12	7.74 (3.73 - 14.17)	278.90 (29.24 - 1440.28)	123.89 (12.20-729.76)	57.36 (ND-331.02)	478.58 (ND-1725.84)	3.03 (ND-12.25)	19.54 (ND-105.95)	9.89 (ND-16.57)

Table 6.2. Estimated BMFs for organisms across river food webs.

Organism	Feeding guild	BMF		
		∑PBDEs	∑PCBs	∑ OCs
Baetis spp.	Grazer	4.04 (1.31-16.53)	1.30 (0.00-3.17)	7.48 (0.76-16.50)
Serratella ignita	Grazer	8.34	0.00	8.95
Ecdyonurus spp.	Grazer	4.37 (0.62-10.89)	6.48 (0.00-13.20)	4.14 (0.42-10.60)
Leuctra spp.	Shredder	14.02 (1.56-29.98)	0.43 (0.00-1.30)	15.34 (1.56-41.63)
Gammarus pulex	Shredder	13.08 (1.46-40.96)	13.05 (0.22-23.23)	12.78 (2.52-39.22)
Hydropsyche spp.	Filterer	8.39 (2.22 - 19.89)	2.11 (0.00 - 7.03)	1.33 (0.25 - 4.58)
Rhyacophila spp.	Predator	10.42 (1.25-25.96)	28.57 (0.20-78.08)	4.04 (0.80-11.88)
Isoperla spp.	Predator	0.61	10.75	2.86
Cottus gobio	Insectivorous	22.93 (10.51-45.09)	21.30 (2.41-47.41)	35.61 (12.66-77.30)
Cinclus cinclus	Insect- and pisc-ivorous	53.07 (4.49-206.79)	247.78 (34.92-546.01)	163.17 (0.01-428.61)

Data are reported as mean (min-max). Further details on the site-specific variation in BMFs are reported in Appendix S12.

All classes of POPs biomagnified across all of the sampled river food webs (Fig. 6.2). As a result, organisms at higher trophic levels consistently accumulated greater concentrations of persistent pollutants, including PCBs, PBDEs and OCs ($R^2 = 0.56$, $F_{17.218} = 18.52$, $p < 0.001$). BMF values, however, varied between taxa, reflecting the position of organisms within the food web ($R^2 = 0.70$, $F_{13,41} = 10.88$, $p < 0.001$; Table 6.2). Specifically, BMFs differed across organism feeding guilds ($F_{4,41} = 29.25$, $p < 0.001$) and were also significantly positively related to the trophic level of the organism ($F_{1,41} = 8.28$, $p = 0.004$). As a result, vertebrate predators exhibited the greatest levels of biomagnification with the highest BMF values (109.68 ± 18.99), followed by invertebrate predators (11.51 ± 3.72) and shredders (12.12 ± 3.35) with intermediate BMF values, and finally followed by the lowest BMF values calculated for grazer and filter feeding invertebrates (5.58 ± 1.06 and 1.59 ± 0.35, respectively).

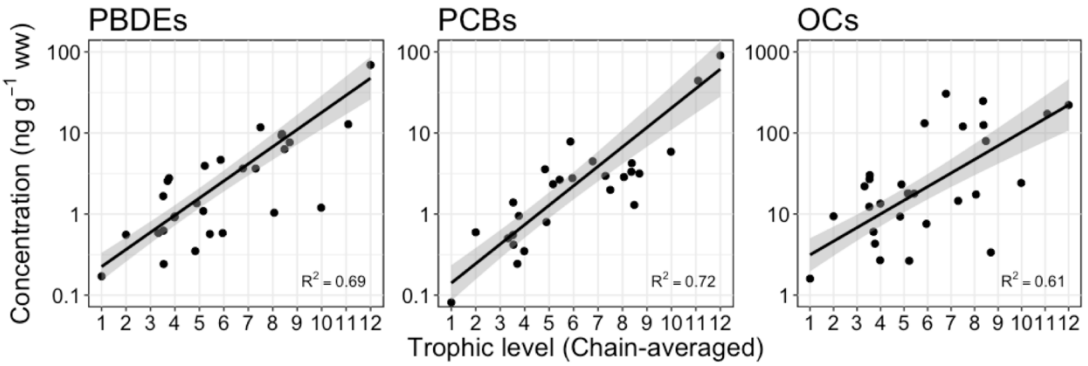


Fig. 6.2. Relationship between chain averaged trophic level and mean concentration of PBDEs, PCBs and OCs for organisms across all sampled river food webs. (A) Mean ΣPBDE concentrations. **(B)** Mean ΣPCB concentrations. **(C)** Mean ΣOC concentrations. Chain-averaged trophic levels were derived from food web models (see Section 6.3). Data are aggregated per trophic level, across all food webs. Adjusted R^2 values for PBDEs, PCBs and OCs are derived from separate post-hoc linear models.

6.4.2. Food web structure, bioaccumulation and magnification

The magnification of POPs at the food web scale, as reflected by TMF values, was related to quantitative food web metrics. TMF values increased with food-web connectance, though the variation in TMF values explained was relatively small (R^2 = 0.11, $F_{1,43}$ = 4.53, p = 0.039; Fig. 6.3). The strength of the relationship between TMF values and connectance, however, was different for different PBDE and PCB congeners, as well as OC chemicals (R^2 = 0.20, $F_{39,130}$ = 2.08, p = 0.001). A number of chlorinated compounds, for example several PCB congeners (138 and 153) and OCs (p,p'-DDD and dieldrin), had stronger relationships between the connectance of the food web and the level of trophic magnification ($F_{19,130}$ = 1.95, p = 0.015).

TMF values were not significantly related to any other food web metrics. However, TMFs appeared to be non-linearly related to chain length (both mean and maximum), food webs with intermediate mean and maximum chain lengths supporting the highest levels of trophic magnification (Fig. 6.3). The

highly variable pollutant-, organism- and site-specific effects on the levels of bioaccumulation and biomagnification (see Section 6.4.1) appeared to obscure any further relationships between TMF and the overall structure of food webs across river systems (Table 6.1 and 6.2).

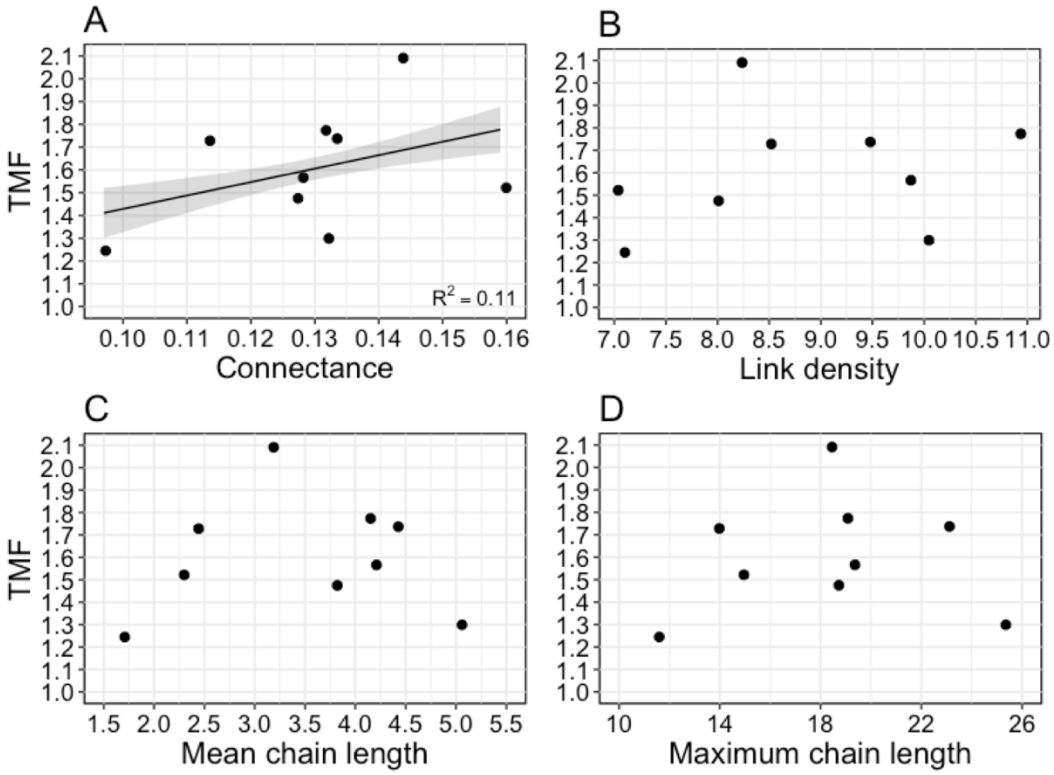


Fig. 6.3. Relationships between food web metrics and TMFs. Mean TMF estimates for PCBs, PBDEs and OCs are presented. **(A)** Connectance of the food web. **(B)** Link density – links per node for food webs. **(C)** Mean and **(D)** Maximum chain length for food webs.

6.4.3. Bioaccumulation and magnification between different POPs

Alongside significant variation between sample organisms and across sample sites, BSAFs were higher for some classes of POPs than others (R^2 = 0.68, $F_{29,146}$ = 103.50, $p < 0.001$; $F_{2,146}$ = 6.25, $p = 0.003$). In general, PCBs and PBDEs had the highest BSAF values, whereas OCs had low accumulation factors as a result of higher concentrations in the basal resources (sediments and biofilms; Table 6.1).

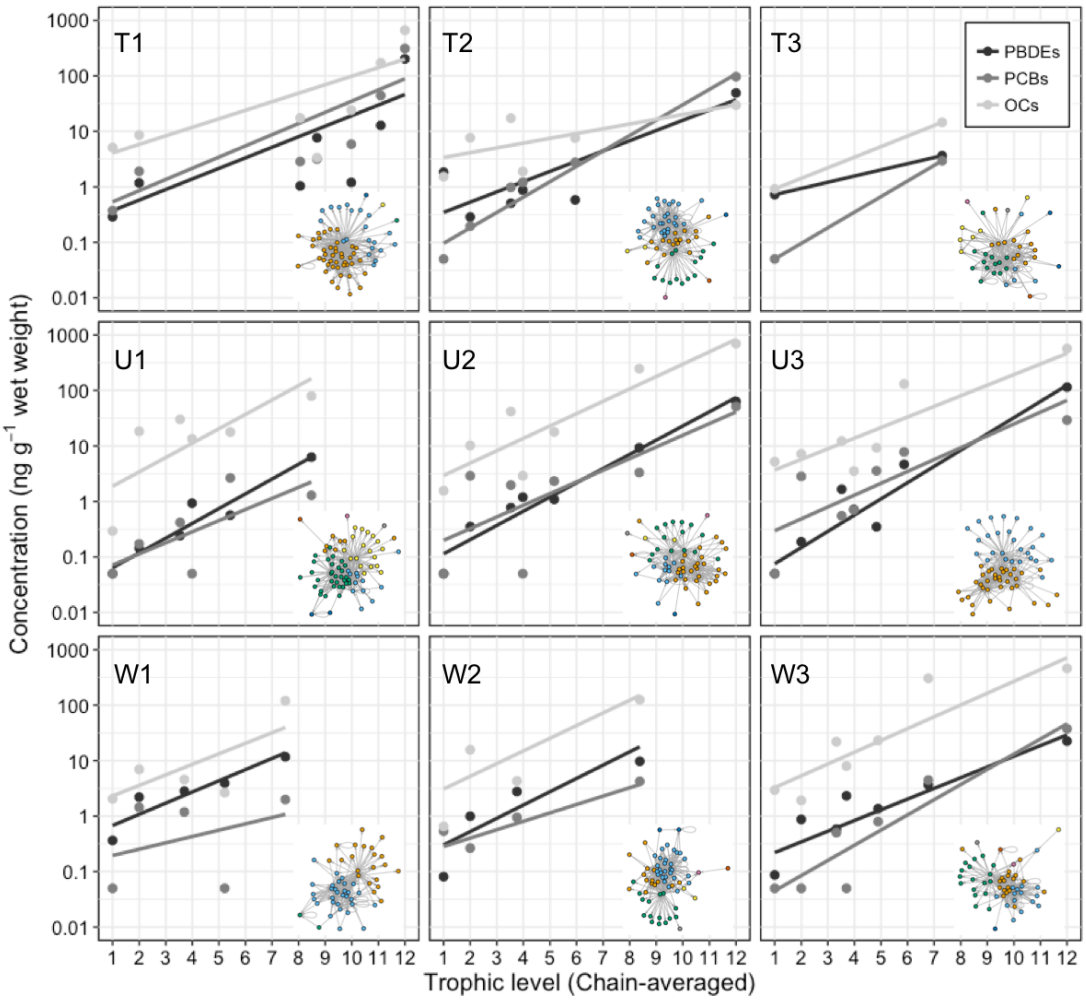


Fig. 6.4. Relationship between trophic level and concentration of PBDEs, PCBs and OCs in organisms across individual river food webs. Site codes are detailed in Fig. 6.1 and Section 6.2. Food webs diagrams represent the invertebrate compartment within river systems and were constructed from taxonomic data collected monthly (2016–2017).

The relationship between organism trophic levels and POP concentrations, which represents the level of magnification in the food web, was not significantly different for pollutants ($F_{8,218}$ = 4.12, p = 0.709), such that different chemicals (PBDEs, PCBs and OCs) did not appear to be consistently magnified to a greater or lesser extent across different sample sites. Nevertheless, there appeared to be variation in the levels of magnification of several pollutants between sites (Fig. 6.4).

The levels of trophic magnification of POPs were also different between PBDE and PCB congeners, and OC chemicals (R^2 = 0.19, $F_{19,150}$ = 1.87, p = 0.021). The highest TMF values across food webs were observed for PCB-153 and 138, BDE-47 and p,p'-DDE (Appendix S12). Although significant differences were observed between POPs, this did not appear to be due to their chemical structure with log K_{OW} values for several widely detected POP congeners (Appendix S8), not related to TMF values across the river food webs (R^2 = 0.28, $F_{5,12}$ = 0.87, p = 0.53). The relationship, although not significant, was nonlinear with increases in TMF values up to log K_{OW} values of approximately 6.5–7.25 then subsequent decreases for higher log K_{OW} values (Fig. 6.5). The low detection frequencies of many PCB and PBDE congeners, and OC chemicals, at the extremes of the potential range of log K_{OW} values (<5.0 and >7.5), however, restricted the total sample size of pollutants available for statistical analysis.

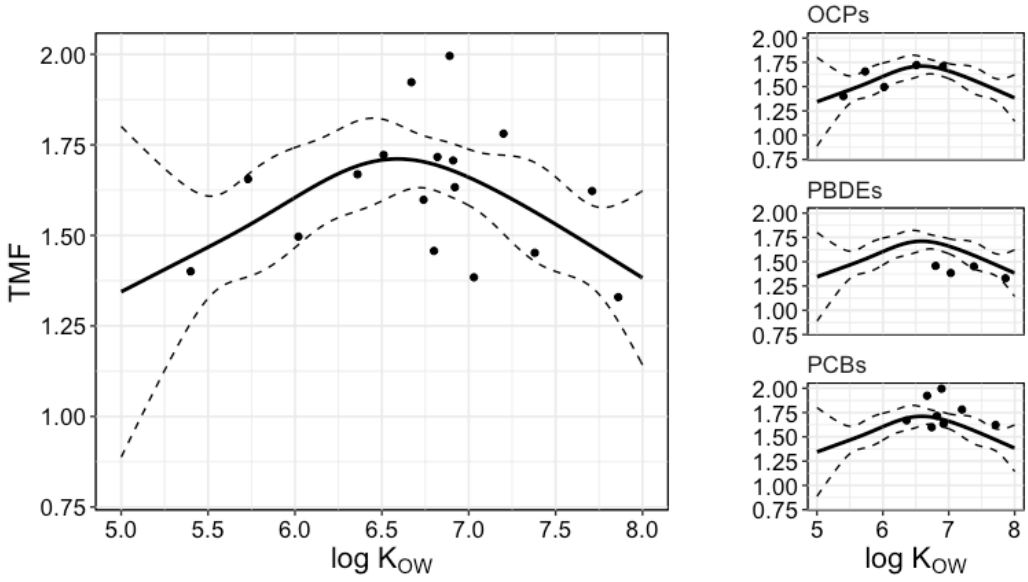


Fig. 6.5. Relationship between TMF and log K_{OW} for persistent pollutants. Labelled plots indicate the relationships for individual POP classes (PBDEs, PCBs and OCs). GAM results were not significant (R^2 = 0.28, $F_{5,12}$ = 0.87, p = 0.53), yet the non-linear relationship conforms to previous research. Points represent congener-specific mean TMFs, summarised for the sampled river food webs in South Wales.

6.4.4. Spatial variation in bioaccumulation and magnification

The bioaccumulation of POPs was variable among different sample sites, although as shown previously, some POP groups had ubiquitously high levels of bioaccumulation than others (R^2 = 0.72, $F_{29,146}$ = 77.61, $p < 0.001$). There was an interactive effect of chemical class and sample site on BSAFs for some POP compounds – with some chemicals having higher BSAFs in certain sample sites than others ($F_{16,146}$ = 3.09, $p < 0.001$). For example, BSAF values for PCBs and PBDEs were greater for biota at sites in both the Wye and Usk catchments, whereas OC BSAF values were the highest in food webs in the Taff and Usk catchments. The marked differences in BSAFs between sample sites appeared to be driven by the low environmental concentrations of POPs at some sites (e.g. T3, U1, W1; Fig. 6.3). The concentrations of PBDEs in sediments across the Usk and Wye catchments (0.23 ± 0.21 ng g^{-1} ww) were relatively low compared to those detected across sediment samples from sites the Taff catchment (8.87 ± 7.15 ng g^{-1} ww), yet concentrations measured in organisms were not markedly different between these catchments. This generated high BSAF values for PBDEs across all organisms occupying the Wye and Usk (762.17 ± 411.23).

Across the majority of sample sites, relationships between the tissue concentrations of POP compounds (PBDEs, PCBs and OCs) and the trophic level of organisms were relatively similar ($F_{8,48}$ = 13.98, p = 0.056; Fig. 6.3). At two sites (U2 and W3), however, the relationships between PBDE and OC concentrations and trophic level were stronger, indicating a greater level of magnification in these river food webs (PBDEs $t_{8,48}$ = 2.28, p = 0.027; OCs $t_{8,48}$ = 2.17, p = 0.035; Fig. 6.3 and Appendix S12).

6.5. Discussion

Trophic magnification of POPs across food webs was significant, generating PBDE, PCB and OC body burdens indicative of potential ecological risks. Bioaccumulation and magnification of POPs was commonplace, and the magnitude of these processes was related to food web characteristics (e.g. connectance), pollutant characteristics and environmental conditions across sample sites. The relationships identified between food web connectance and

TMFs indicate that the structure of the food web may provide a key role in determining the ecological risk of POPs across the aquatic ecosystems. The mechanism underpinning this relationship, however, remains uncertain. Other potentially influential factors, including the chemical structure of POP compounds (e.g. log K_{OW}), did not appear to explain a significant proportion of variation in the levels of bioaccumulation and biomagnification. Yet log K_{OW} values were constrained, as only bioaccumulative POP compounds were measured across organisms (log K_{OW} = 5.5–8.0). Variation in food web structure, environmental concentrations of POPs and site-specific environmental conditions, however, appear to be responsible for confounding any such relationships. In spite of the significant variation in the levels of magnification between sample sites, the overall levels of magnification from basal resources to apex predators was relatively high and the concentrations observed in the tissues of organisms indicates the potential for adverse ecological effects.

Field-based assessment of the transfer and magnification of pollutants across multiple food webs in field-based studies are influenced by several assumptions. Firstly, that target organisms are a representative subsample of the total food web used for the analysis of food web structure and function (Chapter 7); in reality, the sample collected may not necessarily represent the total taxonomic and functional diversity present within systems. Secondly, the diversity of organisms (n = 10) and sites (n = 9) used for assessments was limited relative to the total taxonomic diversity present river systems (albeit this was greater than previous studies on river food webs assessing multiple pollutants, e.g. Ruhí *et al.* 2015). The lack of taxonomic coverage is a pervasive limitation of ecotoxicological research and limits accurate assessments of ecological risk across ecosystems (Chapter 2). Finally, the calculation of TMFs based on aggregated congener concentrations has previously been highlighted as problematic (Fisk, Hobson & Norstrom 2001), due to the aggregation of compounds with different chemical structures (e.g. log K_{OW}) values. Yet, in this study, detection frequencies of individual congeners were relatively low, with approximately 10% of congeners detected in over 50% of samples. These low detection frequencies meant it was

particularly problematic constructing linear models to estimate TMFs (but see Appendix S12 for congener-specific TMFs), yet it was observed that congener-specific TMF values for POP groups (PBDEs, PCBs and OCs) were well represented by the aggregated TMF values and it was therefore deemed suitable to use aggregated values for comparisons of trophic magnification between food webs.

The processes of bioaccumulation and biomagnification generated up to a thousand-fold increase in the concentrations of POPs from basal resources (sediments and biofilms) to apex riverine predators (*Cottus gobio* and *Cinclus cinclus*). Both the accumulation and magnification of pollutants, however, was highly variable between taxa, pollutants and sample sites. These findings support existing findings that indicate the influence of organism-specific biological traits on the transfer and accumulation of pollutants in aquatic ecosystems (Segner 2011; Sidney *et al.* 2016). Here, however, it was shown the highly variable nature of pollutant transfers across different river systems in contrasting catchments. It is apparent that the variable environmental conditions (water quality, hydrology, geomorphology), biological communities, as well as sources and environmental concentrations of pollutants, may influence the accumulation and magnification of pollution. Such findings indicate that biological and environmental variation across different ecosystems may generate differential ecological effects and risk from toxic bioaccumulative pollutants in natural systems, posing challenges for subsequent monitoring and management.

Food web TMFs observed across river systems within this study (1.3–2.0), however, were lower than those recorded for POPs within other reported ecosystem for lakes and marine environments; 1.5–13.7 (Fisk, Hobson & Norstrom 2001; Borgå *et al.* 2004; Walters *et al.* 2011). Nevertheless, the figure corresponds well with the mean TMF of 1.6 calculated in river food webs for ΣPCBs by Walters *et al.* (2008) and for a range of other persistent pollutants, including heavy metals, organochlorine pesticides, PCBs and polycyclic aromatic hydrocarbons (0.33–3.75) (Penland *et al.* 2018). PBDEs have received less attention with regards to biomagnification, yet the low TMFs for PBDEs, relative to PCBs and OCs, have also been observed

previously with relatively low PBDE TMFs in marine systems in comparison to other recalcitrant POPs (e.g. PCBs) in marine food webs (Kelly *et al.* 2008).

Potential explanations for the range, and variation, in TMF values observed across the river food webs examined in this study, include: (1) average chain lengths of sampled food webs; (2) shorter-lived organisms; (3) low environmental concentrations of persistent contaminants and differences between POP groups (PBDEs, PCBs and OCs); and (4) the presence of both aquatic and air-breathing taxa. Firstly, the river food webs appear to be relatively short (mean chain length = 2.1; Trophic levels = 4.0) compared to the longer chain lengths and greater number of trophic levels in food webs (mean chain length = 3.5; Trophic levels = 4.0–5.0) across marine studies (Fisk, Hobson & Norstrom 2001; Hop *et al.* 2002; Hoekstra *et al.* 2003; Borgå *et al.* 2004), as well as other aquatic systems across the globe (Vander Zanden & Fetzer 2007). Such broad characteristics of food webs have been previously identified as potential drivers of differences in the trophic magnification of POPs across food webs (Walters *et al.* 2008). Secondly, organisms within the riverine food webs had far lower lipid concentrations and consisted of relatively short-lived organisms (1–10 years), compared to those in terrestrial and marine ecosystems, for example the longer-lived terrestrial and marine macro-fauna such as cetaceans and pachyderms (10–80 years). Longer-lived organisms are exposed to bioaccumulative pollutants for a longer duration and thus accumulate greater internal concentrations (Borgå *et al.* 2004). Thirdly, the environmental concentrations of pollutants observed within this study are relatively low (Table 6.1), yet extremely heterogeneous across the sampled river systems (Chapter 4). Such spatial variation may lead to inconsistencies and differences in the calculated magnification across river food webs. Finally, the variability in the magnification of different POPs, and relatively low TMF values for some OCs and PBDE congeners (e.g. HEOD [1.4], TDE [1.5], BDE-99 [1.45], BDE-47 [1.46]), may result from differences in the characteristics of the river food webs assess herein, with the presence of both aquatic and air-breathing taxa potentially leading to differences in magnification, in comparison to food webs with either all aquatic or all air-breathing, terrestrial taxa (Kidd *et al.* 2007).

Findings indicate that differences in the structure of food webs, specifically the levels of connectance, might influence the level of POP magnification observed in river ecosystems. This suggests that, as well as the previously observed differences between markedly different food webs (e.g. marine-freshwater and aquatic-terrestrial (Kelly *et al.* 2007; Walters *et al.* 2008)), differences in the structure of food webs within the same ecosystems potentially affect the trophic magnification of persistent pollutants. The exact mechanism responsible for the observed correlation between food web connectance and trophic magnification is uncertain but may relate to either the function of different invertebrate taxa across river food webs, or directly to differences in network structure between sites. An explanation relating to differences in the community composition in river systems, and functional ecology of invertebrate taxa, is that is that the dominance of trophic generalists may enhance the flux of pollutants in river food webs (Chapter 7). Due to their dietary plasticity, these taxa may rapidly expand their ecological niche and use a wider range of basal resources under competitive release resulting from the loss of specialist taxa (Macneil, Dick & Elwood 1997). Such alterations in food web dynamics may potentially increase the flux of pollutants to higher trophic levels, yet this may only be the case if the strength of trophic interactions between the generalist invertebrate taxa and higher trophic levels are also increased in these urban systems. Differences in the relative connectance between food webs, however, were relatively restricted in this study (0.10–0.16), and using broader gradients of connectance, across markedly different food webs, may provide alternative explanations and insights into the effects of food web structure on pollutant transfers. Irrespective of the mechanisms driving differences in the accumulation and magnification of POPs between food webs, the findings further support existing research demonstrating heterogeneity in the interactions between contaminants and biota in aquatic systems (Kelly *et al.* 2007; Walters *et al.* 2008; Coat *et al.* 2011). This further highlights the potential for significant variability in the severity of the ecological risks posed by persistent contaminants across different environments.

The levels of trophic magnification of POPs observed across these river food webs demonstrate that even where POPs occur at undetectable

concentrations in sediments and other environmental compartments, they may still magnify to significant concentrations within organisms and, in turn, pose a significant threat to organism health (Chapter 2). Certainly, the accumulation of pollutants observed within organisms may pose sub-lethal ecological risks, with existing putative physiological and reproductive effects (reduced body condition and depressed triiodothyronine [T3] hormone levels) observed in the nestlings of Eurasian dippers (*C. cinclus*) and related to the concentrations of PCBs and PBDEs measured in their eggs, across these river systems (Morrissey *et al.* 2014). Furthermore, biota Environmental Quality Standards (EQS_{Biota}) for PBDEs (28, 47, 99, 100, 153 and 154; 0.0085 ng g^{-1} ww) and HCB (10 ng g^{-1} ww), and World Health Organisation Toxic Equivalency (WHO_{2005} TEQ) values for dioxin-like PCBs (77, 105, 114, 118, 123, 126, 157, 167, 169, 170, 180, 189; 0.0065 ng g^{-1} ww), were frequently exceeded in the biological samples collected. Although the limits for PCBs and PBDEs were exceeded in a large proportion of invertebrates and fish sampled (65.3% and 100%, respectively), the EQS_{Biota} for HCB was not exceeded in any sample.

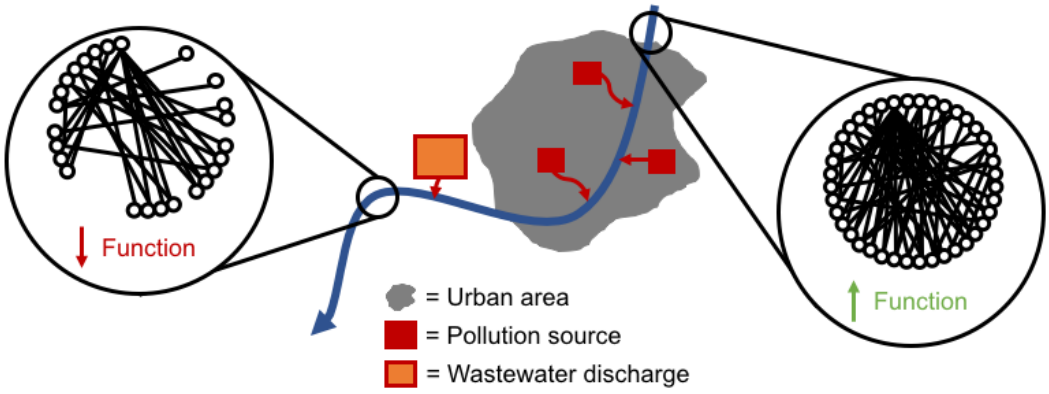
Several gaps in knowledge are highlighted by the results of this study. Firstly, although concentrations of POPs in the environment were relatively low, the accumulation of persistent contaminants in the tissues of aquatic organisms appears to present significant ecological risks. This continued internal exposure pathway, however, is poorly represented by the vast majority of standardised toxicological tests which directly link environmental concentrations to observed effects within organisms (e.g. OECD tests), with additional information regarding predicted bioaccumulation informing the identification of risk thresholds. Thus, developing knowledge relating tissue concentrations to sub-lethal biological effects is required to understand the risk pose by legacy persistent contaminants. Secondarily, a diverse suite of persistent pollutants, from OCs through to PBDE congeners, were shown to bioaccumulate, yet understanding of the relative toxicity of pollutant mixtures, in both the environment and tissues of organisms, remains limited (Spurgeon *et al.* 2010). On top of the mixtures of pollutants observed in the tissues of organisms, there is an absence of research linking body burdens to ecological

effects (Escher & Hermens 2004). The direct exposure of organisms to a range of other current-use chemicals poses a further complication for risk assessments, with individual exposure pathways, and their interactive effects, altering potential ecological risks (Escher *et al.* 2011).

6.6. Conclusions

Differences in food web structure appear to mediate the extent of magnification across sites, indicating that the ecological risks presented by POPs are spatially variable, and not solely related to variation in environmental concentrations. The bioaccumulation and trophic magnification of POPs across sample sites demonstrates the potential for significant accumulation of pollutants, even at sites where concentrations in the environmental compartments/basal resources of the food web are below the detection limits of analysis. Although spatial variation was observed within this study, the net trophic magnification of POPs observed across food webs indicates that legacy POPs may continue to present a widespread threat to river ecosystems.

Chapter 7: Ecological effects of persistent pollutants across river ecosystems in South Wales



This chapter forms the basis for a publication in review at *Water Research*. Data were collected and analysed by FMW, the paper was written by FMW and revisions were provided by MGP, SJO and CRT.

7.1. Abstract

1. Urban areas contribute substantially to xenobiotic contaminant loads in rivers, but the effects have been investigated more for individual organisms and sensitive taxa, rather than across food webs.

2. Here, a replicated, catchment-scale sampling design (18 sites across 3 river catchments in South Wales, UK) with monthly benthic invertebrate samples (2016–2017), and novel multivariate techniques were used to assess whether urban wastewater contaminants affected the structure and function of river food webs. The aim was to assess whether continued contaminant occurrence in river systems might explain the incomplete recovery of urban rivers from past insanitary pollution. Contaminant sources were characterised using remote sensing, water quality data and concentrations of persistent xenobiotic pollutants (PCBs and PBDEs).

3. Urban wastewater had only minor effects on general water quality (temperature, conductivity and dissolved solids) but total concentrations of PCBs and PBDEs in invertebrates increased significantly under increased urban land cover and wastewater discharge.

4. Food webs at highly contaminated urban rivers were characterised by: (1) reduced taxonomic and functional diversity; (2) simplified food web structure with reduced network connectance; and (3) reductions in the abundance of prey important for apex predators such as the Eurasian dipper (*Cinclus cinclus*).

5. Although correlative, these data, using novel bioindicators, are consistent with the hypothesis that bottom-up trophic cascading of urban pollutants might explain significant population, community and ecosystem-level effects in urban river systems, and hence incomplete recovery from past insanitary pollution.

7.2. Introduction

Urban landscapes across the world are linked with effects on water quantity and quality that reflect factors such as increased population density, waste disposal, flow modification, river habitat modification and increased water temperatures (Dudgeon *et al.* 2006; Zimmerman, Mihelcic & Smith 2008; Reid *et al.* 2018). Among these effects, water pollution is one of the most pervasive, and has recognised impacts on river biota (Harding *et al.* 1998; Schulz 2004; Relyea 2005; Moss 2008) while also representing risks to human health (Schwarzenbach *et al.* 2010).

A large number of pollutants reach river systems from point and diffuse sources (Walsh 2000; Bester *et al.* 2008; Heeb *et al.* 2012), and in urban systems these include impervious urban surfaces, stormwater systems, wastewater treatment works (WwTWs), combined sewage overflows and industry (Pitt *et al.* 1995; Feng *et al.* 1998; Phillips *et al.* 2012; Krein *et al.* 2013). While wastewaters are typically dominated by gross organic pollutants, sediments and nutrients, they also contain low levels of chemicals, such as pharmaceuticals, as well as general background pollutants that reach sewers from surface drains. Discharges from industry depend more on the specific processes involved, but often involve low concentrations of toxic substances either of emerging concern or 'legacy' pollutants such as PCBs, PBDEs or non-brominated flame retardants (Owens *et al.* 2001; Fu *et al.* 2003). For all urban pollution sources, the relative discharge and chemical composition is inherently dependent upon population density, demography and the types of anthropogenic activity within the upstream contributing catchment (Taebi & Droste 2004). Consequently, areas with high population density and high urban land cover contribute most effluent and xenobiotic pollutants as a proportion of river runoff, thus also potentially generating the largest ecological effects (Dyer & Wang 2002).

The individual-level effects of xenobiotic contaminants are relatively well understood and include mouthpart deformities, reduced reproductive capability and increased mortality (see Colborn, vom Saal & Soto 1993; Jobling *et al.* 1998; Tyler, Jobling & Sumpter 1998; Watts, Pascoe & Carroll 2003; Segner *et al.* 2003). At higher levels of biological organisation, however,

knowledge is more restricted (Gavrilescu *et al.* 2015; Petrie, Barden & Kasprzyk-Hordern 2015; Chapter 2). Specifically, there is a need for research assessing the effects of xenobiotic chemicals across communities and food webs to assess more accurately the risk of these toxic pollutants in natural systems (Chapter 2).

Assessing the effects of toxic pollutants in field circumstances is not straightforward and requires some understanding of the changing context in which urban pollution is managed. The systematic regulation of conventional and toxic pollutants through national and European Union directives (e.g. Urban Wastewater Treatment Directive 91/271/EEC; Water Framework Directive 2000/60/EC), alongside advances in urban water treatment, have led to significant reductions in the concentration of hazardous organic compounds within the UK and across Europe (Eggen *et al.* 2014). In particular, improvements contemporary sewage treatment, such as the growing use of activated sludge processes, has enabled the more effective removal of organic matter, nitrates, phosphates, suspended sediments and many contaminants from effluents (van Loosdrecht & Brdjanovic 2014; Ahmed *et al.* 2017). Subsequently, there have been improvements over recent decades in water quality and biological diversity in rivers downstream of urban areas – at least in western Europe (Brosnan & O'Shea 1996; Vaughan & Ormerod 2012). In spite of these improvements, a range of xenobiotic compounds still persist in urban runoff at low but toxic concentrations (Purdom *et al.* 1994; Blackburn & Waldock 1995; Zhou *et al.* 2009). Moreover, biological recovery from the effects of past insanitary pollution is still only partial (Vaughan & Ormerod 2012). Some evidence suggests that toxic substances might now affect clean-water organisms, such as the Eurasian dipper (*Cinclus cinclus*), that are recolonising formerly polluted urban river systems (Morrissey *et al.* 2013b; 2014). The aim of this study was to assess whether similar evidence might explain the current status and incomplete recovery of communities of river organisms or the food webs of which they constitute.

Specifically, the objective was to assess the putative effects of persistent xenobiotic pollution on the structure and function of riverine macroinvertebrate communities and food webs, as a potential explanation for the incomplete

recovery of urban rivers in Britain from past insanitary pollution (Vaughan & Ormerod 2012). It was hypothesised that:

1. Food web structure will relate to the levels of urban wastewater contamination within river systems
2. The ecological function of river food webs will be negatively related to the levels of urban pollution
3. Emergent effects resulting from changes in macroinvertebrate community and food web structure are present in urban river systems

7.3. Material and methods

7.3.1. Sample sites

Eighteen sites across South Wales were used to assess the relationships between urban effluents on macroinvertebrate communities (Fig. 7.1). Sample sites were paired upstream or downstream of WwTWs, as well as located across a gradient of urban land use (0.11–33.50 km^2) and other point sources in the upstream catchment (Appendix S6).

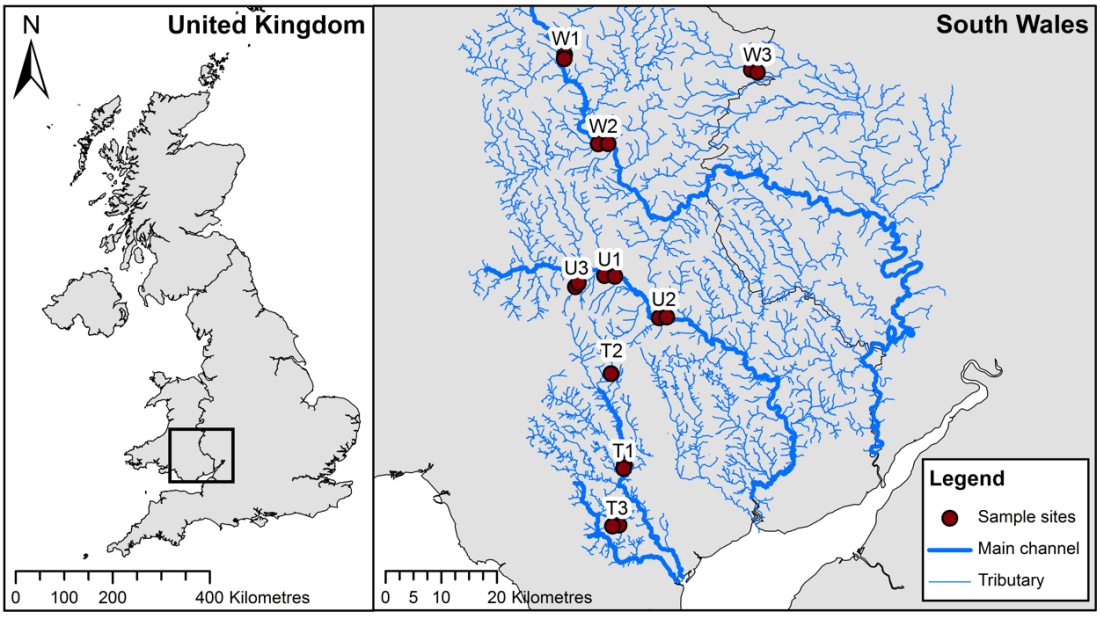


Fig. 7.1. A map of sample locations across South Wales. Site codes correspond to secondary data collated from a range of sources (Table 7.1). Each site code supports two sample locations.

Sites were situated across three hydrological catchments (Taff, Usk and Wye), each of which has a distinct land use history. The Taff catchment has historically supported heavy industrial activity, much of it related to coal mining and gasification (Scullion & Edwards 1980), and the area remains heavily urbanised. In contrast, the Usk and Wye catchments are more rural, with agricultural management practices dominating land use. These inter-catchment differences generate variation in the environmental conditions and allow for different 'environmental contexts' (Burdon *et al.* 2016).

Table 7.1. Physicochemical conditions adjacent to invertebrate sample sites across South Wales. Data are mean values from Environment Agency and Natural Resources Wales datasets over the duration of macroinvertebrate sampling (2015–2017).

Site	pH	Ratio (E:R)	Nitrate (NO_3^-)*	Phosphate (PO_4^{3-})*	DO (mg l^{-1})	CPOM (g dw)	FPOM (g dw)
T1	8.04	0.0109	1.83	0.09	9.66	0.47	0.31
T2	7.99	0.0022	0.27	0.07	11.58	0.69	0.37
T3	7.68	0.0032	1.52	0.06	10.10	0.34	0.30
U1	7.93	0.0043	0.68	0.07	11.29	0.35	0.28
U2	8.12	0.0002	1.52	0.07	11.05	0.49	0.30
U3	8.01	0.0042	0.56	0.04	11.50	0.54	0.31
W1	7.30	0.0045	0.57	0.04	10.88	0.63	0.28
W2	8.05	0.0005	0.64	0.07	11.41	0.36	0.25
W3	7.36	0.0034	0.62	0.05	11.50	0.44	0.35

* Chemical characteristics are all given in mg l^{-1}

** E:R = Ratio of effluent to river discharge, DO = Dissolved oxygen, CPOM = Coarse Particulate Organic Matter, FPOM = Fine Particulate Organic Matter (g dry weight)

Contributing land cover was determined for the upstream catchment of each sample site using JNCC phase 1 habitat classification data (JNCC 2010), in conjunction with the Spatial Tools for the Analysis of River Systems (STARS) and Spatial Stream Network (SSN) tools (Ver Hoef *et al.* 2014). Further to this, the characteristics for WwTW discharge were collated. At sites downstream of WwTWs the ratio of wastewater effluent to river discharge was variable, enabling a gradient of environmental conditions across the landscape (Table 7.1). The invertebrate communities across hydrological catchments have a

similar general structure, dominated by Ephemeroptera, Plecoptera, Trichoptera, Diptera and Coleoptera typical of relatively base-rich stony streams and the regional species pool in South Wales (Appendix S13).

7.3.2. Physicochemical characterisation

A series of physicochemical variables were measured to assess the effects of urban land cover and wastewater effluent on the water quality of stream reaches. Monthly spot measurements of water temperature (°C), Electrical Conductivity (EC), Total Dissolved Solids (TDS) and pH were utilised to assess changes in water chemistry in response to urban wastewater effluent following previous practice (Igbinosa & Okoh 2009). Water quality analyses (2015–2017) by NRW and EA were used; nitrate (NO_3^-), ammonia (NH_4^+) phosphate (PO_4^{3-}) and dissolved oxygen (DO) (Table 7.1; Appendix S6).

The concentrations of PCBs and PBDEs were measured in invertebrates across sample sites downstream of urban areas and effluent discharges (n = 9). For the specific purposes of this paper, body burden data were used to provide a spatio-temporally averaged measure of PCBs and PBDEs contamination, with concentrations of bioaccumulative chemicals measured in organisms assumed to reflect long-term exposure concentrations (Schäfer *et al.* 2015). Over 2016 (May–September), invertebrate taxa (Heptageniidae, Baetidae, Leuctridae, Hydropsychidae, Rhyacophilidae) were sampled to provide an indication of persistent contamination. For each sample (n = 44), multiple individuals of each taxa (n = 50–200) were pooled to collect a 1–2 g of sample. Gas Chromatography Mass Spectrometry (GC/MS) was then used to quantify the concentrations of PCBs and PBDEs within samples of each taxa across the sites. The total concentration of PCBs and PBDEs (ng/g wet weight) was aggregated for invertebrate taxa at each site, providing a total level of persistent contamination within the invertebrate food web. A full description of sample collection and analytical methods is provided in Appendix S7 and Section 4.3.3 (Chapter 4).

7.3.3. Macroinvertebrate community structure

Macroinvertebrate communities were characterised by three pooled Hess (Hess 1941) samples (165 cm^2, 500 μm mesh gauge) per sampling occasion

at each site. These samples collected from the dominant benthic habitats present within each stream reach (Beisel *et al.* 1998). Samples were collected monthly over 2016–2017 and preserved on-site in 70% ethanol before subsequent sorting and identification to the lowest practical taxonomic resolution (minimum of family, and species where possible). The final dataset comprised multivariate, community-level taxonomic data across the 18 sites. Taxonomic data were summarised using common metrics including species richness, total abundance and Simpson's index (SI = *1–D*) (Simpson 1949). To assess taxonomic homogenisation of macroinvertebrate communities in response to WwTW discharges, the Chao index of similarity (Chao *et al.* 2005) was computed for independent pariwise comparisons (upstream–upstream and downstream–downstream of discharge points).

7.3.4. Macroinvertebrate community function

To parameterise macroinvertebrate communty function, a functional dataset was generated through linking taxonomic information to a series of fuzzy-coded trait data derived from the European trait database (Tachet, Bournaud & Usseglio-Polatera 2002). These data were also supplemented by non-fuzzy, categorised feeding guild data for macroinvertebrate taxa of the South Wales (Durance I. & Ormerod S.J., unpublished data). This dataset was used to understand trait variation in response to urban land cover and effluent discharges, but also to calculate the functional diversity of communities. For each community, inter-taxon functional metrics were calculated, with this approach being preferred over the mean-taxon method due to risks in the latter of systematic and detrimental exclusion of important within-taxon functional trait variability (Violle *et al.* 2012). The approach followed Gutiérrez-Cánovas *et al.* (2015) in that a subset of traits related urban land cover and effluent concentrations were selected based on the values of individual Pearson correlations. Traits with an average absolute coefficient of $R \geq |0.40|$ were included for the calculation of functional diversity. For the sub-set of selected traits, assessments of inter-taxon functional diversity were completed by defining a multidimensional Euclidean space, where axes summarised variation in traits. Subsequently, taxon functional richness (tFRic), community

functional richness (FRic), dispersion (FDis), similarity (FSim) and redundancy (FRed), were calculated. Definitions for metrics are provided in Appendix S14.

7.3.5. Macroinvertebrate food webs

See Chapter 6, Section 6.3.3.

7.3.6. Statistical analyses

The potential effects of urban contamination on the structure and function of macroinvertebrate communities were investigated using 'R' statistical software (version 3.2.3) (R Core Team 2015). Prior to analysis data were explored following Zuur, Leno and Elphick (2010) to select statistical tests.

Generalised Linear Models (GLMs) and Generalised Linear Mixed Models (GLMMs), the latter fitted using the package 'lme4' (Bates *et al.* 2015), were used to assess the relationships between taxonomic and functional diversity, food web characteristics and urban pollution. Where required, 'catchment' was included as a random effect to account for broad-scale biogeographical patterns of species distribution (Grönroos & Heino 2012). Chao index values were non-normal, yet sample sizes were equal and between-group variance was homogenous, thus Kruskal-Wallis rank sum tests (Ruxton & Beauchamp 2008) were selected for analysis.

Multivariate analyses were used to assess metrics of invertebrate community structure and function. Non-metric Multidimensional Scaling (NMDS) ordination was utilised to visualise the multivariate relationships. NMDS was computed using the Jaccard index (Jaccard 1908) with a square root transformation and Wisconsin double standardisation to account for variation associated with both common and rare taxa (Kenkel & Orloci 1986). Multivariate GLMs, constructed using the "mvabund" package (Wang *et al.* 2012), were used to assess taxonomic responses and identify the dominant species contributing to observed patterns. The relationships between macroinvertebrate community function and urban pollution sources were assessed using R-mode linked to Q-mode (RLQ) and fourth-corner analysis, following Dray *et al.* (2014). This allowed for links to be drawn between species, traits and environmental data. A modelling framework was not used for RLQ with fourth-corner analysis (see ter Braak, Peres-Neto & Dray, 2017).

7.4. Results

7.4.1. Community structure along a gradient of urban land cover

Macroinvertebrate diversity was related to both urban land-cover and WwTW discharges ($R^2c = 0.76$, $F_{7,180} = 319.41$, $p < 0.001$). Taxonomic diversity was negatively related to the urban land cover upstream of sample sites (-0.029 $\pm$ 0.001 SI per %; $F_{1,180} = 23.45$, $p < 0.001$). Reductions in diversity were also related significantly to the ratio of effluent to river discharge (i.e. increased effluent concentration) ($F_{1,180} = 3.67$, $p = 0.003$). Urban land cover and WwTW discharge interacted to affect diversity ($F_{1,180} = 3.32$, $p = 0.001$), with SI values lowest at sites with greater urban land cover and higher levels of wastewater discharge (Fig. 7.2).

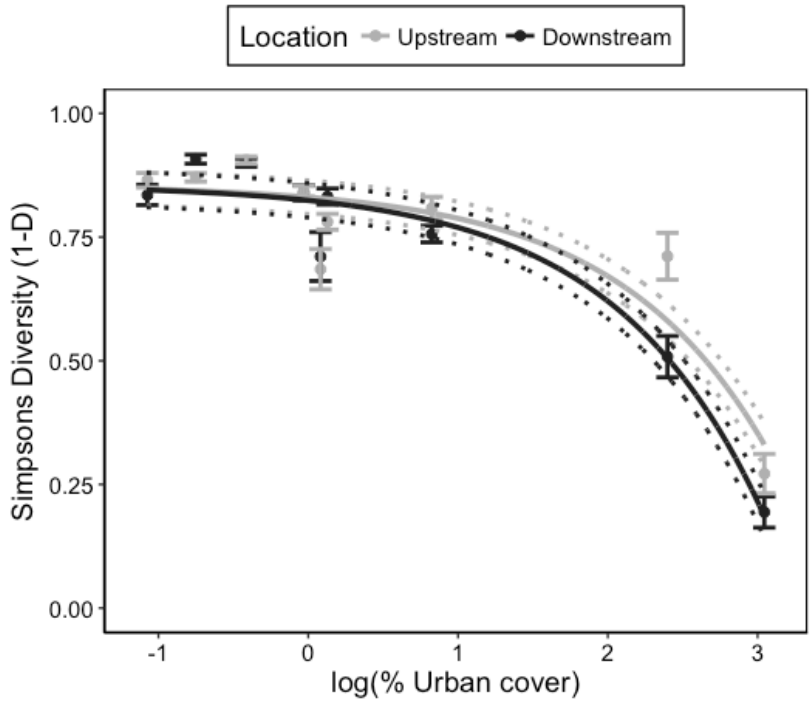


Fig. 7.2. Relationship between macroinvertebrate taxonomic diversity and log^e percentage urban area upstream. Data points are mean annual values upstream and downstream of WwTW discharges (2016–2017). Error bars and dotted lines are $\pm$ 1 standard error. The relationship was derived from a GLMM ($R^2 = 0.76$, $F_{1,180} = 3.32$, $p = 0.001$).

Several taxa varied in abundance as urban land cover increased (Table 7.2). Community structure measured from non-metric multi-dimensional scaling reflected urban land cover and wastewater discharges ($LR_{14,201}$ = 3203.00, $p < 0.001$; see Fig. 7.3), both having effects individually (Urban land cover $LR_{1,213}$ = 615.00, $p < 0.001$; WwTW discharge $LR_{1,214}$ = 168.10, $p = 0.002$) and in interaction ($LR_{1,201}$ = 107.7, $p = 0.037$). Structure also differed upstream and downstream of WwTW discharges, particularly at the most urbanised locations, with >10% urban land in the upstream catchment (e.g. T1 and T3). Community structure also varied among sample dates, as expected from seasonal changes ($LR_{11,202}$ = 2312.10, $p < 0.001$).

Table 7.2. Taxon-specific contributions to communities along a gradient of increasing urban land cover.

Taxon	Effect*	Deviance	*P*-value
Gammarus pulex/fossarum	+	90.55	0.001
Rhithrogena semicolorata	-	58.29	0.001
Tubificidae spp.	+	33.90	0.001
Ancylus fluviatalis	-	27.49	0.001
Amphinemura sulcicollis	-	26.10	0.001
Leuctra moseyli/hippopus	-	21.06	0.004
Limnius volckmari	-	18.65	0.007
Glossosoma conformis	-	18.41	0.007
Agapetus fuscipes	-	17.17	0.010
Baetis rhodani	-	15.16	0.013
Esolus parallelepipedus	-	14.82	0.017
Leuctra inermis	-	14.77	0.017
Sericostoma personatum	-	14.39	0.020
Protonemoura meyeri	-	13.90	0.021
Hydroptila spp.	-	13.66	0.024
Hydropsyche siltalai	-	13.57	0.026

* Direction of change in the relative abundance of taxa

Although there were no significant differences upstream and downstream of WwTWs, there were significant relationships between food web character and urban land cover (Fig. 7.4). Connectance was poorly explained by urban land cover and WwTWs (R^2 = 0.16, $t_{3,14}$ = 0.89, $p = 0.47$). All other models, including urban land cover and wastewater discharges, provided significant

explanation; link density (R^2 = 0.97, $t_{3,14}$ = 266.00, p < 0.001), mean chain length (R^2 = 0.91, $t_{3,14}$ = 54.67, p < 0.001), generality (R^2 = 0.98, $t_{3,14}$ = 293.20, p < 0.001) and vulnerability (R^2 = 0.97, $t_{3,14}$ = 202.00, p < 0.001). Within these models link density ($t_{1,14}$ = -3.36, p = 0.005), mean chain length ($t_{1,14}$ = -3.35, p = 0.003), generality ($t_{1,14}$ = -3.30, p = 0.005) and vulnerability ($t_{1,14}$ = -3.11, p = 0.007) were negatively related to urban land cover but not significantly related to the level of effluent discharge.

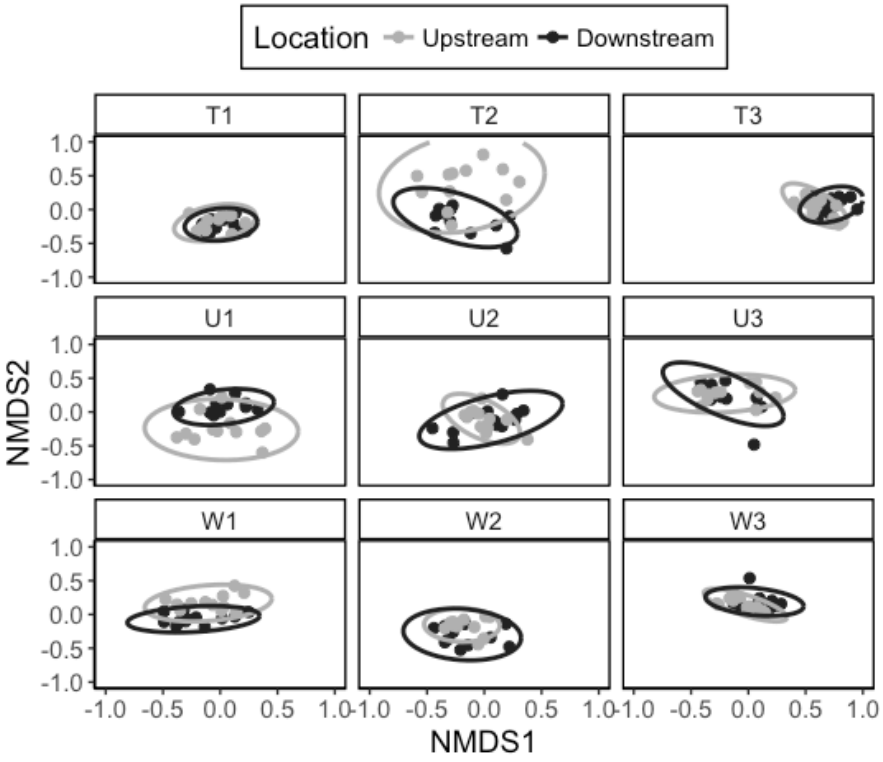


Fig. 7.3. Multivariate relationships between sites and WwTW discharges. Ellipsoids indicate 95% confidence intervals for groups (upstream and downstream of WwTWs). Total stress for the NMDS was 0.16. Statistical analyses of multivariate data are presented in the text (M-GLM), with community structure variable across sites as well as upstream and downstream of WwTW discharges across river systems.

Macroinvertebrate communities downstream of urban wastewater discharges were more similar to one another than upstream sites ($\chi^2_{1,5776}$ = 9.56, p = 0.002; Kruskal Wallis rank sum test), with downstream sites with

consistently lower Chao index values. Although statistically significant, the mean similarity of downstream communities (0.38 ± 0.0028) was only marginally lower than upstream communities (0.39 ± 0.0027).

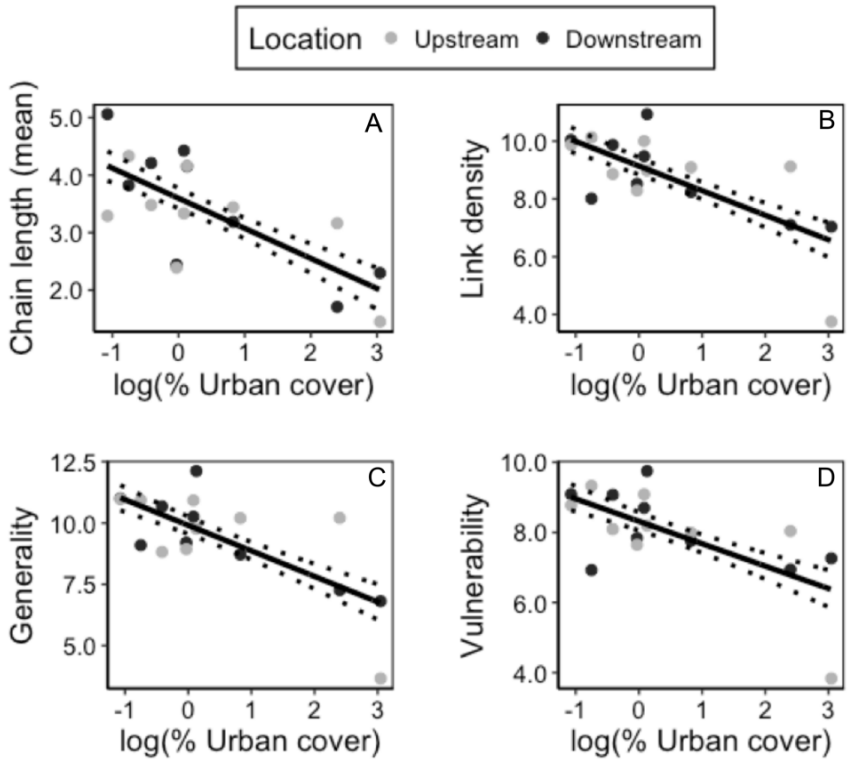


Fig. 7.4. Significant relationships between food web quantitative metrics and log(% urban cover) across sample sites. Location denotes sites upstream or downstream of WwTW discharges. Dotted lines are ± 1 SEM for the linear relationships. Statistics are provided in text.

7.4.2. Community function along a gradient of urban land cover

The functional character of macroinvertebrate communities was significantly related to both urban land cover and WwTW discharges (Fig. 7.5). Although variable in power, all models explained significant variation in functional diversity metrics, including FSim (R^2 = 0.41, $F_{3,212}$ = 48.97, p < 0.001), tFRic (R^2 = 0.30, $F_{3,212}$ = 29.61, p < 0.001), FRic (R^2 = 0.15, $F_{3,212}$ = 12.56, p < 0.001), FDis (R^2 = 0.29, $F_{3,212}$ = 30.29, p < 0.001) and FRed (R^2 = 0.19, $F_{3,212}$ = 17.43, p < 0.001). Of particular note, FSim increased markedly in relation to increasing urban land cover ($t_{1,212}$ = 10.87, p < 0.001), particularly

downstream of WwTW effluent discharges ($t_{1,212} = 4.44$, $p < 0.001$). In contrast, FRic decreased with urban land cover ($t_{1,212} = -5.36$, $p < 0.001$), again most clearly downstream of WwTW discharges ($t_{1,212} = -2.99$, $p = 0.003$).

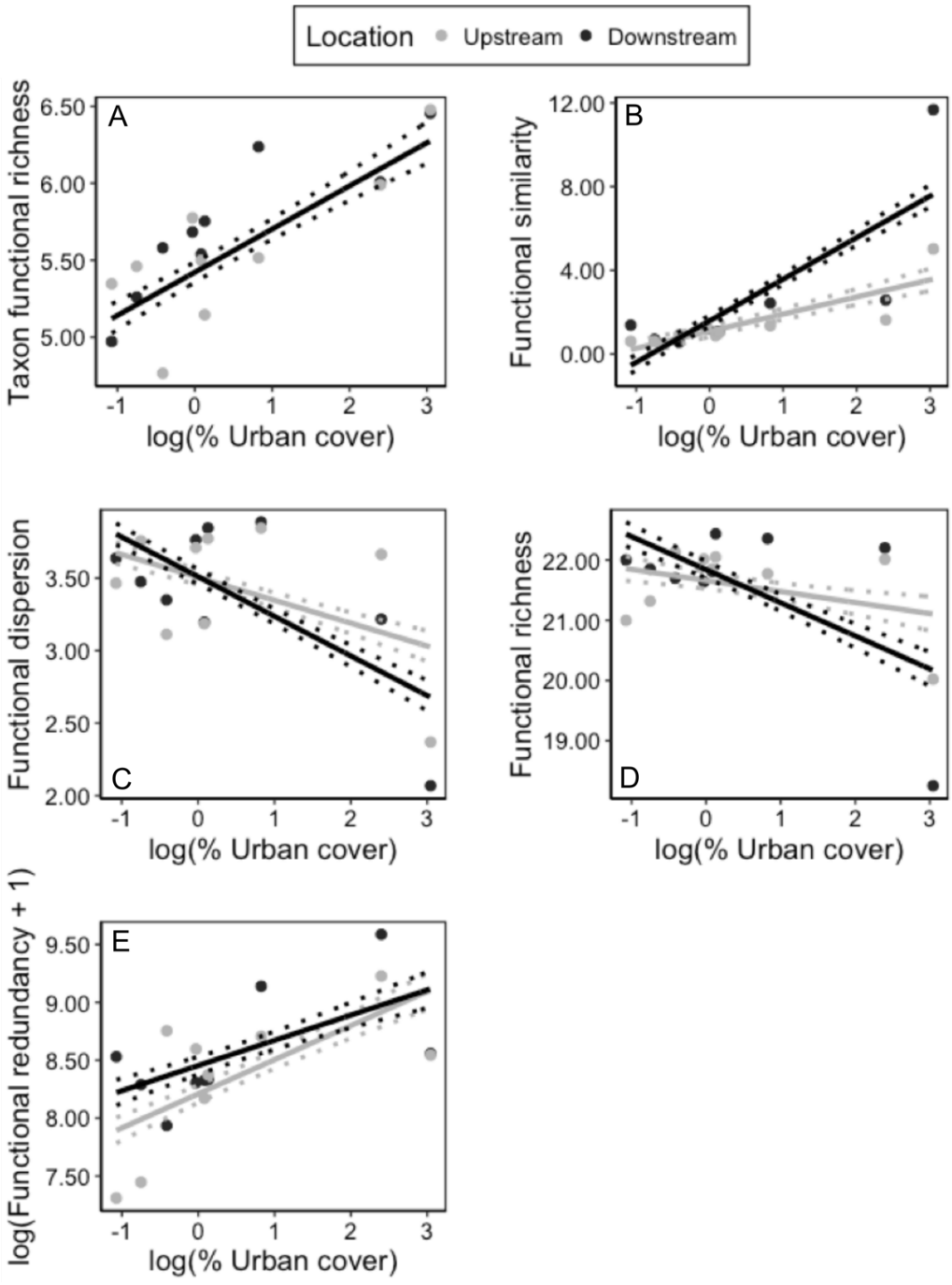


Fig. 7.5. Relationships between functional diversity, urban land cover and WwTW discharges. Location denotes sites upstream or downstream of WwTW discharges. Dotted lines are ± 1 SEM for relationships.

Relationships between macroinvertebrate functional traits and environmental variables are also apparent. Specifically, organisms preferring gravel substrata were not favoured downstream of WwTW discharges (-2.02 $\pm$ 0.04, p = 0.024). Aerial dispersing (-1.71 $\pm$ 0.29, p = 0.019) and uni-voltine taxa (-1.72 $\pm$ 0.30, p = 0.02) both decreased with urban land cover while taxa utilising gills for respiratory processes increased (1.51 $\pm$ 0.31, p = 0.041).

7.4.3. Water quality and contaminants along an urban gradient

Physicochemical conditions varied moderately across sites: water temperature (10.89 $\pm$ 3.46 °C; R^2c = 0.89, $F_{7,200}$ = 42.11, p < 0.001), TDS (66.53 $\pm$ 21.72 ppm; R^2c = 0.53, $F_{7,170}$ = 13.68, p = 0.007) and EC (230.91 $\pm$ 40.98 μS; R^2c = 0.53, $F_{7,170}$ = 13.50, p = 0.009) were explained by wastewater discharges and urban land cover, in conjunction with time of the year at which samples were collected. EC and TDS both increased downstream of WwTWs ($F_{1,170}$ = 5.10, p = 0.03; $F_{1,170}$ = 4.98, p = 0.03, respectively) and water temperature was higher under urban land cover ($F_{1,200}$ = 44.06, p < 0.001).

Total nitrate (NO_3^-) varied among sites, with patterns reflecting urban land cover and effluent discharge (R^2 = 0.13, $F_{3,67}$ = 3.32, p = 0.025). Concentrations were unrelated to urban land cover *per se* ($t_{1,67}$ = 0.47, p = 0.64), but increased with increasing urban land cover in combination with increasing contributions to runoff from effluent discharge ($F_{1,67}$ = 2.03, p = 0.047). Phosphate concentrations (PO_4^{3-}) were not related to urban land cover or effluent discharge from WwTWs (R^2 = 0.01, $F_{3,65}$ = 0.31, p = 0.817).

The concentrations of xenobiotic pollutants across river systems were related to urban land cover and WwTW discharges across sample sites (R^2 = 0.45, $F_{6,55}$ = 6.94, p = 0.05), with total PCB and PBDE concentrations increasing under urban land cover ($t_{1,55}$ = 2.14, p = 0.037). Effects were combined with increasing relative contributions of effluent, such that highly urbanised sites with low dilution rates of WwTW effluent had the highest PCB and PBDE concentrations ($t_{1,55}$ = 1.94, p = 0.057).

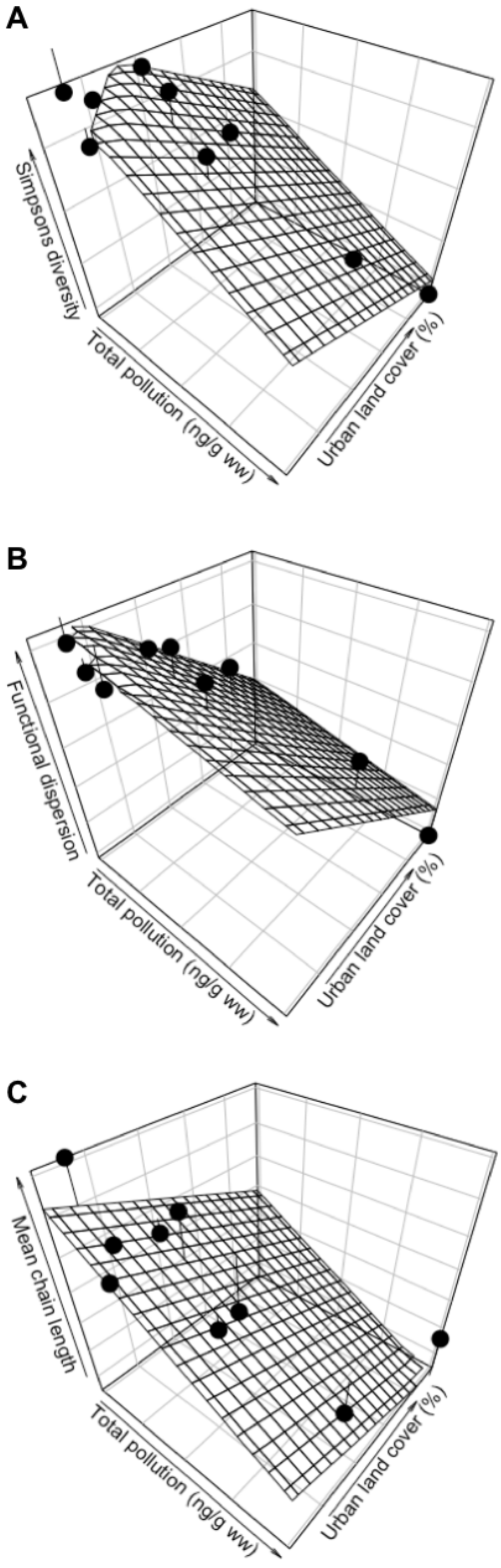


Fig. 7.6. Relationships between metrics describing macroinvertebrate communities, contaminants and urban land cover in South Wales rivers **(A)** Taxonomic diversity. **(B)** Functional dispersion. **(C)** Mean chain length of food webs. Three-dimensional planes are produced from multiplicative linear regressions reported in the main text.

7.4.4. Food webs and contaminants along an urban gradient

Structural (and functional) characteristics of macroinvertebrate food webs were related to persistent contaminants and urban land cover (Fig. 7.6). Taxonomic diversity of macroinvertebrate communities declined significantly with both the total concentration of PCBs and PBDEs within the tissues of invertebrates and the level of urban land cover (R^2 = 0.92, $F_{2,6}$ =46.08, $p < 0.001$). Taxonomic diversity was related most strongly to contaminant concentrations (PCBs and PBDEs; t = -2.93, p = 0.026).

Measures of community functional diversity were also related to both urban land cover and persistent contaminants. As an example, functional dispersion (FDis), was significantly explained by both urban land cover and levels of persistent contaminants in invertebrates in combination (R^2 = 0.81, $F_{2,6}$ = 18.07, p = 0.003), mostly reflecting the apparent effect of persistent contaminant concentrations (t = -2.91, p = 0.021), rather that urban land cover (t = -1.07, p = 0.328). Food web characteristics were also related to the concentrations of persistent contaminants and urban land cover, for example the mean chain length within food webs (R^2 = 0.72, $F_{2,6}$ = 7.58, p = 0.023). Further relationships between a wider range of diversity and food web metrics are presented in Appendix S15 of the Supplementary Materials.

7.5. Discussion

Although there is evidence of recovery in the UK's urban rivers from the past effects of gross pollution, this is only partial (Vaughan & Ormerod 2012), and data confirm that the structure, functional diversity and food web character of invertebrate communities are still impaired in urban locations. Although urban land cover and wastewater discharges did not give rise to significant effects on general water quality in the studied rivers, persistent contaminants (PCBs and PBDEs) were at significantly greater concentrations in invertebrates downstream of urban pollution sources. As a consequence, alterations in macroinvertebrate communities could be linked to these persistent contaminants. In combination, these data are consistent with the hypothesis that contaminant mixtures might explain the incomplete recovery of macroinvertebrate food webs in urban rivers systems from the past effects of

gross pollution, at least in the landscapes of South Wales. Data also add a novel dimension to understanding contaminant effects on river organisms through the assessment of food webs.

As with all field studies of this type, the results are affected by several assumptions. First and most important, the relationships detected between urban land cover, contaminants and macroinvertebrate communities were correlative, and do not necessarily reflect cause-effect links. In particular, urbanisation causes a range of changes in adjacent river ecosystems in addition to pollution, for example altering habitat structure, temperature regimes, discharge and night-time light regimes in ways that all confound straightforward interpretation (Paul & Meyer 2001). In this study at least, the relatively minimal alterations in general water quality (nitrate, phosphate, temperature, EC and TDS) in urban locations at least reduce the likelihood that the effects of contaminants were mistook for the effects of conventional pollution. A related issue is that concentrations of just two groups of chemicals were used to represent contaminants – PCBs and PBDEs – which are likely to be surrogates for a larger array of complex pollutants that occur in urban rivers (Pal *et al.* 2014). Any effects ascribed here to these two groups in reality could be caused by these or other chemicals alone or in combination. At the same time, however, there are major difficulties in experimenting on the emergent effects of pollutants at the levels of biological organisation and spatio-temporal scales achieved in this investigation (Chapter 2). These are well-known problems in resolving pollutant effects on ecosystems in which investigations like this offer insights from field circumstances but cannot confirm any cause-effect mechanisms behind the patterns detected.

A further caveat affecting this correlative study arises from the array of sites at which such a detailed array of biological metrics could be determined. The sample size was small in comparison to previous studies of wastewater effects, for example at national scales (e.g. Dyer & Wang 2002), but investigations with greater spatial coverage often compromise on biological detail by limiting metrics to sensitive taxa or univariate metrics of community structure (e.g. Species At Risk index; Munz *et al.* 2017). Thus, although this study used relatively few sites, a more detailed parameterisation of biological

communities was attained and hence putative ecological responses to pollution. Overall, while data provide an indication of the potential effects of urban pollutants on river food webs, the results should be interpreted the above cautions in mind.

Notwithstanding these caveats, two striking results from the study were the changes in invertebrate community character in urban landscapes affected by wastewater discharge and the accompanying increases in concentrations of PCBs and PBDEs in invertebrates - both groups of persistent, xenobiotic and, in some congeners, toxic pollutants (Chapter 2). These patterns occurred in spite of only minor alterations in water temperature, conductivity, dissolved solids, nitrate and phosphate, or in other words alternative factors that could explain reduced biological diversity in urban river systems (Vaughan & Ormerod 2012). While improvements in wastewater treatment have reduced gross pollution problems across urban river systems in England and Wales sufficiently to allow clean water species such as Atlantic salmon (*Salmo salar*) to recolonise (Mawle & Milner 2008), changes in invertebrate richness and composition do not yet show complete recovery (Maltby & Ormerod 2011; Vaughan & Ormerod 2012), The occurrence of both persistent and current-use pollutants in urban areas from general runoff, storm drains, combined sewer overflows and wastewater discharges, therefore, offer a plausible explanation for current limits on recovery from past gross pollution. Previous data from these rivers show that wastewater discharges are sufficient to change the stable isotopic signatures of river organisms, implying involvement in energy and nutrient additions from wastewater to food webs (Morrissey *et al.* 2013a). Even more relevant, xenobiotic contaminants occur in apex predators such as the Eurasian dipper (*Cinclus cinclus*) along the same rivers at concentrations that have been implicated in negative ecotoxicological effects (Morrissey *et al.* 2013b, 2014). A key question that follows is whether the concentrations of PBDEs, PCBs, or some associated contaminants, were sufficiently high in invertebrates in this study to cause any ecotoxicological effects that might explain food-web impairment or reduced diversity. Toxicological data on the effects of modern or legacy xenobiotic substances on invertebrates are still scarce, however, other than for small numbers of

index species (e.g. Chapter 2). Moreover, where substances such as PCBs are involved, the exact blend of congeners present is important in determining toxicity. PCB congeners typical in Welsh rivers appear to be the widespread PCBs 153, 138 and 180 along with smaller concentrations of the more toxic coplanar PCBs, but these patterns are established only from predatory vertebrates (Ormerod & Tyler 1992). The largest needs overall, therefore, are for fuller understanding of the exact array of contaminants present in these urban rivers, coupled with ecotoxicological assessments of their effects under both field and controlled laboratory conditions.

A further need that follows from this study is to understand more fully the role of any factors affecting urban rivers that might confound that potential effects of xenobiotic substances. This includes not only the direct effects of channel modification, urban thermal regimes, altered discharge and other changes that occur typically where urban land cover exceeds 10–15% (Paul & Meyer 2001), but also interaction between these physical changes and urban pollutant exposure. Such an interaction was detected between urban land cover and the presence of wastewater discharges that highlights the importance appraising the environmental context in which pollution effects occur (see Burdon *et al.* 2016). In this case, urban land cover and wastewater discharge apparently acted together to reduce biodiversity while wastewater discharges in less urbanised sites had more restricted effects on the structure and function of invertebrate communities. This contrasts with instances in which wastewater discharges in more rural stream systems had greater effects – possibly because pollutant loads were dominated by pesticides (Burdon *et al.* 2016; Munz *et al.* 2017).

In addition to identifying a potential role for contaminants in slowing the recovery of urban rivers from gross pollution, a novel aspect of this study was in the assessment of food-web impairment by pollution. Such assessments are infrequent, with studies instead focussing on individual- and population-level responses. Instead, data revealed a series of emergent effects on food webs in response to contamination in urban river systems, including a reduction in connectance and link density that points towards network simplification. Such effects on food webs, although suspected from previous

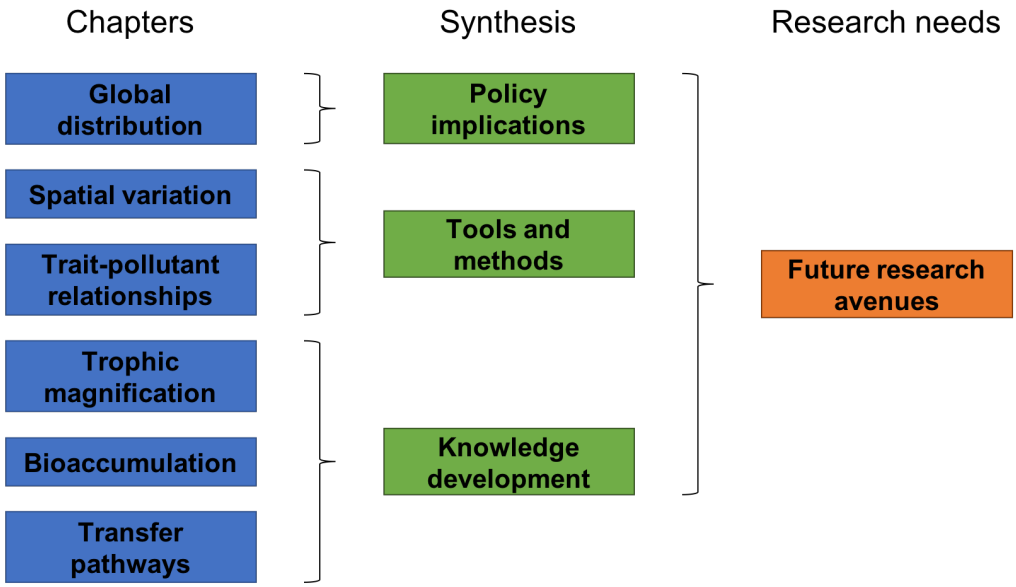
isotope analyses (di Lascio *et al.* 2013), have not previously been shown directly. Additionally, it was identified that urban pollution could cause a range of functional alterations among invertebrate communities through trait-based analyses. This included reductions in the relative abundance of aerially dispersing taxa at polluted, urbanised sites that, if pronounced, could have implications for prey subsidies to riparian zones through reduced matter and energy transfer (Kautza & Sullivan 2015). Such effects on predators, such as bats that prey on insects emerging from rivers, can depend on precise changes in the prey spectrum available (Abbott, Sleeman & Harrison 2009).

As well as consequences for riparian organisms dependent on emergent prey, the simplification of aquatic food webs at polluted urban sites also has the potential to affect aquatic apex predators. A range of clean-water vertebrates rely on macroinvertebrate prey in rivers, including salmonid fish (Bell, Ghioni & Sargent 1994) and river birds that have recently recolonised the UK's recovering urban rivers (Ormerod 1985; Ormerod & Tyler 1991). Some of the macroinvertebrate taxa present at reduced density in urban rivers in south Wales (e.g. *Hydropsyche* spp., *Rhyacophila* spp. and *Leuctra* spp.) would, however, normally form a large component of the diet of the Eurasian dipper (Ormerod 1985). These prey items are nutritionally important for both adults and juveniles, and any reductions in their density in the benthos could carry significant energetic costs (O'Halloran *et al.* 1990). Any such indirect effects of contamination on prey available to dippers, alongside direct effects generated by the enhanced bioaccumulation of toxic xenobiotic pollutants (Morrissey *et al.* 2013b), would be consistent with their inferior body condition along urban rivers in South Wales (Morrissey *et al.* 2014).

7.6. Conclusions

In conclusion, despite improving water quality and increasing invertebrate richness in urban river systems across the UK over the past 30 years, the data provide evidence that xenobiotic contaminants could offer an explanation for the incomplete recovery in the structure and function of biological communities in these same rivers. Simplified macroinvertebrate food webs, alterations in trait character and reduced functional diversity could all have implications for vertebrates that depend on river production, while also offering biological

indications of progress towards recovery that are not normally appraised. There is a need to understand effects like these in more detail, at broader spatial extents, and at sufficient resolution to capture emergent ecosystem effects.


Chapters
Synthesis
Research needs
Global distribution
Spatial variation
Trait-pollutant relationships
Trophic magnification
Bioaccumulation
Transfer pathways
Policy implications
Tools and methods
Knowledge development
Future research avenues

8.1. Synthesis

The studies reported within this thesis aimed to improve understanding of the transfer and emergent ecological effects of persistent xenobiotic pollutants in freshwater ecosystems. Emphasis was placed upon developing knowledge across food webs and contrasting river systems to determine the transfers, ecological interactions and effects of xenobiotic substances to improve risk assessments for freshwater ecosystems more generally. Using a combination of empirical assessments, the thesis highlights the need for continued research into POPs, which were shown to magnify to hazardous levels in freshwater organisms across South Wales and around the globe. Findings from the blend of analyses highlight the importance of interactions among pollutant characteristics, environmental conditions, biological traits and food web structure in generating variation in the distribution of POPs across the natural environment. The exact severity of ecological effects resulting from the distribution and quantity of POPs detected is uncertain, yet POPs appear to continue to provide a range of ecological risks.

Several novel results and advances arose from the individual thesis chapters. These included: (1) the need for ecosystem scale analyses to understand the ecological effects of xenobiotic pollutants; (2) the significant contribution of legacy chemicals to the overall xenobiotic pollutant load in global freshwater ecosystems; (3) the importance of integrated assessments of xenobiotic chemicals using common organisms (shared with other monitoring schemes) at different trophic levels; (4) the role of different basal resources, and subsequent transfer pathways, in understanding the flux of pollutants through freshwater ecosystems; (5) the value of using trait-based methods and food web assessments to appraise the transfer of pollutants; (6) the potential for xenobiotic pollutants to hinder the recovery of freshwater food webs from past gross pollution; and (7) the fact that legacy persistent pollutants remain a potential environmental hazard in freshwater systems and require continued monitoring. These advances are discussed in more detail below.

Current knowledge of the impacts of POPs remains restricted to individual and population levels, and there are disparities between the results of studies at different spatial scales and levels of biological organisation. In this thesis,

these discrepancies were shown to lead to potential misestimations of the ecological risks presented by xenobiotic pollution across the natural environment – particularly pollutants that accumulate and magnify in organisms from very low environmental concentrations. Research at multiple spatial and temporal scales, and across multiple organisms, is therefore important in understanding the effects of pollutants in river systems.

Spatial variation was apparent in pollutant concentrations at a range of scales in the rivers studied, with nested and sometimes confounding spatial, chemical and biological factors influencing the observed bioaccumulation of pollutants. At the catchment scale, data indicate the continued mobilisation of legacy pollutants, as well as the potential for continued low level releases of some chemicals. The widespread distribution of pollutants across a range of spatial scales, across catchments and the globe, demonstrates that xenobiotic pollutants are probably still important biological stressors and agents of ecological change in freshwater ecosystems.

Using organisms, particularly benthic invertebrates, as integrative samplers of bioaccumulative pollutants provides an effective method of identifying spatial variation and potential contemporary sources of pollution within river ecosystems. The heterogeneity in environmental concentrations (water and sediment), in comparison to the relatively consistent concentrations detected in biota, indicates the utility of biomonitoring for understanding how POPs are distributed in ecosystems. The low environmental concentrations detected in this study, in combination with the significant bioaccumulation of pollutants in aquatic organisms, also demonstrates that ecological risk assessments should account for potentially significant internal dosing from several bioaccumulative compounds.

Trait-based approaches were shown to complement existing knowledge regarding the transfer of POPs through ecosystems and between organisms. Biological traits explained variation that was not captured by trophic transfers. Predictive models that used a combination of traits and trophic transfers were able to estimate the relative bioaccumulation of POPs across a range of validation sites with reasonable accuracy. Moreover, trait-based analyses helped in understanding patterns of bioaccumulation in organisms,

irrespective of site-specific conditions across river networks. Further developing such methods may allow for enhanced predictive capabilities with regards to assessing the potential risk of chemicals prior to their introduction into the environment.

Community-level effects of POPs and trophic cascading were identified across food webs, while effluent emitted from urban areas and wastewater discharges were linked to negative effects on aquatic ecosystems, altering both community structure and ecological function. This would suggest that the natural capital and ecosystem services generated by river ecosystems are thus put at risk by the mixture of pollutants emitted from urban areas. The levels of magnification observed in this study appear to be responsible for generating ecological effects across both invertebrate communities and associated riparian vertebrate populations – specifically the Eurasian dipper (*Cinclus cinclus*). These effects illustrate the continued risk provided by legacy POPs across river systems.

Findings across the chapters indicate the continued widespread distribution of xenobiotic pollutants and the continued ecological risk they pose. In general, contemporary monitoring focuses on current-use and emerging pollutants, yet this thesis demonstrates continued interactions between freshwater organisms and legacy pollutants that no longer in active use. This accords with other research indicating the persistent and pervasive effects of organic pollution (Rasmussen *et al.* 2015), and confirms the importance of continued monitoring of legacy pollutants alongside both current-use and emergent chemicals.

8.2. Research design and potential caveats

Caveats and assumptions that affect the interpretation of the work carried out have been outlined in each specific chapter. Here, however, two broader caveats should be acknowledged.

First, the field-based nature of the work reported in the thesis means that findings are influenced by spatial and temporal variations that are characteristic of dynamic freshwater systems: environments vary from location to location, while the fluxes and matter, solutes and pollutants varies with

discharge and season. The diversity of land uses across both the catchments and sites studies at least allows some comparison among locations, while the use of biological sampling ensures some integration of variations through time in hydrology, nutrient concentrations and water chemistry.

Secondly, the work was largely correlative, and so was characterised by weak inference across a range of spatial scales. Where possible, correlative patterns have been linked to causal relationships established from previous work on individuals and populations. Cause-effect links are invariably difficult to establish in ecosystem-scale studies, but findings of this type can still provide useful information on the interaction between pollutants and environmental characteristics across multiple sites. In the case of legacy pollutants, integrative large-scale studies using multiple locations are still relatively scarce within this field of research so that the data produced are still likely to be useful for monitoring and management. As such, assessments such as those presented within this thesis provide data that can be used bridge the gap between experimental or individual-level investigations and broad scale assessments of risk that are useful for management and mitigation across catchments, regions or continents.

8.3. Regulatory and policy implications

A number of important considerations for regulatory organisations and policymakers result from the findings presented in this thesis. Firstly, the combination of low environmental concentrations of legacy pollutants, significant accumulation in organisms, and variable levels of accumulation or magnification among river systems, presents a challenge for monitoring. Commonly utilised risk assessment tools, including no observed effect concentrations (NOECs), predicted no effect concentrations (PNECs) and effect concentration (EC_{50}), use concentrations of pollution in environmental sample media (sediments and water) in conjunction with the chemical characteristics of pollutants (e.g. log K_{OW}) in order to estimate the potential ecological risk. The data here show, however, that in spite of relatively low risk apparent from environmental concentrations, pollutant concentrations in the tissues of organisms are liable to be associated with significant ecological effects. Secondly, contemporary monitoring focuses on priority emergent

pollutants, such as many pharmaceuticals and personal care products (European Commission 2013), but the results here demonstrate the need for continued monitoring of persistent pollutants. Finally, monitoring strategies should encapsulate multiple sample media, assessing concentrations of pollutants in both the environment and organisms, so as to cover the range of pollutants transferred through aquatic ecosystems and through different pathways (Altenburger *et al.* 2015).

Policymakers should not dismiss legacy pollutants due to their low emissions and declining environmental concentrations. In some locations (i.e. certain catchments or geographic regions), these pollutants may even warrant inclusion within contemporary priority listings due to their continued presence in hazardous concentrations.

8.4. Future directions

Findings from this thesis present a number of avenues for future research. In general, an improved focus on persistent pollutants and their low-dose effects is required. Contemporary research focuses instead on generating risk assessments for priority pollutants and pollutants of emerging concern. Understanding the interaction between legacy and emergent pollutants and determining the net ecological effect is urgently required (Altenburger *et al.* 2015).

Although monitoring schemes regularly analyse concentrations of pollutants in the tissues of organisms, there remains a lack of research linking bioaccumulation to specific biological endpoints (Chapter 2). The internal effect concentration paradigm (Sijm & Hermens 2000) and lethal body burdens (van Wezel *et al.* 1996) were constructed to understand the risks from bioaccumulation. Environmental quality standards are in place, yet only for a limited suite of compounds including, PBDEs, HCB and HBCD (European Commission 2013). In general, there has been limited uptake of these concepts within contemporary research. Nevertheless, understanding internal dose-response relationships is required in order to develop knowledge regarding the contemporary effects of many persistent pollutants, especially those which bioaccumulate and biomagnify in organisms.

The ecological effects of different chemicals and chemical mixtures within natural systems remains poorly understood. Recent work has looked to develop bioindicators for signposting effects of environmental pollution (de Lima *et al.* 2018), nevertheless, these indicators generally indicate individual-level biological effects and not at the population or ecosystem scale (Chapter 2). Interactions between individual and population level effects maintain the potential to generate a range of food web and ecosystem scale effects (Chapter 2). Thus, developing novel methods of quantifying population and community level effects within natural systems is required for accurate risk assessments.

Within this study the distribution of pollutants was relatively ubiquitous, yet weakly related to several diffuse and point sources of pollution. There remains, however, a poor understanding of specific sources of persistent pollution – although several compounds, such as HCB and HEOD were observed in higher than expected levels across all catchments. It remains, however, unclear as to whether these high concentrations relate to unlawful contemporary releases or remobilisation from sediments and other secondary stores across catchments. Irrespective of source dynamics, it is apparent that to manage pollution across catchments, both legacy and emergent, need to be understood.

Resolving these knowledge gaps and uncertainties will enable improved assessments of the distribution and severity of ecological risks from persistent pollutants. In turn, this knowledge will facilitate an improved understanding of the role of xenobiotic pollution as an agent of global biological change.

8.5. Conclusions

Persistent pollutants continue to be distributed widely and occur in concentrations that have the potential to generate ecological effects in a range of freshwater organisms across the globe. Data in this thesis signpost a need to assess pollution at broad spatial scales and high levels of biological organisation. The results of this thesis, in conjunction with previous individual-level experimental assessments, demonstrate the continued effects of persistent, legacy pollution within freshwaters. Continuing to monitor these

pollutants, alongside developing an improved knowledge of the relationships between internal concentrations and ecological effects, is required to accurately assess their ecological effects in the natural environment.

Publications

Alongside peer-reviewed publications associated with the chapters of this PhD thesis (highlighted in cover pages of appropriate chapters), parallel research projects have yielded several publications. These are listed below and result from ongoing projects with Co-Is at the University of Birmingham, University of Exeter, University of Plymouth and Centre for Ecology and Hydrology. All publications were submitted, accepted or published over the duration of the research project and PhD studentship are detailed below.

1. Windsor F.M., Grocott M.T. & Milner A.M. (2018) An inter-catchment assessment of macroinvertebrate communities across groundwater-fed streams within Denali National Park, interior Alaska. *Hydrobiologia*, **785**, 373–384. DOI: 10.1007/s10750-016-2944-y

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Supplementary materials

Appendix S1

Toxicity assessments were conducted using biological endpoints linked to population-level effects (Table S1.1). A range of individual- and population-level endpoints are utilised within standardised toxicity tests. The majority of endpoints have clear links to population level processes, and those which were several stages removed were not utilised for the assessment.

Table S1.1. Endpoints used for toxicity assessments. Links to specific population-level effects are derived from the literature.

Endpoint	Measurements	Linked population effects
Growth	Mass (weight), length, volume, growth rate (specific and relative), condition index and limb regeneration	Survival, mortality, reproductive output and population demography
Reproduction	Fertility, clutch production, sperm cell count, progeny counts, time to spawn, fecundity, courtship behaviour, germ cell count, gamete production, copulation, nest attentiveness, motility, viability and reproductive rate	Reproductive output and population demography
Mortality	Mortality, survival, hatch and time to/of death	Population demography
Feeding behaviour	Food consumption, predatory behaviour, feeding time and litter breakdown	Individual growth, development, time to maturity and survival
Development	Stage, deformation, metamorphosis, sexual development, emergence, tissue/organ formation, moulting, maturity and mass	Reproductive output and mortality
Population	Abundance, photosynthesis, population growth rate, chlorophyll A/B concentration (or ratio), sex ratio, biomass, drift, diversity, evenness, carrying capacity,	N/A
Ecosystem processes	Primary production, nitrification and respiration	N/A

Appendix S2

A systematic protocol for data collation was adopted to search peer-reviewed literature databases and collect viable studies. Over a defined period of time (01/12/2016–01/05/2017) several academic search engines (Web of Science, Google Scholar and Scopus) were used to find research articles published between 1990 and 2017 that reported the tissue concentrations of the selected suite of xenobiotic pollutants identified in *Selection of high-risk xenobiotic pollutants*. The following string was used to gather relevant articles: ((bioaccumulat* OR biomagnify OR bioconcentrat* OR concentration OR biomagnification OR) AND (freshwater OR river OR stream OR lake OR pond OR wetland) AND (polychlorinated OR polybrominated OR atrazine OR endosul* OR BPA OR bisphenol OR *osterone OR PCB OR PBDE OR legacy OR emergent OR pesticide OR industrial OR phenol OR *methrin OR organochlorine* OR organohalogen* OR organobromin* OR chlorinat* OR halogenat* OR brominat* OR deltamethrin OR DDT OR DDE OR lindane OR γ-HCH OR HCH OR hexachlorocyclohexane OR gamma-HCH OR dieldrin OR HCB OR hexachlorobenzene OR methoxychlor OR nonyphenol OR octylphenol OR diclofenac OR gemfibrozil OR estrone OR oestrone OR testosterone OR progesterone OR 17α-* OR 17alpha-* OR ethinyloestradiol OR ethinyloestradiol OR ibuprofen OR triclosan OR pharma* OR anti-bacterial OR anti-depressants OR personal care product)). The 27,766 records identified within searchers were listed according to the ‘relevance’ function. Initial screening took the form of assessing the relevance of titles, and abstracts where required. The shortlist of relevant articles was then assessed thoroughly based upon the criteria detailed within Section 3.3.2. Additional observations were collated from the bibliographies of studies. Where available data was identified and recorded.

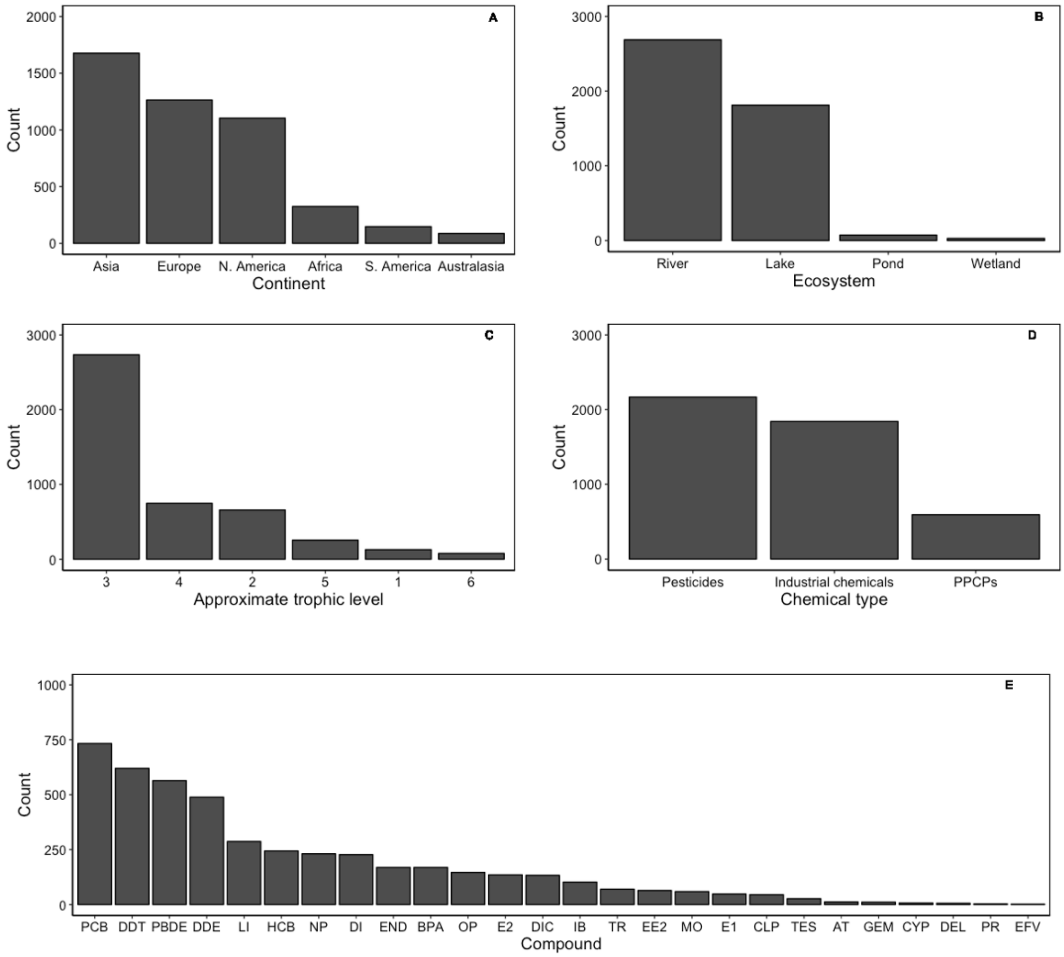

Fig. S2.1. Distribution of observations between spatial and physicochemical categories. A is the continental distribution, B is the ecosystem distribution, C is the trophic distribution, D is the broad compound distribution and E is the specific chemical distribution.

Appendix S3

A similar system to the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) system (Moermond *et al.* 2016), was utilised to assess the reliability of studies for assessing the tissue concentrations of xenobiotics in freshwater organisms. A series of 20 questions are used to rank studies based on their reliability. The same ranking system as presented in Moermond et al. (2016) is utilised (R1–R4). Briefly, R1 indicates that the results of studies can be used without restrictions, R2 some minor flaws are present but results are reliable once these are accounted for, R3 the study is not reliable with clear flaws and R4 suitable information is not provided (e.g. limits of detection or organisms sampled) so that results are unassignable.

The reliability criteria are briefly described in the following text. Factors relating to the study design are important and the collection of samples will influence the quantification and associated variation in measured contamination. Multiple locations and taxa, as well as reference sites, allow for the encapsulation of spatial variation across a region and prevents the biasing of results towards sample locations selected for their significant contamination (1, 2 and 4). Triplicate samples are required to assess heterogeneity within sample sites, resulting from a range of processes (3). Environmental concentrations indicate the relative bioaconcentration and bioaccumulation of contaminants (5). Environmental conditions are important to understand the interactions between biota and contaminants, conditions such as dissolved organic carbon will affect the bioavailability of chemicals (6). The location of sample sites allows for broader spatial patterns to be identified (7). Information regarding the chemicals analysed within studies is important for comparisons. The specific compound of interest is required to compare between studies (8). The risk of a chemical is specific, and in some cases dependent upon the congener analysed, thus this needs to be recorded (9).

Information regarding the nature of the organisms assessed within studies is required for reliable assessments. Greater variation will be derived from individual, compared to pooled samples, but individual samples will enable a greater understanding of variation at each sample unit (10). The feeding

behaviour and trophic level of an organism are important factors influencing the transfer of pollutants, differences between the feeding behaviour of organisms are present at intra- and inter-specific scales, thus system-specific data enhances the reliability of studies (11 and 12). The tissue from which samples are collected influences the observed concentrations of xenobiotic pollutants but also regulates the potential impacts derived from these chemicals (13). Chemical analyses are of critical importance for the reliability of studies. Limits of detection (LOD) and quantification (LOQ), as well as control blanks, are important for identifing low levels of contaminants, they are also variable within and between studies, thus accounting for them is important (14, 15 and 16). Detection frequency is important where studies pool results, or do not provide raw data, as it indicates the relative occurrence of pollutants within the studied samples (17). Data allowing for conversion between lipid and wet weight is critical for studies to allow for the relevant information to be included, and standardisation across studies (18). The levels of variation in pooled results is required for pooled samples to understand data heterogeneity (19). Raw data enables the encapsulation of all variation present within a study, the provision of this allows for the greatest reliability of subsequent meta-analyses (20).

Table S3.1. Scoring criteria for assessing the overall quality of data collection and sample preparation within studies collated for global risk assessment. Criteria indicate publications providing the greatest capability for analysing ecological risk within studied freshwater ecosystem.

Group	Question
Study design	
1	Were multiple locations assessed across the study region?
2	Was more than one taxon assessed at each of the sample sites?
3	Was triplicate, or greater, replicate sampling utilised for each sample unit (e.g. organism per time and sample location)?
4	Was a reference site, or a gradient of potential contamination included?
5	Was the environmental concentration, either water or sediment, reported?
6	Were the environmental conditions within the system reported (e.g. dissolved carbon concentration, suspended solids and pH)?
7	Was the location of sample sites reported (e.g. coordinates)?
Compound	
8	Was the compound(s) and CAS number(s) reported?
9	Were individual congeners reported, or pooled into chemical groups (e.g. PCBs or PCB-60, PCB-118 and PCB-138)?
Organism	
10	Were individuals analysed? The alternative is a pooling samples across multiple individuals?
11	Was feeding niche/behaviour/guild of each sampled organism reported?
12	Was the trophic level of each sampled organism reported?
Chemical analysis	
13	Was the tissue from which samples were taken stated (e.g. liver, muscle or whole body)?
14	Were the limits of detection (LOD) reported for each compound(s)?
15	Were limits of quantification (LOQ) reported for each compound(s)?
16	Were analytical blanks used to account for contamination in analytical procedures?
17	Was detection frequency (%) reported for sample units (e.g. organism per time and sample location)?
18	Were the chemical data reported with appropriate lipid and/or water conversion factors (e.g. % lipid)?

Group	Question
Statistical analysis	
19	Was an estimate of variation provided in pooled results?
Reporting	
20	Was raw data provided in some format? This could be pooled or reported from individuals.

Appendix S4

Table S4.1. Model output and pairwise comparisons from tissue concentration GLMM. Values are derived from the sample model that was included within the main body of text. Only the first set of pairwise comparisons are provided due to the large number of potential combinations.

Variable	Pairwise	Estimate (± SE)	t value	p value
Environmental concentration	NA	0.33 (0.03)	12.91	< 0.001
Trophic level	NA	0.32 (0.07)	4.35	< 0.001
Chemical	AT-BPA	-4.96 (0.92)	-5.37	< 0.001
	AT-CLP	-5.34 (1.18)	-4.52	< 0.001
	AT-DDE	-4.01 (0.93)	-4.28	< 0.001
	AT-DDT	-4.33 (0.93)	-4.63	< 0.001
	AT-DI	-5.82 (0.95)	-6.09	< 0.001
	AT-DIC	-4.42 (0.92)	-4.76	< 0.001
	AT-E1	-4.48 (0.97)	-4.61	< 0.001
	AT-E2	-4.31 (0.93)	-4.61	< 0.001
	AT-EE2	-4.18 (0.95)	-4.39	< 0.001
	AT-EFV	-3.26 (1.99)	-1.64	0.104
	AT-END	-5.61 (0.96)	-5.83	< 0.001
	AT-GEM	-3.97 (1.11)	-3.60	0.003
	AT-HCB	-4.64 (0.97)	-4.78	< 0.001
	AT-IB	-3.68 (0.94)	-3.93	< 0.001
	AT-LI	-5.28 (0.95)	-5.58	< 0.001
	AT-MO	-6.85 (1.02)	-6.68	< 0.001
	AT-NP	-3.37 (0.92)	-3.69	0.002
	AT-OP	-4.56 (0.93)	-4.89	< 0.001
	AT-PBDE	-3.88 (0.97)	-4.02	< 0.001
	AT-PCB	-2.63 (0.94)	-2.81	0.005
	AT-PR	-0.59 (1.48)	-0.40	0.691
	AT-TR	-0.53 (0.97)	-0.55	0.585
Continent	Africa-Asia	0.95 (0.32)	2.88	0.004
	Africa-Europe	0.33 (0.39)	0.85	0.394
	Africa-North America	0.43 (0.37)	1.15	0.252
	Africa-South America	4.36 (0.54)	8.02	< 0.001
Habitat	Lake-Pond	1.32 (0.17)	3.44	< 0.001
	Lake-River	-0.12 (0.17)	-0.73	0.46
	Lake-Wetland	0.38 (0.74)	0.51	0.61

Appendix S5

Table S5.1. Observed and predicted BCFs and BAFs. Predicted factors are derived from the literature, with references provided.

Chemical	Field-based (log BCF/BAF)*	Experimental (log BCF/BAF)	Reference
BPA	0.26 (-3.97, 1.24)	1.08 (-0.43, 1.42)	Wang *et al.* (2017b)
		2.24	Chen *et al.* (2016)
CLP	1.36 (-0.65, 1.90)	1.35 (0.27, 1.76)	Jantunen *et al.* (2008)
DDE	3.11 (-3.23, 4.85)	6.01 (4.48, 7.05)	US EPA (2016)
DDT	2.10 (-3.68, 3.84)	4.23 (1.04, 5.00)	Arnot & Gobas (2006)
		4.47 (3.46, 4.96)	US EPA (2016)
DI	2.66 (-2.67, 4.48)	2.65 (ND, 3.99)	US EPA (2016)
DIC	0.48 (-2.60, 1.41)	2.37 (0.69, 3.43)	Svanfelt *et al.* (2010)
E1	0.16 (-1.55, 0.82)	2.35 (2.22, 2.44)	Gomes *et al.* (2004)
E2	0.48 (-1.10, 1.50)	0.49 (0.18, 0.81)	Specker & Chandlee (2003)
END	1.56 (-0.81, 2.34)	2.39 (1.47, 2.40)	US EPA (NB)
GEM	1.53 (-1.61, 2.30)	2.33 (1.96, 2.70)	Mimeault *et al.* (2005)
HCB	2.13 (-0.05, 3.03)	3.87 (1.81, 5.26)	Arnot & Gobas (2006)
		4.42 (3.08, 5.74)	US EPA (NB)
IB	1.31 (-1.68, 3.27)	-0.15 (-1.09, 0.16)	Nallani *et al.* (2011)
LI	1.53 (-1.02, 3.11)	2.32 (0.52, 3.32)	Arnot & Gobas (2006)
		2.61 (1.39, 4.04)	US EPA (2016)
MO	-0.31 (-4.23, 0.37)	3.12 (0.20, 4.07)	US EPA (2016)
NP	1.80 (-2.70, 3.16)	1.47 (-0.09, 3.53)	Staples *et al.* (1998)
PBDEs	2.09 (-2.05, 3.35)	4.10 (2.90, 5.30)	Wu *et al.* (2008)
PCBs	2.12 (-1.34, 3.91)	5.21 (4.69, 5.87)	Oliver & Nllml (1985)

* Calculated from the global dataset

Appendix S6

Table S6.1. Summary of water quality characteristics and consented discharges at sample sites.

Site	pH	Temp. (°C)	EC (µS/cm)	BOD (mg/l)	Nitrate (NO_3^-)*	Phosphate (PO_4^{3-})*	DO (mg/l)	Copper (µg/l)	Zinc (µg/l)
T1	8.01 (0.04)	10.63 (0.49)	383.73 (11.76)	1.52 (0.09)	1.81 (0.11)	0.07 (0.01)	11.16 (0.22)	1.27 (0.09)	5.35 (0.54)
T2	7.71 (0.04)	10.73 (1.18)	108.64 (4.90)	NA	0.25 (0.06)	0.05 (0.02)	11.27 (0.43)	NA	NA
T3	7.68 (0.03)	10.18 (0.66)	335.31 (12.47)	2.60 (0.27)	2.33 (0.43)	0.19 (0.06)	10.31 (0.10)	1.73 (0.18)	12.38 (2.10)
U1	7.93 (0.03)	9.90 (0.43)	186.44 (6.34)	1.70 (0.30)	0.92 (0.04)	0.05 (0.01)	11.63 (0.14)	1.00 (0.16)	6.91 (0.92)
U2	7.99 (0.03)	10.47 (0.47)	201.55 (8.21)	1.53 (0.17)	1.09 (0.10)	0.04 (0.01)	11. 53 (0.13)	1.27 (0.24)	4.79 (1.18)
U3	8.00 (0.04)	9.17 (0.41)	188.36 (7.27)	1.11 (0.10)	0.80 (0.05)	0.03 (0.01)	11.67 (0.14)	0.86 (0.14)	8.28 (1.91)
W1	7.37 (0.06)	9.16 (0.51)	80.24 (7.44)	0.91 (0.09)	0.62 (0.04)	0.02 (0.01)	11.50 (0.19)	4.25 (3.07)	15.18 (0.76)
W2	7.72 (0.05)	10.45 (0.48)	127.30 (14.67)	0.98 (0.20)	0.65 (0.03)	0.03 (0.01)	11.57 (0.15)	0.97 (0.19)	9.65 (2.85)
W3	8.05 (0.03)	10.09 (0.56)	337.68 (14.11)	0.64 (0.09)	3.43 (0.10)	0.04 (0.01)	11.24 (0.16)	1.17 (0.10)	6.58 (2.00)

* Data are mg/l of the given compounds. Data are from Natural Resources Wales (NRW) routine monitoring (2015–2017). River discharge is the annual average from proximal NRW and EA gauging stations within ~5 km from the sample sites. Data are reported as mean (standard error).

Table S6.2. Summary of landscape characteristics, environmental conditions and biological data collected at sample sites alongside monthly monitoring (2016–2017).

Site	River discharge (m^3/s)	Effluent discharge (m^3/s)*	Ratio (E:R)*	Area (km^2)	Urban (%)	Arable (%)	CPOM (g dw)	FPOM (g dw)	Weighted total POPs (ng/g ww)*	Invertebrate diversity (1 – D)*
T1	20.76	0.231	0.0109	304.44	11.01	1.46	0.47	0.31	42.34	0.54 (0.03)
T2	0.78	0.002	0.0022	32.20	0.34	0.06	0.69	0.37	5.16	0.83 (0.01)
T3	0.89	0.003	0.0032	20.42	20.38	4.75	0.34	0.30	291.31	0.21 (0.02)
U1	18.06	0.079	0.0043	440.49	1.14	2.00	0.35	0.28	18.39	0.83 (0.01)
U2	18.06	0.004	0.0002	582.30	1.09	4.04	0.49	0.30	36.96	0.69 (0.03)
U3	1.03	0.005	0.0042	16.49	0.97	0.73	0.54	0.31	6.60	0.83 (0.01)
W1	6.65	0.030	0.0045	169.53	0.47	4.44	0.63	0.28	5.82	0.88 (0.01)
W2	37.24	0.019	0.0005	1117.66	0.01	6.90	0.36	0.25	3.76	0.86 (0.01)
W3	3.93	0.013	0.0034	108.27	2.28	5.01	0.44	0.35	21.05	0.71 (0.02)

* Data are reported as mean (standard error), where applicable. Reported only for sites downstream of WwTWs.

Appendix S7

Composite samples, including both microbial biofilm and macrophytes, were collected. Across sites, a range of autotrophic communities were present, with significant biofilm communities present in several lowland streams, yet limited algal resources were observed in low order streams where basal resources were dominated by allochthonous carbon inputs. These systems maintain an increased relative abundance of macrophytes. Subsequently, macrophytes and biofilms were collected from sites to analyse pollutant concentrations. All samples were stored on ice (~4 °C) before storage at –80 °C.

Preliminary surveys across sample sites indicated the ubiquitous abundance of several macroinvertebrate taxa. Five target macroinvertebrate genera were selected; *Gammarus pulex* (Amphipoda), *Baetis* spp. (Ephemeroptera), *Ecdyonurus* spp. (Ephemeroptera), *Hydropsyche* spp. (Trichoptera) and *Rhyacophila dorsalis* (Trichoptera). Samples for each genus were collected from stream reaches for spatial analysis. To control for size variation and developmental influences on bioaccumulation, samples were composed of fifth instar individuals and pre-pupae from *Hydropsyche* spp. and *Rhyacophila dorsalis*, final aquatic instars of *Baetis* spp. and *Ecdyonurus* spp. and *Gammarus pulex* individuals over 5 mm in length. Individuals were collected in 200 ml glass jars and transported to the laboratory to confirm field identification. Individuals were kept in river water for 24 hr to allow for gut clearance so as to prevent the overestimation of tissue concentrations (Van Geest *et al.* 2010). Composite samples, including approximately 20–100 individuals per taxon, were stored at –80°C.

European bullhead (*Cottus gobio*) individuals were collected from each sample stream reach (n = 5–10). Both male and female individuals were collected for analysis. Fish were sacrificed through concussion, prior to destruction of the brain before the return of consciousness; a humane technique detailed in Schedule 1 of the Animals in Scientific Procedures Act (1986). Individuals were then dissected, and liver tissue removed. Liver tissue was used due to the preferential accumulation of POPs within this organ (Monosson *et al.* 2003). Samples were stored at –80°C.

Appendix S8

Table S8.1. Limits of detection for Gas Chromatography – Mass Spectrometry analyses. Reported as mean, minimum and maximum for each compound analysed.

Chemical group	Congener	LOD			Detection frequency (%)
		Mean	Minimum	Maximum	
PBDEs	**BDE 30**	0.0531	0.0435	0.1045	0.0000
	BDE 32	0.0531	0.0435	0.1045	0.0000
	BDE 17	0.0531	0.0435	0.1045	0.0000
	BDE 28	0.0531	0.0435	0.1045	1.4925
	BDE 35	0.0531	0.0435	0.1045	0.0000
	BDE 37	0.0531	0.0435	0.1045	0.0000
	BDE 51	0.0531	0.0435	0.1045	0.0000
	BDE 49	0.0531	0.0435	0.1045	1.4925
	BDE 71	0.0531	0.0435	0.1045	0.0000
	BDE 47	0.0531	0.0435	0.1045	83.5821
	BDE 66	0.0531	0.0435	0.1045	0.0000
	BDE 77	0.0531	0.0435	0.1045	0.0000
	BDE 100	0.0531	0.0435	0.1045	31.3433
	BDE 119	0.0531	0.0435	0.1045	7.4627
	BDE 99	0.0531	0.0435	0.1045	53.7313
	BDE 118	0.0531	0.0435	0.1045	0.0000
	BDE 85	0.0531	0.0435	0.1045	2.9851
	BDE 126	0.0531	0.0435	0.1045	0.0000
	BDE 154	0.0531	0.0435	0.1045	2.9851
	BDE 153	0.0531	0.0435	0.1045	13.4328
	BDE 138	0.0531	0.0435	0.1045	0.0000
	BDE 183	0.0531	0.0435	0.1045	0.0000
	BDE 128	0.0531	0.0435	0.1045	0.0000
	BDE 190	0.0531	0.0435	0.1045	0.0000
	BDE 197	0.0525	0.0435	0.0697	1.4925
	BDE 196	0.0525	0.0435	0.0697	1.4925
PCBs	**PCB 8**	0.1180	0.1173	0.1268	0.0000
	PCB 18	0.1180	0.1173	0.1268	0.0000
	PCB 29	0.1070	0.1063	0.1149	0.0000
	PCB 31	0.1180	0.1173	0.1268	0.0000
	PCB 28	0.1180	0.1173	0.1268	1.4925
	PCB 52	0.1180	0.1173	0.1268	2.9851
	PCB 101	0.1180	0.1173	0.1268	14.9254
	PCB 81	0.1180	0.1173	0.1268	25.3731
	PCB 77	0.1291	0.1283	0.1387	14.9254
	PCB 149	0.1180	0.1173	0.1268	5.9701
	PCB 123	0.1180	0.1173	0.1268	0.0000
	PCB 118	0.1180	0.1173	0.1268	32.8358
	PCB 114	0.1180	0.1173	0.1268	8.9552

Chemical group	Congener	LOD			Detection frequency (%)
		Mean	Minimum	Maximum	
	PCB 153	0.1180	0.1173	0.1268	52.2388
	PCB 141	0.1180	0.1173	0.1268	8.9552
	PCB 105	0.1180	0.1173	0.1268	17.9104
	PCB 163	0.1180	0.1173	0.1268	16.4179
	PCB 138	0.1180	0.1173	0.1268	29.8507
	PCB 187	0.1180	0.1173	0.1268	14.9254
	PCB 183	0.1180	0.1173	0.1268	8.9552
	PCB 126	0.1180	0.1173	0.1268	1.4925
	PCB 128	0.1180	0.1173	0.1268	5.9701
	PCB 167	0.1180	0.1173	0.1268	8.9552
	PCB 171	0.1180	0.1173	0.1268	13.4328
	PCB 199	0.1180	0.1173	0.1268	11.9403
	PCB 156	0.1180	0.1173	0.1268	16.4179
	PCB 157	0.1180	0.1173	0.1268	19.4030
	PCB 180	0.1180	0.1173	0.1268	29.8507
	PCB 201	0.1180	0.1173	0.1268	17.9104
	PCB 170	0.1180	0.1173	0.1268	29.8507
	PCB 169	0.1180	0.1173	0.1268	25.3731
	PCB 189	0.1180	0.1173	0.1268	23.8806
	PCB 194	0.1180	0.1173	0.1268	0.0000
	PCB 205	0.1180	0.1173	0.1268	20.8955
	PCB 206	0.1180	0.1173	0.1268	20.8955
	PCB 209	0.1180	0.1173	0.1268	2.9851
OCPs	**α-HCH**	0.1162	0.1155	0.1248	0.0000
	HCB	0.1180	0.1173	0.1268	73.1343
	γ-HCH	0.1180	0.1173	0.1268	0.0000
	DDE	0.1180	0.1173	0.1268	95.5224
	HEOD	0.1180	0.1173	0.1268	83.5821
	TDE	0.1180	0.1173	0.1268	35.8209
	DDT	0.1162	0.1155	0.1248	46.2687

Appendix S9

Table S9.1. Pairwise comparisons between concentrations of POPs measured across invertebrate taxa. Results are derived from GLMs using mean concentrations for invertebrate taxa collected at over six sites.

Chemical	Pairwise comparison	R^2	t	p
ΣPBDEs	**Baetis – Ecdyonurus**	**0.85**	**6.22**	**<0.001**
	Baetis – Hydropsyche	**0.55**	**2.95**	**0.022**
	Baetis – Rhyacophila	**0.75**	**4.58**	**0.003**
	Baetis – Gammarus	0.21	1.37	0.214
	Ecdyonurus – Hydropsyche	**0.47**	**2.51**	**0.041**
	Ecdyonurus – Rhyacophila	**0.79**	**5.07**	**0.001**
	Ecdyonurus – Gammarus	0.29	1.70	0.133
	Hydropsyche – Rhyacophila	0.36	1.98	0.089
	Hydropsyche – Gammarus	0.01	0.24	0.819
	Rhyacophila – Gammarus	0.08	0.81	0.444
ΣPCBs	Baetis – Ecdyonurus	0.18	-1.24	0.254
	Baetis – Hydropsyche	0.01	0.09	0.927
	Baetis – Rhyacophila	0.15	-1.13	0.296
	Baetis – Gammarus	0.09	0.84	0.431
	Ecdyonurus – Hydropsyche	0.43	2.31	0.054
	Ecdyonurus – Rhyacophila	0.24	1.48	0.130
	Ecdyonurus – Gammarus	0.17	1.20	0.271
	Hydropsyche – Rhyacophila	0.01	-0.26	0.800
	Hydropsyche – Gammarus	0.26	1.58	0.157
	Rhyacophila – Gammarus	0.04	0.51	0.623
ΣOCs	Baetis – Ecdyonurus	0.43	2.29	0.561
	Baetis – Hydropsyche	0.07	0.73	0.491
	Baetis – Rhyacophila	0.01	0.04	0.968
	Baetis – Gammarus	0.06	-0.67	0.523
	Ecdyonurus – Hydropsyche	0.16	1.16	0.285
	Ecdyonurus – Rhyacophila	0.13	1.02	0.342
	Ecdyonurus – Gammarus	0.01	0.09	0.934
	Hydropsyche – Rhyacophila	0.23	1.42	0.198
	Hydropsyche – Gammarus	0.01	0.01	0.998
	Rhyacophila – Gammarus	**0.51**	**2.72**	**0.030**

Table S9.2. Pairwise comparisons between concentrations of POPs measured across sample matrices. Results are derived from GLMs using mean concentrations for sample matrices across sites (n = 9).

Chemical	Pairwise comparison	R^2	t	p
ΣPBDEs	Sediment – Biofilm	0.01	-0.04	0.969
	Sediment – Invertebrates	0.04	-0.54	0.607
	Sediment – Fish	**0.57**	**-3.06**	**0.019**
	Biofilm – Invertebrates	**0.78**	**5.03**	**0.002**
	Biofilm – Fish	0.01	0.05	0.962
	Invertebrates – Fish	0.02	0.10	0.925
ΣPCBs	Sediment – Biofilm	0.02	-0.33	0.749
	Sediment – Invertebrates	0.21	1.37	0.213
	Sediment – Fish	**0.66**	**3.69**	**0.008**
	Biofilm – Invertebrates	0.09	-0.82	0.440
	Biofilm – Fish	0.01	0.17	0.869
	Invertebrates – Fish	0.12	0.99	0.355
ΣOCs	Sediment – Biofilm	0.01	0.28	0.788
	Sediment – Invertebrates	0.09	-0.83	0.434
	Sediment – Fish	0.19	1.27	0.244
	Biofilm – Invertebrates	0.37	-2.02	0.083
	Biofilm – Fish	0.02	0.32	0.758
	Invertebrates – Fish	0.01	-0.12	0.911

Appendix S10

Table S10.1. Linear relationships between PBDE, PCB and OCP congeners across samples. DF = degrees of freedom (numerator and denominator).

Chemical group	Congeners	R^2	DF	t	p
PBDEs	BDE 47–99	0.79	1, 35	11.68	<0.0001
	BDE 47–100	0.77	1, 20	8.49	<0.0001
	BDE 99–100	0.74	1, 19	7.61	<0.0001
PCBs	PCB 118–153	0.94	1, 20	18.79	<0.0001
OCPs	HEOD–DDE	0.37	1, 52	5.71	<0.0001
	HEOD–HCB	0.52	1, 39	6.64	<0.0001
	DDE–HCB	0.34	1, 46	5.00	<0.0001
Total (Σ)	PCBs–PBDEs	0.10	1, 64	2.93	0.004
	PCBs–OCPs	0.30	1, 64	5.34	<0.0001
	PBDEs–OCPs	0.17	1, 65	3.81	0.0003

Appendix S11

The flux of biomass along food web links was quantified using the trophic method of production. Biomass flux (F_{ij},g m^{-2} yr^{-1}) from resource *i* to consumer *j* was calculated using the following series of equations:

The proportion of production derived from a food type, *i* (B_i):

1, $B_i = (G_i \times AE_i) / \sum_{Gi=1,\ldots,n}$.

The flow of biomass via food type *i* to consumer *j* (F_{ij}):

2, $F_{ij} = (B_i \times P_j) / (AE_i \times NPE)$

where G*i* is the percentage cover of food type *i* and AE_i is the assimilation efficiency for food *i*, P_j is secondary production for consumer *j* and NPE is the assumed net production efficiency.

Trophic links were assessed using a combination of gut content analysis (n = 545) and modelled links to derive quantitative estimates. The invertebrate food webs here were dominated by herbivore detritivores feeding on amorphous detritus and algae. Gut contents were dissected (x 20 magnification), contents were then placed on slides and contents examined under higher magnification (x 200). A semi-quantitative estimate of proportion of different resources was provided through using an ocular grid (1 cm^2 divided into 100 1 mm^2 cells). Contents were identified as amorphous detritus (matter from biofilms, e.g. polysaccharide matrix), plant fragments and algae, fungi and diatoms were grouped (not identified to a greater taxonomic resolution). The relative proportion of each resource type was calculated using the ocular grid.

Table S11.1. Taxa list for T1 food web. Invertebrates were collected based upon the observed abundance within monthly samples (n = 12) collected across an entire year (2015–2016).

Group	Taxon
Hypnales	Fontinalis spp.
Plecoptera	Leuctra geniculata (Stephens, 1836)
Trichoptera	Polycentropus flavomaculatus (Pictet, 1834)
	Hydropsyche spp.
	Rhyacophila dorsalis (Curtis, 1834)
	Sericostoma personatum (Kirby & Spence, 1826)
	Lepidostoma hirtum (Fabricius, 1775)
Ephemeroptera	Caenis rivulorum (Eaton, 1884)
	Serratella ignita (Poda, 1761)
	Baetis spp.
	Ecdyonurus spp.
	Rhithrogena semicolorata (Curtis, 1834)
	Heptagenia sulphurea (Müller, 1776)
Coleoptera	Platambus maculatus (Linnaeus, 1758)
Amphipoda	Gammarus pulex (Linnaeus, 1758)
Isopoda	Asellus aquaticus (Linnaeus, 1758)
Tricladida	Polycelis spp.
Gastropoda	Radix spp. (Linnaeus, 1758)
Diptera	Simuliidae spp.
Hirudinea	Erpobdella octoculata (Linnaeus, 1758)
Oligochaeta	Naididae spp.
	Eiseniella tetraedra (Savigny, 1826)
Scorpaeniformes	Cottus gobio (Linnaeus, 1758)

Table S11.2. Biological trait data for food web samples. Trait data are from the European trait database (Tachet et al. 2002).

Trophic position	Sample	Size (mm)	Life duration (years)	Life stage	Feeding guild	Voltinism (gen year^{-1})	Habitat
Basal resource	Sediment	-	-	-	-	-	-
	Microbial biofilm	-	-	-	-	-	-
	Fontinalis spp.	-	-	-	-	-	-
Primary consumer	*Rhithrogena semicolorata*	10-20	<1-1	Nymph	Grazer	<1-1	Cobbles
	Heptagenia spp.	10-20	<1-1	Nymph	Grazer	1	Cobbles
	Ecdyonurus spp.	10-20	<1-1	Nymph	Grazer	<1-1	Cobbles
	Caenis rivulorum	5-10	<1	Nymph	Grazer	>1	Mud
	Serratella ignita	5-10	<1	Nymph	Grazer	1	Macrophytes
	Baetis spp.	5-10	<1	Nymph	Grazer	>1	Macrophytes
	Leuctra spp.	5-10	<1	Nymph	Gatherer	1	Cobbles
	Hydropsyche spp.	10-20	1	Larvae	Filterer	1->1	Cobbles
	Lepidostoma hirtum	5-10	>1	Larvae	Gatherer	1	Macrophytes
	Sericostoma personatum	10-20	<1	Larvae	Gatherer	1	Detritus
	Asellus aquaticus	10-20	>1	Adult	Gatherer	>1	Macrophytes
	Gammarus pulex	20-30	>1	Adult	Gatherer	>1	Cobbles
	Radix spp.	20-40	>1	Adult	Grazer	1	Macrophytes
	Eiseniella tetraedra	40-80	>1	Adult	Gatherer	1	Gravel
	Naididae spp.	5-10	<1	Adult	Gatherer	>1	Gravel
	Simuliidae spp.	5-10	>1	Larvae	Filterer	>1	Cobbles

Trophic position	**Sample**	Size (mm)	Life duration (years)	Life stage	Feeding guild	Voltinism (gen year^{-1})	Habitat
Secondary consumer	*Rhyacophila dorsalis*	20-30	>1	Larvae	Predator	1	Cobbles
	Polycentropus spp.	10-20	>1	Larvae	Predator	1	Cobbles
	Polycelis spp.	10-20	>1	Adult	Predator	1	Cobbles
	Erpobdella octoculata	20-40	>1	Adult	Predator	1	Cobbles
	Platambus maculatus	5-10	>1	Adult	Predator	1	Macrophytes

Table S11.3. Relationships between traits and total concentrations of pollutants. R values were used for the selection of traits for modelling of BSAFs across catchments in South Wales.

Trait	Modalities	R		
		PBDEs	PCBs	OCPs
Maximal potential size (mm)	0.25-0.5	**0.30**	**0.34**	**0.44**
	0.5-1	0.02	0.01	0.03
	1-2	0.06	0.24	0.22
	2-4	0.06	0.25	0.00
	4-8	0.02	**0.34**	0.05
Life cycle duration (years)	<1	0.20	**0.34**	0.07
	>1	0.20	**0.34**	0.07
Potential number of life cycles (year-1)	<1	0.10	0.12	0.14
	1	0.25	**0.61**	0.27
	>1	0.25	**0.57**	0.29
Aquatic stage	Egg	0.18	0.20	0.16
	Larva	0.05	0.06	0.20
	Nymph	0.18	**0.33**	**0.31**
	Adult	0.03	0.25	0.08
Reproduction	Ovoviviparity	**0.36**	0.26	0.25
	Egg free	**0.36**	0.20	**0.47**
	Egg cemented	0.29	0.01	0.08
	Clutch cemented	**0.57**	**0.32**	**0.41**
	Clutch free	0.17	0.24	0.16
	Egg vegetated	0.05	0.04	0.02
	Clutch vegetated	0.19	0.03	**0.52**
	Asexual	0.11	0.18	0.11
Dispersal	Aquatic passive	**0.35**	0.00	**0.31**
	Aquatic active	**0.36**	0.21	**0.42**
	Aerial passive	0.04	0.17	0.14
	Aerial active	0.17	0.07	0.17
Resistance forms	Egg	0.12	0.05	0.01
	Cocoons	0.14	0.18	0.18
	Diapause	**0.39**	0.03	**0.31**
	None	0.28	0.17	**0.33**
Respiration	Tegument	0.16	**0.31**	0.28
	Gill	0.24	**0.44**	0.16
	Spiracles	0.19	0.28	0.21
Locomotion and substrate relation	Flier	**0.32**	**0.33**	0.06
	Surface	0.09	0.23	0.19
	Water	0.17	0.02	0.03
	Crawl	0.06	0.02	0.15
	Burrow	0.10	0.01	0.14
	Interstitial	0.17	0.04	0.18
	Temporary attached	0.12	0.14	0.03
Food	Microorganism	0.19	0.28	0.23
	Detritus	**0.38**	0.10	**0.58**

Trait	Modalities	R		
		PBDEs	PCBs	OCPs
	Dead plant	**0.31**	**0.31**	0.04
	Microphytes	0.27	0.26	0.30
	Macrophytes	0.15	0.06	0.01
	Dead animals	0.25	**0.32**	0.16
	Microinvertebrates	**0.37**	0.16	0.19
	Macroinvertebrates	**0.55**	0.18	**0.52**
	Vertebrates	**0.32**	**0.33**	0.06
Feeding habits	Absorber	0.02	**0.34**	0.05
	Deposits	0.22	0.10	0.21
	Shredder	0.26	0.05	0.00
	Scraper	0.17	0.00	**0.32**
	Filterer	0.13	0.03	0.11
	Piercer	**0.32**	**0.33**	0.06
	Predator	**0.34**	0.02	**0.45**
Transversal distribution	Channel	0.02	0.22	0.12
	Banks	0.19	**0.44**	0.05
	Ponds	0.15	0.06	0.10
	Marshes	0.24	0.17	0.14
	Temporary water	0.01	0.05	0.04
	Lakes	0.18	0.30	0.19
	Groundwaters	0.12	**0.33**	0.29
Longitudinal distribution	Crenon	0.25	0.01	0.13
	Epirithron	0.24	0.02	**0.45**
	Metarithron	0.16	0.05	0.09
	Hyporithron	**0.30**	**0.37**	0.07
	Epipotamon	0.01	0.18	0.20
	Metapotamon	0.23	**0.39**	0.20
	Estuary	**0.40**	**0.46**	0.07
	Outside river systems	0.06	0.26	0.04
Altitude	Lowlands	0.12	**0.48**	0.10
	Piedmont	0.11	0.09	0.02
	Alpine	0.15	0.04	0.12
Substrate	Boulder	0.01	0.11	0.02
	Gravel	0.01	0.22	0.21
	Sand	0.09	0.18	0.08
	Silt	0.06	**0.35**	0.05
	Macrophytes	0.01	0.14	0.07
	Microphytes	**0.47**	**0.33**	**0.35**
	Twigs	0.28	0.07	**0.48**
	Detritus	**0.31**	0.12	0.15
	Mud	0.29	0.07	0.18
Current velocity	Null	0.23	0.08	0.07
	Slow	0.01	0.02	0.20
	Medium	0.30	0.06	0.15
	Fast	0.04	0.05	0.25
Trophic status	Oligotrophic	0.06	0.21	0.28

Trait	Modalities	R		
		PBDEs	PCBs	OCPs
	Mesotrophic	0.09	**0.39**	0.11
	Eutrophic	0.00	0.09	0.22
Salinity	Freshwater	0.27	**0.39**	0.09
	Brackish	0.27	**0.39**	0.09
Temperature	Psychophilic	0.07	0.10	0.05
	Thermophilic	0.12	0.08	0.12
	Eurythermic	0.02	0.12	0.03
Saprobity	Xenosaprobic	0.03	0.14	0.11
	Oligosaprobic	0.27	0.05	**0.48**
	β-mesosaprobic	0.09	0.01	0.02
	α-mesosaprobic	0.13	0.13	**0.38**
	Polysaprobic	0.18	0.02	0.24
pH	<4	0.15	0.10	0.23
	>4-4.5	0.22	0.17	0.10
	>4.5-5	0.19	0.26	0.04

Appendix S12

Table S12.1. BMF values for organisms sampled across riverine food webs in South Wales. These values are summarised within the main text.

Site	Organism	BMF		
		∑ PBDEs	∑ PCBs	∑ OCPs
T1	*Leuctra* spp.	1.56	1.30	1.56
	Baetis spp.	1.31	0.97	0.76
	Ecdyonurus spp.	0.62	ND	5.18
	Gammarus pulex	1.46	2.41	4.42
	Hydropsyche spp.	9.05	2.16	0.84
	Rhyacophila dorsalis	1.25	4.31	6.63
	Cottus gobio	12.61	35.70	35.97
	Cinclus cinclus	154.79	136.45	78.57
T2	*Leuctra* spp.	10.55	0.00	2.83
	Baetis spp.	1.82	0.00	4.17
	Ecdyonurus spp.	1.85	ND	4.61
	Gammarus pulex	2.48	23.23	5.52
	Hydropsyche spp.	3.59	6.14	0.24
	Rhyacophila dorsalis	6.53	54.36	1.79
	Cinclus cinclus	43.40	34.92	57.85
T3	*Gammarus pulex*	ND	10.39	39.21
U1	*Baetis* spp.	1.44	0.00	16.50
	Ecdyonurus spp.	2.39	10.85	ND
	Gammarus pulex	4.72	18.44	9.68
	Hydropsyche spp.	14.53	0.00	1.76
	Rhyacophila dorsalis	9.01	78.09	3.43
	Cottus gobio	45.09	5.02	12.66
U2	*Baetis* spp.	3.17	3.17	10.72
	Ecdyonurus spp.	10.89	10.89	2.04
	Gammarus pulex	13.95	13.95	9.33
	Hydropsyche spp.	7.03	7.03	0.27
	Rhyacophila dorsalis	12.06	12.06	2.40
	Cottus gobio	36.26	47.41	36.46
	Cinclus cinclus	206.79	287.58	139.69
U3	*Baetis* spp.	1.94	ND	1.44
	Ecdyonurus spp.	3.80	13.20	0.42
	Gammarus pulex	40.96	0.22	2.52
	Hydropsyche spp.	2.22	0.33	0.79
	Rhyacophila dorsalis	15.99	0.20	0.80
	Isoperla grammatica	0.61	10.75	2.86
	Cottus gobio	23.22	7.76	48.69
	Cinclus cinclus	108.53	75.61	277.39
W1	*Baetis* spp.	2.11	ND	3.90
	Ecdyonurus spp.	0.63	3.87	5.43
	Hydropsyche spp.	3.05	0.80	4.58
	Rhyacophila dorsalis	2.13	ND	1.32

	Cottus gobio	14.83	2.41	77.30
W2	*Leuctra* spp.	29.98	0.00	41.63
	Baetis spp.	16.53	2.34	14.84
	Ecdyonurus spp.	6.65	0.04	10.60
	Serratella ignita	8.34	0.00	8.95
	Hydropsyche spp.	7.80	0.43	0.43
	Cottus gobio	17.98	6.59	21.38
W3	*Ecdyonurus* spp.	8.11	0.00	0.72
	Gammarus pulex	17.62	22.70	18.75
	Hydropsyche spp.	19.89	0.00	1.71
	Rhyacophila dorsalis	25.95	22.38	11.88
	Cottus gobio	10.51	44.20	16.82
	Cinclus cinclus	24.30	125.69	91.26

Table S12.2. Metrics used to calculate TMFs. Statistical data (R^2, slope and error) are derived from linear relationships (Equation 6.2). Total concentration of congeners across the riverine food webs is presented in wet weight.

Congener	log K_{OW}	R^2	Slope	Error	TMF	$\sum$ Congener (ng g^{-1} ww)
BDE-100	7.03	0.59	0.14	0.02	1.38	172.55
BDE-153	7.86	0.66	0.12	0.02	1.33	79.92
BDE-47	6.80	0.67	0.16	0.01	1.46	444.01
BDE-99	7.38	0.63	0.16	0.02	1.45	847.01
PCB-101	6.36	0.70	0.22	0.03	1.67	782.89
PCB-118	6.74	0.74	0.20	0.02	1.60	620.13
PCB-138	6.67	0.73	0.28	0.03	1.92	3480.12
PCB-153	6.89	0.76	0.30	0.03	2.00	5673.63
PCB-163	6.82	0.66	0.23	0.04	1.72	1085.51
PCB-170	7.71	0.67	0.21	0.03	1.62	1254.50
PCB-180	7.20	0.68	0.25	0.03	1.78	3697.12
PCB-187	6.92	0.63	0.21	0.04	1.63	1044.55
TDE	6.02	0.60	0.18	0.03	1.50	491.43
DDE	6.51	0.59	0.24	0.02	1.72	48680.31
DDT	6.91	0.76	0.23	0.02	1.71	4241.92
HCB	5.73	0.75	0.22	0.02	1.66	1223.25
HEOD	5.40	0.43	0.15	0.02	1.40	1765.12

Appendix S13

Table S13.1. Summary of taxonomic data (June 2016-2017) for locations sampled across south-western UK. Abundance codes are as follows: * = 1-10, ** = 10-100, *** = 100+.

Taxa	Site								
	T1	T2	T3	U1	U2	U3	W1	W2	W3
Ephemeroptera									
Baetis rhodani	***	***	***	***	***	***	***	***	***
Baetis scambus	*	**	*			**			
Baetis niger							*	*	*
Alainites muticus		*			*	*	*	*	
Procloeon bifidium				*	*				
Leptophlebia spp.					*	*			
Paraleptophlebia spp.	*	*	*	*	*	**	*	*	*
Habrophlebia spp.				*		*			
Caenis rivulorum	***	***	*	***	***	**	***	***	**
Caenis luctuosa	*			*	*		*	*	
Caenis horaria					*				
Serratella ignita	***	***	*	***	***	***	***	***	***
Ephemera danica		*		*	*			*	*
Ecdyonurus spp.	***	***	*	***	***	***	**	***	***
Rhithrogena semicolorata	**	***	*	***	***	***	***	***	***
Heptagenia sulphurea	***	**	*	**	***	*	*	***	*
Electrogena lateralis	**	*		**	*	*		**	
Plecoptera									
Leuctra geniculata	***	**	*	***	***	***	**	***	**
Leuctra fusca	*			*					
Leuctra moseyli/hippopus	***	***	*	***	***	**	***	***	***
Leuctra nigra	*	*					*		*
Leuctra inermis		***		**	***	**	**	**	**
Nemoura cambrica		**	*	*	*	*	*		*
Nemoura avicularis							*		
Amphinemoura sulcicollis	*	***		*	**	*			
Protonemoura meyeri	*	**			**	*	**	*	
Brachyptera risi		*			*		*	*	*
Chloroperla tripunctata		*		**	**	*	**	**	**
Siphonoperla torrentium	*	*	*	*	*		**	**	**
Dinocras cephalotes		*							
Perla bipunctata		*		*					
Isoperla grammatica		**		*	**	*	**	**	**
Perlodes mortonii	*			*	*		*	*	*
Diura bicaudata							*		*
Trichoptera									
Philopotamus montana					*				
Polycentropus kingi				*		*	*		

Taxa	Site								
	T1	T2	T3	U1	U2	U3	W1	W2	W3
Polycentropus flavomaculatus	**	**	*	**		**	**	***	*
Plectrocnemia conspersa		*		*				*	
Plectrocnemia geniculata	**	**		**		*	*	**	*
Hydropsyche siltalai	**	***		**	**	**	**	***	**
Hydropsyche pellucidula	**	**		*	**	*	*	**	**
Hydropsyche instabilis		*			**	*		*	
Hydropsyche angustipennis		*				*			*
Cheumatopsyche lepida								*	
Agapetus fuscipes	**	**	**	**	*	***	**	***	***
Glossosoma conformis		**		*	*	*	**	*	
Hydroptila spp.	***	*	*	*				*	
Allotrichia pallicornis					*				
Rhyacophila dorsalis	***	**	*	**	**	**	**	**	**
Mystacides spp.	***	**		**	**	**	**	**	*
Athripsodes spp.	**	*	*	*	*	*	*		
Oecetis testacea	*			*			*	*	
Odontocerum albicorne	*	*		*	**	*	*		**
Sericostoma personatum	**	**	*	*	**	*	***	**	**
Drusus annulatus		**	*	*	*	**	*		**
Halesus radiates				*		*			
Limnephilus spp.						*	*		
Potamophylax cingulatus		*							*
Potamophylax latipennis	*			*	*	*	*	*	
Micropterna sequax				*		*	*		
Ecclisopteryx guttulata		*				*			**
Chaetopteryx villosa							*		
Brachycentrus subnubilis								**	
Goera pilosa	*	*			*	*	*	*	*
Silo pallipes		*		*		**	*		*
Psychomyia pusilla	*	**		*	*	*	*	**	*
Metalype fragilis	*								
Tinodes dives		*							
Lepidostoma hirtum	**	**		**	*	*	***	***	**
Ecnomus tennellus								*	
Odonata									
Cordulegaster boltonii		*							
Coleoptera									
Platambus maculatus				**		*	*		*
Elmis aenea	***	***	**	**	**	**	***	**	***
Limnius volckmari	***	***	**	***	***	***	***	***	***
Oulimnius tuberculatus	*	*	*	**	**	*	**	**	*
Esolus parallelepipedus	***	**	**	***	***	***	***	***	***
Gyrinus spp.	*	*		*	*	*	**	**	**
Hydraena gracilis	*	*		*	*	**	*		*
Elodes sp.		*				*	*	*	
Limnichus pygmaeus				*					

Taxa	Site								
	T1	T2	T3	U1	U2	U3	W1	W2	W3
Amphipoda									
Gammarus pulex/fossarum	***	***	***	***	***	***	**	**	***
Isopoda									
Asellus aquaticus	*	*	*	*	*	*	*		*
Arachnida									
Hydracarina	***	**	*	**	**	**	***	**	**
Tricladida									
Polycelis tenuis/nigra	***	***	**	**	***	**	*	*	**
Planaria torva	*					*	*		*
Dugesia spp.	*								
Dendrocoleum lactuem	**								
Gastropoda									
Bithynia leachi	*	*		*	**			*	*
Hydrobia spp.	*	*		*	**			*	*
Bythinella spp.	**	*	*	*	**			**	*
Lymnaea spp.	**	*		*	*			*	
Radix peregra	**	*		*	*		*	*	*
Ancylus fluviatalis	**	**	*	**	**	**	***	***	***
Planorbis spp.		*						*	
Theodoxus fluviatalis	*	*					*	*	
Valvata spp.					*			*	
Bivalvia									
Pisidium spp.	*	*	*	*	**	**		**	*
Diptera									
Chironomidae	***	***	***	***	***	***	***	***	***
Ceratopogonidae	**	**	*	**	**	*	*	**	*
Simuliidae	***	***	***	***	***	***	***	***	***
Dixidae	*			*			*	*	*
Tipulidae	**	**	**	**	**	**	**	**	**
Empididae	**	**	*	*	*	**	*	**	**
Muscidae					*		*		
Athericidae				*	*		*	*	*
Stratiomyidae									*
Psychodidae		*		*		*			*
Hemiptera									
Plea leachi				**					
Arhynchobdellida									
Erpobdella octoculata	*			*	*	*	*		*
Erpobdella testacea			*				*	*	
Glossiphonia complanata		*		*			*	*	
Piscicola geometra	**			*		*		*	
Helobdella spp.				*				*	
Oligochaeta									
Naididae	**	**	**	***	**	**	***	***	***
Tubificidae	***	*	***	***	***	**	**	***	***
Eisienella tetraeda	**	**	*	**	**	**	**	**	**

Appendix S14

Table S14.1. Definitions and notes for functional diversity metrics.

Metric	Definition (notes)
Functional Richness (FRic) *Community-level*	The amount of functional space filled by the community. For multivariate trait data this is the total area, in functional space, covered by all of the taxa within the community.
Taxon Functional Richness (tFRic) *Taxon-level*	The amount of functional space filled by individual taxa within the community. This indicates the ability of the taxa within a community to fill the functional space. The values presented are a mean value for all taxa present within the community.
Functional Dispersion (FDis) *Community-level*	The spread or distribution of traits within a community. Measured by calculating the difference between the traits of taxa from the community from the mean community values for those traits. Higher values indicate a greater variation in the traits of taxa within the community.
Functional Similarity (FSim) *Taxon-level*	The relative amount of overlap in traits by pairs of taxa. Small values indicate dissimilarity, with no overlap in the traits between taxa. Large values indicate that taxa composing the community are functional identical, with limited variation in traits between taxa.
Functional Redundancy (FRed) *Community-level*	The level of niche overlap between taxa in the community functional space. Low values indicate no overlap between pairs, and high values indicate a large amount of overlap between taxa in the community with a high functionally similarity.

Appendix S15

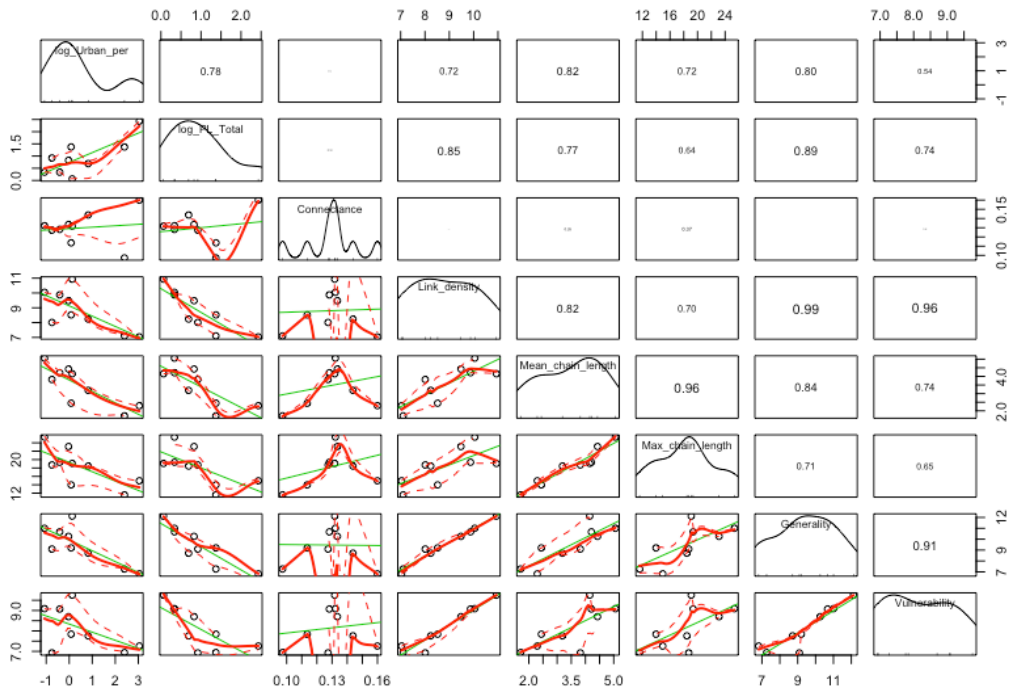

Fig. S15.1. The relationships between urban land cover, total persistent contaminant concentrations in macroinvertebrate samples and quantitative food web metrics. Values in the upper panels are Pearson's correlation coefficients for corresponding relationships displayed in the lower panels.

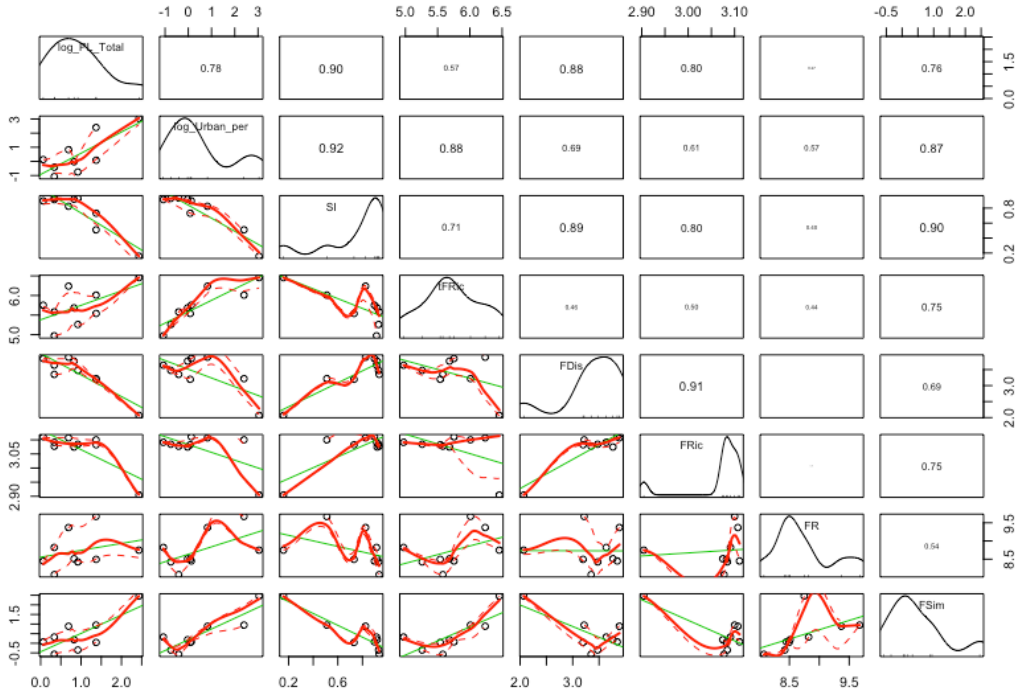

Fig. S15.2. The relationships between urban land cover, persistent contaminants and macroinvertebrate community diversity metrics (structural and functional). Values in the upper panels are Pearson's correlation coefficients for corresponding relationships displayed in the lower panels.