Science of the Total Environment 912 (2024) 169079

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Assessment of the impacts of GABA and AChE targeting pesticides on freshwater invertebrate family richness in English Rivers

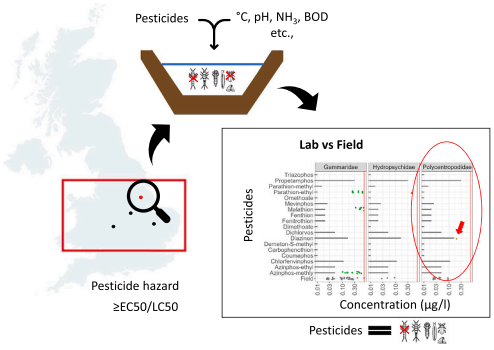
Imogen P. Poyntz-Wright [a,*], Xavier A. Harrison [b], Andrew Johnson [c], Susan Zappala [d], Charles R. Tyler [a,*]

[a] Biosciences, Geoffrey Pope Building, University of Exeter, Stocker Road, Exeter EX4 4QD, UK
[b] Centre for Ecology and Conservation, University of Exeter, Penryn TR10 9FE, UK
[c] Centre for Ecology and Hydrology, MacLean Building, Benson Lane, Crowmarsh Gifford, Wallingford OX10 8BB, UK
[d] JNCC, Quay House, 2 East Station Road, Fletton Quays, Peterborough PE2 8YY, UK

HIGHLIGHTS

- Concentrations of pesticides toxic to invertebrates occur in English rivers.
- AChE/GABA pesticides are associated with riverine invertebrate family richness.
- Pesticide toxicity thresholds differ for invertebrates between laboratory and field.
- AChE/GABA concentrations at riverine sites are generally below lab toxic thresholds.
- Invertebrates most at risk to AChE/GABA pesticides identified in selected rivers.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Daniel Wunderlin

Keywords:
Pesticides
Macroinvertebrates
Pollution
Rivers
Field
Laboratory

ABSTRACT

Globally, riverine system biodiversity is threatened by a range of stressors, spanning pollution, sedimentation, alterations to water flow, and climate change. Pesticides have been associated with population level impacts on freshwater invertebrates for acute high-level exposures, but far less is known about the chronic impact of episodic exposure to specific classes of pesticides or their mixtures. Here we employed the use of the UK Environment Agency's monitoring datasets over 40 years (covering years 1980 to 2019) to assess the impacts of AChE (acetylcholinesterase) and GABA (gamma-aminobutyric acid) receptor targeting pesticides on invertebrate family richness at English river sites. Concentrations of AChE and GABA pesticides toxic to freshwater invertebrates occurred (measured) across 18 of the 66 river sites assessed. For one of the three river sites (all found in the Midlands region of England) where data recorded over the past 40 years were sufficient for robust modelling studies, both AChE and GABA pesticides associated with invertebrate family richness. Here, where AChE total pesticide concentrations were classified as high, 46 of 64 invertebrate families were absent, and where GABA total pesticide concentration were classified as high, 16 of 64 invertebrate families were absent. Using a combination of field evidence and laboratory toxicity thresholds for population relevant endpoints we identify families of invertebrates most at risk in the selected English rivers to AChE and GABA pesticides. We,

* Corresponding authors.
E-mail addresses: ipp203@exeter.ac.uk (I.P. Poyntz-Wright), c.r.tyler@ex.ac.uk (C.R. Tyler).

https://doi.org/10.1016/j.scitotenv.2023.169079
Received 25 August 2023; Received in revised form 30 November 2023; Accepted 1 December 2023
Available online 2 December 2023

furthermore, provide strong evidence that the absence of the invertebrate family Polycentropodidae (caddisfly) from one field site is due to exposure effects to AChE pesticides.

1. Introduction

Globally riverine systems are experiencing a diverse range of stressors including changes in hydro-morphology, nutrient enrichment, invasive species, and toxic substance exposure, that are affecting their ecological status and leading to freshwater biodiversity declines (Dudgeon et al., 2006; Living Planet Report, 2020; Lemm et al., 2021). Of these stressors, chemical pollution is a dominant force driving declines of some freshwater biota (Dudgeon et al., 2006; Vaughan and Ormerod, 2012). Exposure to chemical pollutants including metals, petrochemicals, and pesticides have been shown to cause population level impacts on riverine invertebrate communities (Clements, 1994; Scoggins et al., 2007; Beketov et al., 2009; Byrne et al., 2013). Specifically with regards to pesticides, impacts on invertebrate communities, these have been noted worldwide, including from South America, Africa, USA and Europe (Schriever et al., 2007; Beketov et al., 2013; Chiu et al., 2016; Hunt et al., 2017; Macchi et al., 2018; Beuter et al., 2019; Ganatra et al., 2021). Pesticides shown to impact on riverine invertebrate communities around the world largely belong to the pyrethroid and organophosphate classes (Schriever et al., 2007; Beketov et al., 2013; Chiu et al., 2016; Hunt et al., 2017; Macchi et al., 2018; Ganatra et al., 2021).

In the UK improvement in general water quality due to wastewater treatment regulations invoked in the 1990s by the EU Urban Waste Water Treatment Directive (reducing BOD; Biochemical oxygen demand, metals and ammonia) and EU Nitrate Directive (reducing nitrate) (Vaughan and Ormerod, 2012; Whelan et al., 2022) have led to improvements in freshwater invertebrate family richness. However, chemical stressors may still be impacting upon riverine invertebrate biodiversity, as these assessments compare with baselines from the time of the industrial revolution, where pollution levels were extremely high (Whelan et al., 2022). This in turn may lead to assumptions that chemical pollution is now having little impact on biodiversity (Vaughan and Ormerod, 2012; Outhwaite et al., 2020; Whelan et al., 2022) whereas in fact some measures of water quality (e.g. dissolved organic carbon, nitrogen, phosphorous) in some agricultural catchments are worse now than before the 1960s (Whelan et al., 2022). Furthermore, specific cases of localised deterioration in invertebrate richness have been reported, as in the case for freshwater Mollusc taxa that continue to see declines in Britain (Vaughan and Ormerod, 2012; Outhwaite et al., 2020).

The impact of metals on riverine invertebrates' richness in the UK have been the focus of much research over the past few decades, however, other chemicals, and notably pesticides have received less attention (Dowson et al., 1996; Hirst et al., 2002; Jarvis and Younger, 2006; Amisah and Cowx, 2011; Byrne et al., 2013; Gething et al., 2020; Walker and Hassall, 2021). Most UK freshwater pesticide studies have also centred around spill events (Dowson et al., 1996) with very few UK studies assessing the chronic and/or episodic exposure effects. They have also tended to focus on single chemicals (e.g. the effects metaldehyde determining invertebrate family richness in the Anglian region of the UK; Gething et al., 2020). In Europe, South America, USA and Africa findings on pesticides in freshwater environment have indicated significant impacts on invertebrate family richness and evenness, with losses of taxa up to 42 % (Beketov et al., 2013; Chiu et al., 2016; Hunt et al., 2017; Macchi et al., 2018; Ganatra et al., 2021). Furthermore, some of these effects have been reported to occur for concentrations otherwise reported to be environmentally protective (Beketov et al., 2013). This perhaps is not surprising, given that risk assessment has to date been conducted on a single chemical basis and pesticides in combination can be additive, synergistic or antagonistic in their effects. Building understanding on the effects of pesticides collectively on invertebrate family richness is much needed, albeit the ways in which mixtures of pesticides interact to affect an organism are complicated and difficult to predict (Day and Scott, 1990; Loureiro et al., 2010; LeBlanc et al., 2012; Wang et al., 2015). Investigating pesticides impact on invertebrate family richness from a mode of action perspective offers a useful approach as the relative sensitivity of invertebrates is dependent, at least in part, on these molecular target sites, and this is well documented for arthropods (Rico and Van den Brink, 2015). Factors in addition to the mode of action of pesticides that affect the extent to which freshwater invertebrates may be impacted, include the exposure dose/regime, species, aspects of water physiochemistry and water flow.

Pesticide half-lives vary, as does their application rates to fields over a given season and across farm types. Pesticides such as carbamates, organophosphate (AChE targeting pesticides) have relatively lower level persistence in the environment (Mdeni et al., 2022), whereas organochlorine pesticides (GABA targeting pesticides) tend to be highly persistent (Jayaraj et al., 2016). In many cases organisms will be subjected to repeat exposures to pesticides and this can have a crucial bearing on their relative toxicity (Dohmen et al., 2016). Even for some non-persistent pesticides, such as carbamate and organophosphates, they can be highly toxic to freshwater invertebrates as determined by laboratory toxicity studies. The toxicity of pesticides, however, differs depending on the taxa – with some taxa being more sensitive due to factors including aspects of their life history, traits, genetics etc., (Rico and Van den Brink, 2015; Van Den Berg et al., 2019). Water quality parameters, such as pH, temperature, flow, and salinity can affect both the persistence and potency of pesticide active ingredients, hence the effect of pesticides can be situation (river) specific (Heugens et al., 2008; Stampfli et al., 2013; Bray et al., 2021a, 2021b; Macaulay et al., 2021).

Adopting a case-study type approach here we demonstrate how we can use long-term monitoring datasets and laboratory toxicity information to assess for relationships between exposure to pesticides with specific mode of actions and the invertebrate family richness of riverine sites and determine sensitive/tolerant taxa. The Anglian and Midland regions of England were chosen for these analyses because they have significant levels of arable farmland and where there is potential for significant impact of pesticides on riverine invertebrate communities (Poyntz-Wright et al., 2023). However, the Midland region has experienced lower pesticide application rates than the Anglian region over the past 40 years, and consequently has seen a dramatic improvement in the average number of pesticide sensitive species present compared to Anglia (Poyntz-Wright et al., 2023). The study focused on AChE and GABA acting pesticides that include 3 of the 4 main classes of pesticides used in agriculture in England (Kadiru et al., 2022). We hypothesised that, both AChE and GABA pesticides would play a significant role in determining freshwater invertebrate family richness in sites where upstream land-use was largely dominated by arable farming in England. Further, we sought to identify if certain families were more sensitive to these pesticide groups through a combination of our field-based analyses and published information on laboratory-based data on toxicity thresholds for individual pesticides based on population relevant endpoints. In these analyses we also highlight where data are most needed to build further on this type of approach for understanding interrelationships between invertebrate populations and exposure to specific classes of pesticides.

2. Method

We employed the use of the UK Environment Agencies monitoring data over 40 years, collected between years 1980 to 2019, focusing on the geographical regions of Anglian and the Midlands with the aim of

understanding if pesticide pollution in selected riverine surface waters in England has been a significant factor in determining invertebrate family richness. The Anglian and Midland regions are dominated by arable land-use with higher than average percentages of arable land use in England (78 % and 61 %, respectively) and as such greater overall insecticide (GABA and AChE) application/pollution (Schletterer et al., 2010; Smith et al., 2018; Gov.UK, 2023a, 2023b, 2023c; Poyntz-Wright et al., 2023). Other regions in England, particularly the southeast, more northerly regions and southwest are generally dominated more by pastoral farming and lesser impacted by pesticides, hence not focused on in this study (Smith et al., 2018; Poyntz-Wright et al., 2023). We further use laboratory-based evidence from the peer review literature to assess invertebrate family sensitivity to pesticides to support the case for pesticide impacts on family richness for the field study site findings.

2.1. Data collection

Macroinvertebrate, water quality (air temperature (°C), pH, orthophosphate, ammonia (mg/l), ammoniacal nitrogen (mg/l), nitrite (mg/l), nitrate (mg/l), BOD (mg/l), dissolved oxygen (mg/l), oxygen saturation (mg/l), suspended solids (mg/l) and pesticide (μg/l)) and site characteristic data (altitude, land-use etc.,) for Anglian and Midland regions of England were sourced from ChemPop, CEH BIOSYS database (https://environment.data.gov.uk/ecology/explorer/) and Environment Agency's WIMS databases (https://environment.data.gov.uk/water-quality/view/landing). Laboratory toxicity test information for the pesticide effects on invertebrate families was collected from the ECOTOX database (https://cfpub.epa.gov/ecotox/search.cfm).

2.2. Riverine site selection for pesticides impact assessment

Sites with both invertebrate and pesticide (as well as water quality/site characteristics) data were identified using qGIS mapping (the list of included pesticides is provided in the supplementary material S1). Sites were only included in our analysis if the distance between chemical and biota collected data was <100 m and there was no substantial difference in land-use between these sampling points (i.e. no major differences in road infrastructure, field use, housing development etc.) to avoid, wherever possible, potential compounding factors that may alter physiochemistry of water between the biota and chemical sample site. This approach was adopted to maximise likelihood that the chemical samples were representative of areas for the biota samples. Of the 66 selected sites identified, 30 were from the Anglian region and 36 from the Midlands region; monitored between 1980 and 2019. We classified all pesticides detected at riverine sites into groups based on their main mode of action (MoA), identifying those which were GABA- and AChE-targeting pesticides (see Table S1). GABA pesticides are neurotoxic pesticides, targeting the GABA-gated chloride channel blocking function, and result in reduced neural inhibition leading to hyper-excitation of the nervous system (Gant et al., 1987; Bloomquist, 1993). AChE pesticides, that are also neurotoxic, target the AChE receptor inhibiting its function to degrade acetylcholine (ACh), which is an essential neurotransmitter of the central nervous system (Mladenović et al., 2018).

2.3. Potential pesticide hazard to riverine invertebrates based on laboratory toxicity data

To highlight where a potential risk of pesticides to invertebrates may occur in the English rivers studied, we compared the laboratory derived toxicity data for the target pesticides for relevant invertebrate species, to the concentrations recorded at riverine sites across the Midland and Anglian regions. For this analysis, we gathered all available toxicity data for each pesticide (active ingredient) for the endpoints of growth, development, reproduction and mortality for each invertebrate family found at the riverine study sites. Acute exposure studies were defined as ≤7 days, whereas chronic (and sub-chronic) were >7 days. All life stages and test conditions (pH, temperature etc.) were included. Due to the limited number of studies available for some chemicals, data from studies with both measured and nominal test concentrations were included. For studies with measured pesticide concentrations a prerequisite for inclusion in our analysis was experimental repeats, and mean concentration was used in analysis and only water-borne exposure studies were included in our analyses. We then compared the acute laboratory toxicity concentrations with the maximum concentration of pesticides' measured (i.e. worst case scenarios) from the 66 riverine sites across Midlands and Anglian regions (Environment Agency WIMS database). The laboratory derived effect concentrations were selected based on the minimum concentration for which a significant (≥EC50/LC50) toxic effect on growth, development, reproduction and/or mortality occurred for an invertebrate in each family occurring in English rivers (see Table S3 for all documented chemical toxicity concentrations for the invertebrate families). Due to limited toxicity data, we were not able to apply the 'Criteria for reporting and evaluating ecotoxicity data' (CRED) system that is specifically designed for the evaluation of ecotoxicity data for regulatory use; Moermond et al. (2016).

2.4. In-field analysis of pesticide impact on invertebrate family richness

We chose to average all water quality (including pesticide) data over the 36 days preceding the collections of invertebrate samples to provide 'average water quality exposure conditions' that the invertebrates experienced in days prior to sampling (see Fig. 1). The time period of 36 days was chosen to balance maximizing the dataset (number of coinciding pesticide and biota observations), whilst limiting the possible variation due to seasonal changes in pesticide use and changes in invertebrate presence/absence throughout the year. Pesticide concentrations measured below the LOD (limit of detection), were considered at the LOD for this study. Family richness - the number of unique families present in each sample - was produced from the invertebrate sampling data (see Table S2 for all species and families present at all sites). Studying family richness was adopted as an holistic approach for understanding of interrelationships between exposure to both selected pesticides and other water quality variables' on the entire community, making no assumptions for which taxa may be more or less sensitive to these effects (see: Bray et al., 2021b). Water quality variables' data were averaged over 36 days due to sporadic sampling. We were not able to confidently identify actual maximum or minimum values for pesticides at these sites because of the sporadic sampling across the sites meant these timepoints were likely missed. Thus, average values provide more reliable estimates for assessing variable pressures in study sites.

The limited number sites for which there were matching biota and water quality data resulted in the requirement to remove many riverine sites from subsequent modelling analyses; to provide a good level of confidence for the analyses (reduce type 1 error), only sites with ≥30 observations for biota-water quality data were selected (Warton et al., 2016). This resulted in only 3 sites (all from the Midland region) for modelling the relative influence of pesticide groups (based on MoA) compared with water quality variables on freshwater invertebrate family richness, but these sites provided a high-quality data set. Across the three sites, arable land-use accounted for 59.67 % (site 1), 6.33 % (site 2) and 11.61 % (site 3) of upstream land-use.

2.5. Identification of invertebrate families susceptible to pesticides

Where impact of pesticides on family richness was determined through modelling, we then used laboratory toxicity data (effect concentrations) to determine which families of those recorded at the riverine site were most likely to be sensitive to AChE/GABA pesticides recorded in the river based on population relevant endpoints (reproduction, growth, development and mortality).

Following this, we compared the individual AChE/GABA pesticides'

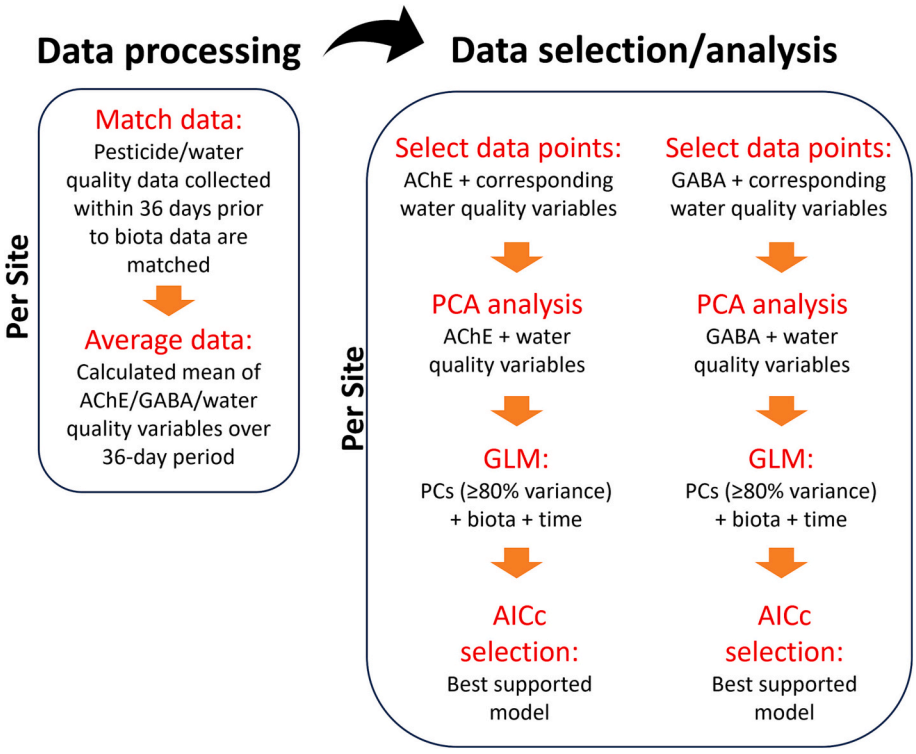


Fig. 1. Methodological diagram detailing the processing, selection and analysis of biota and water quality field data.

laboratory toxicity effect concentration for invertebrate families to field data AChE/GABA pesticides' concentrations (per family) to determine if laboratory-based effect concentrations were exceeded at the riverine site (site 1). Then we looked at the presence/absence of invertebrate families in riverine site against total AChE/GABA riverine concentrations, to determine if the exceedance/non-exceedance of laboratory effect concentrations per family matched the occurrence of family against field site total recorded AChE/GABA concentrations.

This was done to assess whether the pesticide concentrations recorded in rivers might relate to the absence/loss of invertebrate families; i.e. where laboratory based toxicity effect concentrations (both acute and chronic independently) were exceeded in the field, did this coincide with the absence of an invertebrate family.

For some pesticides, laboratory invertebrate toxicity effect data were not available, including isodrin, HCH-alpha/beta/delta and endosulfan (GABA pesticides) and demeton-*s*-methyl and dichlorvos (AChE pesticides) – that all occurred at site 1.

2.6. Statistical analysis

We used R Core Team (2021) version 4.2.1 to conduct all statistical analyses, and QGIS (2022) version 3.10.13 for regional mapping. We used the *R* packages *lme4* (Bates et al., 2015) to fit generalised linear models, *Dharma* (Hartig, 2022) to check model fit and for over-dispersion, *ggplot2* (Wickham, 2016) to produce graphs and *sjmisc* (Lüdecke, 2021), *sjlabelled* (Lüdecke, 2022), *sjPlot* (Lüdecke, 2023), *corrplot* (Hahsler et al., 2008), *FactoMineR* (Husson et al., 2023), *factoextra* (Kassambara, 2020) for PCA analysis and *jtools* to determine 95 % confidence intervals (Long, 2023). All code and analyses required to reproduce these analyses are provided online at https://github.com/ImogenPW/Assessment-of-the-impacts-of-GABA-and-AChE-targeting-pesticides

Principal component analysis (PCA) was carried out for each site per pesticide group, to enable dimension reduction for site-level water quality predictors including pesticides (see Fig. 1). Water quality data was normalised (centred) before calculating components. Site PCA provided components (PC1, PC2 etc.) that were added to a generalised linear model with Poisson errors to determine the extent of which pesticide groups impacted family richness relative to other water quality variables (See Fig. 1). We fitted family richness as response and principal components (≥80 % variance) as fixed effects, along with time (day in year and/or year) as an additional fixed effect. We identified pesticides as important drivers of invertebrate richness where PCs (Principal Components) containing pesticide variables were identified as significant.

We performed model selection using an information theoretic approach to rank models based on their support in the data using AICc. We considered all models within Δ6 AICc unit of the top model to have similar levels of support in the data.

3. Results

3.1. Pesticide concentrations hazardous to riverine invertebrate families

The maximum concentrations for 11 out of 48 AChE and GABA targeting pesticides recorded in the 66 rivers assessed were found to be equal to, or exceed, laboratory concentrations, which cause significant acute toxic (≥EC50/LC50) effects on at least one riverine macroinvertebrate family (Fig. 2, Figs. S1–3). The exceedance of laboratory toxicity concentrations in the Anglian and the Midlands rivers, suggest there is potential for harm from AChE and GABA pesticides. For site 1, fipronil, diazinon, dichlorvos and parathion-methyl concentrations exceeded the EC50's/LC50's, whereas for site 2 and 3, parathion-methyl concentrations exceeded laboratory toxicity threshold concentrations (Fig. 2, and Fig. S4).

3.2. Freshwater invertebrate family richness associations with pesticide exposures

Of the 3 sites assessed, one site in the Midlands region (site 1) indicated that pesticides have an important role in determining freshwater invertebrate family richness. This was the case for both AChE ($n = 44$)

Fig. 2. Maximum pesticide concentrations (µg/l) detected in riverine waters from the Midlands and Anglian. Black dots represent concentrations which exceed a minimum significant acute toxicity concentration of a relevant macroinvertebrate family (laboratory data acquired from ECOTOX database). AChE acting pesticides; Azinophos-methyl, Carbaryl, Diazinon, Dichlorvos, Fenitrothion, Malathion, Mevinphos, Parathion-methyl, Phorate, Propoxur. GABA acting pesticides; Fipronil. Yellow outlined dot is site 1. Blue outlined dot is site 2. Pink outlined dot is site 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and GABA ($n = 59$) pesticides where they were found to be significant in explaining variation in family richness, as illustrated in Fig. 3 and Table 1 (the best supported models in both cases contained PCs dominated by variables associated with pesticide use; Tables S4 and S5). Water quality variables were also factors in determining family richness, alongside pesticides, particularly for AChE (see Fig. 2a). Ammoniacal nitrogen, ammonia, BOD, dissolved oxygen and orthophosphate in combination with AChE pesticides were found to be important in determining invertebrate family richness (Fig. 3, PC1). Temperature too appeared to be an important factor in combination with GABA pesticides in determining family richness (Fig. 3, PC3).

3.3. *Invertebrate families most sensitive to pesticides (based on laboratory exposures)*

Laboratory exposure data for AChE and GABA acting pesticides for

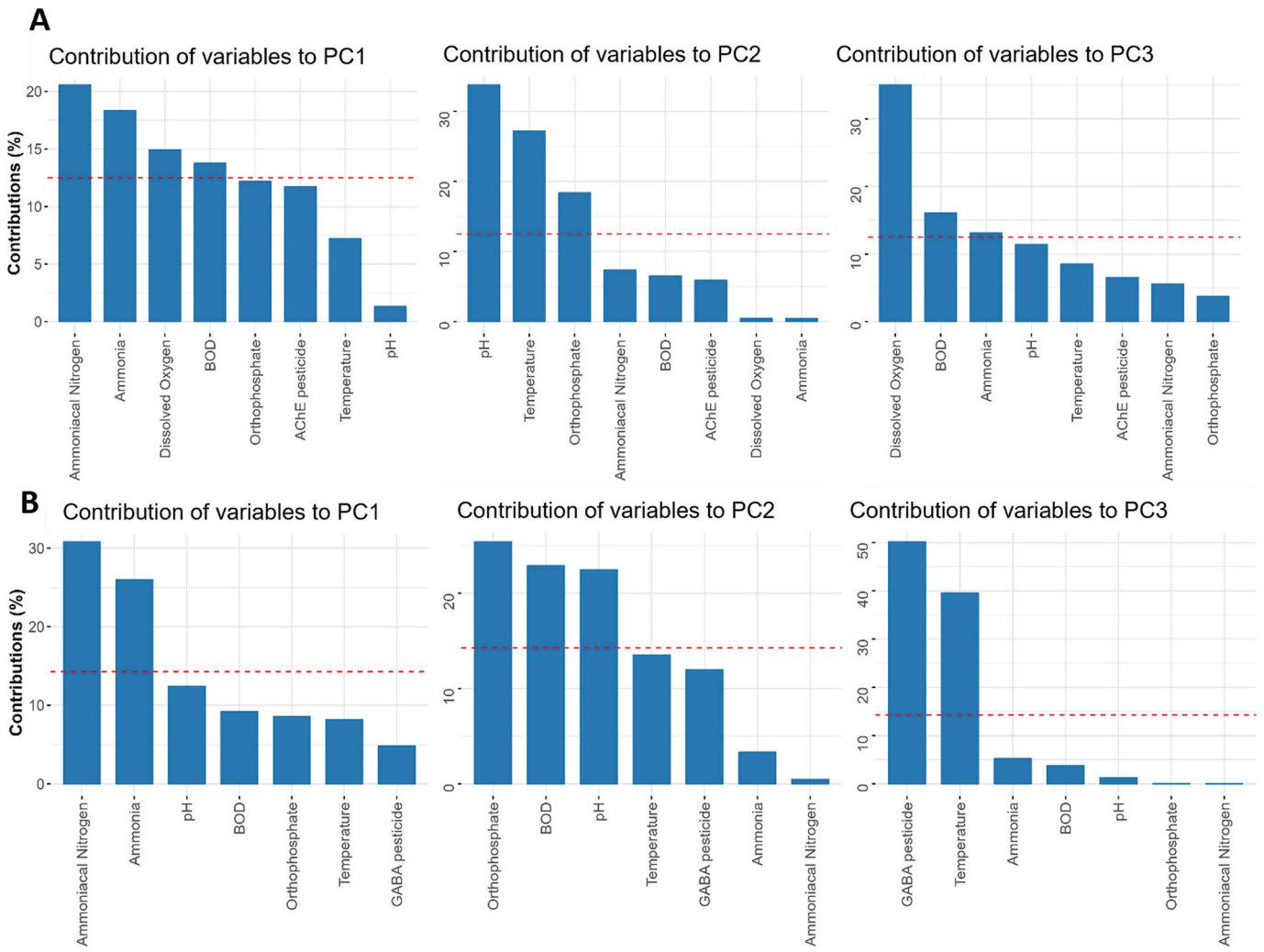


Fig. 3. Contribution of different water quality parameters compared with pesticide group in each principal component at study site 1; A) AChE pesticides, B) GABA pesticides. Variables in addition to pesticides are ammoniacal nitrogen, ammonia, BOD (Biochemical oxygen demand), orthophosphate, temperature, pH and dissolved oxygen. The red dashed lines represent the expected average contribution if all variables were uniform (1/number of variables); average contribution of variables in A) is 12.5%, whereas B) is 14.3%. Best supported model for site 1, supported PC1 for AChE and PC3 for GABA - both of these PCs show greater contribution of pesticide than observed in other PCs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Fixed effect interaction models output for site 1 (linear) – Principal components and Family richness. Parameter estimates from the best supported models examining impact of (A) AChE pesticides principal components and (B) GABA pesticides principal components on family richness. Model selection was supported by AICc (Table S4 and S5).

	Estimate	2.5 % interval	97.5 % interval
AChE pesticides			
Intercept	3.283	3.114	3.454
PC1	−0.075	−0.114	−0.037
Day in year	−0.001	0.002	0.000
GABA pesticides			
Intercept	−30.440	−43.057	−17.827
PC3	−0.113	−0.178	−0.048
Day in year	0.001	0.000	0.002
Year	0.017	0.010	0.023

*AChE best model: AICc is 258.56, weight 0.107 and r^2 (Cragg-Uhler) 0.287, 2nd best model 258.68, weight 0.101 and r^2 (Cragg-Uhler) 0.286. GABA best supported model: AICc is 344.39, weight 0.221 and r^2 (Cragg-Uhler) 0.511, 2nd best model 344.44, weight 0.216 and r^2 (Cragg-Uhler) 0.527.

the invertebrates monitored at site 1 was limited. However, for the data available, we found that toxicity thresholds varied considerably between families, with differences in some cases of several orders of magnitude, as illustrated in Table 2. For AChE acting pesticides, the invertebrate families amongst those most sensitive included Hydropsychidae, Polycentropodiae, Simulidae, Baetidae, Notonectidae, Culicidae, Limphenilidae, Halplidae, Asellidae and Chironomidae. Gammaeridae were particularly sensitive to AChE pesticides, with the exception of dimethoate and fenthion. Importantly, toxicity sensitivity for several families varied considerably too for different individual AChE pesticides (in some cases families were very sensitive to some AChE pesticides and insensitive to others). This was particularly the case for Asellidae, Culicidae, Simulidae, Limphenilidae, Notonectidae and Chironomidae. For GABA acting pesticides, families amongst the most sensitive included, Asellidae, Notonectidae, Halipidae, Gammaridae, Chironomidae, Hydrophilidae, Dystiscidae, Corixidae and Limnephilidae. However, as was the case for AChE pesticides, family sensitivity varied considerably depending on the individual GABA pesticide, notably for the families Asellidae, Halipidae, Gammaridae, Chironomidae and Limnephilidae. Importantly, for both AChE and GABA pesticides, insects seemed to account for the majority of sensitive families.

Table 2
Comparison of toxicity threshold ranges for aquatic invertebrate families occurring at field site 1 – for AChE pesticides and GABA acting pesticides. Data provide the range of toxicity thresholds (e.g., LC50, EC50, NOEC) for population relevant endpoints including mortality, growth, development, and/or reproduction.

	Test	Family	Concentration (μg/l)
AChE pesticide			
Azinphos-methly	LC50 chronic	Asellidae	2.4 (M)
		Baetidae	3.4 (M)
	LC50 acute	Asellidae	4.8–162 (M)
		Baetidae	11.6–16.3 (M)
		Chironomidae	0.37–10 (M)
		Coenagrionidae	26.6–50.2 (M)
		Culicidae	19 (M)
		Gammaridae	0.1–2 (M)
		Planorbidae	123,000–130,000 (M)
	NOEL acute	Baetidae	4.9 (R)
		Dugesiidae	19.2 (R)
		Hydropsychidae	19.2 (R)
		Noteridae	4.9 (R)
		Simuliidae	1 (R)
	NR-ZERO acute	Baetidae	0.2 (M)
		Chironomidae	1000 (M)
		Planorbidae	5000 (M)
	NR-LETH acute	Culicidae	1000 (M)
		Naididae	5000 (M)
Carbophenothion	LC50 acute	Asellidae	1100–1800 (M)
		Gammaridae	5.2–50 (M)
Caumaphos	LC50 acute	Gammaridae	0.074–0.5 (M)
		Hydropsychidae	5.2 (M)
Chlorfenvinphos	LC50 acute	Chironomidae	6–274.36 (M)
		Gammaridae	9.6–27 (M)
Diazinon	LC50/EC50 acute	Baetidae	1.94 (M)
		Chironomidae	10.7–450 (M)
		Culicidae	3–140 (M)
		Dugesiidae	630–11,640 (M)
		Gammaridae	2–47 (M)
		Hydrobiidae	11,000–93,000 (M)
		Hydropsychidae	1–29.4 (M)
		Polycentropodidae	1.1 (M)
		Simuliidae	4.91 (M)
		Unionidae	19,400 (M)
	LOEC chronic	Chironomidae	54 (R)
		Crangonyctidae	34 (R)
		Asellidae	34 (R)
	NOEC chronic	Chironomidae	22 (R)
		Crangonyctidae	6.7 (R)
	NR-ZERO acute	Chironomidae	1000 (M)
		Dugesiidae	6480 (M)
		Gammaridae	2.24 (M)
	NR-LETH acute	Chironomidae	13,900 (M)
		Culicidae	500 (M)
		Dugesiidae	24,550 (M)
Dimethoate	LC50 acute	Baetidae	7 (M)
		Chironomidae	1.29–1290 (M)
		Culicidae	1850–25,000 (M)
		Gammaridae	180–4100 (M)
		Hydropsychidae	23 (M)
		Planorbidae	18,900–23,000 (M)
Fenitrothion	LC50/EC50 acute	Asellidae	1100–1800 (M)
		Culicidae	0.6–29,000 (M)
		Chironomidae	3–13 (M)
		Dugesiidae	1700 (M)
		Gammaridae	2–63 (M)
		Limnephilidae	2.8–610 (M)
		Lymnaeidae	1429 (M)
		Naididae	4342 (M)
		Notonectidae	16.7 (M)
		Simuliidae	82.46–148.35 (M)
		Viviparidae	2399 (M)
	EC50 acute	Culicidae	3.1–105.8 (D)
Fenthion	LC50/EC50 acute	Baetidae	7–13 (M)
		Chironomidae	11–140 (M)
		Culicidae	0.9–4600 (M)
		Gammaridae	5.2–1000 (M)
		Hydropsychidae	2.14 (M)
		Unionidae	23.07–31.66 (M)
Malathion	NR-LETH acute	Chironomidae	1000 (M)
		Culicidae	50 (M)
		Asellidae	3000–6000 (M)
	LC50/EC50 acute	Baetidae	6 (M)
		Chironomidae	0.44–36,000 (M)
		Culicidae	1.6–61,090 (M)
		Dugesiidae	4400 (M)
		Gammaridae	0.33–3.8 (M)
		Haliplidae	1000–6800 (M)
		Hydropsychidae	–32 (M)
		Limnephilidae	1.3–6.8 (M)
		Notonectidae	70.7–220 (M)
		Planorbidae	94,780–468,650 (M)
		Simuliidae	54.2 (M)
		Unionidae	80–667,000 (M)
	NOEL chronic	Chironomidae	300 (D)
		Planorbidae	9.6 (G/M/R)
	NR-ZERO acute	Culicidae	100–600 (M)
		Naididae	4000 (M)
	NR-LETH acute	Culicidae	3500–6000 (M)
		Naididae	4000 (M)
		Notonectidae	300 (M)
Mevinphos	LC50 acute	Asellidae	56–1500 (M)
		Gammaridae	2.8–650 (M)
Parathion-ethyl	LC50/EC50 acute	Asellidae	12–5600 (M)
		Baetidae	1.7–2.6 (M)
		Chironomidae	0.17–660 (M)
		Culicidae	2.2–140 (M)
		Dytiscidae	1.8–28 (M)
		Gammaridae	0.25–12.8 (M)
		Haliplidae	7–10 (M)
		Hydrophilidae	17–40 (M)
		Hydropsychidae	0.43–7 (M)
		Limnephilidae	2.4–37.9 (M)
		Notonectidae	7–20 (M)
	LC50 chronic	Asellidae	4.8 (M)
		Baetidae	0.38 (M)
		Chironomidae	2.2–2.9 (M)
		Gammaridae	0.07–0.09 (M)
		Limnephilidae	0.2–2.2 (M)
	NR-LETH acute	Culicidae	200–600 (M)
		Naididae	500 (M)
		Coenagrionidae	33–120 (M)
Parathion-methyl	LC50 acute	Culicidae	2.2–35 (M)
		Dugesiidae	2600–4100 (M)
		Erpobdellidae	4000–5000 (M)
		Gammaridae	2.52–16 (M)
		Naididae	500 (M)
		Planorbidae	9300–22,900 (M)
		Unionidae	20,000–50,000 (M)
GABA pesticide			
Aldrin	LC50 acute	Asellidae	8–50 (M)
		Gammaridae	4300–56,000 (M)
Dieldrin	LC50 acute	Asellidae	5–20 (M)
		Coenagrionidae	12 (M)
		Culicidae	2.6–500 (M)
		Gammaridae	600–1800 (M)
		Haliplidae	2–4 (M)
		Notonectidae	1 (M)
	LC50 chronic	Chironomidae	1.1–500 (M)
		Lymnaeidae	30–120 (M)
	NR-ZERO chronic	Chironomidae	0.1 (M)
		Naididae	4000 (M)
Heptachlor	LC50/EC50 acute	Baetidae	32 (M)
		Chironomidae	149 (M)
		Dytiscidae	29.9–63.6 (M)
		Gammaridae	29–180 (M)
		Hydrophilidae	35.9 (M)
		Naididae	3700 (M)
		Notonectidae	2.55 (M)
		Physidae	1450 (M)

(*continued on next page*)

Table 2 (*continued*)

	Test	Family	Concentration (µg/l)
		Asellidae	10–375 (M)
		Baetidae	50–92 (M)
		Chironomidae	2–330 (M)
		Corixidae	3.9 (M)
		Culicidae	45–3700 (M)
		Gammaridae	5.1–225 (M)
	LC50/EC50 acute	Haliplidae	20–100 (M)
		Hydropsychidae	330 (M)
		Limnephilidae	9.6 (M)
		Lymnaeidae	1200–7300 (M)
		Naididae	6233 (M)
		Notonectidae	3–7 (M)
		Viviparidae	1050–8700 (M)
		Unionidae	400 (M)
		Chironomidae	2–13 (M)
	LC50/EC50 chronic	Gammaridae	7 (M)
		Limnephilidae	0.8 (M)
		Lymnaeidae	230 (M)
Lindane		Unionidae	400 (M)
	EC50 chronic	Lymnaeidae	65–250 (R)
	ET50 chronic	Chironomidae	0.1–9.9 (D)
		Gammaridae	0.09–8.3 (D)
	LOEC chronic	Chironomidae	9.9 (D)
		Gammaridae	3.1–6.11 (R, G)
		Baetidae	0.8 (R)
	NOEC chronic	Chironomidae	1.1 (D)
		Gammaridae	0.8 (R, G)
		Chironomidae	24–74 (M)
	NR-LETH acute	Culicidae	400–10,000 (M)
		Naididae	4000 (M)
	NR-LETH chronic	Chironomidae	24 (M)
		Lymnaeidae	2200 (M)
		Chironomidae	0.8–100 (M)
		Culicidae	10–1000 (M)
	NR-ZERO	Hydropsychidae	500–10,000 (M)
		Limnephilidae	10,000 (M)
		Naididae	4000 (M)

*LC50; Lethal concentration of 50 %, EC50; Effect concentration of 50 %, ET50; Exposure time to effect 50 %, LOEC; lowest observed effect concentration, NOEC; no observed effect concentration, NR-LETH: occurrence of 100 % mortality., NR-ZERO; occurrence of 0 % mortality, NOEL; No observed effect level. G; Growth, D; development, R; reproduction, M; mortality endpoints. (To access all study citations use the ECOTOX database (https://cfpub.epa.gov/ecotox/search.cfm) and use the search terms - chemical, endpoint (LC50 etc.,) and effect (development etc.,), then download dataset and filter taxa by family information outlined in Section 2.3 of the methods section).

3.4. Associations between GABA and AChE targeting pesticide toxicity and freshwater invertebrate family richness

At site 1, 46 of 64 families were absent where total concentration of AChE pesticides exceeded 0.7 µg/l and 16 of 64 families absent where total concentration of GABA acting pesticides exceeded 0.045 µg/l (the maximum concentrations recorded in rivers). The families absent were from orders, Amphipoda (2), Architaeioglossa (1), Archyaeogastropoda (1), Archynchobdellida (1), Coleoptera (4), Diptera (7), Ephemeroptera (3), Haploscerida (1), Harpacticoida (1), Hemiptera (5), Heterostropha (1), Hygrophila (1), Littorinimorpha (1), Lumbriculida (1), Mysida (1), Odonata (1), Poduromorpha (1), Stylommatophora (1), Trichoptera (7), Tricladida (2), Unionida (1) for AChE pesticides, and orders, Amphipoda (1), Anthoathecate (1), Diptera (5), Ephemeroptera (1), Haplosclerida (1), Haplotaxids (1), Hemiptera (2), Lumbriculida (1), Mysida (1), Poduromorpha (1), Tricladida (1) for GABA pesticides (numbers in brackets indicate the number of families absent). The majority of families absent for GABA and AChE pesticides belong to insect orders.

Only 5 of the 46 families absent at the highest AChE total field concentrations had laboratory toxicity thresholds for the individual AChE acting chemicals that were lower than the total AChE field concentration (0.72 µg/l; see Figs. 4 and 5). All other invertebrate families and chemicals had laboratory effect concentrations greater than the maximum total field concentrations of GABA and AChE pesticides (0.0475 µg/l and 0.72 µg/l respectively; see Figs. S7–10). Hence, for the vast majority of pesticide chemicals, laboratory toxicity data do not directly support the absence of those families at the highest measured field concentrations (i.e. neither individual effects nor additive effects of these chemicals on those invertebrates are likely). However, even for these 5 families where the laboratory chemical effect concentrations for any individual AChE pesticide was lower than, or equal to, the measured field concentrations of total AChE pesticides (≤0.72 µg/l), only one family (Polycentropodidae) had concentrations recorded in the field which were close to a laboratory effect concentration (for mortality). For the other 4 families field concentrations for AChE pesticides were several fold lower than laboratory effect concentrations, as illustrated in Figs. 3 and 4. The absence of Polycentropodidae, where the total AChE pesticide field concentrations were 0.72 µg/l, was supported by the data for laboratory effect concentrations; diazinon's maximum riverine concentration of 0.16 µg/l, and a corresponding lab based LC10 acute toxicity concentration of 0.2 µg/l, see Fig. 4.

4. Discussion

Through a case-study on riverine sites in England we have combined the use of field monitoring data and laboratory toxicity data to assess the impacts of pesticide based on their MoA on invertebrate populations. In our analysis we found that pesticides targeting AChE and GABA receptors have been measured at concentrations in rivers across the Midlands and Anglian regions of England at levels that are acutely toxic (≥EC50/LC50) to invertebrate families. The different timings of biota and chemical collection from these rivers meant we were able to undertake robust assessments for the impact of pesticides on invertebrate family richness for three of these study sites only. At these three sites however, for one (site 1) we found that both GABA and AChE acting pesticides had an important role, alongside other water quality parameters, in determining family richness of riverine macroinvertebrates spanning over the past 40 years. Importantly, at this site 44 families of the 64 monitored were absent when the total concentration of AChE acting pesticide (carbamates and organophosphates) exceeded 0.7 µg/l in the river and 16 families were absent when total collective GABA pesticide (organochlorine) concentration exceeded 0.045 µg/l. Interestingly, lab-based toxic thresholds for individual pesticides (both chronic and acute) were several orders of magnitude higher than those measured in the field for associated effects. For one family however, Polycentropodidae, their absence from the field sites with the highest measured total concentration of AChE acting pesticides was supported by the lethal toxicity threshold for diazinon (LC10; AChE acting pesticide) determined from laboratory-based studies. We thus conclude that pesticides are playing an important role in determining the presence/absence of invertebrate families for this English river. Furthermore, we show that many other rivers have received pesticides at levels that exceed the toxic threshold for effects on selected invertebrate species, based on laboratory testing, indicating a high likelihood for impacts in those rivers, but with insufficient data for our modelling work to assess this. Our analyses also show that ammoniacal nitrogen, ammonia, BOD, dissolved oxygen, orthophosphate in combination with AChE pesticides, and temperature in combination with GABA pesticides, were important in determining freshwater invertebrate family richness.

4.1. Pesticides exposure versus riverine invertebrate family richness

Pesticides are applied (kg/ha) to UK arable areas on average at a rate more than three times that used in other countries in Europe, and it is therefore perhaps not surprising that concentrations of pesticides recorded in English rivers have been found to exceed toxic thresholds for invertebrates (≥EC50/LC50) relevant to population level endpoints (growth, development, reproduction and mortality) (Sharma et al., 2019). In other European countries which use lower amounts (kg/ha) of

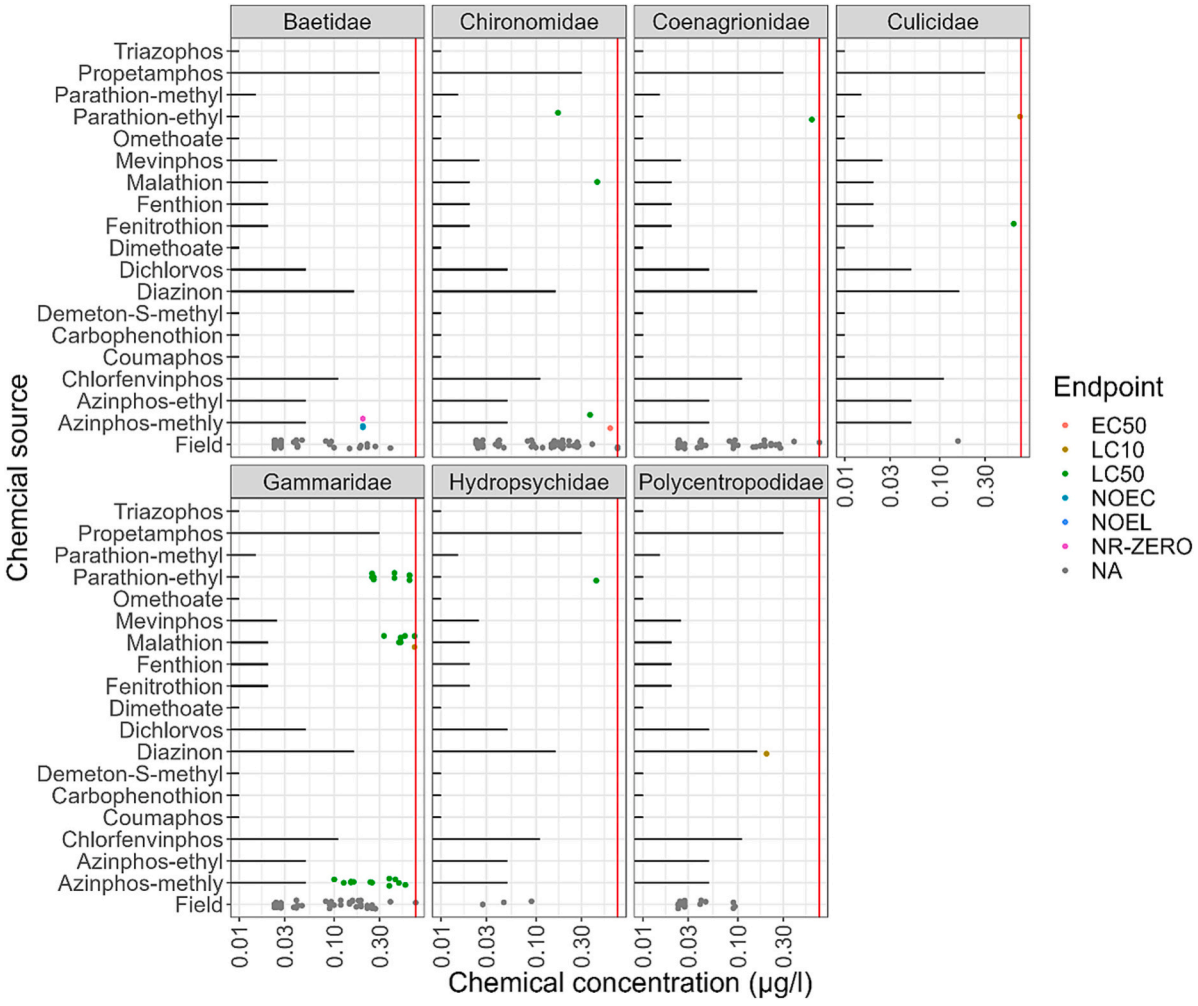


Fig. 4. Family presence/absence in the river compared to recorded river concentrations of individual AChE chemicals (site 1) and laboratory **acute toxicity effect concentrations**. Black lines represent chemical concentrations measured in the river. Coloured dots indicate the laboratory toxicity endpoints. **EC50**: effect concentration of 50% of individuals, **LC10**: lethal concentration of 10% of individuals, **LC50**: lethal concentration of 50% of individuals, **LOEL**: lowest dose whereby an adverse effect is observed, **NOEC**: highest concentration whereby there is no observed toxic effect, **NOEL**: highest dose that does not produce a toxic effect, **NR-ZERO**: concentration at which there is no mortality. Red line represents maximum concentration of total AChE acting pesticides measured in the river. Field (y-axis) – shows presence/absence of the family at recorded total AChE concentrations in the river, demonstrating if taxa present at maximum concentration. Red line represents maximum concentration of total AChE pesticides measured in the river. Field (y-axis) – shows presence/absence of the family at recorded total AChE concentrations in the river, demonstrating if taxa present at maximum concentration (red line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pesticides than those used in England, it has been reported that pesticides have negatively impacted riverine invertebrate communities (Berenzen et al., 2005; Beketov et al., 2009). In one such study assessing 6 rivers in Lower Saxony, Germany in agriculturally intense regions, all sites showed pesticides to be the main stressor impacting invertebrate family richness (Berenzen et al., 2005). In our study for one river (site 1) in an area in the Midlands with a high intensity of upstream agriculture, we also show pesticides have adversely impacted invertebrate family richness - see Fig. 3 and Table 1, in accordance with the high concentrations of fipronil, diazinon, dichlorvos and parathion-methyl recorded at that site over the 40-year period (Fig. 2). In contrast, we found no significant effects of pesticides on invertebrate family richness for riverine sites in areas with relatively low levels of upstream arable farming (<12 %). So, whilst pesticides in rivers in urban settings have in some cases been reported to be associated with impairment to invertebrate assemblages (Carpenter et al., 2016; Waite et al., 2019), our finding more support the general literature which indicates that stressors other than pesticides including, road-runoff (organic PAH; Polycyclic Aromatic Hydrocarbons) and/or general poor water quality from wastewater effluents discharges are likely more the key drivers in determining invertebrate family richness in such urban areas, particularly in England (Beasley and Kneale, 2002; Langford et al., 2009). This is further supported by our finding that maximum concentrations of individual GABA and AChE pesticides did not exceed toxic concentrations in rivers over the 40 years for the sites (2 and 3) at which they have been monitored; the only exception for this being for parathion-methyl.

The water quality variables including ammoniacal nitrogen, ammonia, BOD, dissolved oxygen and orthophosphate were also important in determining invertebrate family richness, and this was particularly the case when in combination with AChE targeting pesticides (see Fig. 3). Various other water quality variables have been determined to impact invertebrate family richness directly (e.g. sediment type) alone or in conjunction with pesticides, for example water turbidity; increased organic load and suspended solids in turbid water tends to reduce he bioavailability of pesticides and therefore toxicity of pesticides to invertebrates (Hall et al., 1986; Benson and Long, 1991; Kadlec and Benson, 1995; Overmyer et al., 2005). These combination effects of environmental parameters on pesticide toxicity likely help

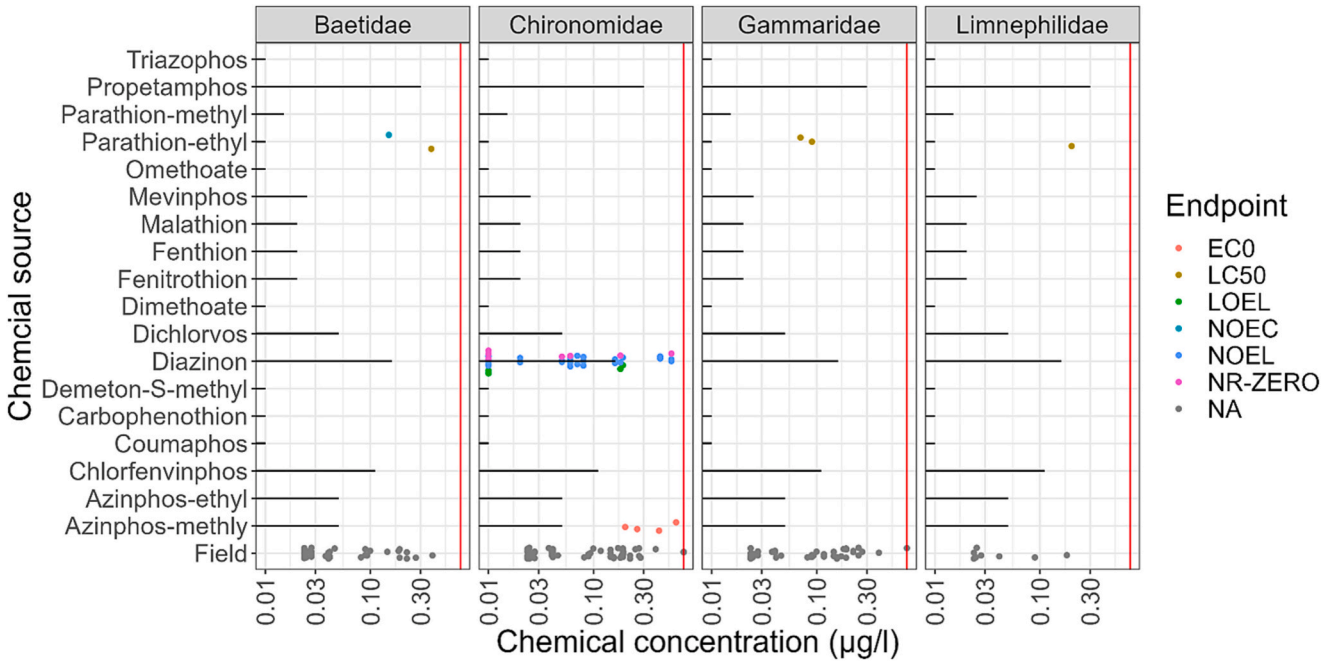


Fig. 5. Family presence/absence in the river compared to recorded river concentrations of individual AChE chemicals (site 1) and laboratory chronic toxicity effect concentrations. Black lines represent individual concentrations of chemicals measured in the river. Coloured dots indicate the laboratory toxicity endpoints. **EC0**: effect concentration of 0 % of individuals, **LC50**: lethal concentration of 50 % of individuals, **LOEL**: lowest dose whereby an adverse effect is observed, **NOEC**: highest concentration whereby there is no observed toxic effect, **NOEL**: highest dose that does not produce a toxic effect, **NR-ZERO**: concentration at which there is no mortality. Red line represents maximum concentration of total AChE pesticides measured in the river. Field (y-axis) – shows presence/absence of the family at recorded total AChE concentrations in the river, demonstrating if taxa present at maximum concentration (red line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

explain the disparity seen between some of the field data and laboratory derived testing data, where there are associations between invertebrate absences across riverine sites for AChE and GABA acting pesticide concentrations that are below those for the toxic thresholds for these chemicals based on laboratory data. Overall, it is clear, however, that AChE and GABA acting pesticides have played a key role in determining the family richness of invertebrates at site 1. For other site locations where the concentrations of AChE and GABA acting pesticides exceed acutely toxic laboratory effect concentrations (Fig. 2) invertebrate populations may also be impacted, but data deficiency has prohibited their investigation. Further, it should be noted that our study was unable todid not consider potential interactive effects between AChE and GABA pesticides. This limitation was due to disparity in the timing and locations for the monitoring of these two pesticide classes.

4.2. *Invertebrate family sensitivity to pesticides*

Family richness was impacted by AChE and GABA pesticides at site 1, however which families are most sensitive differs depending on the individual pesticides as determined from laboratory toxicity data (focusing on mortality, reproductive, growth and development endpoints) and as illustrated in Table 2. Overall, however, based on acute and chronic laboratory toxicity data, certain families found at site 1 were found consistently to be the more sensitive to AChE pesticides, including, Hydropsychidae (Caddisfly), Polycentropodiae (Caddisfly), Baetidae (mayfly), and Halplidae (beetle) and other families, notably, the Notonectidae (true bugs), Hydrophilidae (beetle), Dystiscidae (beetle), Corixidae (true bugs) consistently shown to be sensitive to GABA receptor pesticides. It is perhaps not surprising that all these families are insects as both AChE and GABA acting pesticide modes of actions are designed to target and kill terrestrial insects (Jayaraj et al., 2016; Struger et al., 2016). Invertebrate families most sensitive to GABA and AChE pesticides in the field, based on absence at highest recorded GABA and AChE concentrations (46 of 64 and 16 of 64 families absent, respectively) included the orders deemed to be most sensitive based on laboratory toxicity studies. An exception to this was the order Coleoptera which were not associated with high sensitivity to GABA based on field data despite laboratory toxicity data for aquatic beetles suggesting this taxa is highly sensitivity to GABA pesticides (Table 2). Overall, these analyses evidence that the invertebrate taxa most sensitive to AChE and GABA pesticides from the field studies include freshwater insects and this is largely supported by the available laboratory toxicity data.

4.3. *Relationship between absence of invertebrate families in the field and pesticide lab toxicity data*

The absence at site 1 of numerous invertebrate families at the highest measured riverine pesticide concentrations, correlated with the toxic threshold for Polycentropodidae only based on laboratory exposures. For the highest recorded field concentration of total AChE pesticides (0.72 µg/l), diazinon in that mixture was close to the LC10 acute toxicity threshold (0.02 µg/l, Fig. 4). Furthermore, the maximum concentration of diazinon measured at site 1 (not matched to biota sample due to 36-day threshold period) was 0.27 µg/l which exceeds its acute LC10 (0.2 µg/l). The high sensitivity of the Polycentropodidae (caddisfly) corresponds with it being defined as “Species at risk” to pesticide pollution based on trait analyses (according to SPEARpesticide index; Liess and Von Der Ohe, 2005; Environment Agency, 2008).

The absences of other invertebrate families could not be explained by the highest total riverine concentrations of AChE or GABA pesticides (0.72 and 0.0475 µg/l, respectively) as in some cases they were several orders of magnitude lower than those shown to induce acute/chronic toxicity effects in laboratory studies (Figs. 4, 5 and Figs. S7–10). This contrasts with our determinations for effect of these pesticides (AChE and GABA) on family richness (Table 1). These differences are hard to reconcile but it is more than plausible that other factors in the riverine

settings compound pesticide effects compared with that as determined under laboratory conditions, where exposure scenarios are generally highly simplistic and optimal in terms of supporting the health and nutrition of the organisms being exposed (Hall et al., 1986; Benson and Long, 1991; Kadlec and Benson, 1995; Kreuger, 1998; Lydy et al., 1999; Overmyer et al., 2005; Willming et al., 2013). In natural environments organisms are exposed to a range of stressors which can act in combination to affect chemical effects susceptibility and sensitivity (Coors and De Meester, 2008; Bray et al., 2021a, 2021b). This was well illustrated in one study where withholding food resulted in an increased toxicity of prochloraz to *Daphina magna* (Shahid et al., 2019). Toxicity thresholds derived from laboratory studies may, therefore, not necessarily accurately represent those for freshwater invertebrates in their natural environments and in some instances underestimate the threat of pesticide exposures. Furthermore, some pesticides have been shown to have interactive effects (acting additively, synergistically, and/or antagonistically; Anderson and Lydy, 2002; Pham et al., 2018) further complicating extrapolation between laboratory based data for single chemical exposures and threshold effect concentrations in natural environments. Illustrating this AChE targeting pesticides (carbaryl, carbofuran, parathion, demeton-*S*-methyl, and aldicarb) have been shown to additive in their effects on AChE activity in an enzyme bioassay (Mwila et al., 2013), and a mixture of chlorpyrifos, dimethoate (organophosphates) and imidacloprid produced a synergistic effect in a laboratory exposure with *Chironomus dilutus* larvae (LeBlanc et al., 2012). These interactive effects are not limited to pesticides, but other chemical types too, such as metals, that can also interact with pesticide (e.g. organophosphate, carbamate) to affect (including to enhance) their toxicity to aquatic invertebrates (Joe et al., 1999). Hence AChE and GABA pesticides may have a contributing effect to the decline of invertebrate families at lower concentrations in the field than for their effects determined in individual chemical toxicity test studies. Such data, however, are lacking and the 'chemical mixtures' and combined stressors questions for pesticides remains largely unanswered for studies on freshwater invertebrate populations.

4.4. Focus on monitoring efforts

Despite the availability of one of the most comprehensive monitoring datasets worldwide for freshwater invertebrates and water chemistry data, we found, for the most part, these data sets were still lacking for use in understanding the role of pesticides in determining the status (richness) of riverine invertebrate populations in English rivers. The lack of consistency in pesticide monitoring efforts over time including relating to the number of pesticide measured across the different sites, together with the lack of consistency in the monitoring of other physicochemical parameters over time, and the disparity between where pesticide/physicochemical samples had been collected versus that for the invertebrate biota, meant that only a subset of sites qualified for use in our analyses. Furthermore, many sites for the chemical and biota sampling on any given river stretch were distant to one another and as such the water physicochemistry at these different sampling points is likely to differ due to different proximate land-use and land-use changes over time. We thus emphasise that to better dissect out the roles of pollution (and other environmental factors) in the changing status of aquatic wildlife populations greater compatibility is needed between the locations of the chemical and biota sampling sites and in the timing of theses collections for long term monitoring. Ephemeroptera, Plecoptera, and Trichoptera (EPT) rather than species richness as a whole are often used as measures of water quality, most notably as bioindicators of organic pollution. However, for the occurrence data at sites 1–3, there were fewer than 30 observations for EPT alongside pesticide and water quality data. In turn this did not provide a sufficient dataset for a robust statistical analysis underscoring the importance of ensuring sampling during seasonal periods when pollution-sensitive taxa, such as EPT, are present in rivers. This is crucial for validating observed responses in total family richness at a finer scale, here specifically for EPT richness.

5. Conclusion

Riverine sites across the Midlands and Anglian regions of England have experienced levels of both AChE and GABA pesticides that are toxic to riverine invertebrates. For one of the three sites assessed in the Midlands region, where data were sufficient for a comprehensive analysis, we show a significant relationship between pesticide pollution (AChE and GABA receptor targeting pesticides) and reduced riverine invertebrate family richness. At this site certain families of invertebrates were absent during the periods of the higher pesticide concentrations in the river. In one of these cases diazinon appears to be a key driver in the absence of the Polycentropodidae family of freshwater invertebrates. Through this case study in England we have shown how monitoring data and laboratory toxicity data can be effectively combined to assess the impact of selected pesticides on riverine invertebrate communities. However, more tightly coordinated site and temporal sampling for biota and chemistry would provide considerably more powerful data sets for establishing possible associations between pesticides (and other chemicals) and riverine biota populations and should be applied in future environmental monitoring.

CRediT authorship contribution statement

Imogen P. Poyntz-Wright: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Xavier A. Harrison:** Writing – review & editing, Methodology. **Andrew Johnson:** Writing – review & editing, Supervision. **Susan Zappala:** Writing – review & editing. **Charles R. Tyler:** Writing – review & editing, Supervision, Investigation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Imogen Poyntz-Wright reports financial support was provided by Natural Environment Research Council. Imogen Poyntz-Wright reports financial support was provided by United Kingdom Department for Environment Food and Rural Affairs. Susan Zappala reports a relationship with JNCC Support Co that includes: employment.

Data availability

Data will be made available on request.

Acknowledgments

Authors' contribution statement using CRediT with degree of contribution:

Thanks to the ChemPop (NE/S000100/1) team for assimilation of Environment Agency data and to Ioanna Katsiadaki (Centre for the Environment, Fisheries and Aquaculture Sciences) for comments on a draft of this paper.

Funding sources

This work was supported by the Natural Environmental Research Council (NERC) ECORISC DTP (NE/V013041/1); Department for Environment, Food and Rural Affairs, UK Government; and Joint Nature Conservation Committee. For the purpose of open access, the author has applied a 'Creative Commons Attribution (CC BY) licence to any Author Accepted Manuscript version arising.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.169079.

References

Amisah, S., Cowx, I.G., 2011. Impacts of abandoned mine and industrial discharges on fish abundance and macroinvertebrate diversity of the Upper River Don in South Yorkshire, UK, 15 (2), 237–250. https://doi.org/10.1080/02705060.2000.9663741.

Anderson, T.D., Lydy, M.J., 2002. Increased toxicity to invertebrates associated with a mixture of atrazine and organophosphate insecticides. Environ. Toxicol. Chem. 21 (7), 1507–1514. https://doi.org/10.1002/ETC.5620210724.

Bates, D., et al., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67 (1), 1–48. https://doi.org/10.18637/JSS.V067.I01.

Beasley, G., Kneale, P., 2002. Reviewing the impact of metals and PAHs on macroinvertebrates in urban watercourses, 26 (2), 236–270. https://doi.org/10.1191/0309133302PP334RA.

Beketov, M.A., et al., 2009. SPEAR indicates pesticide effects in streams – comparative use of species- and family-level biomonitoring data. Environ. Pollut. 157 (6), 1841–1848. https://doi.org/10.1016/J.ENVPOL.2009.01.021.

Beketov, M.A., et al., 2013. Pesticides reduce regional biodiversity of stream invertebrates. Proc. Natl. Acad. Sci. 110 (27), 11039–11043. https://doi.org/10.1073/PNAS.1305618110.

Benson, W.H., Long, S.F., 1991. Evaluation of humic-pesticide interactions on the acute toxicity of selected organophosphate and carbamate insecticides. Ecotoxicol. Environ. Saf. 21 (3), 301–307. https://doi.org/10.1016/0147-6513(91)90069-2.

Berenzen, N., et al., 2005. Macroinvertebrate community structure in agricultural streams: impact of runoff-related pesticide contamination. Ecotoxicol. Environ. Saf. 60 (1), 37–46. https://doi.org/10.1016/J.ECOENV.2003.10.010.

Beuter, L.K., et al., 2019. Testing effects of pesticides on macroinvertebrate communities in outdoor stream mesocosms using carbaryl as example test item. Environ. Sci. Eur. 31 (1), 1–17. https://doi.org/10.1186/S12302-019-0185-1/FIGURES/8.

Bloomquist, J.R., 1993. Toxicology, mode of action and target site-mediated resistance to insecticides acting on chloride channels. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 106 (2), 301–314. https://doi.org/10.1016/0742-8413(93)90138-B.

Bray, J., et al., 2021a. Sub-organism (acetylcholinesterase activity), population (survival) and chemical concentration responses reinforce mechanisms of antagonism associated with malathion toxicity. Sci. Total Environ. 778, 146087 https://doi.org/10.1016/J.SCITOTENV.2021.146087.

Bray, J.P., et al., 2021b. Can SPEcies at risk of pesticides (SPEAR) indices detect effects of target stressors among multiple interacting stressors? Sci. Total Environ. 763, 142997 https://doi.org/10.1016/J.SCITOTENV.2020.142997.

Byrne, P., Reid, I., Wood, P.J., 2013. Changes in macroinvertebrate community structure provide evidence of neutral mine drainage impacts. Environ Sci Process Impacts 15 (2), 393–404. https://doi.org/10.1039/C2EM30447C.

Carpenter, K.D., et al., 2016. Storm-event-transport of urban-use pesticides to streams likely impairs invertebrate assemblages. Environ. Monit. Assess. 188 (6), 1–18. https://doi.org/10.1007/S10661-016-5215-5/FIGURES/7.

Chiu, M.C., Hunt, L., Resh, V.H., 2016. Response of macroinvertebrate communities to temporal dynamics of pesticide mixtures: a case study from the Sacramento River watershed, California. Environ. Pollut. 219, 89–98. https://doi.org/10.1016/J.ENVPOL.2016.09.048.

Clements, W.H., 1994. Benthic invertebrate community responses to heavy metals in the Upper Arkansas River basin, Colorado. J. N. Am. Benthol. Soc. 13 (1), 30–44. https://doi.org/10.2307/1467263.

Coors, A., De Meester, L., 2008. Synergistic, antagonistic and additive effects of multiple stressors: predation threat, parasitism and pesticide exposure in Daphnia magna. J. Appl. Ecol. 45 (6), 1820–1828. https://doi.org/10.1111/J.1365-2664.2008.01566.X.

Day, K.E., Scott, I.M., 1990. Use of acetylcholinesterase activity to detect sublethal toxicity in stream invertebrates exposed to low concentrations of organophosphate insecticides. Aquat. Toxicol. 18 (2), 101–113. https://doi.org/10.1016/0166-445X(90)90021-G.

Dohmen, G.P., et al., 2016. Population-level effects and recovery of aquatic invertebrates after multiple applications of an insecticide. Integr. Environ. Assess. Manag. 12 (1), 67–81. https://doi.org/10.1002/IEAM.1676.

Dowson, P., et al., 1996. The environmental impact of a chemical spill from a timber-treatment works on a Lowland River system. Water Environ. J. 10 (4), 235–244. https://doi.org/10.1111/J.1747-6593.1996.TB00041.X.

Dudgeon, D., et al., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81 (2), 163–182. https://doi.org/10.1017/S1464793105006950.

Environment Agency, 2008. Freshwater Biological Indicators of Pesticide Contamination-adaptation of the SPEAR Approach for the UK. Available at: www.environment-agency.gov.uk. (Accessed 19 May 2022).

Ganatra, A.A., et al., 2021. Calibration of the SPEARpesticides bioindicator for cost-effective pesticide monitoring in east African streams. Environ. Sci. Eur. 33 (1), 1–15. https://doi.org/10.1186/S12302-021-00497-9/FIGURES/6.

Gant, D.B., Eldefrawi, M.E., Eldefrawi, A.T., 1987. Cyclodiene insecticides inhibit GABAA receptor-regulated chloride transport. Toxicol. Appl. Pharmacol. 88 (3), 313–321. https://doi.org/10.1016/0041-008X(87)90206-7.

Gething, K., et al., 2020. The effects of metaldehyde on non-target aquatic macroinvertebrates: integrating field and laboratory-based evidence. Environ. Pollut. (Barking, Essex : 1987) 265 (Pt A). https://doi.org/10.1016/J.ENVPOL.2020.115015.

Gov.UK, 2023a. Agricultural facts: East Midland Region. Available at: https://www.gov.uk/government/statistics/agricultural-facts-england-regional-profiles/agricultural-facts-east-midland-region. (Accessed 24 August 2023).

Gov.UK, 2023b. Agricultural facts: East of England Region. Available at: https://www.gov.uk/government/statistics/agricultural-facts-england-regional-profiles/agricultural-facts-east-of-england-region. (Accessed 24 August 2023).

Gov.UK, 2023c. Agricultural Facts: West Midland Region. Available at: https://www.gov.uk/government/statistics/agricultural-facts-england-regional-profiles/agricultural-facts-west-midland-region. (Accessed 24 August 2023).

Hahsler, M., Hornik, K., Buchta, C., 2008. Getting things in order: an introduction to the R package seriation. J. Stat. Softw. 25 (3), 1–34. https://doi.org/10.18637/JSS.V025.I03.

Hall, W.S., et al., 1986. Effects of suspended solids on the bioavailability of chlordane to Daphnia magna. Arch. Environ. Contam. Toxicol. 15 (5), 529–534. https://doi.org/10.1007/BF01056566/METRICS.

Hartig, F., 2022. Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. Comprehensive R Archive Network (CRAN). Available at: https://cran.r-project.org/package=DHARMa. (Accessed 27 October 2022).

Heugens, E.H.W., et al., 2008. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment, 31 (3), 247–284. https://doi.org/10.1080/20014091111695.

Hirst, H., Jüttner, I., Ormerod, S.J., 2002. Comparing the responses of diatoms and macro- invertebrates to metals in upland streams of Wales and Cornwall. Freshw. Biol. 47 (9), 1752–1765. https://doi.org/10.1046/J.1365-2427.2002.00904.X.

Hunt, L., et al., 2017. Species at risk (SPEAR) index indicates effects of insecticides on stream invertebrate communities in soy production regions of the argentine pampas. Sci. Total Environ. 580, 699–709. https://doi.org/10.1016/J.SCITOTENV.2016.12.016.

Husson, F., et al., 2023. FactoMineR: Multivariate Exploratory Data Analysis and Data Mining. Available at: https://cran.r-project.org/web/packages/FactoMineR/index.html. (Accessed 28 March 2023).

Jarvis, A.P., Younger, P.L., 2006. Dominating chemical factors in mine water induced impoverishment of the invertebrate fauna of two streams in the Durham Coalfield, UK, 13 (4), 249–270. https://doi.org/10.1080/02757549708035531.

Jayaraj, R., Megha, P., Sreedev, P., 2016. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. Interdiscip. Toxicol. 9 (3–4), 90. https://doi.org/10.1515/INTOX-2016-0012.

Joe, J., et al., 1999. Joint action of pollutant combinations (pesticides and metals) on survival (LC50 values) and acetylcholinesterase activity of Tigriopus brevicornis (Copepoda, Harpacticoida). Environ. Toxicol. Chem. 18 (5), 912–918. https://doi.org/10.1002/ETC.5620180514.

Kadiru, S., Patil, S., Souza, R.D., 2022. Effect of pesticide toxicity in aquatic environments: a recent review. Int. J. Fish. Aquat. Stud. 10 (3), 113–118. https://doi.org/10.22271/FISH.2022.V10.I3B.2679.

Kadlec, M.C., Benson, W.H., 1995. Relationship of aquatic natural organic material characteristics to the toxicity of selected insecticides. Ecotoxicol. Environ. Saf. 31 (1), 84–97. https://doi.org/10.1006/EESA.1995.1047.

Kassambara, A., 2020. Factoextra: Extract and Visualize the Results of Multivariate Data Analyses [R package factoextra version 1.0.7]. Comprehensive R Archive Network (CRAN). Available at: https://cran.r-project.org/package=factoextra. (Accessed 28 March 2023).

Kreuger, J., 1998. Pesticides in stream water within an agricultural catchment in southern Sweden, 1990–1996. Sci. Total Environ. 216 (3), 227–251. https://doi.org/10.1016/S0048-9697(98)00155-7.

Langford, T.E.L., et al., 2009. Long-term recovery of macroinvertebrate biota in grossly polluted streams: re-colonisation as a constraint to ecological quality. Ecol. Indic. 9 (6), 1064–1077. https://doi.org/10.1016/J.ECOLIND.2008.12.012.

LeBlanc, H.M.K., et al., 2012. Single versus combined lethal effects of three agricultural insecticides on larvae of the freshwater insect Chironomus dilutus. Arch. Environ. Contam. Toxicol. 63 (3), 378–390. https://doi.org/10.1007/S00244-012-9777-0/FIGURES/6.

Lemm, J.U., et al., 2021. Multiple stressors determine river ecological status at the European scale: towards an integrated understanding of river status deterioration. Glob. Chang. Biol. 27 (9), 1962–1975. https://doi.org/10.1111/GCB.15504.

Liess, M., Von Der Ohe, P.C., 2005. Analyzing effects of pesticides on invertebrate communities in streams. Environ. Toxicol. Chem. 24 (4), 954–965. https://doi.org/10.1897/03-652.1.

Living Planet Report, 2020. Living Planet Report 2020. Bending the Curve of Biodiversity Loss, Wwf. Available at: https://wwflac.awsassets.panda.org/downloads/living_planet_report_2020.pdf. (Accessed 6 October 2021).

Long, J., 2023. Analysis and Presentation of Social Scientific Data.

Loureiro, S., et al., 2010. Toxicity of three binary mixtures to Daphnia magna: comparing chemical modes of action and deviations from conceptual models. Environ. Toxicol. Chem. 29 (8), 1716–1726. https://doi.org/10.1002/ETC.198.

Lüdecke, D., 2021. Data and Variable Transformation Functions [R Package sjmisc Version 2.8.9]. Available at: https://cran.r-project.org/package=sjmisc. (Accessed 28 March 2023).

Lüdecke, D. (2022) Package 'sjlabelled' Title Labelled Data Utility Functions. Available at: https://orcid.org/0000-0002-8895-3206 (Accessed: 28 March 2023).

Lüdecke, D., 2023. Package 'sjPlot' Title Data Visualization for Statistics in Social Science.

Lydy, M.J., Belden, J.B., Ternes, M.A., 1999. Effects of temperature on the toxicity of M-parathion, chlorpyrifos, and pentachlorobenzene to Chironomus tentans. Arch. Environ. Contam. Toxicol. 37 (4), 542–547. https://doi.org/10.1007/S002449900550/METRICS.

Macaulay, S.J., et al., 2021. Time-cumulative effects of neonicotinoid exposure, heatwaves and food limitation on stream mayfly nymphs: a multiple-stressor experiment. Sci. Total Environ. 754, 141941 https://doi.org/10.1016/J.SCITOTENV.2020.141941.

Macchi, P., et al., 2018. The impact of pesticides on the macroinvertebrate community in the water channels of the Río Negro and Neuquén Valley, North Patagonia (Argentina). Environ. Sci. Pollut. Res. 25 (11), 10668–10678. https://doi.org/10.1007/S11356-018-1330-X/FIGURES/4.

Mdeni, N.L., et al., 2022. Analytical evaluation of carbamate and organophosphate pesticides in human and environmental matrices: a review. Molecules 27 (3). https://doi.org/10.3390/MOLECULES27030618.

Mladenović, M., et al., 2018. The targeted pesticides as acetylcholinesterase inhibitors: comprehensive cross-organism molecular modelling studies performed to anticipate the pharmacology of harmfulness to humans in vitro. Molecules 23 (9). https://doi.org/10.3390/MOLECULES23092192.

Moermond, C.T.A., et al., 2016. CRED: criteria for reporting and evaluating ecotoxicity data. Environ. Toxicol. Chem. 35 (5), 1297–1309. https://doi.org/10.1002/ETC.3259.

Mwila, K., et al., 2013. The effect of mixtures of organophosphate and carbamate pesticides on acetylcholinesterase and application of chemometrics to identify pesticides in mixtures. Environ. Monit. Assess. 185 (3), 2315–2327. https://doi.org/10.1007/S10661-012-2711-0/TABLES/4.

Outhwaite, C.L., et al., 2020. Complex long-term biodiversity change among invertebrates, bryophytes and lichens. Nat. Ecol. Evol. 4 (3), 384–392. https://doi.org/10.1038/s41559-020-1111-z.

Overmyer, J.P., Noblet, R., Armbrust, K.L., 2005. Impacts of lawn-care pesticides on aquatic ecosystems in relation to property value. Environ. Pollut. 137 (2), 263–272. https://doi.org/10.1016/J.ENVPOL.2005.02.006.

Pham, B., et al., 2018. Assessing interactive mixture toxicity of carbamate and organophosphorus insecticides in the yabby (Cherax destructor). Ecotoxicology 27 (9), 1217–1224. https://doi.org/10.1007/S10646-018-1973-X/TABLES/3.

Poyntz-Wright, I.P., et al., 2023. Pesticide pollution associations with riverine invertebrate communities in England. Sci. Total Environ. 903, 166519 https://doi.org/10.1016/J.SCITOTENV.2023.166519.

QGIS, 2022. QGIS Geographic Information System. QGIS Association. Available at: http://www.qgis.org. (Accessed 27 October 2022).

R Core Team, 2021. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Available at: https://www.scirp.org/(S(czeh2tfqw2orz553k1w0r45))/reference/referencespapers.aspx?referenceid=3131254. (Accessed 28 June 2023).

Rico, A., Van den Brink, P.J., 2015. Evaluating aquatic invertebrate vulnerability to insecticides based on intrinsic sensitivity, biological traits, and toxic mode of action. Environ. Toxicol. Chem. 34 (8), 1907–1917. https://doi.org/10.1002/ETC.3008.

Schletterer, M., et al., 2010. Testing the coherence of several macroinvertebrate indices and environmental factors in a large lowland river system (Volga River, Russia). Ecol. Indic. 10 (6), 1083–1092. https://doi.org/10.1016/J.ECOLIND.2010.03.004.

Schriever, C.A., et al., 2007. Agricultural intensity and landscape structure: influences on the macroinvertebrate assemblages of small streams in northern Germany. Environ. Toxicol. Chem. 26 (2), 346–357. https://doi.org/10.1897/05-629R.1.

Scoggins, M., et al., 2007. Occurrence of polycyclic aromatic hydrocarbons below coal-tar-sealed parking lots and effects on stream benthic macroinvertebrate communities. J. N. Am. Benthol. Soc. 26 (4), 694–707. https://doi.org/10.1899/06-109.1/ASSET/IMAGES/LARGE/I0887-3593-26-4-694-F04.JPEG.

Shahid, N., Liess, M., Knillmann, S., 2019. Environmental stress increases synergistic effects of pesticide mixtures on Daphnia magna. Environ. Sci. Technol. 53 (21), 12586–12593. https://doi.org/10.1021/ACS.EST.9B04293/ASSET/IMAGES/LARGE/ES9B04293_0002.JPEG.

Sharma, A., et al., 2019. Worldwide pesticide usage and its impacts on ecosystem. SN Appl. Sci. 1 (11), 1–16. https://doi.org/10.1007/S42452-019-1485-1/TABLES/4.

Smith, L.G., et al., 2018. Modelling the production impacts of a widespread conversion to organic agriculture in England and Wales. Land Use Policy 76, 391–404. https://doi.org/10.1016/J.LANDUSEPOL.2018.02.035.

Stampfli, N.C., et al., 2013. Two stressors and a community – effects of hydrological disturbance and a toxicant on freshwater zooplankton. Aquat. Toxicol. 127, 9–20. https://doi.org/10.1016/J.AQUATOX.2012.09.003.

Struger, J., et al., 2016. Occurrence and distribution of carbamate pesticides and metalaxyl in southern Ontario surface waters 2007–2010. Bull. Environ. Contam. Toxicol. 96 (4), 423. https://doi.org/10.1007/S00128-015-1719-X.

Van Den Berg, S.J.P., et al., 2019. Modeling the sensitivity of aquatic macroinvertebrates to chemicals using traits. Environ. Sci. Technol. 53 (10), 6025–6034. https://doi.org/10.1021/ACS.EST.9B00893/SUPPL_FILE/ES9B00893_SI_003.XLSX.

Vaughan, I.P., Ormerod, S.J., 2012. Large-scale, long-term trends in British river macroinvertebrates. Glob. Chang. Biol. 18 (7), 2184–2194. https://doi.org/10.1111/J.1365-2486.2012.02662.X.

Waite, I.R., et al., 2019. Effects of urban multi-stressors on three stream biotic assemblages. Sci. Total Environ. 660, 1472–1485. https://doi.org/10.1016/J.SCITOTENV.2018.12.240.

Walker, J.R., Hassall, C., 2021. The effects of water chemistry and lock-mediated connectivity on macroinvertebrate diversity and community structure in a canal in northern England. Urban Ecosyst. 24 (3), 491–500. https://doi.org/10.1007/S11252-020-01053-8/FIGURES/4.

Wang, Y., et al., 2015. Assessing joint toxicity of four organophosphate and carbamate insecticides in common carp (Cyprinus carpio) using acetylcholinesterase activity as an endpoint. Pestic. Biochem. Physiol. 122, 81–85. https://doi.org/10.1016/J.PESTBP.2014.12.017.

Warton, D.I., et al., 2016. Three points to consider when choosing a LM or GLM test for count data. Methods Ecol. Evol. 7 (8), 882–890. https://doi.org/10.1111/2041-210X.12552.

Whelan, M.J., et al., 2022. Is water quality in British rivers "better than at any time since the end of the industrial revolution"? Sci. Total Environ. 843, 157014 https://doi.org/10.1016/J.SCITOTENV.2022.157014.

Wickham, H., 2016. Ggplot2: Elegant Graphics for Data Analysis. Springer International Publishing. Available at: https://www.bibguru.com/r/how-to-cite-r-package-ggplot2/. (Accessed 27 October 2022).

Willming, M.M., Qin, G., Maul, J.D., 2013. Effects of environmentally realistic daily temperature variation on pesticide toxicity to aquatic invertebrates. Environ. Toxicol. Chem. 32 (12), 2738–2745. https://doi.org/10.1002/ETC.2354.