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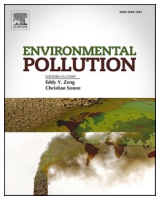

Widespread pesticide pollution in two English river catchments of contrasting land-use: from sediments to fish☆

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ABSTRACT

Water, sediments, fish and invertebrates were collected along two English rivers (R. Tone, Sommerset and R. Wensum, Norfolk) and analysed for 52 pesticides to assess source to sea spatial distribution and track bioaccumulation within wildlife. Chemical risk assessments, using Toxic Units, Risk Quotients, and Microtox® solid phase tests were applied to understand threats to river health. Widespread pesticide pollution was detected in the water and sediments of both rivers, often forming complex mixtures containing numerous pesticides. Hydrophobic pesticides, such as Fipronil and Propiconazole, were also observed widely bioaccumulating in fish. The veterinary pesticide Fipronil was measured in the highest concentrations, up to 87.7 ng/g in fish muscle and 322 ng/g in invertebrates. Of particular concern were neonicotinoids in water, which frequently exceeded environmental quality standards (detected ranges: Imidacloprid <1.2–97.1 ng/L; Clothianidin <28.7–63.4 ng/L) and presented a significant risk to aquatic invertebrates and overall river health. Chronic sub-lethal risks to fish resulting from pesticide exposure were also identified. In sediments, Fipronil regularly exceeded likely-effect benchmarks by up to 256 % (0–0.355 ng/g OC; 0–12.6 ng/g). The findings highlight the potentially negative impact of pesticide pollution on river health in England, and emphasise the need for stricter regulation of the most high-risk pesticides, particularly those used in veterinary care.

1. Introduction

England has historically been one of the largest pesticide users globally (Zhang, 2018). There are over three thousand pesticide products and 451 active substances authorised for use in England (HSE, 2023). Government data suggests that reliance on agricultural pesticides has increased over the past two decades, leading to a 24 % rise in active ingredients per hectare of arable land since 2000 (DEFRA, 2020; FERA, 2023; Poyntz-Wright et al., 2023). Across the UK, pesticides are also widely used domestically to combat weeds, pests, fungi (Tassin de Montaigu and Goulson, 2023), and pets are regularly treated for ectoparasites with toxic pesticides such as neonicotinoids, which are otherwise banned in agriculture (Perkins and Goulson, 2023). Consequently, pesticide usage is a potentially major source of environmental pollution to air, soils, and waterways nationwide.

Pesticide pollution can be extremely damaging to the environment. Pesticides are intrinsically toxic chemicals capable of inflicting a wide range of effects on wildlife, which can in turn cause lasting damage to wildlife populations and ecosystems (Ito et al., 2020; Werner et al., 2021; Beaumelle et al., 2023; Dutta et al., 2024; Wan et al., 2025). When pesticides occur in mixtures (Casado et al., 2019; Khurshid et al., 2024), toxicity can accumulate through additive and synergistic interactions (Cedergreen, 2014; Hernández et al., 2017), making it harder to assess and predict risks accurately, potentially leading to an underestimation (Schuijt et al., 2021). In England, pesticide usage is suspected to be partly responsible for significant declines in bee, butterfly and bird diversity and abundance (Gilburn et al., 2015; Goulson, 2013; Goulson, 2019; Tassin de Montaigu and Goulson, 2023). Many ecologically-important rivers have suffered widespread aquatic invertebrate diversity losses, potentially due to pesticide pollution,

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particularly neonicotinoids (Measham, 2015, 2021; Sánchez-Bayo et al., 2016). There are concerns that these declines will further affect the ecological structure and function of aquatic ecosystems (Sánchez-Bayo et al., 2016; Goulson, 2019; Whelan et al., 2022).

Despite these concerns, relatively little is known about pesticide pollution in English rivers. EU Water Framework Directive (WFD) monitoring data has previously identified chronically elevated concentrations of neonicotinoids in rivers across England, highlighting significant health risks to wildlife (BugLife, 2017; Perkins et al., 2021). However, monitoring is typically limited to "Priority Substances" or to chemicals in the WFD "Watch-Lists" (EU Commission, 2013; Pietrzak et al., 2019), leaving many other potentially harmful pesticides unmonitored. National monitoring efforts have also declined markedly over the past decade (Environment Agency, 2025b). Beyond government data, few independent studies have quantitatively measured pesticide concentrations in English surface waters (Townsend et al., 2018; Taylor et al., 2021).

Even less is known about pesticide pollution in other environmental matrices in England. Many pesticides are hydrophobic and preferentially bind to sediments and organic matter, where they may persist for many years (Mathers et al., 2017; Pathak et al., 2022; Shah and Parveen, 2023; Bizeul et al., 2024). Yet sediments remain overlooked by most monitoring programs (Chiaia-Hernandez et al., 2013). Bioaccumulation in aquatic organisms is also rarely assessed, despite evidence in fish (Belenguer et al., 2014; Masiá et al., 2015; Ccanccapa et al., 2016; Michel et al., 2016; Pico et al., 2019) and invertebrates (Lauper et al., 2022). Unlike water sampling, which may miss transient pesticide peaks (Stehle et al., 2013), sediment and tissue sampling provides a time-integrated measure of contamination (Adams et al., 2011; Shahid et al., 2018; Vane et al., 2022). Widespread bioaccumulation can also indicate chronic exposure and increased risks of adverse effects (Van Der Oost et al., 2003). No previous study has measured contemporary pesticides in sediments or biota in England, leaving the extent of contamination and associated risks largely unknown.

The aim of this study was to comprehensively assess: i) the distribution of pesticide pollution in the waters and sediments of two typical but contrasting English rivers catchments, the River Tone (Somerset) and the River Wensum (Norfolk), ii) the bioaccumulation within multiple levels of the trophic web, and iii) to determine the potential ecological risks associated with these pesticides. The data generated by this study represents one of the most comprehensive assessments of pesticide pollution in any English river catchment to date and is widely applicable to other river catchments across the UK and beyond at risk from similar pressures.

2. Materials and methods

2.1. Study areas

The River Tone is 33 km long from its source to its confluence with the River Parrett, draining a 414 km^2 catchment (Fig. 1), with a mean flow of 3.00 m^3/s (UK NRFA, 2025). Its catchment contains several urban settlements, industries, and a mix of arable land, woodland, and grazing grassland (Fig. A.1). Previous concerns about mercury, organic contaminants (Environment Agency, 2025a), and raw sewage pollution (The Rivers Trust, 2024) led the local council to declare an ecological emergency in 2020 (Stevens, 2023).

The River Wensum is a large chalk river of high ecological importance due to its rich biodiversity. It has a mean flow of 4.10 m^3/s (UK NRFA, 2025). Land-use over its 675 km^2 of catchment area primarily consists of intensive agricultural, with 390 km^2 of cropland area (58 % of total catchment area) compared to 92.3 km^2 (22 %) in the Tone catchment (Fig. A.1; Marston et al., 2022). Over 99 % of its habitat is considered as "poor", and there have long been concerns about aquatic wildlife declines due to poor water quality. Three consecutive invertebrate health surveys have ranked the Wensum as the worst of all 12 major chalk rivers (Measham, 2015, 2021).

2.2. Sample collection

Water and sediment samples were collected from along the River Tone (n = 17) and River Wensum (n = 16) catchments in Oct. 2021 (Fig. 1; Table B.1). Composite sediment samples were obtained by pushing a polycarbonate tube (20 cm L) fitted with a stainless-steel basket catcher into the river bed to a depth of about 8–10 cm (Vane et al., 2007). Composite surface water samples were collected from the middle of the river 30 cm below the surface using a plastic beaker attached to a long pole and were stored at 4 °C in 1 L amber glass bottles. Sediment and water samples were frozen at −18 °C upon return to the laboratory. Six additional water and sediment samples were later taken from the six fish sampling sites (see below), on the Wensum in May 2022 and on the Tone in Aug/Sept. 2022 (Fig. 1; Table B.1).

Fish (*Rutilus rutilus* – common roach) were sampled from three sites on each river using a combination of seine netting and electrofishing (Fig. 1; Table B.1). In total, 52 roach were sampled from the Tone and 65 from the Wensum. White muscle was sampled and stored in plastic vials at 4 °C prior to freezing at −20 °C. Scales were collected and sent to the EA's National Fisheries Laboratory (Brampton, England) for ageing and growth analysis.

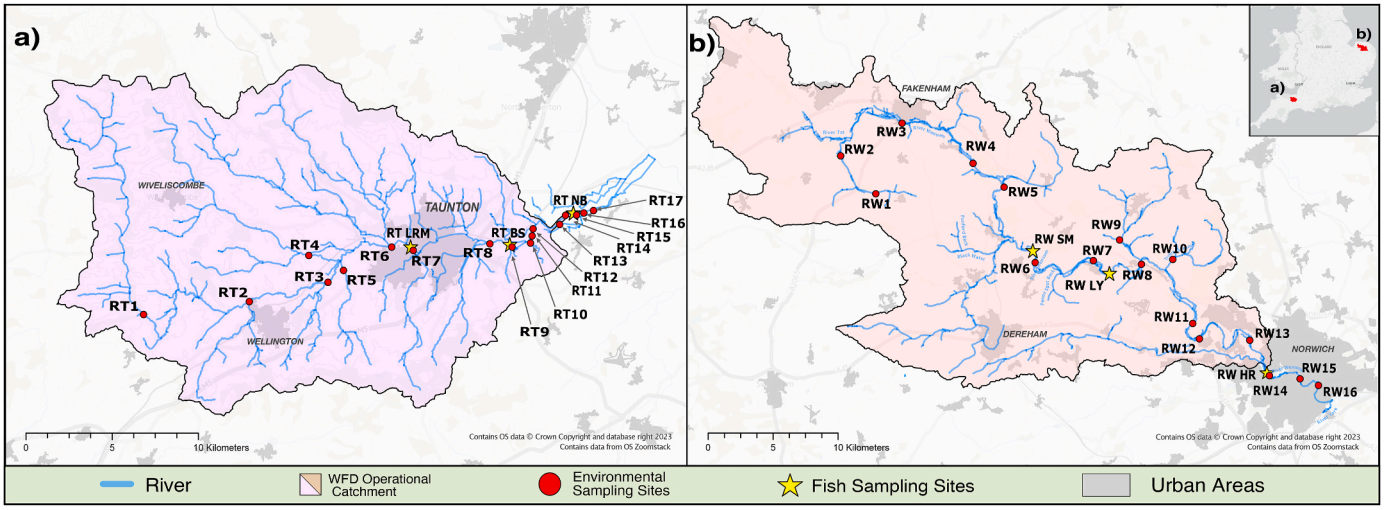

Fig. 1. River Tone (a) and Wensum (b) catchment maps. See Appendix C for site selection information. Maps created using ArcGIS Pro version 2.5.0.

Invertebrates were collected from the Wensum only, at RW SM, LY and HR (Fig. 1), in June 2022, following the Anglers' Riverfly Monitoring Initiative (ARMI) method. After identification to taxonomic Family level (Dobson et al., 2011), invertebrates from each site were pooled and separated (Table B.2), and kept at 4 °C until freezing at −20 °C back at the laboratory.

2.3. Sample processing

Water samples were passed through a 1 μm glass fibre pre-filter and 0.45 μm nylon membrane filter. 200 mL of filtered water were spiked with 20 ng of each internal standard (Appendix C). Spiked samples were passed through preconditioned SPE Oasis HLB cartridges at 2 mL/min and left to dry for 10 min, then eluted with 10 mL of dichloromethane/methanol (50:50 v/v). Eluates were dried under N_2 gas, reconstituted in 1 mL acetonitrile, and filtered through a 0.45 μm PFTE syringe filter into an LC vial for analysis.

Sediment samples were freeze-dried, sieved to <2 mm, and ball-milled to <250 μm (Vane et al., 2020a; Vane et al., 2022). Combined invertebrate samples from each site were freeze-dried and ground in a ball mall using zirconium oxide beads. Fish muscle tissues were freeze-dried, broken up using scissors and ground to a powder using a mortar and pestle. Prepared sediment, invertebrate and fish samples were extracted using a slightly modified version of the QuEChERS method developed by Anastassiades et al. (2003) (Appendix C; Fig. A.2). 1 mL of the cleaned extract was then filtered through a 0.45 μm PTFE syringe filter into an LC vial for analysis. Matrix-matched procedural blanks – free of pesticides – were treated in an identical fashion.

Total organic carbon (TOC) was determined on 300 mg of sediment using an Elementar VarioMax C, N analyser after acidification with hydrochloric acid (HCl, 5.7 mol/L). The limits of quantification were 0.18 % (wt/wt) (Vane et al., 2009). See Table B.3 for TOC values.

2.4. LC-MS/MS determination

Pesticides were analysed using a uHPLC UltiMate 3000 (Thermo Scientific) coupled to a triple-stage quadrupole (TSQ) Quantiva tandem mass spectrometer (UPLC-MS/MS) fitted with a guard column and a Hypersil GOLD C18 column (150 mm × 3 mm, 3 μm particle size, Thermo Fisher Scientific, Waltham, USA). MS/MS was performed in selected reaction monitoring (SRM) mode using electro-spay ionisation (ESI) in positive mode (Table B.4). Samples were injected at a flow rate of 0.4 mL/min. The gradient programme was initiated with a mobile phase consisting of 70:30 mix of water/acetonitrile, both with 0.1 % formic acid, increased to 95 % acetonitrile at 5.6 min and maintained for 2 min followed by equilibration for 15 min at 70:30 water/acetonitrile. Detection and quantification were based on precursor and product ions of the analytes using 7-level calibrations for all matrices, in conjunction with the seven deuterated internal standards. All data were processed using Thermo Xcalibur software.

Fifty-two pesticides (Table B.5) were analysed in sediments and fish, and 50 in water (Malathion and Terbutryn were not analysed in water). Identification parameters and limits of detection (LOD) and quantification (LOQ) are presented in Table B.6 and B.7. Quality control was achieved by analysing soil CRMs; ERA 925 and ERA 926; all CRM compounds fell within the certified QC values (Table B.8). Recoveries and precision were tested on a subset of 16 pesticides; recovery was between 73.9 and 100 % and variation between each of five replicates was below 20 % (Table B.9). All analytical data from this study can be found in Ramage et al. (2024).

2.5. Risk assessments

Pesticide concentrations were first compared to relevant guidelines to identify exceedances; these include water annual-average environmental quality standards (AA-EQS) established under the EU WFD (Directive, 2000/60/EC; EU Commission, 2000; Table B.10) and sediment benchmarks developed by the United States Geological Survey (USGS; Nowell et al., 2016; Table B.11).

Ecotoxicological risk to fish, invertebrates, and algae, based on water pesticide concentrations, was calculated using the toxic unit (TU) and risk quotient (RQ) approach, as described in Ccanccapa et al. (2016). Sediment-associated TU were also calculated (Ccanccapa et al., 2016). These tests assume a Concentration Addition (CA) toxicity model and are routinely applied to toxicity risk assessments of pesticide mixtures (Gustavsson et al., 2017). Acute toxicity thresholds (96h LC50, 48hr EC50, 72hr growth EC50 for fish, invertebrate and algae, respectively – Table B.12 and Appendix C) were used for TU calculations. Meanwhile, Predicted No-Effect Concentrations (PNEC) were used in the RQ calculations (Table B.12 and Appendix C). Algae RQ were not calculated due to a lack of available data. Both approaches were used in parallel to provide a higher-end (TU) and a more conservative (RQ) estimate of toxicity risk.

Whole effluent sediment toxicity was estimated using the Microtox® solid phase test (SPT) according to (Vane et al., 2020a; Vane et al., 2020b). A subset of 12 sites per river were selected for this assay. The assay outputs were used to create a dose response curve and an EC50 value in mg/L. Benchmark toxicity values for this test are as follows: >10000 mg/L indicates non-toxic sediment, 10000-5000 mg/L indicates moderately toxic sediment, and <5000 mg/L indicates acutely toxic sediment (Kwan and Dutka, 1995; Vane et al., 2020b).

2.6. Data analysis

Data were analysed in R and R Studio version 2023.06.1 (R Core Team, 2023), and plots were created using ggplot2 (Wickham, 2016) unless otherwise stated. Summary statistics were computed in R using the non-parametric Kaplan-Meier (KM) method in the NADA package (Lee, 2020), enabling adjustments of data containing values below LOD (Hladik et al., 2024). For values below LOQ but above LOD, instrument-generated data were used, acknowledging the higher uncertainty associated with these values (Gustavsson et al., 2017). ΣTU and RQ_{mix} calculations were adjusted for censored data using the NADA package according to Helsel (2010). For manipulations which required log10 transformation, a negligeable constant was added to the data to counter null values. For group comparisons, parametric tests (t-tests, ANOVA with Tukey's HSD) were used when assumptions were met, and non-parametric alternatives (Wilcoxon rank sum, Kruskal-Wallis with post-hoc multiple comparisons and Bonferroni corrections) were applied otherwise. General linear model (GLM) fit was evaluated by checking the distribution of residuals, and model refinement was checked based on a change in Akaike Information Criterion (AIC) of >2. Tests requiring the use of censored data were computed using the NADA package (Lee, 2020). A significance level of 95 % was used ($p < 0.05$) for all analyses unless stated otherwise. See Appendix C for a full description of methods.

3. Results and discussion

3.1. Water

All water samples were contaminated by multiple pesticides. Several pesticides were detected in nearly all water samples (Table 1). Herbicide and fungicide water concentrations were generally in agreement with past studies in England (Taylor et al., 2021), and were also within the same range as the concentrations reported in other European rivers (e.g. Masiá et al., 2013, 2015; Moschet et al., 2014; Casado et al., 2019). However, very few quantitative studies exist in England for comparison.

Many of the detected pesticides, including Azoxystrobin, Tebuconazole, Imidacloprid, and Clothianidin are included in the EU WFD Priority Substances List and 4th Watch List, and are suspected to present a significant risk to the aquatic environment (EU Commission, 2022).

Table 1
Pesticide concentrations in water samples from the Rivers Tone and Wensum. Summary statistics were calculated using KM analysis to handle values below LOD (Hladik et al., 2024). n(C) represents the number of censored values (<LOD). The frequencies represent detection rates (>LOD), which may be influenced by the varying detection limits for each compound (Table B.7). The single highest detected concentration in each river is shown in bold. Pesticides not detected in any sample are not shown. All concentrations are presented in ng/L.

Pesticide	River Tone (n = 20)					River Wensum (n = 18)				
	n (C)	Min	Mean ± SD	Max	Freq.	n (C)	Min	Mean ± SD	Max	Freq.
Acetamiprid	2	0	<LOD	<LOQ	15 %	2	0	0.188 ± 0.351	1.23	28 %
Atrazine	4	<LOD	2.76 ± 1.38	7.94	80 %	2	<LOD	7.85 ± 3.83	16.6	89 %
Azoxystrobin	0	<LOQ	6 ± 4.52	18.2	100 %	0	1.08	15.1 ± 28.4	**126**	100 %
Boscalid	3	0	5.21 ± 3.51	9.89	80 %	12	<LOD	3.98 ± 3.47	16.3	33 %
Carbaryl	5	0	<LOD	<LOD	0 %	3	0	<LOD	<LOD	0 %
Carbendazim	0	<LOD	1.2 ± 0.77	3.45	95 %	0	<LOQ	1.36 ± 0.669	2.81	100 %
Carbetamide	0	0	6.8 ± 19.4	79.3	15 %	0	0	6.35 ± 15.8	56.5	17 %
Chlorfenvinphos	15	0	0	<LOD	0 %	7	0	0	<LOD	0 %
Chlorotoluron	2	0	0.0342 ± 0.153	0.684	5 %	0	0	0.273 ± 0.285	0.833	61 %
Clethodim	6	0	<LOD	<LOD	0 %	3	0	<LOD	<LOQ	6 %
Clothianidin	6	0	14.3 ± 11.9	36.9	65 %	9	<LOD	15.2 ± 13.3	63.4	50 %
Diazinon	2	<LOD	0.485 ± 0.303	1.69	90 %	5	<LOD	3.41 ± 2.8	7.03	72 %
Difenoconazole	5	0	0.303 ± 0.267	0.889	70 %	8	0	0.464 ± 1.31	5.5	50 %
Dimethomorph (isomer 1)	18	0	<LOD	<LOD	0 %	10	0	0	<LOD	0 %
Dimethomorph (isomer 2)	15	0	<LOD	<LOQ	15 %	16	0	0	<LOQ	6 %
Diuron	15	0	<LOD	<LOQ	5 %	9	0	0	<LOD	0 %
Epoxiconazole	2	<LOD	0.264 ± 0.254	5.14	90 %	0	1.12	3.07 ± 2.22	10.4	100 %
Fenazaquin	4	0	<LOD	<LOD	0 %	4	0	<LOD	<LOD	0 %
Fenbuconazole	8	0	<LOD	<LOQ	10 %	6	0	<LOD	<LOQ	11 %
Fenuron	2	0	0.539 ± 0.711	2.48	55 %	0	0	2.52 ± 3.08	10	72 %
Fipronil	15	0	<LOD	<LOQ	15 %	13	0	0	<LOD	0 %
Flufenacet	1	0	2.14 ± 1.28	4.17	85 %	1	<LOD	7.8 ± 14.1	63.5	94 %
Fluoxastrobin	7	0	<LOD	<LOQ	5 %	4	0	0.0246 ± 0.0404	1.57	44 %
Fluquinconazole	0	0	0	0	0 %	0	0	0	2.23	11 %
Imazalil	0	0	0.807 ± 0.632	2.09	75 %	2	0	0.273 ± 0.254	0.69	67 %
Imidacloprid	1	<LOD	25.8 ± 20.9	**97.1**	95 %	0	0	15.2 ± 10.3	34	94 %
Mandipropamid	2	0	0.361 ± 0.558	1.85	65 %	6	0	0.162 ± 1.27	5.34	28 %
Metalaxyl-M	15	0	0	<LOD	0 %	16	0	0	<LOD	0 %
Nitenpyram	8	0	0	<LOD	0 %	11	0	0	<LOD	0 %
Picoxystrobin	3	0	<LOD	<LOD	0 %	3	0	<LOD	<LOQ	6 %
Prochloraz	3	0	0	0	0 %	2	0	0	<LOD	0 %
Propamocarb	2	0	0.572 ± 0.831	2.54	55 %	2	0	0.248 ± 0.291	0.822	56 %
Propiconazole	1	<LOD	2.37 ± 1.26	4.63	95 %	0	<LOQ	1.27 ± 0.468	2.3	100 %
Propyzamide	2	0	0.803 ± 0.844	2.2	75 %	0	0	2.51 ± 1.45	6.99	94 %
Prothioconazole	10	0	<LOD	<LOQ	20 %	7	0	<LOD	<LOQ	17 %
Pymetrozine	0	0	0.381 ± 0.703	2.14	35 %	3	0	0.477 ± 1.66	7.06	11 %
Pyraclostrobin	6	0	<LOD	<LOQ	10 %	8	0	<LOD	<LOD	0 %
Pyrimethanil	6	0	<LOD	<LOQ	40 %	7	0	<LOD	<LOQ	0 %
Spiroxamine	8	0	<LOQ	<LOQ	55 %	16	0	<LOD	<LOD	0 %
Tebuconazole	1	<LOD	22.7 ± 14.3	44.5	95 %	0	4.95	9.41 ± 5.82	30.5	100 %
Thiabendazole	20	<LOD	0	<LOD	0 %	16	0	0	<LOD	0 %
Thiacloprid	0	0	0	0	0 %	0	0	<LOD	<LOQ	6 %
Thiamethoxam	3	0	0	<LOD	0 %	3	0	<LOD	<LOQ	6 %
Tricyclazole	13	0	0	<LOD	0 %	7	0	0	<LOD	0 %
Trifloxystrobin	2	0	<LOD	<LOD	0 %	1	0	<LOD	<LOD	0 %
Zoxamide	5	0	0.225 ± 0.51	1.8	20 %	1	0	<LOD	<LOQ	6 %

Two pesticides, Imidacloprid and Clothianidin, exceeded their AA-EQS (Table B.10). Imidacloprid exceeded AA-EQS (6.8 ng/L) at 17 of 20 sites on the River Tone (10.6–97.1 ng/L; mean: 25.8 ± 20.9 ng/L) and at 14 of 18 sites on the River Wensum (7.03–34.0 ng/L; mean: 15.2 ± 10.3 ng/L). Clothianidin exceeded AA-EQS (10 ng/L) at 13 sites on the Tone and 8 on the Wensum; however, concentrations were above LOQ in only two samples.

Total neonicotinoid concentrations were also summed due to their shared mode of action and known additive toxicity and compared to acute and chronic invertebrate toxicity thresholds proposed by Morrissey et al. (2015). No sample exceeded the acute toxicity threshold of 200 ng/L. The chronic threshold (35 ng/L) was exceeded throughout both rivers (Fig. 2a and b), at 12 sites on the Tone and four on the Wensum, with a maximum of 97.1 ng/L at RT NB. These frequent EQS and toxicity threshold exceedances highlight a continued concern over neonicotinoid pollution in England and beyond (Morrissey et al., 2015; Sánchez-Bayo et al., 2016; BugLife, 2017). However, since our data represents one-time sampling, the comparison to chronic thresholds (derived using longer-term exposure data) should be viewed with the understanding that chronic adverse effects would require sustained concentrations over time.

The two pesticides exceeding their AA-EQS likely have different sources and entry pathways. Clothianidin and its parent compound Thiamethoxam, banned in 2018, were widely used as seed treatments on crops like cereals and oilseed rape. Since then, emergency authorisations have allowed Thiamethoxam use on sugar beet to combat beet yellow virus. The high Clothianidin concentrations at RW1 (63.4 ng/L; c.f. Fig. 1) likely reflect this use, as sugar beet is widely cultivated in the upper-Wensum (Marston et al., 2022). Imidacloprid, although banned in agriculture, remains approved for veterinary use, with 68 products currently authorised in the UK (Veterinary Medicines Directorate, 2023). These products, usually applied topically to pets, can enter waterways through pet bathing, runoff during rainfall, or washing of contaminated bedding (Teerlink et al., 2017). A key pathway is via the sewage network (Sadaria et al., 2017; Teerlink et al., 2017; Perkins et al., 2021). Higher concentrations in the Tone, particularly downstream of Taunton (Figs. 1 and 2a-b), may reflect a larger human population and greater sewage system inefficiencies; untreated wastewater

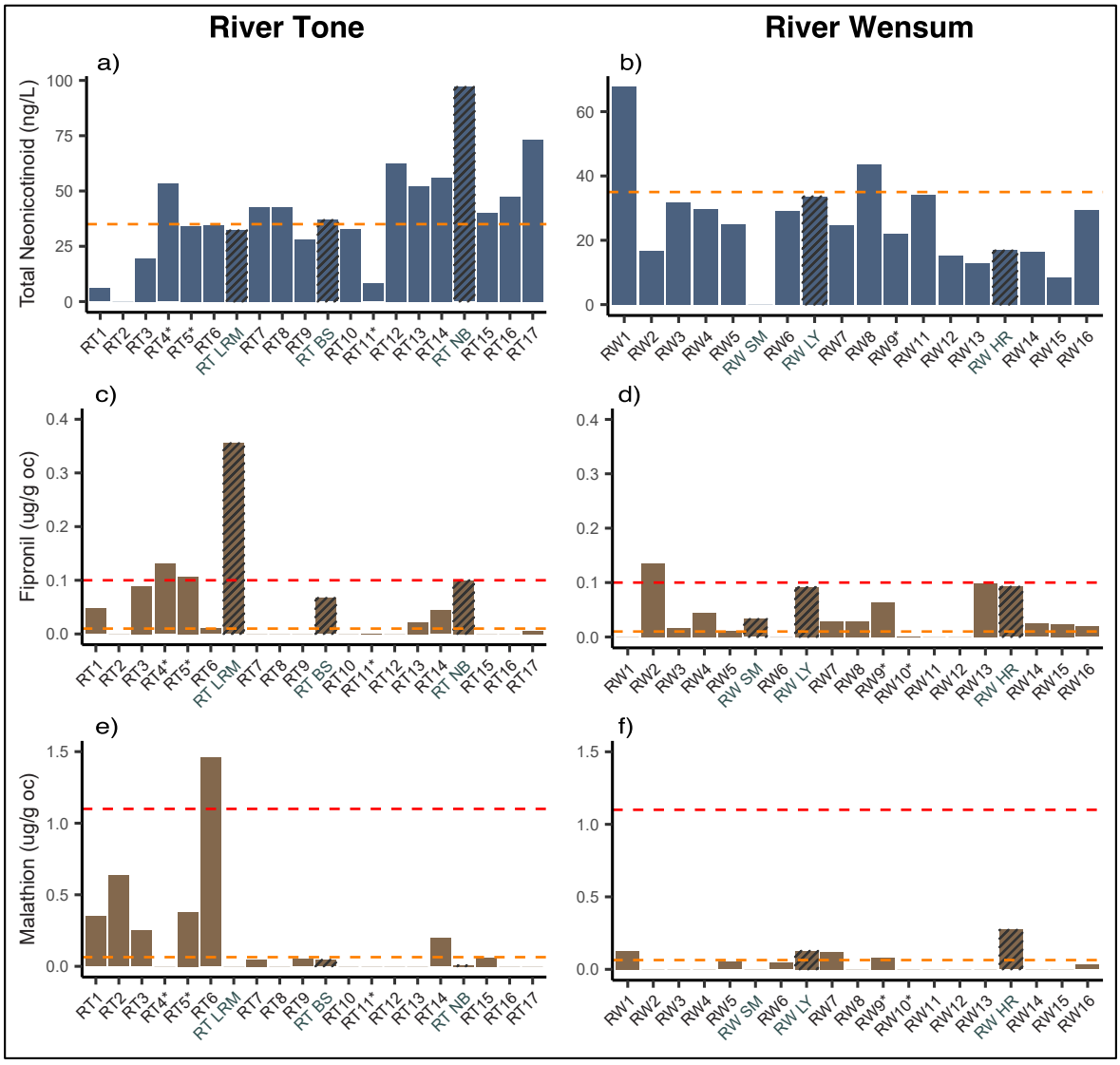

Fig. 2. Pesticides and pesticide groups exceeding toxicity thresholds. Blue bars represent water concentrations; brown bars represent sediments. Panels a–b show total neonicotinoids, summed using Kaplan-Meier adjustments for censored data (Helsel, 2010; Lee, 2020), compared against chronic invertebrate toxicity thresholds (Morrissey et al., 2015). Panels c–f show organic carbon–normalised Fipronil and Malathion concentrations, alongside sediment benchmarks from Nowell et al. (2016): orange lines denote Threshold Effect Benchmarks (TEB; adverse effects unlikely), and red lines denote Likely Effect Benchmarks (LEB; high probability of effects on benthic invertebrates). Sites are positioned from source to mouth. Sampling sites noted with asterisks (*) are tributaries to the main river. Striped bars indicate sites sampled in Summer (2022); solid bars were sampled in Autumn (2021).

was discharged into the Tone over 4500 h in 2022, compared to just 107 h in the Wensum (The Rivers Trust, 2024).

Because both rivers fall within Drinking Water Protected Areas (DWPA) and Drinking Water Safeguard Zones (DWSZ), pesticide concentrations were also compared to EU drinking water quality guidelines. No water sample exceeded the generic guideline for total pesticides of 500 ng/L, with the highest total being 277 ng/L at RW1 (c.f. Fig. 1). However, the systemic fungicide Azoxystrobin was detected above the EU guideline of 100 ng/L for single pesticides, reaching 126 ng/L at RW11 (c.f. Fig. 1). This site is located directly downstream of a golf course, which may serve as a point source of pollution. In the UK, Azoxystrobin is commonly applied to golfing greens throughout the warmer months to treat fungal diseases (Garthwaite et al., 2023).

3.2. Sediments

Sediments from both rivers contained complex pesticide mixtures, with over 83 % of samples containing 16 pesticides or more. Such complex mixtures have been reported previously in other countries (Allinson et al., 2015; Moran et al., 2020; Khurshid et al., 2024). One study, which analysed 164 pesticides in European river sediments, frequently detected 10–20 pesticides, with the highest number in a single sample (n = 48) recorded in the Czech Republic (Khurshid et al., 2024). To the best of our knowledge, this is the first study to measure contemporary pesticides in river sediments in the UK.

While there was some overlap between the pesticides most frequently detected in water and in sediment, several were far more common in sediment (Table 1 and B.13). These pesticides tended to be more hydrophobic, with higher log P values (Lewis et al., 2016) and a stronger affinity for organic matter. Total organic carbon bore a significant positive influence on total pesticide concentrations in sediments ($F_{1,35} = 66.4$, $p < 0.01$), explaining 64.5 % of total pesticide concentration variation.

Two pesticides exceeded sediment benchmarks (Table B.11; Nowell et al., 2016): the organophosphorus insecticide Malathion and the phenylpyrazole ectoparasiticide Fipronil (Fig. 2c–f). Malathion

exceeded the Likely Effect Benchmark (LEB)—indicating a high probability of adverse effects—once, at RT6 (1.46 ng/g OC). Fipronil exceeded the LEB five times: once on the Wensum (RW2: 0.135 ng/g OC) and four times on the Tone, including at RT LRM, a designated bathing water area in Taunton, where concentrations reached 0.355 ng/g OC (12.6 ng/g), a 256 % benchmark exceedance. RT LRM is a popular dog bathing spot, likely explaining the elevated Fipronil. Fipronil, much like Imidacloprid, is exclusively used in veterinary medicine (Veterinary Medicines Directorate, 2023). Similar associations between high ectoparasiticide concentrations and dog bathing activity have previously been reported in London swimming ponds (Yoder et al., 2024).

River sediments form an important dietary component for omnivorous fish such as roach (Jamet, 1994). The ingestion of contaminated sediments serves as a key pathway for lipophilic pesticides such as Fipronil, which tend to accumulate in sediments, to expose non-target organisms, potentially causing toxic effects (Baird et al., 2013; Santillán Deiú et al., 2021). Fipronil concentrations below those detected in this study have been shown to cause harm to invertebrates (Maul et al., 2008) and fish (Santillán Deiú et al., 2021). These findings thus highlight an environmental hazard that might have gone undetected without sediment monitoring, as Fipronil was rarely detected above LOD in water, and exemplify the important role of sediment monitoring in assessing contamination and environmental risks.

3.3. Bioaccumulation in fish and invertebrates

Fish age varied between 2 and 9 years (median 4 years). There was a non-significant negative association between total pesticide burden and length when controlling for age (GLM controlling for length, age class and the interaction between the two; $p > 0.05$).

Forty-eight of the 52 pesticides analysed were detected in at least one fish (Fig. A.3), averaging nine pesticides per fish. Bioaccumulation was widespread, with no significant differences in total pesticide concentration between rivers or sites. In the Wensum, Propiconazole bioaccumulation was significantly greater upstream ($df = 2$, $\chi^2 = 7.15$, $p < 0.05$), while the same was true for Boscalid and Spiroxamine in the Tone (Boscalid: $\chi^2 = 9.65$, $p < 0.01$; Spiroxamine: $\chi 2 = 11.4$, $p < 0.005$).

The most frequently detected pesticide in fish was Propiconazole, detected in 92 % of fish (0.863 ± 0.868 ng/g). Spiroxamine was detected in every single River Tone fish (1.25 ± 0.826 ng/g) and in 43 % of River Wensum fish (0.0929 ± 0.185 ng/g), while Fipronil was detected in 80 % (4.69 ± 5.08 ng/g) and 83 % (9.36 ± 15.2 ng/g) of fish in the Tone and Wensum respectively, reaching 83.7 ng/g in one roach at RW LY (c.f. Fig. 1), the highest single pesticide concentration detected in fish (Fig. A.3). Diazinon, Epoxiconazole, Boscalid, Atrazine, Terbutryn and Fenbuconazole were also frequently detected (Table B.14). These pesticides are all characterised by moderate-to-high log P values. Indeed, pesticides detected in fish samples had significantly higher log P values (median 3.50, IQR 2.45–3.50) than those not detected (median 2.84, IQR 0.72–2.84; Mann-Whitney *U* test, $p < 0.01$), suggesting that pesticides with higher log P values are more likely to be detected and bioaccumulate in wildlife (Fig. A.4). Log P is a critical predictor of how well an organic compound will be absorbed and transported, where it will be distributed in the body, and of toxicity potential (Czerwinski et al., 2006; Pico et al., 2019; Ivanović et al., 2020; Yukawa and Naven, 2020). However, a pesticide's bioaccumulation is also governed by its resistance to metabolic or soil degradation; compounds that are more resistant to degradation tend to persist and accumulate more in organisms over time (Cui et al., 2019; Chen et al., 2021).

Invertebrates were collected from the River Wensum only. The highest pesticide concentration was recorded for Fipronil at RW HR (322 ng/g; Fig. A.5), the most downstream sampling site, located within Norwich (c.f. Fig. 1). Fipronil concentrations were considerably lower further upstream at LY (7.37 ng/g) and SM (1.53 ng/g). Excluding Fipronil (which drove most of the between-site variation), total invertebrate pesticide concentrations followed a decreasing spatial trend.

This study is the first to measure contemporary pesticides in fish or invertebrates in the UK, with past studies focusing solely on legacy OCPs (Jürgens et al., 2016). Many more pesticides were detected in fish from this study than in comparable studies from Spain (Belenguer et al., 2014; Masiá et al., 2015; Pico et al., 2019). Mean Fipronil concentrations were similar to those measured in fish from Southern Brazil (Miranda et al., 2008) and Southern France (Roche et al., 2009), but lower than in European eels from the same location in France (Ribeiro et al., 2005). The concentrations detected by Roche et al. (2009) in benthic macroinvertebrates were much higher than those in fish (similarly to our findings at RW HR), reaching 506 ng/g dw in pink shrimp. Generally, however, few studies have measured contemporary pesticides in aquatic biota, and comparisons are further hindered by sampling and analytical differences (e.g., tissue measured, normalisation method) (Swanson et al., 2018; Kraus et al., 2021; Scully-Engelmeyer et al., 2021; Lauper et al., 2022).

Bioaccumulation monitoring provides one approach for assessing exposure to chemicals in their bioavailable form (Van Der Oost et al., 2003). These results clearly indicate that non-target organisms are bioaccumulating numerous pesticides, in particular lipophilic pesticides such as Fipronil, in much higher concentrations than those found in water or sediment. Furthermore, the widespread bioaccumulation of certain pesticides, affecting as much as 100 % of fish in some cases, is a significant cause for concern. Together, these results indicate that aquatic wildlife in the Tone and Wensum are chronically exposed to numerous pesticides and are at risk of suffering from adverse health effects. These risks were estimated in water and sediment using TUs, RQs, and Microtox® SPT.

3.4. Risk assessment

3.4.1. C.D.A. water risk assessment using a TU and RQ approach

ΣTU values were below 1 for fish, invertebrates, and algae at all sites, suggesting no risk of acute toxicity from pesticide mixtures to either trophic level (Ccanccapa et al., 2016). Invertebrate ΣTU were highest, ranging from 5.54×10^{-4}-0.486 in the Tone and 3.26×10^{-4}-0.339 in the Wensum. Neonicotinoids were responsible for nearly all risk. In the Tone, the highest risks were detected below Taunton (c.f. Fig. 1) due to high Imidacloprid concentrations, whereas in the Wensum, RW1 (c.f. Fig. 1) had the highest risk due to elevated Clothianidin.

According to the RQ calculations, which used PNEC rather than EC50s, pesticide mixtures presented medium risks of chronic toxicity to fish ($0.1 < RQ_{mix} < 1$) at one site on the Wensum, RW1 ($RQ_{mix} = 0.115$) and at one site on the Tone, RT12 ($RQ_{mix} = 0.104$) (Figs. 1 and 3). Risks in the Wensum were significantly higher at RW1 compared to the rest of the catchment area, where no clear spatial pattern was observed. In contrast, risks in the Tone increased steadily from RT3 ($RQmix = 0.048$) to RT12 (Fig. 3). In both rivers, much of the risk was driven by Tebuconazole and Carbendazim. Carbendazim, a fungicide banned in 2017 due to its mutagenic and teratogenic properties, was frequently detected in water and sediments of both rivers (Table 1 and B.13). Despite the ban, it remains a common wastewater contaminant due to its use in household and industrial products (e.g., kitchen and toilet paper, textiles, construction materials), and has also been widely detected in groundwater (Lapworth et al., 2018; Merel et al., 2018). It can also form from the degradation of certain fungicides such as Thiophanate-methyl; both thiophanate-methyl and carbendazim are still used in cosmetic products but are being phased out. Together, these sources likely contribute to Carbendazim's widespread presence in both catchments. Tebuconazole is the third most sprayed fungicide on arable crops in the UK (FERA, 2023) and was detected throughout the Tone catchment, suggesting widespread agricultural usage and diffuse entry into the river system. Short-term exposure to Tebuconazole has been shown to cause disease in fish (Altenhofen et al., 2017; Li et al., 2019; Macirella et al., 2022), albeit at concentrations significantly higher than those detected in this study. However, the effects of chronic, long-term exposure to

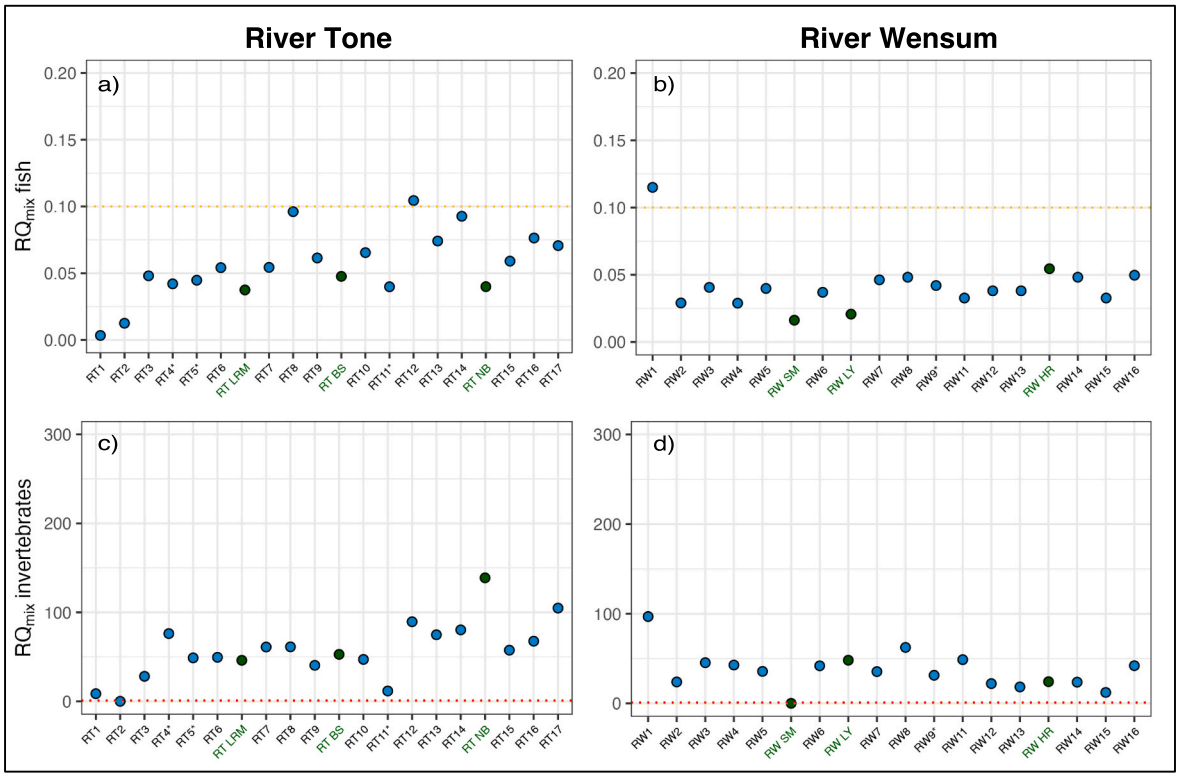

Fig. 3. RQ_{mix} values for fish (a–b) and invertebrates (c–d) in water samples from the Rivers Tone and Wensum. Values were calculated with Kaplan-Meier adjustments for censored data using the NADA package in R (Helsel, 2010; Lee, 2020). The orange dotted lines represent the threshold above which medium risks of chronic toxicity are present, while the red dotted lines represent the threshold above which high risks of chronic toxicity are present. Sites are positioned from source to mouth. Sampling sites noted with asterisks (*) are tributaries to the main river. Sites in green were sampled in Summer (2022), while all others were sampled in Autumn (2021).

Tebuconazole are less known, and further research is needed (EFSA, 2008). Chronic risks to fish were much lower in the Tone in Summer (2022) sampling (Fig. 3), primarily due to lower Tebuconazole concentrations.

Invertebrate $RQ_{mix} > 1$ were detected at all but two sites, ranging from 0.0289 to 139 in the River Tone and 0.0262–96.8 in the River Wensum (Fig. 3). Neonicotinoids were responsible for nearly all risk in both catchments. These values suggest that, if the measured neonicotinoid concentrations are sustained over extended periods, aquatic invertebrates in both rivers are at high risk of chronic toxicity from neonicotinoid exposure. Long-term invertebrate data from 2015 to 2017, quantified using SPEAR scores (Species at Risk Index; Liess and von der Ohe, 2005), further support this, indicating that invertebrate communities in the Wensum are under chronic chemical pressure affecting community composition (WildFish, 2019). Over time, such pressures can result in significant alterations to invertebrate populations and communities (Beketov et al., 2013) and may exasperate biodiversity loss and ecological decline throughout freshwater ecosystems.

3.4.2. C.D.B. sediment risk assessment using sediment TU and Microtox® assay

Sediment ΣTU exceeded the acute risk threshold (>1) for benthic invertebrates (Ccanccapa et al., 2016) once in the Tone, at RT6 (ΣTU = 2.03) near the Taunton urban area (Fig. 1), and once in the Wensum, at RW14 (ΣTU = 2.82) in the Norwich suburbs (Fig. 1). Malathion was the main contributor to sediment ΣTU in the Tone, while both Malathion and Dimethoate dominated risk in the Wensum (Fig. A.6), despite their infrequent detection (Table B.13).

The Microtox® whole-effluent toxicity assay indicated, in contrast to the sediment ΣTUs, that all sediments in the River Tone presented low risk of acute toxicity (Microtox EC50 values > 10,000 mg/L). In contrast, three sites in the upper-Wensum, RW1, RW4 and RW5 (c.f. Fig. 1), exhibited EC50 values of 2709 mg/L, 3645 mg/L, and 4452 mg/L respectively, indicating that these sediments were acutely toxic to life (Fig. A.6). The values from the upper-Wensum are lower (more toxic) than sediments from the River Thames (Vane et al., 2020b) and Conwy estuary (Vane et al., 2020a), but similar to sediments from the River Mersey (Vane et al., unpublished results), and highlight a significant cause for concern. As this assay provides a measure of whole-effluent toxicity, i.e. caused by the combination of all chemicals present in each sample, few inferences can be made about the primary drivers of toxicity. The Microtox values did not correlate with any of any of the 52 pesticides tested, neither did they align with sediment ΣTU values. These discrepancies may indicate that the pesticides measured in the sediment samples were not the only chemicals contributing to overall sediment toxicity. Sediment toxicity at RW1, located in a catchment area almost exclusively used for intensive agriculture and with limited domestic sources (Fig. A.1), is likely caused by agrochemicals or agriculture-related stressors. In contrast, RW4 and RW5, situated downstream of Fakenham, may also be affected by industrial or domestic pollutants, contributing to the observed toxicity.

4. Summary and future directions

Widespread pesticide pollution, containing complex mixtures, was detected in the water, sediments, and in the aquatic wildlife of two typical English river catchments. Several pesticides exceeded legally-binding EQS in water, as well as sediment toxicity guidelines, indicating potential risks to aquatic wildlife. These risks were estimated in water and sediments using TUs, RQs, and Microtox® SPT. Most notably, neonicotinoid water concentrations, driven by Imidacloprid and Clothianidin, exceeded chronic thresholds for aquatic invertebrates at one

third of sites sampled, in some cases by up to a factor of three (Fig. 2). Risk quotient calculations supported these findings, suggesting a high potential for chronic toxicity to aquatic invertebrates throughout both catchments if neonicotinoid concentrations persist over time. Medium risks of chronic toxicity were also occasionally detected for fish, driven by several pesticides in mixture.

When interpreting this pesticide data, it is important to acknowledge the numerous uncertainties that accompany it. For example, the random-event single spot water sampling methodology employed in this study must be considered. Pesticide exposure is known to be closely linked to rainfall events (Casado et al., 2019), and there have been suggestions that event-triggered, or flow-proportional water sampling should preferably be employed to capture peak exposure (Stehle et al., 2013; Bundschuh et al., 2014). This claim is partially supported by long-term EA data from the River Wensum (Fig. A.7 and A.8), which shows several of the pesticides – but not all – measured here exhibiting significant concentration peaks during high flow periods. In the present study, the detection of pesticide concentrations exceeding EQS and calculated to pose risks of chronic toxicity to aquatic wildlife, despite a random-event spot sampling approach and minimal rainfall in the preceding week (Hollis et al., 2024), raises concerns about the potential extent of additional pesticide pollution that was not captured. Moreover, this study analysed fewer than 10 % of all *currently-approved* pesticides in the UK, leaving many extensively used pesticides—such as pyrethroid insecticides, 2,4-D, and Glyphosate (FERA, 2023)—unmeasured. Transformation products, which can be more persistent and toxic than their parent compounds, were also not analysed (Michel et al., 2016). Therefore, the pesticide concentrations, ΣTU and RQ_{mix} presented here may in fact underestimate the actual risks. This is in part supported by the Microtox® results, which suggest that acute toxicity in sediments from the upper-Wensum was likely caused by pesticides or other unmonitored chemicals.

The pesticides identified in this study have diverse potential origins, including agricultural, domestic, and veterinary. Historically, agriculture has shouldered the blame for the majority of pesticide pollution (Rasmussen et al., 2015; Pascual Aguilar et al., 2017). However, more recent research has shed light on the important contributions of domestic sources (Tassin de Montaigu and Goulson, 2023) as well ectoparasiticides used in domestic veterinary care (Perkins et al., 2021). Interestingly, despite the major differences between both catchments (section 2.1), the primary pesticides contributing to risks to aquatic wildlife were broadly identical. Two ectoparasiticides, Imidacloprid and Fipronil, were responsible for some of the highest risks to aquatic wildlife in this study, but many other veterinary ectoparasiticides (e.g. afoxolaner, selamectin, and fluralaner) may pose similar environmental hazards (Perkins et al., 2021; Wells and Collins, 2022). Ectoparasiticide sales in the UK have increased 40-fold between 1997 and 2017, with an estimated 90 % of the 21 million dog and cat owners applying them at least once a year (PDSA, 2019; Perkins and Goulson, 2023). Additional protective measures should strongly be considered, including raising awareness of environmental impacts (Yoder et al., 2024), promoting more environmentally-sustainable practices (British Veterinary Association, 2021), and introducing stricter regulation.

CRediT authorship contribution statement

Calum I. Ramage: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Raquel Alfama Lopes dos Santos:** Writing – review & editing, Software, Resources, Methodology, Formal analysis, Conceptualization. **Lisa Yon:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Matthew F. Johnson:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Christopher H. Vane:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Calum Ramage reports financial support was provided by UK Research and Innovation Natural Environment Research Council. Calum Ramage reports financial support was provided by Environment Agency and the Broadland Catchment Partnership. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.126371.

Data availability

Data will be made available on request.

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