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Supporting Information for

Environment and food web structure interact to alter the trophic magnification of persistent chemicals across river ecosystems

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The Supporting Information contains additional information on the analytical and statistical methods.

Appendix S1

Sample collection

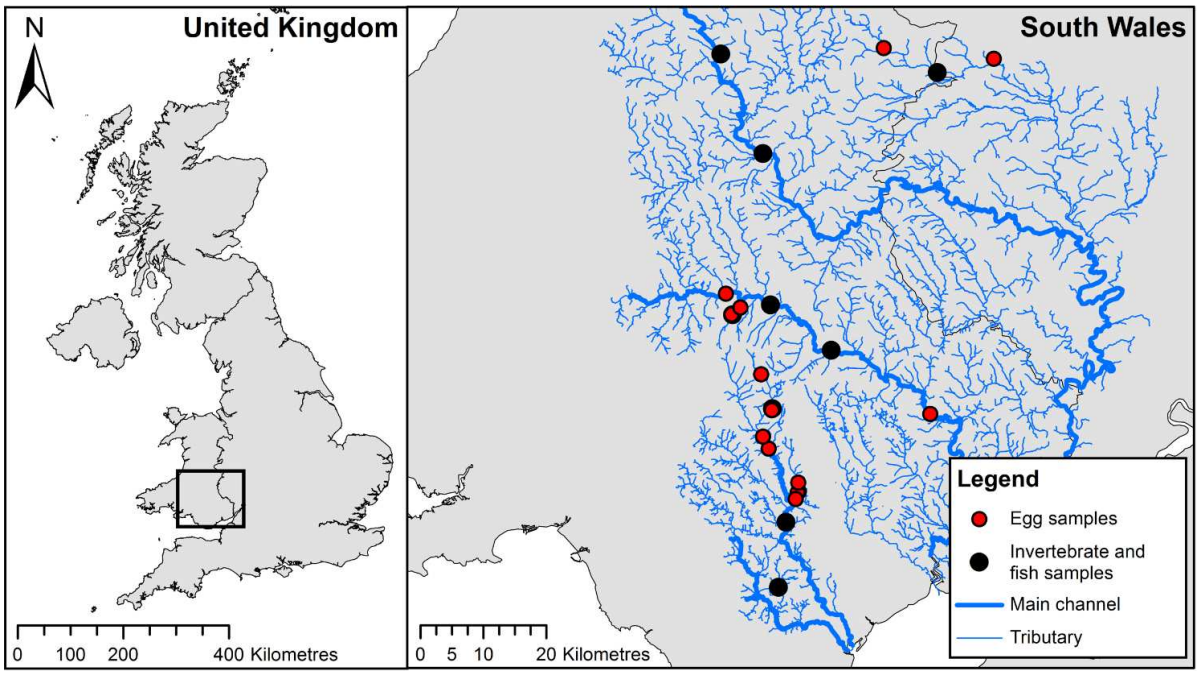

Fig. S1. The location of sample sites across contrasting river catchments in South Wales (United Kingdom). Sites for invertebrates and fish samples (black; n = 9), as well as egg samples (red; n = 12), were distributed across the Taff, Usk and Wye catchments. Some red and black markers are overlapping.

Collection of biofilms and macrophytes

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

Collection of macroinvertebrates

Preliminary surveys across sample sites indicated the ubiquitous abundance of several macroinvertebrate taxa. Five target macroinvertebrate genera were selected; *Gammarus pulex* (Amphipoda: Fabricius, 1775), *Baetis* spp. (Ephemeroptera),

Ecdyonurus spp. (Ephemeroptera), *Hydropsyche* spp. (Trichoptera) and *Rhyacophila dorsalis* (Trichoptera: Curtis, 1834). Samples for each genus were collected from stream reaches for spatial analysis. To control for size variation and developmental influences on bioaccumulation, samples were composed of fifth instar individuals and pre-pupae from *Hydropsyche* spp. and *Rhyacophila dorsalis*, final aquatic instars of *Baetis* spp. and *Ecdyonurus* spp. and *Gammarus pulex* individuals over 5 mm in length. Individuals were collected in 200 ml glass jars and transported to the laboratory to confirm field identification. Individuals were kept in river water for 24 hr to allow for gut clearance so as to prevent the overestimation of tissue concentrations [1]. Composite samples, including approximately 20–100 individuals per invertebrate taxon, were stored at –80°C prior to chemical analysis.

Collection of fishes

European bullhead (*Cottus gobio*; Linnaeus, 1758) individuals were collected from each sample stream reach (n = 5–10). Both male and female individuals were collected for analysis. Fish were sacrificed through concussion, prior to destruction of the brain before the return of consciousness; a humane technique detailed in Schedule 1 of the Animals in Scientific Procedures Act (1986). Individuals were then dissected, and liver tissue removed. Liver tissue was utilised as the sample tissue due to the preferential accumulation of POPs within this organ [2]. Composite liver tissue samples were frozen (–80°C) until analysis.

Collection of dipper eggs

The more dispersed breeding distribution of Eurasian dippers (*Cinclus cinclus*; Linnaeus, 1758), meant that egg samples were collected more opportunistically (under licence from NRW) from adjacent breeding sites across the three catchments in 2008–2010 (see Morrissey et al. [3]). Sample sites for dipper eggs were matched to the closest sample sites for the other components of the river food webs (biofilms, macroinvertebrates and fish). Nests were followed from building to laying and a single random egg was collected during the first 7–10 days of incubation. Eggs were candled to determine fertility at the time of egg collection. All eggs were transported on ice and frozen in the shell at -20°C. The contents of eggs were transferred to hexane/acetone (1:1 v/v) rinsed jars within 6 weeks and were stored at -80°C until further chemical analysis.

Appendix S2

All samples were initially thawed, accurately weighed (0.5–2 g), ground with sand, dried with anhydrous sodium sulphate, spiked with internal recovery standards (^{13}C OCs, ^{13}C PCBs and ^{13}C PBDEs), and Soxhlet-extracted with dichloromethane for 16 hours. A small proportion of the extract was subsampled and evaporated to zero volume under N and lipid content was determined gravimetrically. The remaining extract was cleaned using automated size exclusion chromatography followed by filtering through an alumina glass column packed with pre-treated alumina (12 hours at 550 °C) deactivated using deionised water 5% (w/w). The extract was divided into two, with one fraction spiked with labelled OCs and PCBs, and the other with PBDEs (internal standards). An aliquot of the extract was injected into the gas chromatograph–mass spectrometer (Agilent, Wokingham, UK) using a 50 m (OCs and PCBs) or 25 m (PBDEs) HT8 column (SGE, Milton Keynes, UK) and programmable temperature vaporization. The injector temperature was 250 °C and helium was the gas carrier (2.0 ml min^{-1}). An isothermal temperature regime was programmed at 50 °C for 2 min, then ramped at 45 °C min^{-1} to 200 °C, 1.5 °C min^{-1} to 240 °C, 2 °C min^{-1} to 285 °C, 50 °C min^{-1} to 325 °C and 350 °C for 10 minutes. Compounds were detected in electron ionisation mode. The internal standard method was used to quantify residues and calibration curves of standards for PCBs and OCs (Greyhound Ltd, Birkenhead, UK) and PBDEs (LGC Ltd., Teddington, UK). A series of procedural blanks were concurrently run, and samples were corrected based on recovery spikes and blank samples. Recovery values were 85.8–103.9% and detection limits, minimum concentration of internal standard detected, averaged 0.04–0.11 ng g^{-1} wet weight (Appendix S2).

Table S2. Limits of detection for chemical analyses. Reported as mean, minimum and maximum for each compound analysed.

Chemical group	Congener	LOD			Detection frequency (%)
		Mean	Minimum	Maximum	
PBDEs	BDE 30	0.0531	0.0435	0.1045	0.0000
	BDE 32	0.0531	0.0435	0.1045	0.0000
	BDE 17	0.0531	0.0435	0.1045	0.0000
	BDE 28	0.0531	0.0435	0.1045	1.4925
	BDE 35	0.0531	0.0435	0.1045	0.0000
	BDE 37	0.0531	0.0435	0.1045	0.0000
	BDE 51	0.0531	0.0435	0.1045	0.0000
	BDE 49	0.0531	0.0435	0.1045	1.4925
	BDE 71	0.0531	0.0435	0.1045	0.0000
	BDE 47	0.0531	0.0435	0.1045	83.5821
	BDE 66	0.0531	0.0435	0.1045	0.0000
	BDE 77	0.0531	0.0435	0.1045	0.0000
	BDE 100	0.0531	0.0435	0.1045	31.3433
	BDE 119	0.0531	0.0435	0.1045	7.4627
	BDE 99	0.0531	0.0435	0.1045	53.7313
	BDE 118	0.0531	0.0435	0.1045	0.0000
	BDE 85	0.0531	0.0435	0.1045	2.9851
	BDE 126	0.0531	0.0435	0.1045	0.0000
	BDE 154	0.0531	0.0435	0.1045	2.9851
	BDE 153	0.0531	0.0435	0.1045	13.4328
	BDE 138	0.0531	0.0435	0.1045	0.0000
	BDE 183	0.0531	0.0435	0.1045	0.0000
	BDE 128	0.0531	0.0435	0.1045	0.0000
	BDE 190	0.0531	0.0435	0.1045	0.0000
	BDE 197	0.0525	0.0435	0.0697	1.4925
	BDE 196	0.0525	0.0435	0.0697	1.4925
PCBs	PCB 8	0.1180	0.1173	0.1268	0.0000
	PCB 18	0.1180	0.1173	0.1268	0.0000
	PCB 29	0.1070	0.1063	0.1149	0.0000
	PCB 31	0.1180	0.1173	0.1268	0.0000
	PCB 28	0.1180	0.1173	0.1268	1.4925
	PCB 52	0.1180	0.1173	0.1268	2.9851
	PCB 101	0.1180	0.1173	0.1268	14.9254
	PCB 81	0.1180	0.1173	0.1268	25.3731
	PCB 77	0.1291	0.1283	0.1387	14.9254
	PCB 149	0.1180	0.1173	0.1268	5.9701
	PCB 123	0.1180	0.1173	0.1268	0.0000
	PCB 118	0.1180	0.1173	0.1268	32.8358
	PCB 114	0.1180	0.1173	0.1268	8.9552
	PCB 153	0.1180	0.1173	0.1268	52.2388
	PCB 141	0.1180	0.1173	0.1268	8.9552
	PCB 105	0.1180	0.1173	0.1268	17.9104
	PCB 163	0.1180	0.1173	0.1268	16.4179

	PCB 138	0.1180	0.1173	0.1268	29.8507
	PCB 187	0.1180	0.1173	0.1268	14.9254
	PCB 183	0.1180	0.1173	0.1268	8.9552
	PCB 126	0.1180	0.1173	0.1268	1.4925
	PCB 128	0.1180	0.1173	0.1268	5.9701
	PCB 167	0.1180	0.1173	0.1268	8.9552
	PCB 171	0.1180	0.1173	0.1268	13.4328
	PCB 199	0.1180	0.1173	0.1268	11.9403
	PCB 156	0.1180	0.1173	0.1268	16.4179
	PCB 157	0.1180	0.1173	0.1268	19.4030
	PCB 180	0.1180	0.1173	0.1268	29.8507
	PCB 201	0.1180	0.1173	0.1268	17.9104
	PCB 170	0.1180	0.1173	0.1268	29.8507
	PCB 169	0.1180	0.1173	0.1268	25.3731
	PCB 189	0.1180	0.1173	0.1268	23.8806
	PCB 194	0.1180	0.1173	0.1268	0.0000
	PCB 205	0.1180	0.1173	0.1268	20.8955
	PCB 206	0.1180	0.1173	0.1268	20.8955
	PCB 209	0.1180	0.1173	0.1268	2.9851
OCs	α-HCH	0.1162	0.1155	0.1248	0.0000
	HCB	0.1180	0.1173	0.1268	73.1343
	γ-HCH	0.1180	0.1173	0.1268	0.0000
	DDE	0.1180	0.1173	0.1268	95.5224
	HEOD	0.1180	0.1173	0.1268	83.5821
	TDE	0.1180	0.1173	0.1268	35.8209
	DDT	0.1162	0.1155	0.1248	46.2687

Appendix S3

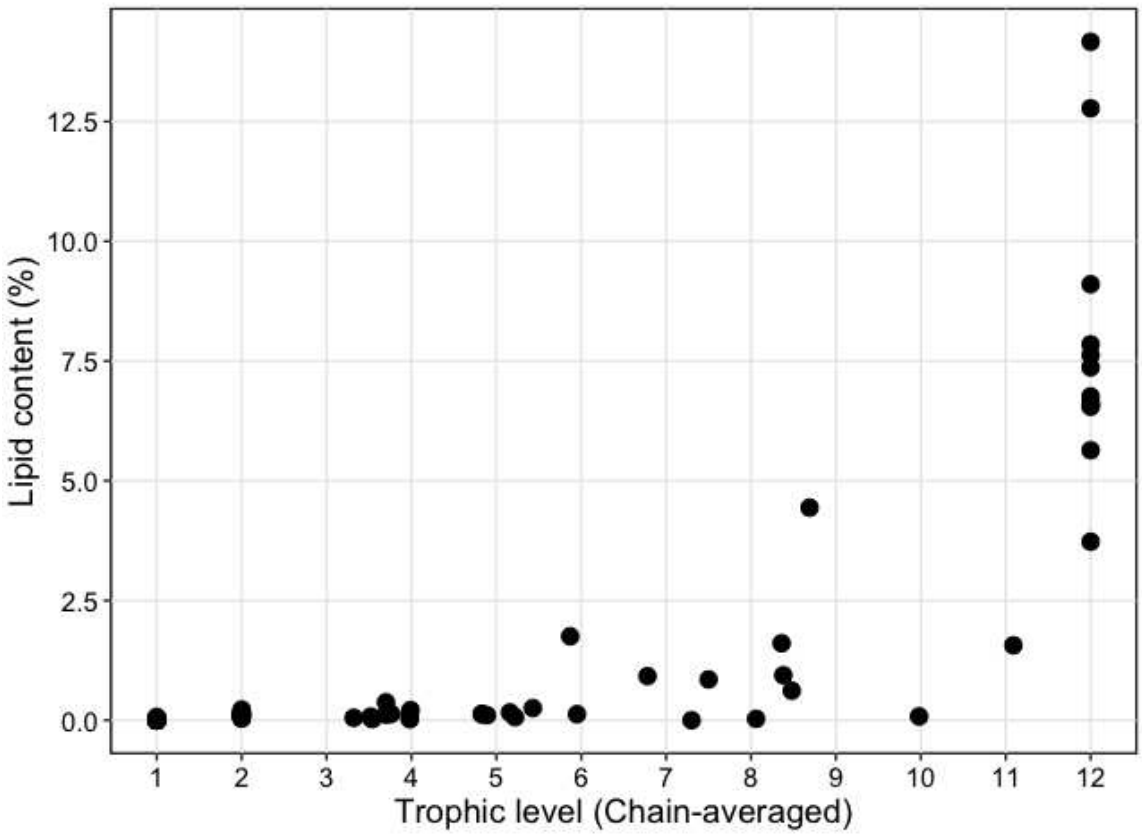

Fig. S4. Relationship between trophic level and lipid content in organisms from river food webs. Individual points represent individual samples from unique species across all nine river food webs. Trophic level is chain-averaged for organisms in each food web.

Appendix S4

Table S4. Metrics used to calculate TMFs. Statistical data (R^2, slope and error) are derived from linear relationships (Equation 2). Total concentration of congeners across the riverine food webs is presented in wet weight.

Congener	log K_{OW}	R^2	Slope	Error	TMF
BDE-100	7.03	0.59	0.14	0.02	1.38
BDE-153	7.86	0.66	0.12	0.02	1.33
BDE-47	6.80	0.67	0.16	0.01	1.46
BDE-99	7.38	0.63	0.16	0.02	1.45
PCB-101	6.36	0.70	0.22	0.03	1.67
PCB-118	6.74	0.74	0.20	0.02	1.60
PCB-138	6.67	0.73	0.28	0.03	1.92
PCB-153	6.89	0.76	0.30	0.03	2.00
PCB-163	6.82	0.66	0.23	0.04	1.72
PCB-170	7.71	0.67	0.21	0.03	1.62
PCB-180	7.20	0.68	0.25	0.03	1.78
PCB-187	6.92	0.63	0.21	0.04	1.63
TDE	6.02	0.60	0.18	0.03	1.50
DDE	6.51	0.59	0.24	0.02	1.72
DDT	6.91	0.76	0.23	0.02	1.71
HCB	5.73	0.75	0.22	0.02	1.66
HEOD	5.40	0.43	0.15	0.02	1.40

Appendix S5

A series of generalised linear and additive models (GLMs and GAMs) were used to analyse the concentration data within the study. These are detailed in the following table.

Table S5. GLM and GAM structures used to understand variation in POP concentration data.

Model type	Subject	Dependent variable	Independent variables	Model family (link)
GLM	TMF vs site characteristics	TMF	Site (categorical) Chemical (categorical) Site : Chemical	Gaussian (identity)
GLM	Level of trophic magnification vs site characteristics	POP concentrations	Trophic level Chemical (categorical) Chemical : Trophic level	Gaussian (log)
GAM	TMF vs chemical characteristics	TMF	Chemical congener Log K_{OW}	Gaussian
GLM	Level of trophic magnification vs landscape characteristics	POP concentrations	Trophic level Proportion of arable land Proportion of urban land	Gaussian (log)
GLM	TMF vs food web metrics	Food web metrics	Food web metric (connectance, modularity, number of clusters, mean chain length)	Gaussian (identity)

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