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

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13 **Abstract**

14 Legacy organic pollutants persist in freshwater environments, but there is limited  
15 understanding of how their trophic transfer and effects vary across riverine  
16 ecosystems with different land use, biological communities and food webs. Here, we  
17 investigated the trophic magnification of polybrominated diphenyl ethers (PBDEs),  
18 polychlorinated biphenyls (PCBs) and a suite of organochlorines (OCs) across nine  
19 riverine food webs in contrasting hydrological catchments across South Wales (United  
20 Kingdom). Pollutants biomagnified through the food webs in all catchments studied, in  
21 some cases reaching levels sufficient for biological effects on invertebrates, fish and  
22 river birds such as the Dipper (*Cinclus cinclus*). Trophic magnification differed across  
23 food webs depending on pollutant characteristics (e.g. octanol-water partitioning  
24 coefficient) and site-specific environmental conditions (e.g. land use, water chemistry  
25 and basal resource composition). The trophic magnification of PBDEs, PCBs and OCs  
26 also reflected food-web structure, with greater accumulation in more connected food  
27 webs with more generalist taxa. These data highlight interactions between pollutant  
28 properties, environmental conditions and biological network structure in the transfer  
29 and biomagnification of POPs in river ecosystems. We advocate the need for further  
30 investigations of system-specific transfers of contaminants through aquatic food webs  
31 as these factors appear to have important implications for risk assessment.

32 **Keywords:** ecotoxicology, food webs, legacy pollution, network ecology, pesticides,  
33 trophic magnification, urban streams, xenobiotics.

## 34 **Introduction**

35 Xenobiotic pollutants include a wide range of synthetic chemicals that are widely  
36 distributed in the aquatic environment. Some of these are associated with significant  
37 exposure risks for individuals through a wide range of mechanisms (Tyler, Jobling &  
38 Sumpter, 1998; Petrović *et al.*, 2004; Malaj *et al.*, 2014; Solecki *et al.*, 2017).  
39 Chemicals can also affect populations, communities and ecosystems, for  
40 example through transfer between prey and predators following tissue accumulation  
41 (Windsor, Ormerod & Tyler, 2018). Persistent organic pollutants (POPs), in particular,  
42 generate effects at the higher trophic levels of aquatic food webs (Hutchinson *et al.*,  
43 2013) and can accumulate to levels in urban watercourses sufficient to offset biological  
44 recovery from historical sanitary and industrial pollution (Windsor, 2019).

45 Evidence that the structure of biological communities might influence pollutant transfer  
46 comes from the variable levels of trophic magnification across food webs (Walters *et*  
47 *al.*, 2008, 2011, 2016). An extreme example is the difference in magnification between  
48 terrestrial and aquatic food webs for various moderately hydrophobic chemicals, such  
49 as  $\beta$ -HCH (Kelly *et al.*, 2007). In air-breathing taxa in terrestrial ecosystems,  
50 respiratory elimination reduces levels of biomagnification compared with that in  
51 aquatic food webs (Kelly *et al.*, 2007). In marine environments, food-chain length,  
52 tissue lipid content and organism-specific feeding ecology are the major factors that  
53 determine trophic magnification (Borgå *et al.*, 2004). Data from freshwater ecosystems  
54 indicate that the broad structure of food webs (e.g. chain length) and their constituent  
55 characteristics (e.g. species composition and ecology) can influence pollutant transfer  
56 (Walters *et al.*, 2008), leading to substantial differences in trophic magnification factors  
57 (TMFs) across compounds (e.g. mercury (Lavoie *et al.*, 2013)). Nevertheless,  
58 research comparing the magnification of POPs across food webs is limited. We

59 hypothesized that variations in levels of magnification may be explained by food web  
60 structure (connectance and modularity), water chemistry, land cover, chemical  
61 mixtures, as well as other site-specific factors (Windsor *et al.*, 2018).

62 In previous research, we illustrated how biological traits and differential partitioning of  
63 organic pollutants altered the tissue concentrations of pollutants observed in aquatic  
64 organisms (Windsor *et al.*, 2019c). Here, we build on this evidence by assessing how  
65 variation in broad-scale ecological processes across multiple river systems may  
66 influence trophic transfer and potential effects of persistent pollutants. Specifically, we  
67 investigate the bioaccumulation and trophic magnification of POPs across sediment  
68 to river bird food-webs in catchments across South Wales (United Kingdom). To  
69 understand variation in trophic magnification of POPs, we tested three hypotheses: (i)  
70 trophic magnification is related to the chemical characteristics of pollutants (e.g. log  
71 Kow) across different food webs); (ii) the levels of magnification observed within food  
72 webs are influenced by the environmental context (e.g. land use composition, water  
73 chemistry and basal resources); and (iii) pollutant magnification through river  
74 ecosystems is influenced by food-web structure.

## 75 **Methods and Materials**

76 Pooled samples of sediments (n = 9), biofilms (n = 9), aquatic invertebrates (n = 42),  
77 fish (n = 6) and eggs from a predatory bird, the Dipper (*Cinclus cinclus*) (n = 12) were  
78 collected from 9 sites across three catchments (Taff, Usk and Wye) in South Wales  
79 (2010–2017; Appendix S1). Regional variation in environmental conditions (e.g. land  
80 use, hydrology and water chemistry; Table 1 in Windsor *et al.* (2019c)) across the 9  
81 sites (T1, T2, T3, U1, U2, U3, W1, W2, W3), the ‘environmental context’ (e.g. land  
82 use, water chemistry and basal resources), allowed for the testing of the hypothesis:

83 trophic magnification of persistent organic pollutants differs between ecosystems in  
84 response to the interactions between environmental and biological factors.

85 All sample collection was licensed by Natural Resources Wales. Freshly collected  
86 samples were placed in acetone-hexane (1:1 v/v) rinsed glass vials, transported at  
87 4°C and subsequently stored at –80 °C until chemical analyses (see Appendix S1 for  
88 collection protocols). Samples were then analysed for chemical contaminants (DDT,  
89 DDE, dieldrin and hexachlorobenzene, 36 PCB congeners and 23 PBDE congeners),  
90 at the Centre for Ecology and Hydrology, Lancaster. Detailed methods are provided  
91 in Appendix S2 of the Supporting Information. Briefly, chemicals were Soxhlet-  
92 extracted, and lipids removed in two stages (size-exclusion chromatography and  
93 deactivated alumina columns). The remaining extracts were then analysed using Gas  
94 Chromatography Mass Spectrometry (Agilent, Wokingham, UK). All values are  
95 reported in ng g<sup>-1</sup> and corrected based on recovery and calibration standards.

96 Wet weight (ww) concentrations of POPs and their congeners were used for several  
97 reasons. First, across a large proportion of samples (~30%), lipid concentrations were  
98 <0.05%, and calculated for whole body samples not specific tissues – potentially  
99 generating errors in lipid normalisation (Hebert & Keenleyside, 1995; Muir & Sverko,  
100 2006). Second, although samples varied in lipid content, from invertebrates and fish  
101 (0–4.43%) to river bird eggs (3.73–14.17%), the relationship with organism trophic  
102 level was non-linear and highly variable (Appendix S3), limiting the effectiveness of  
103 lipid normalisation (Hebert & Keenleyside, 1995). Third, it is arguable that wet weight  
104 concentrations may provide a more precise and accurate evaluation for both food web  
105 transfer and ecological risk assessments, considering that predators within these  
106 systems consume the entire prey organism (Ross & Birnbaum, 2003). Finally, other  
107 studies highlight the fact that, for a range of chemicals with different hydrophobicities,

108 it cannot be assumed that all chemicals are bound to lipids (Powell *et al.*, 2018). Even  
109 for POPs, which are among the chemicals most likely to bind to lipids, not all  
110 compounds and congeners behave in this way and the bioaccumulation of POPs is  
111 affected by a range of biochemical, physicochemical and environmental factors  
112 (Elskus, Collier & Monosson, 2005).

113 Aquatic food webs were constructed from community data at each location sampled  
114 monthly over 2016–2017 producing 97,308 individuals from 139 invertebrate taxa  
115 (Windsor, 2019). An empirical method, explained in detail by Gray *et al.* (2015), was  
116 used to assemble food webs based on observed trophic interactions (<sup>15</sup>N and <sup>13</sup>C  
117 stable isotopes, observational and dietary analyses) across the UK. Once food webs  
118 were constructed, the trophic level (TL) of organisms (chain-averaged (Martinez,  
119 1991)) was determined from the inferred food webs and a range of other metrics  
120 calculated. Metrics included connectance (ratio of observed to potential links), link  
121 density (number of links per node), maximum chain length (number of links in the  
122 longest chain), mean chain length (average number of links for chains) and modularity  
123 (degree of clustering in the network) (Warren, 1994; Bersier, Banašek-Richter &  
124 Cattin, 2002).

125 To assess the levels of magnification in food webs, trophic magnification factors  
126 (TMFs) were calculated using Equation 1 and 2 (Fisk, Hobson & Norstrom, 2001;  
127 Walters *et al.*, 2011):

$$(1) \quad \log_{10} \text{POP}_{\text{ww}} = b + (m \times \text{TL})$$

$$(2) \quad \text{TMF} = 10^m$$

128 The slope ( $m$ ) of the relationship between  $\log_{10}$  transformed concentrations ( $\text{ng g}^{-1}$   
129 ww) and TL is used to calculate TMF. Factors were calculated for compounds detected  
130 in >50% of samples, and total PBDEs, PCBs and OCs (Appendix S4).

131 All statistical analyses were completed using R statistical software (version 3.6.1) (R  
132 Core Team, 2019) and data were initially explored using the framework by Zuur *et al.*  
133 (2010) to understand the data (heteroscedasticity, normality, outliers) and to inform  
134 the selection of further statistical tests. In this study, Generalised linear models (GLMs)  
135 (Nelder & Baker, 2006) were used to model variation in TMFs and tissue  
136 concentrations, and Generalised Additive Models (GAMs) (Zuur, Leno & Smith, 2007)  
137 for chemical-specific TMFs which often exhibit non-linear relationships. The model  
138 structures are detailed in Appendix S5 and were used to assess the potential  
139 influences on trophic magnification of pollutants across sampled food webs. Model  
140 validation followed the series of procedures detailed in Zuur *et al.* (2007) and Thomas  
141 *et al.* (2015). Briefly, residual normality was assessed using QQ plots, homogeneity of  
142 variance was determined by plotting residuals against fitted values and influential  
143 observations were investigated using Cook's leverage distances.

## 144 **Results and discussion**

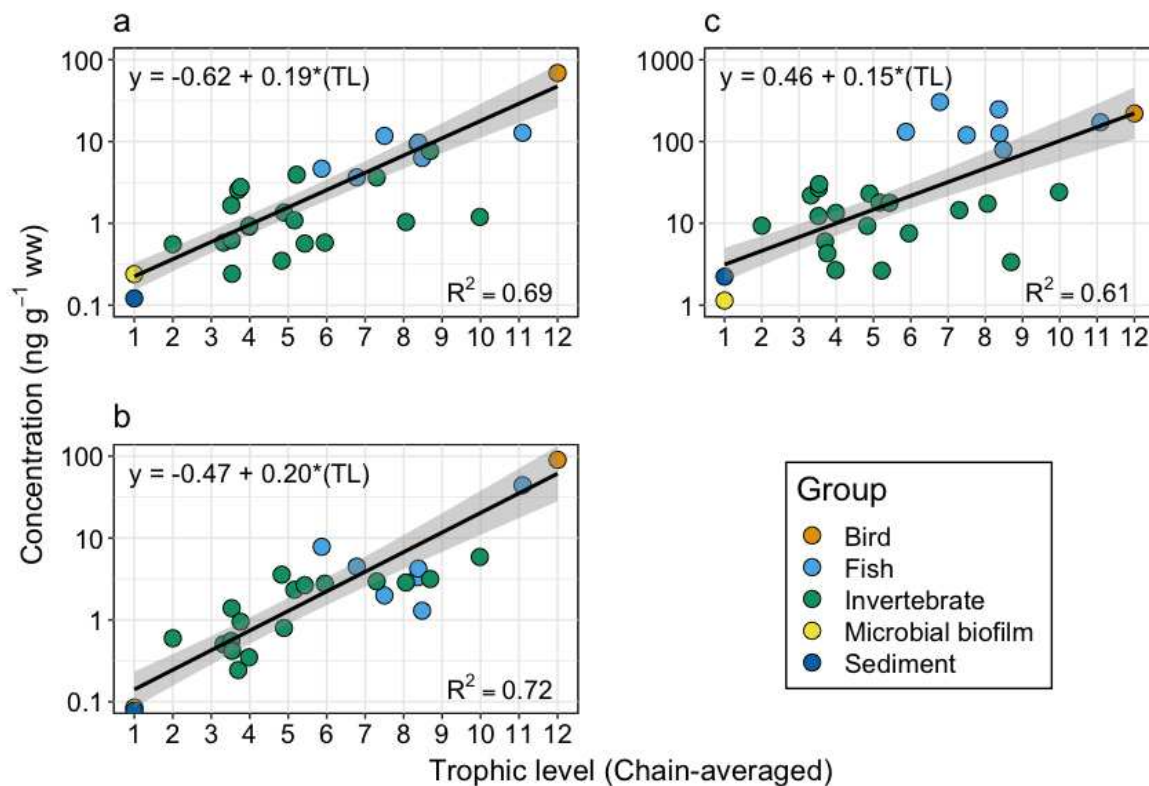
145 Trophic magnification occurred for all POPs, averaged across all 9 stream food webs  
146 (T1–T3, U1–U3 and W1–W3; Fig. 1 & 2). Trophic magnification values ranged from  
147 1.3–2.0, which are relatively low in comparison to other studies in lake and marine  
148 food webs (TMFs = 1.5–13.7 (Fisk *et al.*, 2001; Borgå *et al.*, 2004; Walters *et al.*,  
149 2011)) but do fall within the range of those recorded for POPs across a variety of  
150 freshwater ecosystems, including North American rivers (TMFs = 0.33–3.75 (Walters  
151 *et al.*, 2008; Penland *et al.*, 2018)). The levels of trophic magnification varied for  
152 different POPs and across the different food webs, with the interactive effect of both  
153 variables explaining a significant amount of variation in TMFs (Gaussian GLM;  
154  $R^2 = 0.58$ ,  $F_{12,32} = 3.75$ ,  $p = 0.002$ ). With regards to TMFs, most variation occurred  
155 across the different food webs ( $F_{8,32} = 5.12$ ,  $p < 0.001$ ), yet when site-variation was



156 accounted for there was limited variation in the TMFs between contaminants  
157 ( $F_{4,40} = 0.99, 0.426$ ). The slope of the relationship between POP concentrations and  
158 the chain-averaged trophic level of organisms, a secondary proxy for trophic  
159 magnification, was also different between food webs but also between different  
160 chemicals (Gaussian GLM;  $R^2 = 0.68, F_{53,183} = 10.49, p < 0.001$ ). Specifically, PBDEs  
161 and PCBs had steeper slopes, indicating greater levels of magnification in comparison  
162 to OCs ( $F_{2,223} = 3.73, p = 0.025$ ; Fig. 1). Across the majority of food webs, relationships  
163 between the tissue concentrations of POPs and the trophic level of organisms were  
164 relatively similar ( $F_{8,48} = 13.98, p = 0.056$ ; Fig. 2). Yet, at two sites (U2 and W3), the  
165 relationships for PBDEs and OCs were more significant, indicating a greater level of  
166 magnification for these compounds (PBDEs  $t_{8,48} = 2.28, p = 0.027$ ; OCs  $t_{8,48} = 2.17,$   
167  $p = 0.035$ ; Fig. 2).

168 Compound properties are likely to explain some of the observed variation in trophic  
169 magnification. Certainly, some of the highest TMFs across food webs were observed  
170 for specific recalcitrant compounds; PCB-153 and 138, BDE-47 and *p,p'*-DDE (2.0,  
171 1.92, 1.46 and 1.72, respectively). Furthermore, there appeared to be a non-linear  
172 relationship between TMF and log  $K_{ow}$ , with increases in TMF values up to log  $K_{ow}$   
173 values of approximately 6.5–7.25 then subsequent decreases for higher log  $K_{ow}$   
174 values – yet this relationship was not significant (Gaussian GAM:  $R^2 = 0.28,$   
175  $F_{5,12} = 0.87, p = 0.53$ ). This contradicts results from previous studies which shows that  
176 levels of POP accumulation and magnification in organisms are linearly related to the  
177 log  $K_{ow}$  (Russell, Gobas & Haffner, 1999; Walters *et al.*, 2008, 2011). This discrepancy  
178 is likely due to a restricted sample size of log  $K_{ow}$  values in this study (restricted to  
179 chemicals with intermediate log  $K_{ow}$  values), which is a result of the low detection

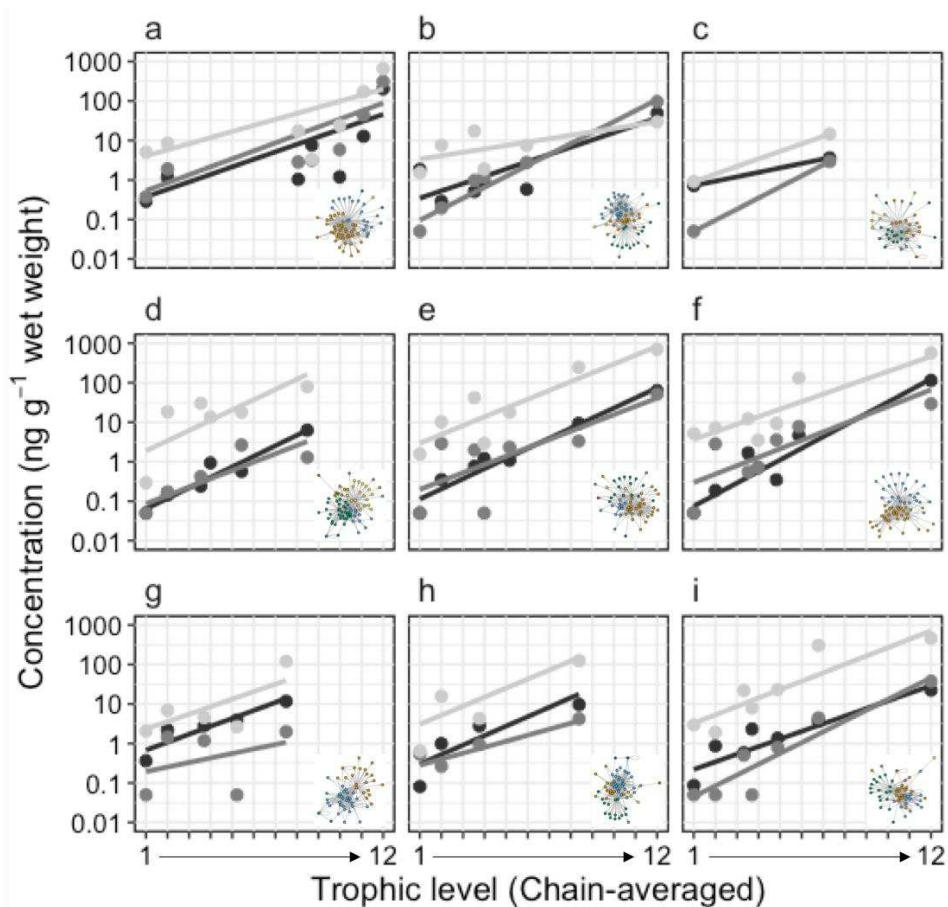
180 frequencies of many PCB and PBDE congeners, and OC chemicals, at the extremes  
181 of the range of log  $K_{ow}$  values (<5.0 and >7.5).



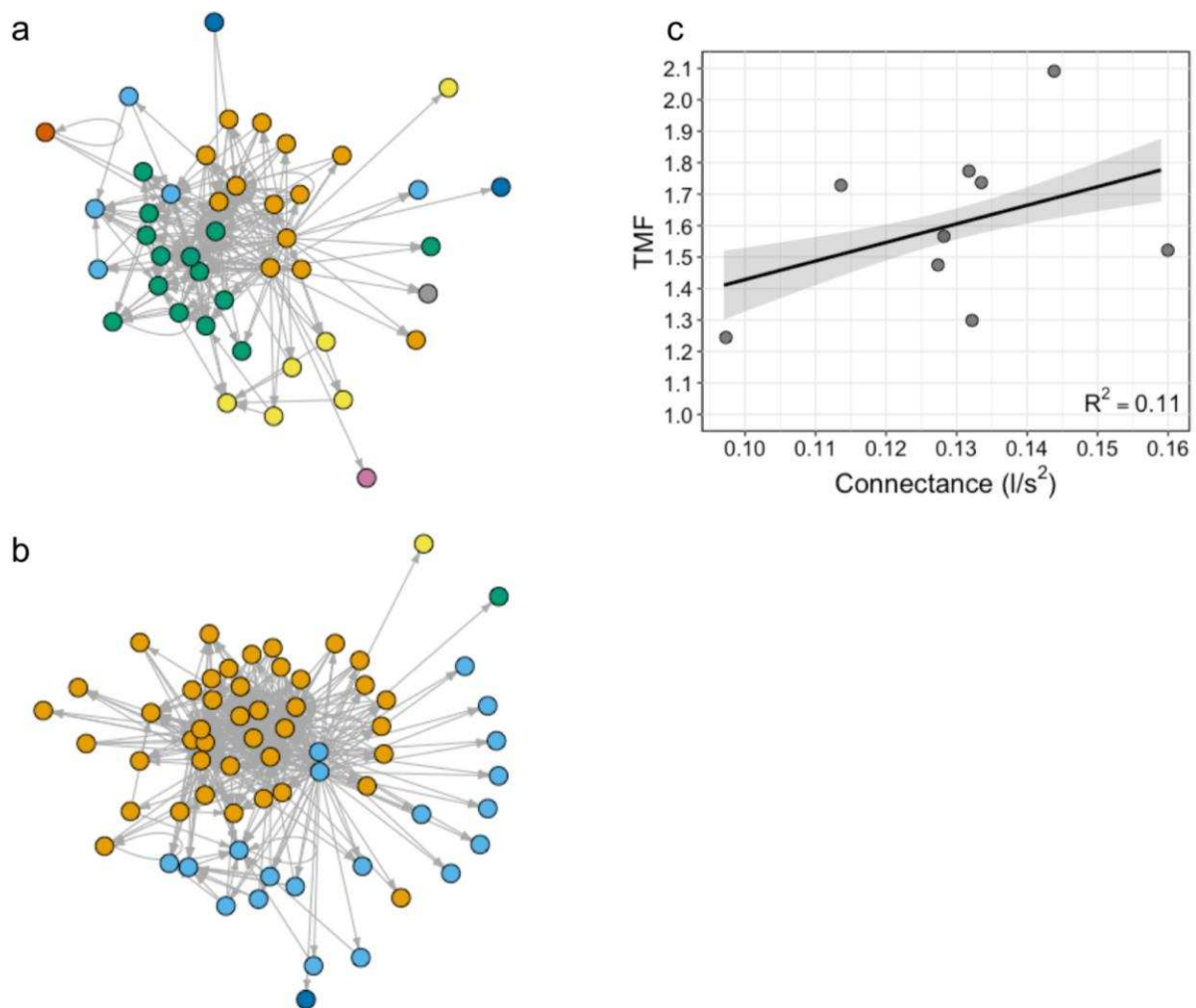
**Fig. 1. Relationship between trophic level (chain-averaged) and mean concentration of PBDEs, PCBs and OCs for organisms in river food webs. (a)** Mean  $\Sigma$  PBDE concentrations. **(b)** Mean  $\Sigma$  PCB concentrations; **(c)** Mean  $\Sigma$  OC concentrations. Data are aggregated for chain-averaged trophic levels (for prey-averaged values see the supplementary data), across all food webs ( $n = 9$ ). Adjusted  $R^2$  values were derived from separate post-hoc linear models.

182 Inter-site variation in the trophic magnification could result from land-use, water  
183 chemistry and hydrology. Land use (total catchment area, urban and arable land  
184 cover) appeared to influence the relationships between concentrations of PBDEs,  
185 PCBs and OCs and the organisms' trophic level (Log Gaussian GLM:  $R^2 = 0.72$ ,  $F_{17,219}$   
186  $= 32.92$ ,  $p < 0.001$ ). Specifically, the level of OC magnification was lower in more  
187 urbanised river systems ( $F_{3,225} = 3.19$ ,  $p = 0.002$ ) and PCB magnification was greater  
188 in river systems with greater urban land cover ( $F_{3,225} = 4.34$ ,  $p = 0.005$ ). Otherwise,

189 variation in trophic magnification between sample sites was not significantly related to  
190 other land use variables, including the total upstream area and the proportion  
191 coverage of improved grassland. Although not measured in this study, variation in  
192 dissolved and particulate organic matter within the water column has the potential to  
193 further alter the transfer and magnification of POPs (Coat *et al.*, 2011). This effect also  
194 depends on other physicochemical characteristics (e.g. flow rate, temperature).



**Fig. 2. Relationship between trophic level and total concentrations of PBDEs (black), PCBs (dark grey) and OCs (light grey) in organisms across nine river food webs. (a) T1. (b) T2. (c) T3. (d) U1. (e) U2. (f) U3. (g) W1. (h) W2. (i) W3. For site codes see the methods and supporting information. Network diagrams represent the invertebrate compartment in food webs, constructed from data collected in 2016–2017 (Windsor, 2019).**



**Fig. 3. Relationships between food web connectance and TMFs. (a)** A network with high connectance ( $C = 0.15$ ) and intermediate modularity ( $Q = 0.13$ , clusters = 13). **(b)** A network with lower connectance ( $C = 0.09$ ) and intermediate modularity ( $Q = 0.13$ , clusters = 5). **(c)** Relationship between connectance and TMF values. **(a-b)** The colours of nodes in the food webs indicate modules from random walk algorithms. Mean TMF values are calculated based on the concentrations of frequently detected PCBs, PBDEs and OC chemicals summarised for each aquatic food web ( $n = 9$ ).

195 Inter-site variation in trophic magnification might also reflect food web structure and  
 196 connectance. POP magnification, as reflected by TMF values across sites, increased  
 197 with food-web connectance (Gaussian GLM:  $R^2 = 0.11$ ,  $F_{1,43} = 4.53$ ,  $p = 0.039$ ; Fig. 3).  
 198 The relationship between TMF values and connectance strengthened further when  
 199 values were calculated for the most frequently detected congeners, rather than as

200 mean TMF values across sites (Gaussian GLM:  $R^2 = 0.20$ ,  $F_{39,130} = 2.08$ ,  $p = 0.001$ ).  
201 Effects apparently differed among PBDE and PCB congeners, as well as OCs  
202 ( $F_{19,130} = 1.95$ ,  $p = 0.015$ ). In particular, several recalcitrant compounds, including PCB  
203 congeners (138 and 153) and OCs (*p,p'*-DDD and dieldrin), showed stronger  
204 relationships between the level of trophic magnification and food-web connectance.  
205 TMF values were not related to any other food web metrics but there was a suggestion  
206 that food webs with intermediate mean and maximum chain lengths had the greatest  
207 trophic magnification.

208 Our findings indicate that differences in the structure of food webs, specifically the  
209 levels of connectance, might influence the level of POPs magnification observed in  
210 river ecosystems. This suggests that, as well as the previously observed differences  
211 between markedly different food webs, e.g. marine-freshwater and aquatic-  
212 terrestrial (Kelly *et al.*, 2007; Walters *et al.*, 2008), differences in the inherent structure  
213 of food webs within the same ecosystems potentially affect the trophic magnification  
214 of persistent pollutants. The exact mechanism responsible for the observed  
215 relationship between food web connectance and trophic magnification is uncertain but  
216 may relate to either the functional traits (e.g. diet, habitat affinities, voltinism, dispersal  
217 strategy) of different invertebrate taxa across river food webs (Windsor *et al.*, 2019a),  
218 or directly to differences in network structure between sites.

219 For example, differences in the community composition in river systems, and  
220 functional ecology of invertebrate taxa, may reveal dominance of trophic generalists  
221 that may enhance the transfer of pollutants in river food webs (Windsor, 2019). Due to  
222 their dietary plasticity, these taxa may rapidly expand their ecological niche and use a  
223 wider range of basal resources under competitive release resulting from the loss of  
224 specialist taxa (Macneil, Dick & Elwood, 1997). Such alterations in food web dynamics

225 may potentially increase the transfer of pollutants to higher trophic levels, yet this may  
226 only be the case if the strength of trophic interactions between the generalist  
227 invertebrate taxa and higher trophic levels are also increased in these urban systems.  
228 We emphasize, however, that differences in the relative connectance between food  
229 webs were relatively restricted in this study to similar river communities (0.09–0.16),  
230 and that using broader gradients of connectance, across markedly different food webs,  
231 may strengthen the explanations and insights into the effects of food web structure on  
232 pollutant transfer. Irrespective of the mechanisms driving differences in the  
233 accumulation and magnification of POPs between food webs, our findings further  
234 support existing research on the heterogeneity in the interactions between  
235 contaminants and biota in diverse aquatic and terrestrial systems (Kelly *et al.*, 2007;  
236 Walters *et al.*, 2008; Coat *et al.*, 2011). This ecological variability in network structure  
237 highlights the potential for modified ecological risks posed by persistent contaminants  
238 even within similar riverine ecosystems.

## 239 **Conclusions**

240 The bioaccumulation and trophic magnification of OCs, PCBs and PBDEs across  
241 riverine sites demonstrates the potential for significant accumulation of legacy and  
242 more recent pollutants, even where concentrations in the environmental  
243 compartments/basal resources of the food web are low. Although spatial variation  
244 related to several environmental and biological factors was observed in this study, the  
245 net trophic magnification of POPs observed across food webs confirms that legacy  
246 POPs are strongly affected by food web structure.

247 **Supporting Information**

248 Site information, sample collection (Appendix S1), chemical extraction and analysis  
249 from environmental and biological samples, limits of detection for gas  
250 chromatography-mass spectrometry (Appendix S2 and S3), TMF calculation data  
251 (Appendix S4), and generalised linear and additive model structures (Appendix S5).

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