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

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Abstract

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

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

Introduction

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

Evidence that the structure of biological communities might influence pollutant transfer comes from the variable levels of trophic magnification across food webs (Walters *et al.*, 2008, 2011, 2016). An extreme example is the difference in magnification between terrestrial and aquatic food webs for various moderately hydrophobic chemicals, such as β-HCH (Kelly *et al.*, 2007). In air-breathing taxa in terrestrial ecosystems, respiratory elimination reduces levels of biomagnification compared with that in aquatic food webs (Kelly *et al.*, 2007). In marine environments, food-chain length, tissue lipid content and organism-specific feeding ecology are the major factors that determine trophic magnification (Borgå *et al.*, 2004). Data from freshwater ecosystems indicate that the broad structure of food webs (e.g. chain length) and their constituent characteristics (e.g. species composition and ecology) can influence pollutant transfer (Walters *et al.*, 2008), leading to substantial differences in trophic magnification factors (TMFs) across compounds (e.g. mercury (Lavoie *et al.*, 2013)). Nevertheless, research comparing the magnification of POPs across food webs is limited. We hypothesized that variations in levels of magnification may be explained by food web structure (connectance and modularity), water chemistry, land cover, chemical mixtures, as well as other site-specific factors (Windsor *et al.*, 2018).

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

Methods and Materials

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

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

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

Wet weight (ww) concentrations of POPs and their congeners were used for several reasons. First, across a large proportion of samples (~30%), lipid concentrations were <0.05%, and calculated for whole body samples not specific tissues – potentially generating errors in lipid normalisation (Hebert & Keenleyside, 1995; Muir & Sverko, 2006). Second, although samples varied in lipid content, from invertebrates and fish (0–4.43%) to river bird eggs (3.73–14.17%), the relationship with organism trophic level was non-linear and highly variable (Appendix S3), limiting the effectiveness of lipid normalisation (Hebert & Keenleyside, 1995). Third, it is arguable that wet weight concentrations may provide a more precise and accurate evaluation for both food web transfer and ecological risk assessments, considering that predators within these systems consume the entire prey organism (Ross & Birnbaum, 2003). Finally, other studies highlight the fact that, for a range of chemicals with different hydrophobicities, it cannot be assumed that all chemicals are bound to lipids (Powell *et al.*, 2018). Even for POPs, which are among the chemicals most likely to bind to lipids, not all compounds and congeners behave in this way and the bioaccumulation of POPs is affected by a range of biochemical, physicochemical and environmental factors (Elskus, Collier & Monosson, 2005).

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

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

(1) $$\log_{10} POP_{ww} = b + (m \times TL)$$

(2) $$TMF = 10^{m}$$

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

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

Results and discussion

Trophic magnification occurred for all POPs, averaged across all 9 stream food webs (T1–T3, U1–U3 and W1–W3; Fig. 1 & 2). Trophic magnification values ranged from 1.3–2.0, which are relatively low in comparison to other studies in lake and marine food webs (TMFs = 1.5–13.7 (Fisk *et al.*, 2001; Borgå *et al.*, 2004; Walters *et al.*, 2011)) but do fall within the range of those recorded for POPs across a variety of freshwater ecosystems, including North American rivers (TMFs = 0.33–3.75 (Walters *et al.*, 2008; Penland *et al.*, 2018)). The levels of trophic magnification varied for different POPs and across the different food webs, with the interactive effect of both variables explaining a significant amount of variation in TMFs (Gaussian GLM; $R^2 = 0.58$, $F_{12,32} = 3.75$, $p = 0.002$). With regards to TMFs, most variation occurred across the different food webs ($F_{8,32} = 5.12$, $p < 0.001$), yet when site-variation was accounted for there was limited variation in the TMFs between contaminants ($F_{4,40}$ = 0.99, 0.426). The slope of the relationship between POP concentrations and the chain-averaged trophic level of organisms, a secondary proxy for trophic magnification, was also different between food webs but also between different chemicals (Gaussian GLM; R^2 = 0.68, $F_{53,183}$ = 10.49, p < 0.001). Specifically, PBDEs and PCBs had steeper slopes, indicating greater levels of magnification in comparison to OCs ($F_{2,223}$ = 3.73, p = 0.025; Fig. 1). Across the majority of food webs, relationships between the tissue concentrations of POPs and the trophic level of organisms were relatively similar ($F_{8,48}$ = 13.98, p = 0.056; Fig. 2). Yet, at two sites (U2 and W3), the relationships for PBDEs and OCs were more significant, indicating a greater level of magnification for these compounds (PBDEs $t_{8,48}$ = 2.28, p = 0.027; OCs $t_{8,48}$ = 2.17, p = 0.035; Fig. 2).

Compound properties are likely to explain some of the observed variation in trophic magnification. Certainly, some of the highest TMFs across food webs were observed for specific recalcitrant compounds; PCB-153 and 138, BDE-47 and *p,p'*-DDE (2.0, 1.92, 1.46 and 1.72, respectively). Furthermore, there appeared to be a non-linear relationship between TMF and log K_{OW}, with increases in TMF values up to log K_{OW} values of approximately 6.5–7.25 then subsequent decreases for higher log K_{OW} values – yet this relationship was not significant (Gaussian GAM: R^2 = 0.28, $F_{5,12}$ = 0.87, p = 0.53). This contradicts results from previous studies which shows that levels of POP accumulation and magnification in organisms are linearly related to the log K_{OW} (Russell, Gobas & Haffner, 1999; Walters *et al.*, 2008, 2011). This discrepancy is likely due to a restricted sample size of log K_{OW} values in this study (restricted to chemicals with intermediate log K_{OW} values), which is a result of the low detection frequencies of many PCB and PBDE congeners, and OC chemicals, at the extremes of the range of log K_{OW} values (<5.0 and >7.5).

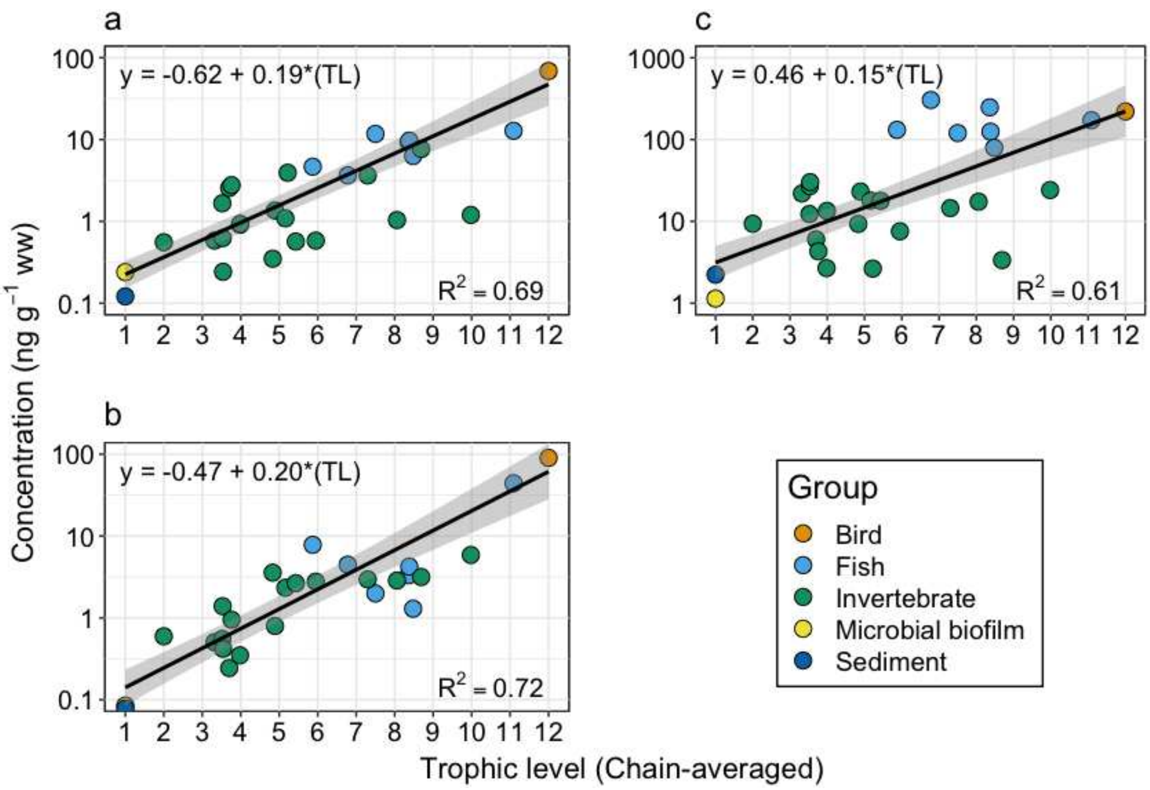

Fig. 1. Relationship between trophic level (chain-averaged) and mean concentration of PBDEs, PCBs and OCs for organisms in river food webs. (a) Mean Σ PBDE concentrations. **(b)** Mean Σ PCB concentrations**; (c)** Mean Σ OC concentrations. Data are aggregated for chain-averaged trophic levels (for prey-averaged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

Inter-site variation in the trophic magnification could result from land-use, water chemistry and hydrology. Land use (total catchment area, urban and arable land cover) appeared to influence the relationships between concentrations of PBDEs, PCBs and OCs and the organisms' trophic level (Log Gaussian GLM: $R^2 = 0.72$, $F_{17,219} = 32.92$, $p < 0.001$). Specifically, the level of OC magnification was lower in more urbanised river systems ($F_{3,225} = 3.19$, $p = 0.002$) and PCB magnification was greater in river systems with greater urban land cover ($F_{3,225} = 4.34$, $p = 0.005$). Otherwise, variation in trophic magnification between sample sites was not significantly related to other land use variables, including the total upstream area and the proportion coverage of improved grassland. Although not measured in this study, variation in dissolved and particulate organic matter within the water column has the potential to further alter the transfer and magnification of POPs (Coat *et al.*, 2011). This effect also depends on other physicochemical characteristics (e.g. flow rate, temperature).

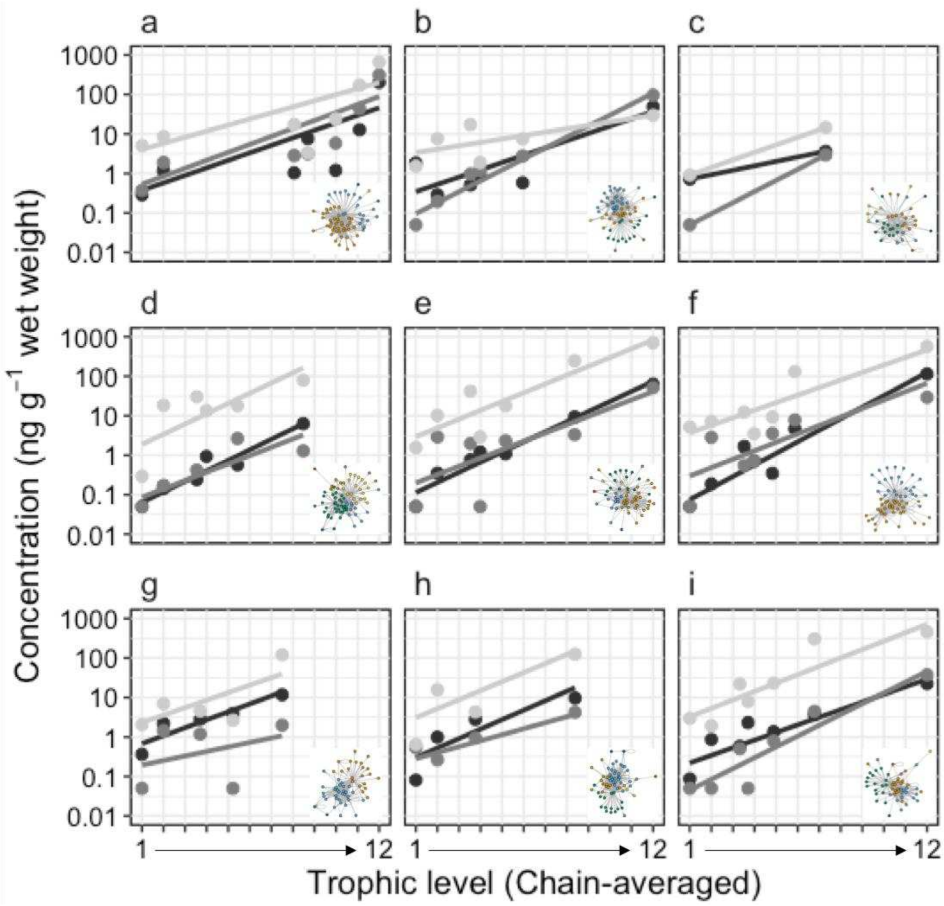

Fig. 2. Relationship between trophic level and total concentrations of PBDEs (black), PCBs (dark grey) and OCs (light grey) in organisms across nine river food webs. (a) T1. (b) T2. (c) T3. (d) U1. (e) U2. (f) U3. (g) W1. (h) W2. (i) W3. For site codes see the methods and supporting information. Network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

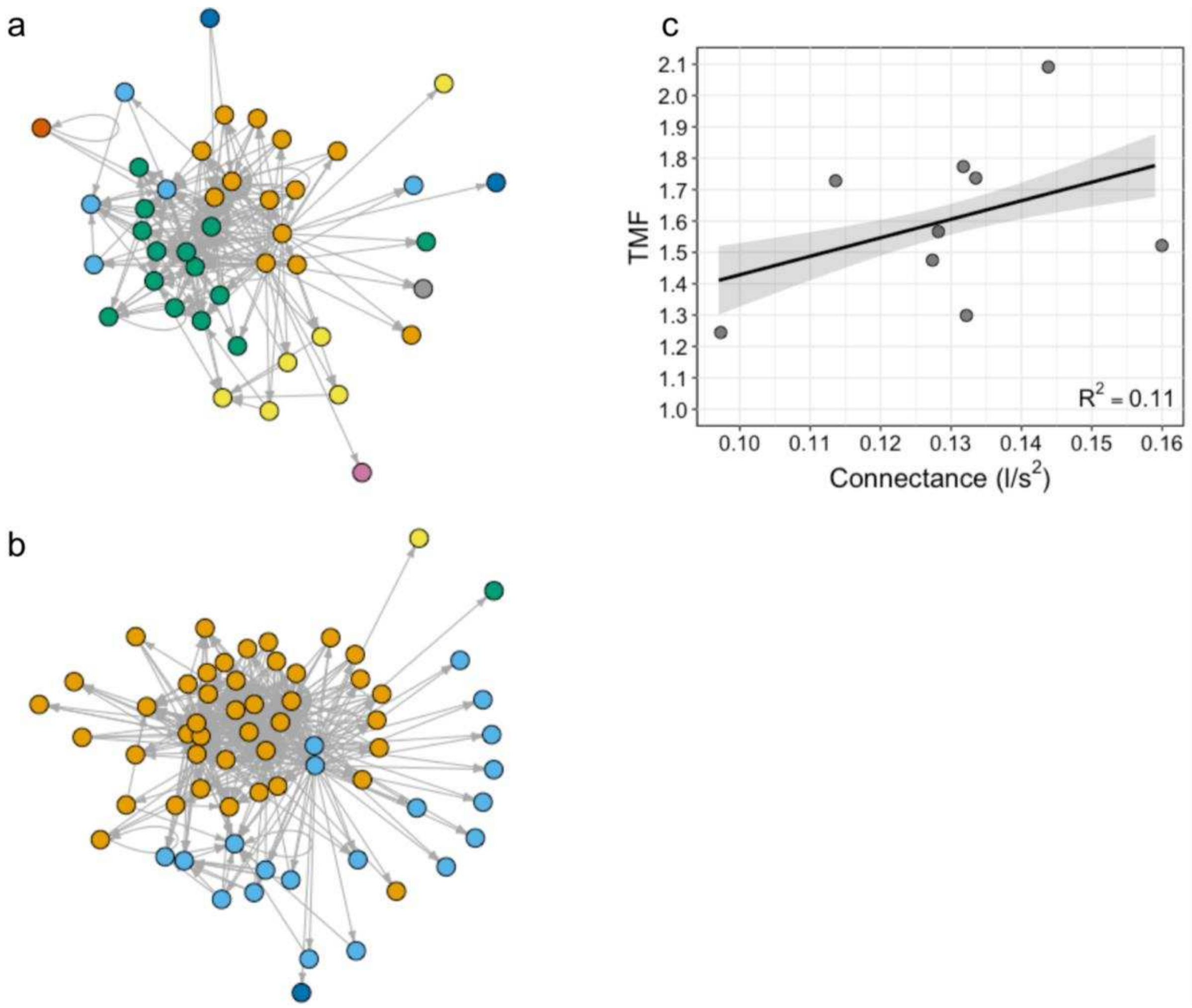

Fig. 3. Relationships between food web connectance and TMFs. (a) A network with high connectance (C = 0.15) and intermediate modularity (Q = 0.13, clusters = 13). **(b)** A network with lower connectance (C = 0.09) and intermediate modularity (Q = 0.13, clusters = 5). **(c)** Relationship between connectance 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).

Inter-site variation in trophic magnification might also reflect food web structure and connectance. POP magnification, as reflected by TMF values across sites, increased with food-web connectance (Gaussian GLM: R^2 = 0.11, $F_{1,43}$ = 4.53, p = 0.039; Fig. 3). The relationship between TMF values and connectance strengthened further when values were calculated for the most frequently detected congeners, rather than as mean TMF values across sites (Gaussian GLM: R^2 = 0.20, $F_{39,130}$ = 2.08, p = 0.001). Effects apparently differed among PBDE and PCB congeners, as well as OCs ($F_{19,130}$ = 1.95, p = 0.015). In particular, several recalcitrant compounds, including PCB congeners (138 and 153) and OCs (*p,p'*-DDD and dieldrin), showed stronger relationships between the level of trophic magnification and food-web connectance. TMF values were not related to any other food web metrics but there was a suggestion that food webs with intermediate mean and maximum chain lengths had the greatest trophic magnification.

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

For example, differences in the community composition in river systems, and functional ecology of invertebrate taxa, may reveal dominance of trophic generalists that may enhance the transfer of pollutants in river food webs (Windsor, 2019). Due to their dietary plasticity, these taxa may rapidly expand their ecological niche and use a wider range of basal resources under competitive release resulting from the loss of specialist taxa (Macneil, Dick & Elwood, 1997). Such alterations in food web dynamics may potentially increase the transfer of pollutants to higher trophic levels, yet this may only be the case if the strength of trophic interactions between the generalist invertebrate taxa and higher trophic levels are also increased in these urban systems. We emphasize, however, that differences in the relative connectance between food webs were relatively restricted in this study to similar river communities (0.09–0.16), and that using broader gradients of connectance, across markedly different food webs, may strengthen the explanations and insights into the effects of food web structure on pollutant transfer. Irrespective of the mechanisms driving differences in the accumulation and magnification of POPs between food webs, our findings further support existing research on the heterogeneity in the interactions between contaminants and biota in diverse aquatic and terrestrial systems (Kelly *et al.*, 2007; Walters *et al.*, 2008; Coat *et al.*, 2011). This ecological variability in network structure highlights the potential for modified ecological risks posed by persistent contaminants even within similar riverine ecosystems.

Conclusions

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

Supporting Information

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

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