



Trophic fate and biomagnification of organic micropollutants from staple food to a specialized predator

Frauke Ecke^{a,b,*}, Oksana Golovko^c, Birger Hörnfeldt^b, Lutz Ahrens^c

^a Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, PO Box 65, FIN-00014, University of Helsinki, Finland

^b Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences (SLU), SE-901 83, Umeå, Sweden

^c Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences (SLU), SE- 750 07, Uppsala, Sweden

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ABSTRACT

The environmental burden of organic micropollutants has been shown in aquatic ecosystems, while trophic fate of many compounds in terrestrial food chains remains highly elusive. We therefore studied concentrations of 108 organic micropollutants in a common European mammal, the bank vole (*Clethrionomys glareolus*), and 82 of the compounds in a specialized predator, Tengmalm's owl (*Aegolius funereus*) relying to >90 % on voles as its prey. We studied compounds in whole voles (n = 19), pools of 4–8 bank voles (n_{pools} = 4), owl blood (n = 10) and in owl eggs (n = 10) in two regions in Sweden. For comparison, we also included previously published data on 23 PFAS (per- and polyfluoroalkyl substances) in bank vole liver (n_{pools} = 4) from the same regions. In voles, concentrations of the organic micropollutants caffeine (max_{Individual} 220 ng/g ww) and DEET (N,N-diethyl-m-toluamide) (max_{Pool} 150 ng/g ww) were 2–200 times higher in voles relative to owl blood and eggs. Conversely, concentrations of nicotine, oxazepam, salicylic acid, and tributyl citrate acetate were 1.3–440 times higher in owls. Several PFAS showed biomagnification in owls as revealed by maximum biomagnification factors (BMFs); PFNA (perfluorononanoate) BMF = 5.6, PFTeDA (perfluorotetradecanoic acid) BMF = 5.9, and PFOS (perfluorooctane sulfonate) BMF = 6.1. Concentrations of organic micropollutants, alongside calculated BMFs, and Tengmalm's owl's heavy reliance on bank vole as staple food, suggest, despite small sample size and potential spatio-temporal mismatch, accumulation of PFAS (especially PFNA, PFTeDA, and PFOS) in owls and biomagnification along the food chain. Concentrations of PFAS in owl eggs (e.g., 21 ng/g ww PFOS) highlight the likely pivotal role of maternal transfer in contaminant exposure for avian embryos. These concentrations are also of concern considering that certain predators frequently consume owl eggs, potentially leading to additional biomagnification of PFAS with yet undetermined consequences for ecosystem health.

1. Introduction

Organic micropollutants (OMPs) are an increasing burden to animal and ecosystem health (Evich et al., 2022). OMPs are a large group of compounds which includes pharmaceuticals, cosmetics, personal care products, artificial sweeteners, pesticides, hormones and per- and polyfluoroalkyl substances (PFAS) (Nilsen et al., 2019). While there are encouraging declines in the concentrations of several legacy OMPs including organochlorine DDT (dichlorodiphenyltri-chloroethane) and PCBs (polychlorinated biphenyls) in, for example, Arctic biota, levels of emerging OMPs are increasing (Ecke et al., 2020; Rigét et al., 2019).

The environmental burden of OMPs has shown high spatial variation

and is difficult to predict. Some compounds are emitted via point sources and locally deposited, while others show long-range water and atmospheric transport or are translocated via migratory wildlife, or via a combination of multiple transport pathways (Evich et al., 2022). For example, multiple PFAS have been emitted locally at fire training sites with further transport of the compounds to aquatic recipients and the atmosphere (Ahrens and Bundschuh, 2014; Evich et al., 2022; Koch et al., 2019). A recent study has shown the importance of studying field data of OMPs at large spatial scale to reveal potential environmental hazards far from source areas (Malnes et al., 2023).

Generally, OMPs enter aquatic and terrestrial food chains via sediment, soil and/or water where they can be taken up by primary

* Corresponding author. Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, PO Box 65, FIN-00014, University of Helsinki, Finland.

E-mail address: Frauke.Ecke@helsinki.fi (F. Ecke).

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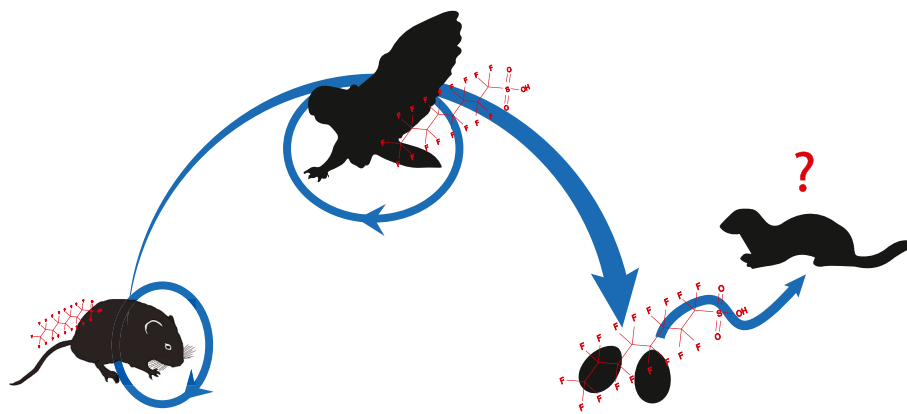


Fig. 1. Schematic illustration of the expected fate of PFAS (per- and polyfluoroalkyl substances) in the food chain involving bank vole (*Clethrionomys glareolus*) as staple food of the specialized predator Tengmalm's owl (*Aegolius funereus*) and egg nest predators like mustelids. In this study, we focused on bank voles and Tengmalm's owl including owl eggs.

producers. Further transfer along food chains and in food webs occurs via consumers including predators with subsequent re-entrance at lower levels of food chains upon organisms' death and decomposition. While many OMPs, including PFAS, bioaccumulate in organisms during their lifetime, OMPs like PFAS also biomagnify, i.e., their concentrations increase along the food chain, with profound negative effects on ecosystem and wildlife health (Evich et al., 2022). In especially birds, maternal transfer (i.e., the transfer of substances from the mother to eggs and hence offspring) of OMPs contributes to maintaining the substances at trophic levels (Gaur et al., 2022). Bioaccumulation can then further contribute to the increase of OMP concentrations in the organisms.

Small mammals are keystone species for the functioning of boreal ecosystems (Ericson, 1977; Hansson, 1988), in particular since rodents such as voles, lemmings and mice are staple food for multiple mammalian and avian predators (Hörnfeldt et al., 1990; Krebs and Myers, 1974). OMPs that accumulate in rodents through their feeding habits (for PFAS see Grønnestad et al., 2019) can potentially biomagnify when these rodents are consumed by predators. Indeed, in tawny owls (*Strix aluco*), a generalist predator preferably consuming voles but having a broad food spectrum, PFOS (perfluorooctanesulfonate) concentrations were highest in years with high vole abundance (Bustnes et al., 2015). The trophic fate of OMPs should be even better understood if the compounds were studied in a predator-prey system where the predator depends on one or a few food sources.

Herein, we have studied the relationship between OMPs in Europe's most common mammal, viz. the bank vole (*Clethrionomys glareolus*) (Mitchell-Jones et al., 1999) and Tengmalm's owl (*Aegolius funereus*), a specialist predator whose diet especially in northern regions is dominated, and made up >90 %, by voles of the genera *Clethrionomys* and *Microtus* (Hörnfeldt et al., 1990; Koivunen et al., 1996). We performed the study in northern Sweden, where bank voles constitute >90 % of the food items cached by Tengmalm's owl early in the season of the increase phase of the vole cycle (Hörnfeldt et al., 1990; personal information). We hypothesised that persistent OMPs like PFAS biomagnify from bank voles to Tengmalm's owl and that OMPs also show maternal transfer (Fig. 1).

2. Material and methods

2.1. Field sampling

We performed our study in the county of Västerbotten, northern Sweden in spring (April–May) 2020, at the transition between two vole cycles during the beginning of the increase phase (1st year) of the new vole cycle (for definitions see Hörnfeldt (1994) and update in Khalil et al. (2019)). The study areas comprise Ammannäs (65°57'N 16°12'E) in

the Swedish mountains as part of the National Environmental Monitoring Programme of Small Mammals (Ecke and Hörnfeldt, 2024) and an area close to the coastal city of Umeå (Dåvamyrån (63°52'N 20°23'E) and Alvik (63°47'N 20°17'E)) (Fig. S1). Ammannäs represents a remote mountain area without any industrial point sources for OMPs, while the sampling sites close to the city of Umeå were in the vicinity (ca. 1 km) of both an airport and a combustion plant for municipal waste, which are potential sources of OMPs (Ahrens et al., 2015; Liu et al., 2021, 2022).

Small mammals were snap-trapped by opportunistically placing traps in forested areas. The trapping effort was limited since we aimed for a minimum of five and maximum of ten bank voles per locality. Trapped bank voles ($n = 8$ and $n = 13$ in Ammannäs and Umeå, respectively) were sealed in plastic bags and frozen at $-20\text{ }^{\circ}\text{C}$ during field work and stayed frozen until processing when they were also weighed and sexed.

For owl sampling, we took advantage of the network of ca. 500 nest boxes for Tengmalm's owl (*Aegolius funereus*) in the county of Västerbotten (Hörnfeldt et al., 2005). We took blood samples from the brachial wing vein of owl females and juveniles. The amount of blood taken from the owls depended on their weight but never exceeded 1 mL. In total, we gained $n = 8$ blood samples from Ammannäs and Umeå, respectively. We also sampled non-viable eggs from the nest boxes with an unfortunate spatial mismatch ($n = 1$ in Ammannäs and $n = 13$ in Umeå).

From a previous study comprising bank voles from 2017 to 2018 in the regions of Ammannäs and Vindeln (ca. 50 km north-west of Umeå; Fig. S1), we had PFAS data from livers (Ecke et al., 2020) (Ammannäs: one pool with 10 voles, Vindeln: three pools with 10 voles each), which we used for comparison and calculation of biomagnification factors. Due to the scarcity of PFAS data from terrestrial food chains, we used the vole liver data for the biomagnification factors despite the temporal mismatch of 2–3 years between collection of vole liver and owl data. For simplicity, and also considering that the distance of the trapping sites near Umeå were only ca. 20 km from the nearest sites in the Vindeln area, we refer to all voles in this study as originating from Umeå.

2.2. Sample preparation

Ecotoxicological studies are generally performed on specific organs or tissue. Owls consume whole prey specimens such as voles and subsequently excrete fur and bones as pellets. We were therefore interested in the whole body vs. liver concentrations of OMPs in the voles and their respective role for biomagnification in owls. Whole voles were milled with Retsch® Knife Mill Grindomix GM 200 and further homogenized with a stand mixer. From each specimen we used ca. 3 g of homogenate for the chemical analyses. Specimens were analysed individually, and

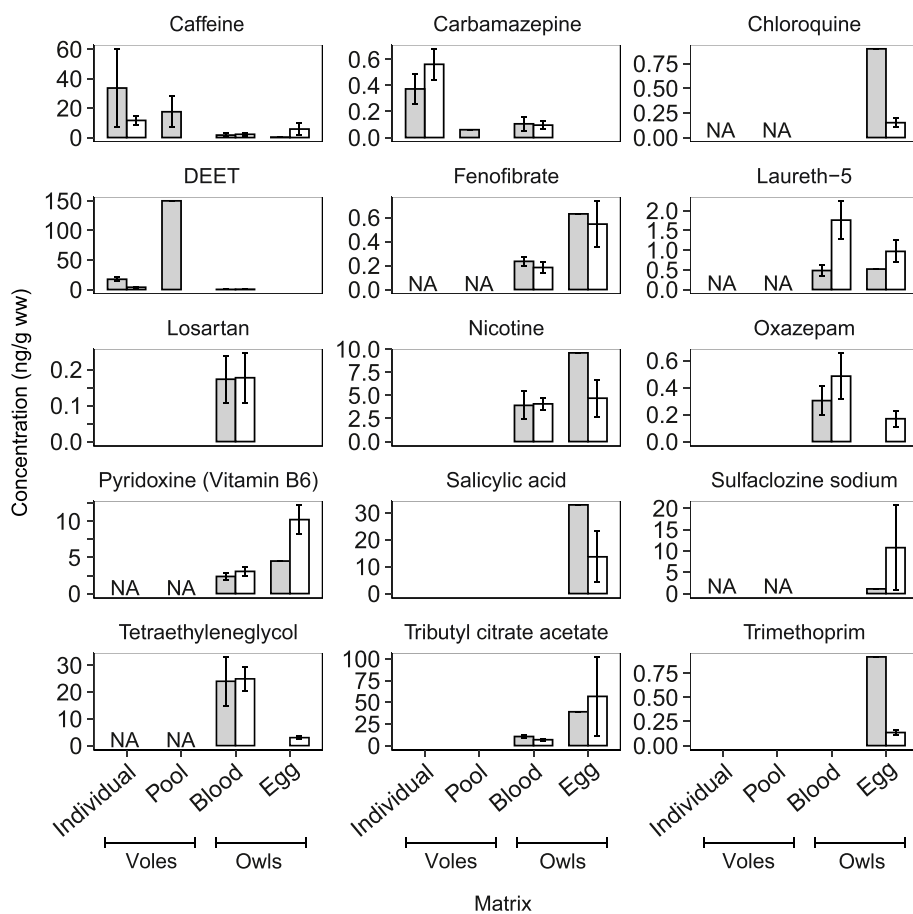


Fig. 2. Mean concentrations (\pm SE) of organic micropollutants in different samples of bank voles (individual samples and pooled samples) and Tengmalm's owls (blood and eggs) divided by region (grey and white bars represent Amarnäs and Umeå, respectively, in Sweden). Carbamazepine, carbamazepine 10, 11-epoxide; DEET, N,N-diethyl-m-toluamide. Only concentrations above limit of quantification are shown. NA, not analysed. For n and raw data, see [Table S1](#) but note that n = 1 for the egg in Amarnäs.

we also analysed pools of voles with specimens being pooled per sampling region (Amarnäs and Umeå, respectively), resulting in four pools consisting of 4–8 specimens with the number of specimens per pool depending on number of trapped voles per area. All samples were stored in a freezer ($-20\text{ }^{\circ}\text{C}$) in acid-washed plastic containers.

2.3. Chemicals

In total, 108 OMPs were included in this study including PFAS (n = 13), pharmaceuticals (n = 72), personal care products (n = 4), pesticides (n = 2), stimulants (n = 3), parabens (n = 3), industrial chemicals (n = 9), fatty acids (n = 1), and food additives (n = 1) ([Table S1](#)). All analytical standards used for analysis were of high purity grade ($>95\%$). Native standards originate from Sigma-Aldrich (Sweden). Isotopically labelled standards (IS) (n = 28; atenolol, D₇, oxazepam, D₅, N,N-diethylmeta-toluamide (DEET), D₁₀, bezafibrate, D₄, diazepam, D₅, caffeine, ¹³C₃, codeine, D₃, mefenamic acid, ¹³C₆, propylparaben, D₇, furosemide, D₅, diltiazem, D₄, irbesartan, D₇, losartan, D₄, metronidazole, D₄, trimethoprim, D₉, cis-Sertraline, D₃, venlafaxine, D₆, isotroturon, D₃, Fluoxetine, D₅, ¹³C₈-FOSA, ¹³C₂-PFHxA, ¹³C₄-PFOA, ¹³C₅-PFNA, ¹³C₂-PFDA, ¹³C₂-PFUnDA, ¹³C₂-PFDoDA, ¹⁸O₂-PFHxS, ¹³C₄-PFOS) for quantification were obtained from Wellington Laboratories (Canada), Teknolab AB (Kungsbacka, Sweden), Sigma-Aldrich and Toronto Research Chemicals (Toronto, Canada). Detailed information about internal and native standards can be found elsewhere ([Rostvall et al., 2018](#)). Ultrapure water was generated by a Milli-Q Advantage Ultrapure Water purification system and filtered through a 0.22 μm Millipak

Express membrane and LC-Pak polishing unit (Merk Millipore, Billerica, MA). Methanol, acetonitrile, isopropanol, formic acid and ammonium acetate of high analytical grade were acquired from Sigma-Aldrich (Sweden).

2.4. Biota sample pre-treatment and extraction

Biota samples were extracted by using a method described by [Grabicova et al. \(2018\)](#). Briefly, 0.5 g of biota samples were spiked with lab IS mixture (10 ng absolute per IS) and 0.5 mL of extraction solvent was added (acetonitrile and isopropanol acidified with 1% of formic acid (3:1)), followed by homogenization at 1800 rpm for 5 min (Precellys Evolution Homogenizer, Bertin Technologies, Paris, France). The extract was then centrifuged at 3900 \times g for 15 min (Eppendorf Centrifuge 5424 R Hamburg, Germany). The supernatant was filtered through a syringe filter (0.45 mm pore size, regenerated cellulose) and frozen at $-20\text{ }^{\circ}\text{C}$ for 24 h to ensure protein precipitation. The final extract was then centrifuged again at 10 000 \times g for 3 min and an aliquot was taken for analysis. Plasma samples (0.25 g) were spiked with an IS mixture (10 ng absolute per IS) and 0.25 mL of extraction solvent was added (acetonitrile acidified with 1% of formic acid), vortexed and then frozen at $-20\text{ }^{\circ}\text{C}$ for 24 h. Finally, the samples were extracted in the same way as the rest of biota samples.

2.5. Instrumental analysis

The biota sample extracts were analysed by a DIONEX UltiMate 3000

ultra-high pressure liquid chromatography (UPLC) system (Thermo Scientific, Waltham, MA, USA) coupled to a triple quadrupole mass spectrometer (MS/MS) (TSQ QUANTIVA, Thermo SCIENTIFIC, Waltham, MA, USA). An Acquity UPLC BEH-C18 column (Waters, 100 mm × 2.1 i.d., 1.7 µm particle size from Waters Corporation, Manchester, UK) was used as an analytical column (for details see [Rehrl et al., 2020](#)). The mobile phase consisted of Milli-Q water with the addition of 5 mM ammonium acetate (phase A) and acetonitrile (phase B). The same linear gradient was used in both ionization modes, with a flow rate of 0.5 mL/min. The gradient started at 2 % of phase B and increased to 99 % from 0.5 min to 10.0 min. This composition of the mobile phase was maintained for 3 min until 13.0 min. Finally, the gradient returned to the initial conditions at 13.1 min and was maintained until the end of the analytical run (15 min).

2.6. Statistical analyses

For statistical analyses, concentrations that were below limit of quantification (LOQ) were replaced by half of LOQ. To compare concentrations in owl blood plasma (ng/L) with concentrations in tissues and whole body (ng/g wet weight (ww)), we multiplied owl blood concentration by 1.03 to account for blood plasma density ([Elert, 2003](#)), which resulted in all concentrations being expressed as ng/g ww. We only included OMPs in the statistical analyses including visualisations that were detected in at least two samples (n = 24). To meet assumptions for parametric tests, concentrations were Box-Cox-transformed prior analyses. We computed main effects ANOVA with associated Tukey's post-hoc comparisons on concentrations of PFAS and other OMPs to analyse if concentrations varied among matrices (vole liver, vole individuals, pools of voles, owl blood, owl egg) and between regions (Umeå/Vindeln and Ammarnäs). We calculated biomagnification factors (BMFs) for PFAS from vole liver to owl blood and owl eggs by dividing concentrations found in the owl blood and eggs by the concentrations in bank vole liver according to [Gobas et al. \(2016\)](#). For these analyses, we only used PFAS that were analysed and found in both species. In these analyses we could unfortunately not avoid the temporal mismatch (2–3 years) between vole livers and owl samples. To better understand the potential maternal transfer of OMPs from owls to their offspring, we calculated maternal transfer ratios (MTRs) for all OMPs that were detected in at least two owl samples by dividing the mean concentrations of OMPs in eggs by those found in blood ([Lee et al., 2023](#)). Statistical analyses were performed in R ([R Development Core Team, 2022](#)) using the packages 'car' and 'stats'.

2.7. Ethics

Small mammal and owl sampling was approved by the Animal Ethics Committee in Umeå (Dnr A 18–2019, A 19–2019, A 27–19, A 2–18), and the Swedish Environmental Protection Agency (NV-07483-19 and NV-03606-19). All applicable institutional and national guidelines for the use of animals were followed.

3. Results

In total, 24 of 108 analysed OMPs were detected in at least two samples. ∑OMP concentrations varied among matrices and ranged from 0.04 to 220 ng/g ww in whole voles, 0.03–53 ng/g ww in blood of owls and 0.04–420 ng/g ww in eggs of owls ([Table S1](#)). Dominant OMPs were caffeine and PFDA (perfluorodecanoate) in whole voles, caffeine, sulfaclozine sodium, tetraethyleneglycol, tributyl citrate acetate, and PFOS in blood of owls and caffeine, nicotine, pyridoxine (vitamin B6), salicylic acid, sulfaclozine sodium, tributyl citrate acetate, and PFOS in eggs of owls. The concentrations of the OMPs caffeine, a central nervous system stimulant, carbamazepine 10, 11-epoxide, a metabolite of the drug carbamazepine, and DEET (N,N-diethyl-m-toluamide), the active ingredient in many insect repellents, were 2–200 times higher in voles

Table 1

Main effects ANOVA (sum of squares, SS; degrees of freedom, *df*; *F*-statistic, *F*; and *p*-values, *P*) on the effect of matrix (vole liver, vole individuals, pool of voles, owl blood, owl eggs), region (Umeå and Ammarnäs), and their interaction on concentrations (see [Fig. 2](#)) of organic micropollutants.

Compound	Factor	SS	df	F	P ^a
Caffeine	Intercept	2.78	1	1.77	0.192
	Matrix	30.13	3	6.39	0.001
	Region	2.32	1	1.48	0.232
	Matrix × Region	2.93	3	0.62	0.606
	Region				
Carbamazepine 10, 11-epoxy	Intercept	54.66	1	37.05	0.000
	Matrix	8.10	3	1.83	0.160
	Region	0.07	1	0.05	0.829
	Matrix × Region	0.90	3	0.20	0.894
	Region				
Chloroquine	Intercept	49.04	1	134.11	0.000
	Matrix	7.63	1	20.86	0.000
	Region	0.00	1	0.00	1.000
	Matrix × Region	4.00	1	10.94	0.004
	Region				
DEET (N,N-diethyl-m-toluamide)	Intercept	21.00	1	20.98	0.000
	Matrix	19.42	3	6.47	0.001
	Region	2.57	1	2.57	0.118
	Matrix × Region	5.66	3	1.89	0.150
	Region				
Fenofibrate	Intercept	14.79	1	14.26	0.002
	Matrix	1.28	1	1.24	0.282
	Region	0.66	1	0.64	0.435
	Matrix × Region	0.02	1	0.02	0.902
	Region				
Laureth-5	Intercept	4.65	1	2.19	0.159
	Matrix	0.06	1	0.03	0.865
	Region	1.40	1	0.66	0.429
	Matrix × Region	0.30	1	0.14	0.711
	Region				
Losartan	Intercept	26.37	1	182.05	0.000
	Matrix	2.37	3	5.45	0.003
	Region	0.00	1	0.00	0.965
	Matrix × Region	0.00	3	0.00	1.000
	Region				
Nicotine	Intercept	1.06	1	2.04	0.162
	Matrix	9.47	3	6.06	0.002
	Region	1.39	1	2.68	0.111
	Matrix × Region	2.60	3	1.66	0.193
	Region				
Oxazepam	Intercept	15.99	1	17.79	0.000
	Matrix	3.38	3	1.25	0.305
	Region	0.42	1	0.46	0.500
	Matrix × Region	4.83	3	1.79	0.167
	Region				
Pyridoxine (Vitamin B6)	Intercept	2.58	1	18.66	0.001
	Matrix	0.33	1	2.36	0.144
	Region	0.11	1	0.77	0.392
	Matrix × Region	0.07	1	0.51	0.485
	Region				
Salicylic acid	Intercept	11.36	1	13.90	0.001
	Matrix	35.92	3	14.66	0.000
	Region	0.00	1	0.00	1.000
	Matrix × Region	4.95	3	2.02	0.129
	Region				
Sulfaclozine sodium	Intercept	17.14	1	13.12	0.002
	Matrix	3.16	1	2.42	0.140
	Region	0.00	1	0.00	1.000
	Matrix × Region	0.11	1	0.09	0.772
	Region				
Tetraethyleneglycol	Intercept	21.52	1	57.21	0.000
	Matrix	1.62	1	4.31	0.054
	Region	0.43	1	1.14	0.302
	Matrix × Region	0.03	1	0.08	0.776
	Region				
Tributyl citrate acetate	Intercept	17.40	1	58.08	0.000
	Matrix	10.32	3	11.49	0.000
	Region	0.18	1	0.62	0.437
	Matrix × Region	0.66	3	0.73	0.540
	Region				
Trimethoprim	Intercept	49.04	1	449.42	0.000

(continued on next page)

Table 1 (continued)

Compound	Factor	SS	df	F	P ^a
	Matrix	32.56	3	99.45	0.000
	Region	0.00	1	0.00	1.000
	Matrix × Region	4.97	3	15.19	0.000

^a Values 0.000 denote that P-values have been <0.0005.

compared to owls (Fig. 2, Table 1, Tables S2–3). In contrast, the concentrations of the following OMPs were 1.3–440 higher in owls compared to voles: The stimulant nicotine, oxazepam (benzodiazepine anxiolytic drug), the non-steroidal anti-inflammatory drug salicylic acid, tributyl citrate acetate (plasticizer), and trimethoprim (antibiotic). Losartan, an antihypertensive pharmaceutical was detected in owl blood but not in voles and owl eggs (Fig. 2, Table 1, Tables S1–3).

Overall, PFAS concentrations were higher in vole liver than in whole vole specimens as shown by several PFAS being < LOQ in the whole specimens, but not in liver, e.g., PFDoDA (perfluorododecanoate) and PFNA (perfluorononanoate). In contrast, PFDA concentrations were surprisingly on average 30 times higher in whole individuals and pools (26.26 ng/g ww) than in liver (0.86 ng/g ww) (Fig. 3, Table 2, Tables S1, S4–5). Except for PFOA (perfluorooctanoate) and PFDA, PFAS concentrations were higher in owls than in voles, with concentrations of PFNA (perfluorononanoate), PFDA, PFUnDA (perfluoroundecanoate), PFDoDA, PFTeDA (perfluorotetradecanoate) and PFOS being higher in owl eggs than in owl blood (Fig. 3, Table 2, Tables S4–5). PFDA (80 % of \sum PFAS) and PFOA (20 % of \sum PFAS) were the only PFAS detected in the whole vole specimens (individual and pooled samples) and hence dominated the relative PFAS profiles in these matrices. In contrast, PFOS was the dominating PFAS in vole liver, and the owls (Fig. 4), with the

proportion of PFOS in vole liver (46–52 %) being lower than the contribution in owl blood (57 %) and owl eggs (60–65 %).

The concentrations of the analysed compounds were only negligibly affected by the sex of the bank voles. Of the four compounds with concentrations lower than LOQ (PFDA, caffeine, carbamazepine 10, 11-epoxy and DEET) in vole individuals, only caffeine showed a difference with higher concentrations in females compared to males (Table S6). This result is however likely caused by one female outlier (220 ng/g ww) (Table S1).

Overall, biomagnification factors in owl blood and eggs indicate bioaccumulation and biomagnification of PFAS (BMFs >1). In owl blood PFNA, PFTeDA, and PFOS showed biomagnification from voles (BMFs 1.7–2.2). In contrast, all PFAS showed biomagnification from voles to owl eggs (especially PFNA, PFDA, PFTeDA, and PFOS) in both the Umeå and Ammarnäs area BMFs (1.3–6.1) (Fig. 5, Table S1).

Most of the studied OMPs (15 out of 22; 68 %) are likely maternally transferred, i.e., transferred from the owls to offspring as shown by the MTRs of caffeine, chloroquine, fenofibrate, nicotine, pyridoxine, salicylic acid, sulfaclozine sodium, tributyl citrate acetate, trimethoprim, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, and PFOS (Table S1, Figs. 2 and 3).

4. Discussion

Overall, concentrations of the studied OMPs were low (no compound was detected >420 ng/g ww), except for caffeine in voles ($\max_{\text{Individual}}$ 220 ng/g ww), DEET in voles (\max_{Pool} 150 ng/g ww), and tributyl citrate acetate in owls ($\max_{\text{Owl egg}}$ 420 ng/g ww). Currently, we lack knowledge on the toxic relevance of the found concentrations. Some of the studied OMPs showed only bioaccumulation in voles (e.g.,

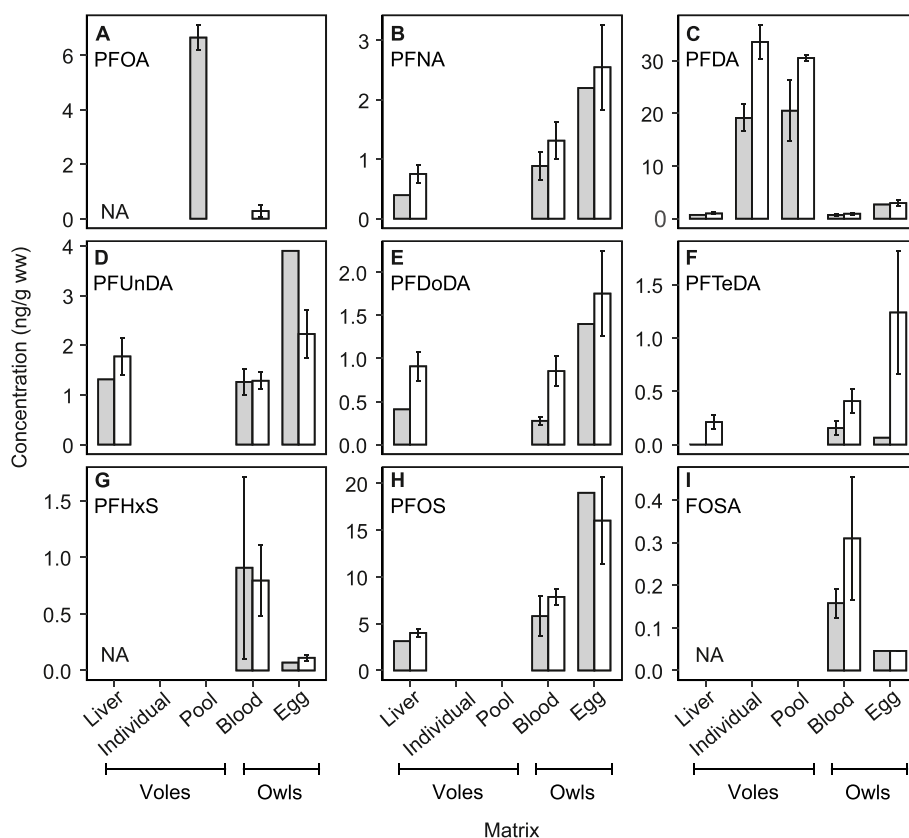


Fig. 3. Mean concentrations (\pm SE) of PFAS (per- and polyfluoroalkyl substances) in different samples of bank voles (liver, individual samples and pooled samples) and Tengmalm's owls (blood and eggs) divided by region and white bars represent Ammarnäs and Umeå, respectively, in Sweden). Only concentrations above limit of quantification are shown. PFOA, PFHxS, and FOSA were not analysed (NA) in vole liver. For n and raw data, see Table S1 but note that n = 1 for the egg in Ammarnäs.

Table 2

Main effects ANOVA (sum of squares, SS; degrees of freedom, *df*; *F*-statistic, *F*; and *p*-values, *P*) on the effect of matrix (vole liver, vole individuals, pool of voles, owl blood, owl eggs), region (Umeå and Ammarnäs), and their interaction on concentrations (see Fig. 3) of PFAS (per- and polyfluoroalkyl substances).

PFAS	Factor	SS	<i>df</i>	<i>F</i>	<i>P</i> ^a
PFNA	Intercept	0.87	1	53.48	0.000
	Matrix	0.37	4	5.75	0.001
	Region	0.03	1	1.69	0.201
	Matrix × Region	0.04	4	0.58	0.682
PFDA	Intercept	0.79	1	68.84	0.000
	Matrix	0.81	4	17.76	0.000
	Region	0.01	1	0.77	0.387
	Matrix × Region	0.01	4	0.23	0.919
PFUnDA	Intercept	1.35	1	72.78	0.000
	Matrix	0.28	4	3.80	0.011
	Region	0.00	1	0.03	0.875
	Matrix × Region	0.04	4	0.51	0.730
PFDoDA	Intercept	0.22	1	18.68	0.000
	Matrix	0.10	4	2.22	0.085
	Region	0.12	1	10.15	0.003
	Matrix × Region	0.10	4	2.13	0.097
PFOA	Intercept	31.70	1	198.08	0.000
	Matrix	42.32	3	88.14	0.000
	Region	0.70	1	4.37	0.044
	Matrix × Region	3.42	3	7.13	0.001
PFTeDA	Intercept	0.08	1	4.29	0.045
	Matrix	0.04	4	0.62	0.650
	Region	0.05	1	2.84	0.101
	Matrix × Region	0.10	4	1.35	0.271
PFHxS	Intercept	0.25	1	15.57	0.000
	Matrix	0.56	3	11.40	0.000
	Region	0.05	1	2.94	0.095
	Matrix × Region	0.03	3	0.68	0.570
PFOS	Intercept	2.77	1	187.51	0.000
	Matrix	0.42	4	7.07	0.000
	Region	0.01	1	0.99	0.326
	Matrix × Region	0.02	4	0.40	0.808
FOSA	Intercept	0.09	1	28.59	0.000
	Matrix	0.02	3	2.47	0.078
	Region	0.01	1	3.92	0.056
	Matrix × Region	0.01	3	0.95	0.429

^a Values 0.000 denote that *P*-values have been <0.0005.

caffeine, DEET, carbamazepine, and PFDA), while others also suggest biomagnification from staple food (bank vole) to the predator (Tengmalm's owl) (e.g., PFNA and PFOS) with associated potential uncertainty of these results due to the temporal mismatch (2–3 years difference) between vole liver and owl samples.

Caffeine has been detected in wastewater, surface water, and groundwater worldwide and has been used as an anthropogenic marker (Buerge et al., 2003), which we also found in the terrestrial animals. DEET is one of the most frequently detected OMPs in wastewaters and is used primarily as an insect repellent in humans but also in agriculture for treatment of livestock and animal houses (Aronson et al., 2011). DEET found in the voles could therefore be a result of environmental contamination since insect repellents were not used during field work. The plasticizer tributyl citrate acetate demonstrates low-dose effects, i. e., physiological effects are not apparent at high doses (Li et al., 2024). It is unknown if the found concentrations of tributyl citrate acetate in owl eggs in Ammarnäs and Umeå could induce such low-dose effects and if they pose potential health concern for the owls.

Surprisingly, we found oxazepam in owl blood and eggs. The concentrations in our study were 1/10 of the ones found in muscle of European perch (*Perca fluviatilis*) in effluent-influenced surface water and that altered behaviour in the fish (Brodin et al., 2013). Adult mice that were prenatally exposed to oxazepam showed increased frequency of grooming and reduced walking (Fiore et al., 1995), i.e., behavioural effects that could potentially increase their exposure to predation. The concentrations of oxazepam that we found in owl blood and eggs were low (0.2–1.0 ng/g ww and 0.3–0.6 ng/g ww, respectively). However, in

fish, already low oxazepam concentrations (mean 3.6 ng/g ww in muscle) affected behaviour (Brodin et al., 2013). Fish and mammalian physiology differ substantially. It is therefore important to test if and at which concentrations oxazepam and other psychoactive substances induce sedation in bank voles. Such behavioural changes could increase predation risk and present a plausible biomagnification mechanism from voles to owls.

The detection of several OMPs in the owls but not in voles is not surprising and does not refute voles as the source of these OMPs. For example, concentrations of oxazepam were below LOQ in voles and in the owls higher in blood than in eggs. For OMPs, we only analysed whole vole bodies, while most of the studied substances accumulate in certain tissue and organs, which in turn results in low whole-body concentrations. For example, oxazepam showed high concentrations in especially liver and brain of rats (Sindo et al., 1971), and losartan in kidneys (Xu et al., 2009). PFAS has shown to accumulate mainly in liver (Costello et al., 2022). Also in our study, PFAS concentrations were highest in liver except for PFDA which was highest in whole specimens. The observed PFAS profiles support our conclusion that the PFAS detected in the owls originate from the voles. PFAS in vole liver, owl blood and owl eggs showed overall similar relative concentrations between the species (Fig. 4). All PFAS for which we calculated BMFs have a log octanol–water partition coefficient (K_{OW}) > 5 (Wang et al., 2011), supporting our interpretations of bioaccumulation and magnification of these PFAS as compounds with a log K_{OW} > 4 are suggested to be bioaccumulative (Caliman and Gavrilescu, 2009; >5 according to SSC, 2018). However, it is important to keep in mind that in blood, PFAS rather sorb to water-soluble proteins than to lipophilic proteins (Forsthuber et al., 2020). This also explains why water-soluble albumin is the most important carrier protein of PFAS and why PFAS concentrations are highest in blood-rich organs like the liver (Forsthuber et al., 2020).

Furthermore, during the nestling period of approximately four weeks, a Tengmalm's owl nestling consumes, depending on vole cycle phase, i.e., vole availability, 19–29 voles (Hakkarainen and Korpimäki, 1994). Considering the lifespan of the owls (in many cases >5 years; Hörnfeldt and Eklund, 1990), it is therefore reasonable to assume that the adult, >1 year old, owls that we sampled in our study have consumed many more voles, up to several hundred during their lifetime. Low concentrations of OMPs in voles and even concentrations below LOQ can therefore potentially biomagnify from staple food to a predator. Bank voles make up >90 % of the food cache of Tengmalm's owls in the study area (Hörnfeldt et al., 1990; personal information). This feeding ecology in combination with the calculated BMFs for PFAS suggest that the voles are the main source of the OMPs found in the owls. Tengmalm's owls also occasionally prey on passerine birds (Hörnfeldt et al., 1990; Rajkovic, 2018), which at northern latitudes is, however, mainly the case at low vole availability (Hörnfeldt et al., 1990; personal observation). Tengmalm's owls, especially females as studied here, are nomadic (Löfgren et al., 1986) and may therefore forage in more or less contaminated areas. It is probably likely that in our study performed at the transition between cycles there may well have been an influx of females from other areas (sensu Löfgren et al., 1986). During such years or conditions of low vole abundance, OMPs in passerine birds could also be a potential additional source of OMPs in the owls. Tracking the movement of the owls in future studies would help us to evaluate if the here calculated BMFs are biased by the birds' potential migratory behaviour.

The concentrations of PFOS in owl eggs were similar to those found in eggs of tawny owls in Norway 1986–2009 (Bustnes et al., 2015). PFOS concentrations in the environment were generally much higher in the past (Ahrens et al., 2011). Considering this general decreasing trend in PFOS, the PFOS concentrations that we found in the owls in this study could be judged as comparatively high (cf. study by Bustnes et al. (2015)). Consequently, the vicinity to the airport and combustion plant for municipal waste in the Umeå area, generally known point sources for PFAS (Ahrens and Bundschuh, 2014), could be reflected in the detected

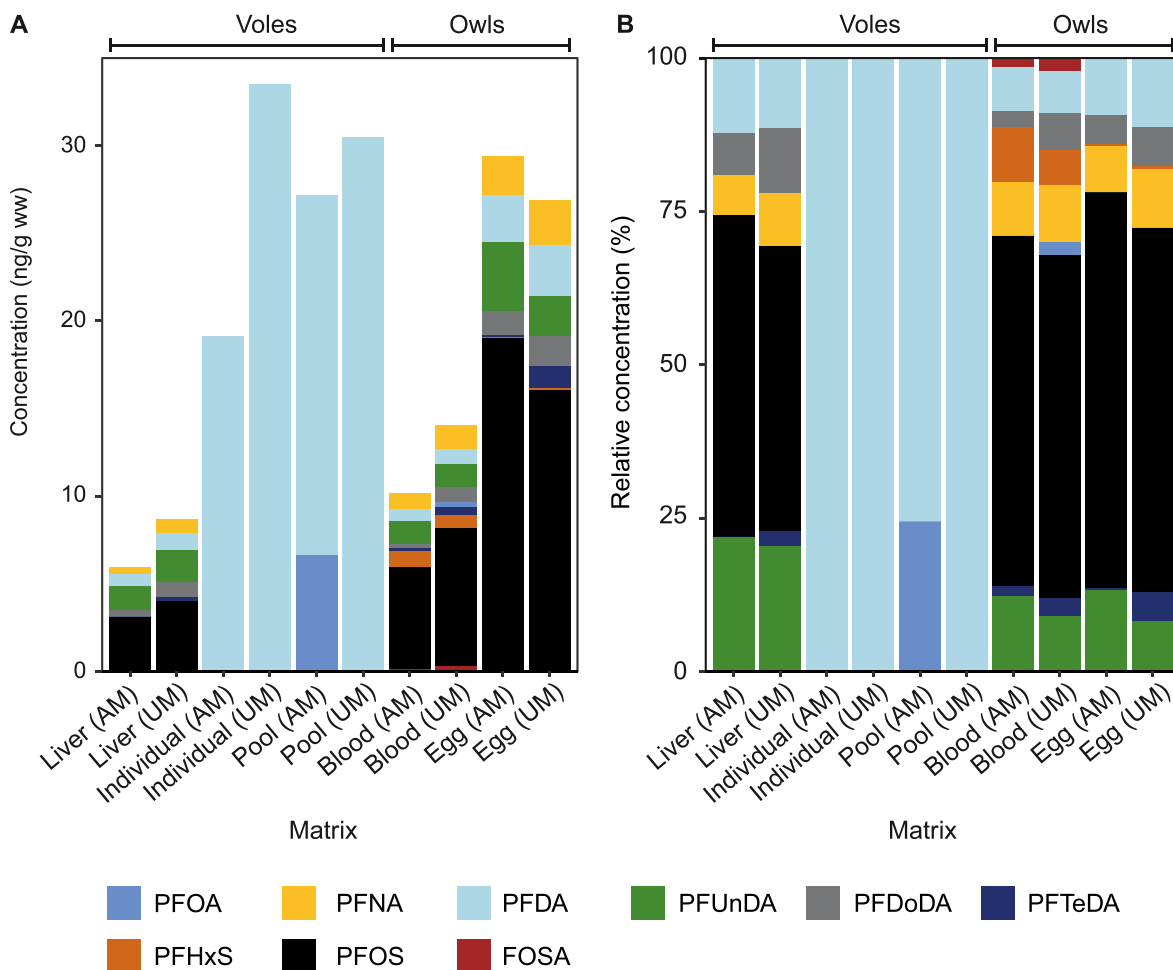


Fig. 4. Sum PFAS (per- and polyfluoroalkyl substances) concentrations divided by PFAS concentration (left), and relative PFAS profiles (right) in different samples of bank voles and Tengmalm’s owls divided by region (AM Ammanräs, UM Umeå). FOSA and PFHxS were not analysed in vole liver. Only concentrations above limit of quantification are shown. For n and raw data, see Table S1 but note that n = 1 for the egg in Ammanräs.

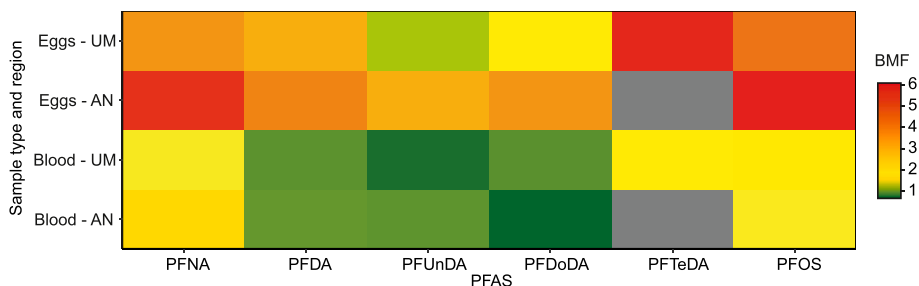


Fig. 5. Biomagnification factors (BMFs) represented as a colour ramp for six PFAS from livers of bank voles (*Clethrionomys glareolus*) to blood and eggs of Tengmalm’s owl (*Aegolius funereus*) in the regions of Ammanräs (AN) and Umeå (UM). Grey boxes represent missing data. For n and raw data, see Table S1 but note that n = 1 for the egg in Ammanräs.

PFAS concentrations at these sites. This does however only partly explain the differences between PFAS in owl samples from the Umeå area and the point source-free study area Ammanräs.

The composition profile of PFNA, PFDA, PFDoDA, PFTeDA, and PFOS in liver found in our study was comparable to that found in voles at a skiing area in Norway where PFAS-containing ski wax has been used (Grønnestad et al., 2019). The relatively high concentrations of PFDA in whole-voles specimens but not in vole liver and in owl blood and eggs is puzzling. This finding could potentially indicate that PFDA in the environment might cause surface contamination and sticks to the skin

and fur of the voles; hence high concentrations (>30 ng/g ww) in whole specimens. When owls consume voles, they usually do not digest fur and skin but excrete these as pellets, which we in turn hypothesize leads to and explains the low PFDA concentrations in owl blood and eggs. This hypothesis could be “tested” and falsified/rejected by finding markedly different PFDA concentrations in pellets and whole voles. However, owl pellets are rarely found in the boxes, as they are usually vomited outside the box. Hence, the comparison is not easy to do. An alternative, but less likely explanation could be that PFDA taken up by voles via their diet is excreted by the voles before the voles are preyed upon by the owls. In

rats, the serum half-life of PFDA is 40–60 days (D'Eon and Mabury, 2010). This is for a short-living vole a rather long-time period, which makes this explanation therefore less likely. A short half-life in combination with excretion could on the other hand apply to caffeine, carbamazepine 10, 11-epoxide, and DEET. Indeed, for example >90% DEET in mammals is eliminated in urine and the half-life degradation in rodents is 6–19 h (reviewed by Legeay et al., 2018).

Our study showed generally low concentrations of OMPs in the voles and owls but demonstrates despite low sample size biomagnification of several OMPs along the terrestrial food chain. To increase our understanding of the trophic fate of OMPs along the terrestrial food chain, future studies should include additional tissues and organs like muscle, kidney, liver, and blood. Preferably, such studies should include the same tissues and/or organs for the calculation of BMFs, e.g., liver in owls and voles. Currently, it is however unknown if the measured elevated concentrations of, for example, tributyl citrate acetate and several PFAS in the owls are of health concern for the owls. Maternal transfer is an important source of contaminants in eggs (Ackerman et al., 2016) and neonate chicks (Zheng et al., 2015). The calculated MTRs as well as detection and partly high concentrations of certain OMPs in eggs (e.g., salicylic acid, sulfaclozine sodium, tributyl citrate acetate and PFOS) highlight the critical role of maternal transfer in contaminant exposure for avian embryos also in our study. The non-maternal transfer of oxazepam is supported by its short half-life and high recovery in urine (Sonne et al., 1988).

The OMPs studied here along with other so far unstudied ones might further biomagnify in predators such as pine martens (*Martes martes*) and corvids (e.g., Western Jackdaw (*Coloeus monedula*)) that commonly prey on owl eggs. Such accumulation and biomagnification have so far unknown consequences for wildlife fitness and ecosystem health.

CRedit authorship contribution statement

Frauke Ecke: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Oksana Golovko:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Data curation. **Birger Hörnfeldt:** Writing – review & editing, Writing – original draft, Conceptualization. **Lutz Ahrens:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data are included in the Supplementary Information

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2024.119686>.

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