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Legacy and emerging organohalogenated compounds in feathers of Eurasian eagle-owls (*Bubo bubo*) in Norway: Spatiotemporal variations and associations with dietary proxies (δ^{13}C and δ^{15}N)

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ABSTRACT

The occurrence of organohalogenated compounds (OHCs) in wildlife has received considerable attention over the last decades. Among the matrices used for OHCs biomonitoring, feathers are particularly useful as they can be collected in a minimally or non-invasive manner. In this study, concentrations of various legacy OHCs –polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs)–, as well as emerging OHCs –per- and polyfluoroalkyl substances (PFAS) and organophosphate ester flame retardants (OPEs)– were determined in feathers of 72 Eurasian eagle-owls (*Bubo bubo*) from Norway, with the goal of studying spatiotemporal variation using a non-invasive approach. Molted feathers were collected at nest sites from northern, central and southern Norway across four summers (2013–2016). Additionally, two museum-archived feathers from 1979 to 1989 were included. Stable carbon (δ13C) and nitrogen isotopes (δ15N) were used as dietary proxies. In total, 11 PFAS (sum range 8.25–215.90 ng g^{-1}), 15 PCBs (4.19–430.01 ng g^{-1}), 6 OCPs (1.48–220.94 ng g^{-1}), 5 PBDEs (0.21–5.32 ng g^{-1}) and 3 OPEs (4.49–222.21 ng g^{-1}) were quantified. While we observed large variation in the values of both stable isotopes, suggesting a diverse diet of the eagle-owls, only δ13C seemed to explain variation in PFAS concentrations. Geographic area and year were influential factors for δ15N and δ13C. Considerable spatial variation was observed in PFAS levels, with the southern area showing higher levels compared to northern and central Norway. For the rest of OHCs, we observed between-year variations; sum concentrations of PCBs, OCPs, PBDEs and OPEs reached a maximum in 2015 and 2016. Concentrations from 1979 to 1989 were within the ranges observed between 2013 and 2016. Overall, our data indicate high levels of legacy and emerging OHCs in a top predator in Norway, further highlighting the risk posed by OHCs to wildlife.

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1. Introduction

Organohalogenated compounds (OHCs) have been of great concern for several decades. Legacy persistent organic pollutants (POPs) including many polybrominated diphenyl ethers (PBDEs) were banned several years ago and despite decreasing levels over time, still occur at high concentrations in wildlife (Bytingsvik et al., 2012; Espín et al., 2016; Jepson et al., 2016; Law and Jepson, 2017; Leat et al., 2019). The widespread occurrence of novel OHCs, such as organophosphate ester flame retardants (OPEs) and per- and polyfluoroalkyl substances (PFAS), have also raised concerns surrounding their impacts on ecosystem, human, and wildlife health (D'Hollander et al., 2010; Groffen et al., 2019, 2018; Guigueno and Fernie, 2017; Van den Eede et al., 2011). Despite extensive production over the last 60 years (e.g., for PFAS; Buck et al., 2011), the vast majority of studies examining PFAS and OPEs in wildlife are from the last 20 years (Gómez-Ramírez et al., 2017; Greaves and Letcher, 2017; Guigueno and Fernie, 2017; Monclús et al., 2019).

Due to their environmental persistence and extensive application in industrial and consumer products, emerging OHCs occur globally in the environment, including remote Arctic ecosystems (Butt et al., 2010; Lau et al., 2007; Salamova et al., 2014). Similar to legacy OHCs, diet is considered the key exposure pathway for PFAS (Trudel et al., 2008) and may be an important pathway for OPEs (Hou et al., 2016). Once ingested, these compounds exhibit different physicochemical properties. PFAS encompass thousands of individual substances, ranging from strongly hydrophobic/lipophobic (e.g., fluoropolymers) to amphipathic (e.g., long chain perfluoroalkyl acids) and highly water soluble (e.g., trifluoroacetic acid; López and Salazar, 2013). Some amphipathic PFAS like perfluorooctane sulfonic acid (PFOS) bind strongly to serum proteins and tend to accumulate in protein-rich tissues (Jones et al., 2003). In contrast, OPEs are mostly lipophilic (Van der Veen and de Boer, 2012) and some studies have showed they undergo rapid metabolism in wildlife (Briels et al., 2019; Greaves et al., 2016a; Su et al., 2014). While some PFAS have demonstrated bioaccumulation and biomagnification (Kelly et al., 2009), there is no clear evidence that OPEs can biomagnify in food webs (Greaves et al., 2016b; Hou et al., 2016; Pantelaki and Voutsa, 2020) nor bioaccumulate to the same degree as PBDEs (Lu et al., 2017). However, OPEs are considered compounds of special concern, in part because of their presence in biota (Guo et al., 2018; Su et al., 2015) and their potential toxicity (Greaves and Letcher, 2017).

Birds, especially birds of prey, are often used as biomonitoring species to reflect pollution from the environment due to their longevity and high position in the food web (Ahrens et al., 2011; Furness, 1993; Sun et al., 2020). Biomonitoring studies in these species have revealed spatial and temporal contaminant variations. For instance, Ahrens et al. (2011) evaluated the temporal trends of PFAS in eggs of tawny owls (*Strix aluco*) in Norway and found a decrease in PFOS and increase in ∑PFASs over time (1986–2009). Similar results were found in eggs of peregrine falcon (*Falco peregrinus*) (1974–2007) in Sweden (Holmström et al., 2007). While this drop in PFOS over time could be explained by the voluntary phase-out of perfluorooctane sulfonyl fluoride-based compounds by 3M (including PFOS) in 2001 (see for instance Butt et al., 2007), PFOS was added to the Stockholm Convention on POPs in 2009 (UNEP, 2009). The only other restricted PFAS are perfluorooctanoic acid (PFOA), which was listed in the Stockholm Convention in 2019, and the perfluorohexane sulfonic acid (PFHxS) which is under review (UNEP, 2019a, 2019b). Some other PFAS are starting to be regulated under the EU chemicals legislation and the Stockholm Convention ([ECHA] European Chemicals Agency, 2021; UNEP, 2021). However, there is still an important concern related to the high ubiquity of PFAS in the environment and their potential exposure-related biological effects (González-Rubio et al., 2021).

In 2012, the Norwegian Institute for Nature Research (NINA) reported the highest PFAS concentration ever recorded in Norway in an egg of Eurasian eagle-owl (*Bubo bubo;* hereafter eagle-owl) (1000 ng g^{-1} ww; Nygård and Polder, 2012). The same report also described high concentrations of POPs in eagle-owl eggs sampled between 1975 and 2010 (Nygård and Polder, 2012). The eagle-owl is an ideal sentinel to monitor local pollution as it is an apex predator, highly territorial and long-living (Eriksen and Wabakken, 2018). As an opportunistic hunter, its diet depends on the availability of prey which may differ between habitats, ranging from terrestrial (small mammals and amphibians) to marine (seabirds and fish) (Obuch and Bangjord, 2016). Temporal changes in the abundance and availability of prey may also produce changes in eagle-owl foraging behavior (e.g., Korpimäki et al., 1990). This offers a unique opportunity to study variations in contaminants depending on dietary input in this species.

In this study, we examined spatiotemporal differences in concentrations of various legacy (PCBs, OCPs and PBDEs) and emerging OHCs (PFAS and OPEs) in feathers of eagle-owls collected across Norway from 2013 to 2016 (with some records from 1979 to 1989). There is currently limited knowledge about pollutants in raptors in Norway (Briels et al., 2019; Eulaers et al., 2014; Gómez-Ramírez et al., 2017; Løseth et al., 2019), and as far as the authors' knowledge, the present study is the first biomonitoring pollutants in feathers of eagle-owls. We sampled molted feathers at the nest sites as their use offers a non-destructive and non-invasive sampling method highly suitable to investigate contaminants in endangered species (Eulaers et al., 2014; Gómez-Ramírez et al., 2017; Jaspers et al., 2013; Monclús et al., 2018a). We also investigated contaminant concentrations in relation to stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) to elucidate the impact of possible dietary variations.

2. Material and methods

2.1. Study species and data collection

The eagle-owl is considered the largest owl in the world (Penteriani and Delgado, 2019) and is currently classified as endangered in the Norwegian Red List for Species (Henriksen and Hilmo, 2015) and of Least Concern in the IUCN Red List (BirdLife International, 2021). The eagle-owl is a residential and territorial bird, highly adaptive and can be found in different environments (Penteriani and Delgado, 2019). In Norway, it is spread along the coast from Østfold in the southeast (58°S) to Nordland in the north (67°N; Eriksen and Wabakken, 2018), and scattered in the inland. The core areas of the distribution are in Nordland, Trøndelag, Hordaland, Rogaland and Agder (Øien et al., 2014; Penteriani and Delgado, 2019).

The present study was performed within the framework of the project 'Management plan for the eagle-owl in Norway' (Norwegian Environment Agency, 2009). Molted flight and covert feathers from eagle-owls were collected from known nest sites around Norway (Fig. 1). Eagle-owls show a partial molt where only a few feathers are replaced each molting season, which takes place from June until October when they are at the breeding grounds (Penteriani and Delgado, 2019). Head, body, and wing covert feathers grow at the same time, usually starting at early June to mid-July. Although breeding dispersal (moving from one successful breeding area to another in successive years) has been reported for some eagle-owls, most of them stay in the same or vicinity breeding areas in consecutive years (Penteriani and Delgado, 2019). Contaminant concentrations in the feathers reflect the circulating concentrations during growth of the feathers, which is on the breeding grounds.

Only adult feathers, from both males and females, were collected. These were identified by their distinct plumage patterns with thicker darker bands and rounder tip than juvenile feathers (Solheim, 2011). A total of 72 feathers collected between 2013 and 2016 from five different counties in Norway were included and in downstream analyses pooled by area; southern (n = 13), central (n = 22) and northern Norway (n = 37) (Fig. 1; Table A1 Supplementary Information SI). Central Norway included both coast (n = 18) and inland (n = 4) (Fig. 1). Feathers from two individuals sampled in 1979 and 1989 were also included in the descriptive analyses (Table A1 SI) but excluded from the statistical

Fig. 1. Map displaying sampling areas; southern Norway (red), central Norway coastal and inland (blue), and northern Norway (green) (map downloaded from Kartverket). For a better understanding of the data, we have included here the four different areas collected, but because of small sample size central Norway inland and central Norway coast were pooled together for further statistical analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

analyses.

2.2. Feather pre-treatment

Sample treatment was performed according to previously described methods (Jaspers et al., 2007a, 2007b). Briefly, the calamus was removed and kept for molecular sexing (see Section 2.3). Then, the feather length was measured, and feathers were washed thoroughly in MilliQ-water to remove dust and particles. Tweezers were used to carefully separate the barbs. After washing, the feathers were placed on clean lab paper, covered with tissue paper and dried overnight at ambient room temperature. Dried feathers were weighed, cut into pieces of ~1 mm^2 with scissors and transferred to analytical glass recipients. Clean stainless steel and glass tools were utilized to wash and cut the feathers. Between individual samples, tools were thoroughly rinsed with acetone (for POPs and OPEs) or methanol (for PFAS).

2.3. Molecular sexing

Molecular sexing of feathers was performed at NINA in Trondheim (Norway) according to the method described by Kleven et al. (2013). Briefly, genomic DNA was extracted from the feather calamus using a semi-automated system (Maxwell®16 Research System, Promega) and the Maxwell 16 tissue DNA purification kit following the manufacturer's protocol. Sex was determined using the primers M5 (Bantock et al., 2008), MP and NP (Ito et al., 2003). Females amplified two fragments (228 and 237 base-pairs) and males a single fragment (228 base-pairs).

2.4. Chemical analysis

The target compounds analyzed were 15 PCB congeners (CB-99, -101, −105, −118, −138, −153, −156, −170, −171, −177, −180, −183, −187, −206, −209), 7 PBDEs (BDE-28, -47, −99, −100, −153, −154, −183), 12 OCPs, amongst which dichlorodiphenyltrichloroethane (*p,p'*-DDT) and dichlorodiphenyldichloroethylene (*p,p'*-DDE), hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs: *α*-, *β*- and *γ*-HCHs) and chlordanes (CHLs: *cis*-, *trans*- and *oxy*-chlordane, *cis*- and *trans*-nonachlor), as well as 4 OPEs, being tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCiPP), tri-phenyl phosphate (TPhP), tris(1,3-dichloro-2-propyl) phosphate (TDCiPP). Five classes of PFAS, i.e., perfluoroalkyl carboxylic acids (PFCAs), fluorotelomer acids (FTAs), perfluoroalkyl sulfonic acids (PFSAs), (N-alkyl substituted) perfluorooctane sulfonamides (FASAs), fluorotelomer sulfonate (FTSs), composing 31 target substances, were also evaluated (Table A2 SI).

Feathers were extracted and analyzed for POPs (including PCBs, OCPs and PBDEs) and OPEs according to Monclús et al. (2018a), and for PFAS according to Jaspers et al. (2013) (see Annex A1 and A2 SI). Feathers were first cut, homogenized, and mass measured. When the mass was >0.2 g, feathers were used for POPs and OPEs as well as PFAS extraction. When the mass of the homogenized feather was ≤0.2 g, PFAS extraction was prioritized. Therefore, all 72 feathers collected were used for PFAS analysis, while only 49 could be used for PCBs, OCPs, PBDEs and OPEs in addition to PFAS (Table A1 SI). The extraction of all compounds was performed at the Norwegian University of Science and Technology (Trondheim, Norway), and the extracts were sent to analytical laboratories. The quantification of POPs and OPEs was performed at the University of Antwerp (Antwerp, Belgium) and the quantification of PFAS at Stockholm University (Stockholm, Sweden). Analyses for POPs and OPEs were performed using a gas chromatograph coupled to a mass spectrometer (GC/MS). For PFAS analyses, a Waters ultra-performance liquid chromatography (UPLC) system coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer was used and operated in negative ion electrospray ionization mode. More details on sample preparation, instrumental analysis, quantification, and QA/QC measured, are given in SI.

2.5. Stable isotopes analysis

The stable isotope analysis of feathers (n = 72) was performed at the University of Koblenz-Landau (Landau, Germany). A subsample of homogenized cleaned feather material from each individual feather (mean ± standard deviation *SD*: 1.51 ± 0.26 mg) was wrapped into a tin combustion cup and analyzed for its elemental and isotopic composition using a Flash 2000 HT elemental analyzer coupled via a ConFlo IV interface to a Delta V Advantage isotope ratio mass spectrometer (all Thermo Fisher Scientific, Bremen, Germany). The reported stable carbon and nitrogen isotope values are expressed as δ (‰) relative to the international reference standards Vienna PeeDee Belemnite and atmospheric nitrogen, respectively. An internal reference material (i.e., casein) was measured in duplicate every tenth samples revealing an imprecision (±1 *SD*) of 0.06 and 0.03‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.6. Statistics

Only compounds with a detection frequency over 60% of the measurements above the limit of quantification (LOQ) were included for further analyses (Table A2 and A3 SI). For those compounds, samples that showed concentrations below LOQ were imputed using the Solver add-in for Microsoft Excel (Microsoft Excel) following John (1998) (see more information in Annex A3 SI). This approach was used to reduce potential data bias introduced by substitution of non-detects with a fixed value (John, 1998). Statistical analyses were then performed using R software version 3.2.2 (R Development Core Team 2015). For statistical modelling, compounds (including imputed values) were summed ($\sum$) per group ($\sum_{11}$PFAS, $\sum_{15}$PCBs, $\sum_{6}$OCPs, $\sum_{5}$PBDE, $\sum_{3}$OPE). All variables were investigated for outliers, normality, and homoscedasticity (Zuur et al., 2010). Variables that were not normally distributed were ln-transformed to meet criteria of parametric statistics. To ensure normality of the residuals of the model, outliers were identified and excluded from further analysis. Outliers were considered as individuals showing extremely high values numerically distant from the rest of the data, i.e., one male from central Norway for PCBs (430 ng g^{-1}), OCPs (220 ng g^{-1}) and PBDEs (5.3 ng g^{-1}), two females from southern and northern Norway for PFAS (up to 216 ng g^{-1}) and three females from northern Norway for OPEs (up to 2000 ng g^{-1}). Significance levels were

set at $P < 0.05$ (*) and <0.01 (**). A *P-value* between <0.1 and ≥ 0.05 was considered a trend (^{T}).

Differences in contaminant concentrations between geographic areas and years, and their relationship with dietary proxies were investigated using linear models. Sex was included in all models as a fixed factor. No interactions between variables could be included because of poor distribution of data across years and geographic areas. In all cases, the initial models contained all the fixed factors. Akaike's Information Criterion (AIC) and Akaike Weight (Wi) were used to rank models in each set (Burnham and Anderson, 2002). Model selection was performed on models fitted with maximum likelihood, while parameters were estimated using restricted maximum likelihood. The model with the lowest AIC value indicated the most parsimonious model and thus was selected for further examination (Table A4 SI). Then, we examined further differences by post-hoc Tukey's HSD Test. Finally, because the stable isotope values were not normally distributed, differences in δ^{15}N and δ^{13}C values between years and geographic areas were investigated using Kruskal-Wallis analyses, followed by a Dunn's post-test of multiple comparisons with 'Bonferroni' correction. Correlations between δ^{15}N and δ^{13}C isotopes and between stable isotopes and contaminants were investigated by Spearman's rank correlation for each year and geographic area separately and combined.

3. Results and discussion

3.1. Occurrence and concentrations of contaminants

A total of 15 PCBs, 11 PFAS, 6 OCPs, 5 PBDEs and 3 OPEs were quantified above LOQ in over 60% of the collected feather samples (Table A2 and A3 SI). The compound groups with the highest median concentrations in feathers were $\sum_{15}$PCBs (37.2 ng g^{-1}) > $\sum_{11}$PFAS (31.6 ng g^{-1}) > $\sum_{3}$OPEs (21.4 ng g^{-1}) > $\sum_{6}$OCPs (14.1 ng g^{-1}) > $\sum_{5}$PBDEs (0.8 ng g^{-1}). From all the groups, the compounds with the highest concentrations were *p,p'*-DDE > CB-153 > TCiPP > CB-138 > L-PFOS (Table 1).

The overall dominance of the compounds *p,p'*-DDE, CB-153 and CB-180 is similar to previous studies investigating pollutants in feathers of several bird of prey species; e.g., nestling cinereous vultures (*Aegypius monachus*) in Spain (Monclús et al., 2018a), nestling and adult white-tailed eagles (*Haliaeetus albicilla*) in Norway (Eulaers et al., 2014; Sun et al., 2020) as well as adult spotted owlets (*Athene brama*) and black kites (*Milvus migrans*) in Pakistan (Abbasi et al., 2017).

In contrast, OPEs were observed as the dominant compound group in feathers of nestling northern goshawks (*Accipiter gentilis*) and white-tailed eagles in Norway (Briels et al., 2019; Løseth et al., 2019). Because those feathers were sampled from nestlings still in the nests, they are probably not comparable to the adult molted feathers used in the present study. The high concentrations of OPEs in the former studies could rather reflect an external contamination than internal accumulation, as indicated by the low detection frequencies of OPEs found in plasma and preen oil compared to feathers (Briels et al., 2019; Løseth et al., 2019) and the apparent lack of association between OPEs in feathers and in plasma (Eulaers et al., 2014; Løseth et al., 2019). Regarding PFAS, L-PFOS was the predominant compound detected in all feather samples, contributing 61% to $\sum_{11}$PFAS. The dominance of L-PFOS has been observed earlier in several bird of prey species (Eriksson et al., 2016; Jaspers et al., 2013; Løseth et al., 2019; Sletten et al., 2016) and may be due to preferential uptake of the linear isomers and their bioaccumulation and biomagnification (Gebbink and Letcher, 2010).

The median concentrations of PCBs detected in eagle-owl feathers were higher than the ones reported in some previous studies, in Norway [i.e., nestling northern goshawks ($\sum_{10}$PCBs range 8.04–27.8 ng g^{-1} Briels et al., 2019; $\sum_{8}$PCBs range 6.78–140 ng g^{-1} Eulaers et al., 2011), nestling white-tailed eagles ($\sum_{12}$PCBs range 14.2–95.9 ng g^{-1} Eulaers et al., 2011; $\sum_{9}$PCBs range 2.00–5.79 ng g^{-1} Løseth et al., 2019)] and elsewhere [i.e., nestling cinereous vulture ($\sum_{10}$PCBs range 1.28–10.11 ng g^{-1}; Monclús et al., 2018a) and adult red kites (*Milvus milvus*) in Spain ($\sum_{5}$PCBs range 0.56–122.44 ng g^{-1}; Monclús et al., 2018b)]. Concentrations of OPEs were in the range as previously reported in nestling white-tailed eagles (median concentrations range 4.3–110 ng g^{-1}; Eulaers et al., 2014) and adult northern goshawks (median concentrations range 25.5–206 ng g^{-1}; Briels et al., 2019) in Norway, and much higher than described in nestling cinereous vultures in Spain (median concentrations ranges 2.3–13.4 ng g^{-1}; Monclús et al., 2018a). Concentrations of PFAS were in the same range as adult Norwegian white-tailed eagles sampled from 1971 to 2015 (PFOS: 5.5 ng g^{-1}; Sun et al., 2019) but three times higher than concentrations found in nestling white-tailed eagles sampled in 2014 in northern Norway ($\sum$PFASs 12.28 ng g^{-1}; Gómez-Ramírez et al., 2017). The overall contaminant concentrations in the present study were generally high compared to previous studies performed in the Norwegian environment, possibly being explained by the age factor as we analyzed molted feathers from adult individuals in contrast to previous studies that mostly used samples from nestlings.

Table 1
Concentrations (mean ± *SE*; median; min-max; ng g^{-1} dw), of PCBs, OCPs, PBDEs, OPEs and PFAS quantified in feathers of Eurasian eagle-owls (*Bubo bubo*) sampled during 2013–2016. The sample size was 72 feathers for PFAS and 49 for PCBs, OCPs, PBDEs and OPEs. Limits of quantification and detection frequency are shown in SI (Table A2 and A3).

	Mean	*SE*	Median	min - max
$\sum_{15}$PCBs	62	11	37	4.2–430
CB-99	2.4	0.40	1.5	0.16–16
CB-101	0.75	0.47	0.47	0.09–4.2
CB-105	1.4	0.22	0.90	0.12–7.6
CB-118	4.3	0.59	2.8	0.38–18
CB-138	12	2.4	6.9	0.73–89
CB-153	19	3.9	11	1.4–161
CB-156	0.97	0.16	0.59	0.06–4.7
CB-170	4.1	0.77	2.5	0.17–26
CB-171	0.54	0.09	0.32	0.03–3.4
CB-177	0.33	0.06	0.23	0.03–1.9
CB-180	8.8	1.7	5.6	0.38–64
CB-183	1.6	0.33	0.91	0.10–12
CB-187	3.3	0.57	2.2	0.22–23
CB-206	0.21	0.03	0.15	0.03–1.1
CB-209	0.12	0.01	0.08	0.01–0.44
$\sum_{6}$OCPs	21	4.9	14	1.5–220
p,p'-DDE	20	4.8	13	1.2–218
β-HCH	0.29	0.03	0.22	0.06–1.4
γ-HCH	0.09	0.02	0.07	0.03–1.0
HCB	0.39	0.03	0.34	0.12–0.94
OxC	0.15	0.02	0.09	0.02–1.0
CN	0.15	0.02	0.10	0.02–0.67
$\sum_{5}$PBDEs	1.2	0.16	0.77	0.21–5.3
BDE-47	0.54	0.09	0.32	0.05–2.7
BDE-99	0.23	0.03	0.13	0.02–0.89
BDE-100	0.21	0.03	0.15	0.02–0.89
BDE-153	0.12	0.02	0.07	0.01–0.57
BDE-154	0.07	0.01	0.05	0.01–0.27
$\sum_{3}$OPEs	36	6.8	21	4.5–222
TCEP	9.0	2.7	2.3	0.06–96
TCiPP	19	4.4	9.1	0.48–160
TPhP	8.4	1.5	5.8	1.5–29
$\sum_{11}$PFAS	37	3.6	31	8.3–215
PFOA	1.5	0.17	1.1	0.20–7.8
PFNA	0.66	0.04	0.58	0.13–2.1
PFDA	0.73	0.06	0.59	0.11–2.7
PFUnDA	2.7	0.23	2.2	0.50–10
PFDoDA	1.5	0.17	1.1	0.20–7.8
PFTriDA	3.9	0.41	2.9	0.57–19
PFTeDA	0.84	0.11	0.54	0.08–4.6
PFPeDA	0.48	0.07	0.23	0.02–2.8
L-PFOS	11	1.3	8.8	0.10–74
Br-PFOS	0.96	0.09	0.84	0.11–6.5
L-FOSA	0.33	0.06	0.19	0.03–2.9

3.2. Dietary proxies

In the present study, there was large variation in $\delta^{15}N$ and $\delta^{13}C$ values, suggesting a diverse diet of eagle-owls in Norway. A positive $\delta^{15}N$ to $\delta^{13}C$ correlation was observed in the eagle-owls from all areas (all $P < 0.01$; Table 2), although it was only a tendency towards significance for the owls from the south ($P = 0.07$; Table 2). Both stable isotopes in the analyzed feathers differed among years ($\delta^{15}N$: $\chi^2 = 10.98$, $P = 0.01$; and $\delta^{13}C$: $\chi^2 = 8.50$, $P = 0.04$). The feathers tended to display lower $\delta^{15}N$ values in 2015 and 2016 compared to 2013 (Bonferroni post-hoc test: $P = 0.06$; Fig. A1), while the $\delta^{13}C$ values were only significantly lower in 2016 compared to 2013 (Bonferroni post-hoc test: $P = 0.03$; Fig. A2). Overall, these between-year variations were not strong enough to indicate that diet changed markedly between years for this species.

The statistical analyses also indicated geographic area as a significant factor in explaining the variation of $\delta^{15}N$ ($\chi^2 = 10.82$, $P < 0.01$) and a tendency of significance for $\delta^{13}C$ ($\chi^2 = 5.88$, $P = 0.05$). Feathers collected in southern Norway showed significantly lower $\delta^{15}N$ values than in northern (Bonferroni post-hoc test: $P < 0.01$) and central Norway (Bonferroni post-hoc test: $P = 0.03$; Fig. A3). This difference in $\delta^{15}N$ values could be explained by a different trophic level or diet composition, although own observations suggest the eagle-owls mainly feed on rodents in these locations. Eagle-owls from northern Norway and parts of central Norway originated from coastal areas consisting mainly of islands of various sizes, and a few samples from inland sites. Water voles (*Arvicola amphibius*) constitute most of the diet of eagle-owls from northern Norway (Heggøy and Shimmings, 2020; Wabakken et al., unpublished data). Small mammals are also an important part of the diet of eagle-owls from central Norway. On smaller islands in coastal central Norway, the diet tends towards being marine, including seabirds and fish. In southern Norway, eagle-owls also prey upon rodents [e.g., short-tailed vole (*Microtus agrestis),* lemmings (*Lemmus lemmus)* and bank voles (*Myodes glareolus)*; Heggøy et al., 2020]. Due to similarity of trophic level in diet composition among areas, differences observed in $\delta^{15}N$ values could rather be explained by nitrogen fluxes in the ecosystems, which can be affected by local emissions of fossil fuels, precipitations, agricultural use of fertilizers or differences in N2-fixing plant communities (Fry, 2006). In the case of $\delta^{13}C$, no significant differences were found among areas ($P > 0.05$, Fig. A4). However, large variation can be observed in $\delta^{13}C$ suggesting a large variability in carbon source, spanning both terrestrial and marine primary producers (Fig. 2). Some eagle-owls from north and central Norway showed $\delta^{13}C$ values higher than −20‰, which could suggest marine dietary carbon input in their diet (Fry, 2006).

3.3. Exposure variation depending on years, geographic areas, and dietary proxies

The most parsimonious models for variation in PCBs, OCPs, PBDEs and OPEs in the eagle-owl feathers included the variables year and $\delta^{13}C$, while PFAS variation was best explained by the variables geographic area and $\delta^{13}C$ (Table 3).

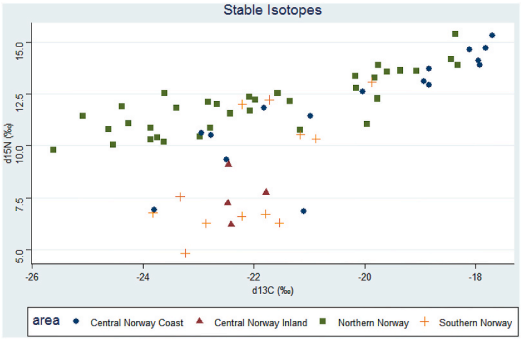

Fig. 2. Values of δ13C and δ15N in individual Eurasian eagle-owl (*Bubo bubo*) feathers collected in four areas across Norway. The individual stable isotope values are represented by points in different shapes and colors according to the areas where feathers were collected. For better understanding of the data, we have included here the four different areas collected, but because of small sample sizes Central Norway Inland and Central Norway Coast were pooled together for further statistical analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

PCB, OCP, PBDE and OPE concentrations were all significantly higher in feathers collected in 2015 compared to 2013 (Tukey post-hoc test: estimate ± *SE*, 2013–2015 = −1.25 ± 0.35 PCBs, −0.97 ± 0.32 OPCs, −1.06 ± 0.26 PBDEs, −1.31 ± 0.39 OPEs, all $P < 0.05$, Fig. 3, Table A5), while significantly higher PBDE and OPE concentrations were also detected in feathers collected in 2016 compared to those from 2013 (Tukey post-hoc test: estimate ± *SE*, 2013–2016 = −0.78 ± 0.27 PBDEs, −1.12 ± 0.39 OPEs, all $P < 0.05$, Fig. 3, Table A5) and PCBs and PBDEs were higher in 2015 than 2014 (Tukey post-hoc test: estimate ± *SE*, 2014–2015 = −1.29 ± 0.40 PCBs, −0.94 ± 0.29 PBDEs, all $P < 0.05$, Fig. 3, Table A5). Annual variation in PCB, OCP and PBDE concentrations in feathers have been previously reported in Norwegian northern goshawk and white-tailed eagle nestlings (Briels et al., 2019; Løseth et al., 2019). However, the general trend of POPs reported in Norwegian biota indicated a decline in concentrations over the last 20–40 years (Andersen et al., 2015; Nygård et al., 2019; Sun et al., 2020), as observed for the Arctic environment (Hung et al., 2016; Rigét et al., 2019). When studied more specifically for the years 2013–2016, reports on atmospheric concentrations of PCBs, OCPs and PBDEs in Norway displayed annual variations (Bohlin-Nizzetto et al., 2014, 2015, 2017; Bohlin--Nizzetto and Aas, 2016). However, those atmospheric variations do not coincide with the results of the present study. The short time frame of this monitoring dataset (2013–2016) does not have sufficient power to identify temporal trends (Rigét et al., 2019) and the here observed between-year variations are small and random compared to the general trend. In this line, Vorkamp et al. (2019) found small variations of PFAS and polychlorinated naphthalenes between consecutive years while observing a general decline when a 28 year-period was considered. In

Table 2
Spearman's rank correlations between contaminants (PCBs, OCPs, PBDEs, OPEs and PFAS) and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). Significant values ($P < 0.05$*, $P < 0.01$**) and tendencies ($0.10 > P \geq 0.05^T$) are shown in bold.

	northern Norway				central Norway				southern Norway			
	$\delta^{13}C$		$\delta^{15}N$		$\delta^{13}C$		$\delta^{15}N$		$\delta^{13}C$		$\delta^{15}N$	
	Rho	P	Rho	P	Rho	P	Rho	P	Rho	P	Rho	P
PCBs	−0.29	0.17	**−0.44**	**0.03***	−0.29	0.29	−0.25	0.37	−0.20	0.78	−0.20	0.78
OCPs	−0.30	0.15	**−0.42**	**0.04***	−0.20	0.47	−0.18	0.53	−0.20	0.78	−0.20	0.78
PBDEs	−0.30	0.15	**−0.40**	**0.05^T**	−0.27	0.33	−0.23	0.40	−0.20	0.78	−0.20	0.78
OPEs	−0.30	0.15	−0.19	0.36	−0.13	0.66	0.09	0.73	−0.80	1.33	−0.80	1.33
PFAS	**0.46**	**<0.01****	**0.52**	**<0.01****	**0.54**	**0.01****	**0.72**	**<0.01****	−0.12	0.72	0.04	0.90
δ13C			**0.81**	**<0.01****			**0.83**	**<0.01****			**0.53**	**0.07 ^{T}**
$\delta^{15}N$	**0.81**	**<0.01****			**0.83**	**<0.01****			**0.53**	**0.07^T**		

Table 3
Model estimates from the selected models explaining the variation of $\sum_{11}$PFAS, $\sum_{15}$PCBs, $\sum_{6}$OCPS, $\sum_{5}$PBDES, $\sum_{3}$OPES in feathers of Eurasian eagle-owls (*Bubo bubo*) from Norway. The table includes the model intercept (β_0), the model estimates (β_x), significance values (*P*-values) and R^2.

	Explanatory variables	β_0	β_1	β_2	β_3	β_4	β_5	*P*-values	ΔAIC	R^2
$\sum_{11}$PFAS	~ δ^{13}C + area	***Int.***	***δ13C***	***Loc. N***	***Loc. S***			**<0.01****; 0.39; **<0.01****	0.00	0.37
		6.09	0.13	0.11	0.68					
$\sum_{15}$PCBs	~ year + δ^{13}C	***Int.***	***Y 2014***	***Y 2015***	***Y 2016***	***δ13C***		0.81; **<0.01****; 0.21; 0.75	0.00	0.32
		2.55	0.09	1.36	0.51	−0.02				
$\sum_{6}$OCPs	~ year + δ^{13}C	***Int.***	***Y 2014***	***Y 2015***	***Y 2016***	***δ13C***		0.78; **0.01***; 0.45; 0.53	0.00	0.22
		1.28	0.10	0.96	0.29	−0.04				
$\sum_{5}$PBDEs	~ year + δ^{13}C	***Int.***	***Y 2014***	***Y 2015***	***Y 2016***	***δ13C***		0.58; **<0.01****; **0.03***; 0.37	0.00	0.37
		−1.67	0.16	1.06	0.66	−0.04				
$\sum_{3}$OPEs	~ year + δ^{13}C + δ^{15}N	***Int.***	***Y 2014***	***Y 2015***	***Y 2016***	***δ13C***	***δ15N***	**0.01***; **<0.01****;**<0.01****; **0.07**^{T}; 0.16	0.00	0.35
		−3.04	1.05	1.34	1.27	−0.17	0.13			

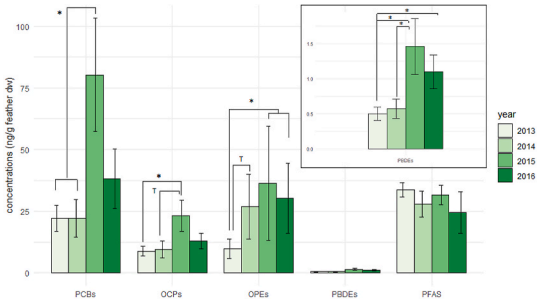

Fig. 3. Concentrations of PCBs, OCPs, OPEs, PBDEs and PFAS in feathers of Eurasian eagle-owls (*Bubo bubo*) sampled in different years (2013, 2014, 2015, 2016). Asterisks show significances ($P < 0.05$) and T shows tendencies ($0.10 > P \geq 0.05$).

another long-term study, Sun et al. (2020) did not find any variation in DDT levels between 1992 and 2015 in the Norwegian white-tailed eagle population and authors attributed this result to a relatively small sample size for the large area studied. In the present study, concentrations of PCBs, OCPs, OPEs and PFAS from two samples from 1979 to 1989 were highly variable but within the ranges observed between 2013 and 2016 (Table A6). This result does therefore not depict a decreasing trend between decades, although we cannot conclude on this due to the small sample sizes.

Although identified in the most parsimonious models for both legacy and emerging OHCs, a significant association between contaminant concentrations and δ^{13}C values was only found for PFAS ($P < 0.01$; Table 3). The PFAS model indicated significantly higher concentrations in feathers of birds feeding on a ^{13}C-enriched diet ($\beta = 0.13$, $P < 0.01$; Table 3), which may reflect marine carbon input from seabirds and fish (Kelly, 2000). The most parsimonious PFAS model also indicated differences among areas ($P < 0.01$; Table 3, Fig. 4). When explored with post-hoc analyses, the southern area showed statistically higher PFAS concentrations than northern (Tukey post-hoc test: estimate ± *SE* = −0.24 ± 0.07, t = −3.19, $P < 0.01$) and central Norway (Tukey post-hoc test: estimate ± *SE* = −0.19 ± 0.08, t = −2.44, $P = 0.04$), although concentrations did not significantly vary between central and northern Norway (Tukey post-hoc test: $P = 0.78$) (Table A7). Southern Norway is a more densely populated area, and the sampling locations were in closer vicinity to urban areas and continental Europe, which could explain the higher PFAS concentrations in these feathers. Also, southern Norway has a high frequency and magnitude of point-source emissions, such as airports and fire-drill fields, which could also contribute to higher PFAS levels.

Surprisingly, only the most parsimonious OPE model included the trophic proxy δ^{15}N as an explanatory variable, although it was non-significant ($P = 0.16$; Table 3). The correlation analyses indicated that PCBs, OCPs and PBDEs were negatively correlated to δ^{15}N in feathers collected in northern Norway (PCBs and OCPs $P < 0.05$, PBDEs $P = 0.05$, Table 2), while no correlations were found for the feathers from southern or central Norway (all $P > 0.05$, Table 2). These results indicate that δ^{15}N is not a clear explanatory variable for the present dataset. Finally, sex was not included in any of the best candidate models in explaining the variation of contaminants in the feathers (Table A4). This is consistent with the general lack of sex differences when legacy and emerging compounds are measured in feathers (Gómez-Ramírez et al., 2017; Monclús et al., 2019).

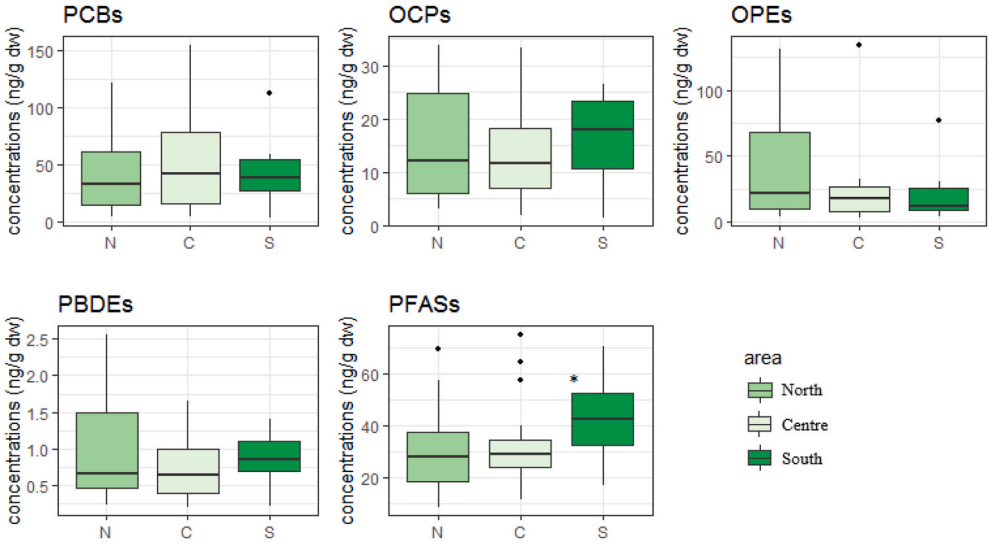

Fig. 4. Concentrations of PCBs, OCPs, OPEs, PBDEs and PFAS in feathers of Eurasian eagle-owls (*Bubo bubo*) from different areas of Norway (N=Northern, C=Central, S=Southern). Asterisks show significances ($P < 0.05$). Two high values were eliminated for better visualization of the graphs but were included in the statistical analysis. Boxplots include the median value (thick line in the middle of the box), the 25th-75th interquartile range (top and bottom of the box) and the maximum and minimum values within the 1.5 interquartile range (whiskers). Note that range of concentrations are different among compounds.

3.4. Limitations of the present study

While feathers have been validated several times to quantify POPs (Jaspers et al., 2007b; Løseth et al., 2019), their validity as a biomonitoring tool for PFAS and OPEs remains unclear (Jaspers et al., 2019). Feathers seem to be an appropriate matrix to quantify PFOS, as explained by the strong correlation between levels in liver and feathers (Jaspers et al., 2013). However, some inconsistencies have been found for other PFAS (e.g., PFDA, PFDoDA, PFTrDA; see Gómez-Ramírez et al., 2017; Løseth et al., 2019), suggesting that the suitability of feathers for PFAS analyses could be compound-specific (Sun et al., 2019). Regarding OPEs, it is still unclear whether the detected concentrations are deposited inside the feather or on its surface. Feathers could be, in fact, potential useful passive air samplers for atmospheric OPEs rather than for internal burdens, though this issue has, to our best knowledge, not been investigated yet. In addition, there are numerous biological factors which may influence the concentrations of environmental contaminants detected in feathers. Previous studies have found that there can be large variations in contaminant concentrations between different feather types from the same individual bird (Jaspers et al., 2011; Monclús et al., 2018a). As we sampled different molted feather types in the present study, this could indeed be an influential factor. Standardizing feather type collection in a biomonitoring program should therefore be a priority in future studies. However, it is worth noticing that the present study was the first investigating legacy and emerging OHCs in molted feathers of eagle-owls, collected from or near the nest and without disturbing the animals, which constitutes a purely non-invasive biomonitoring approach. This method does not only provide valuable information of the status of this endangered species, but also results from long and extensive ecological monitoring studies to establish nest distribution, which is highly valuable and not always available for all species and regions.

4. Conclusions

There is scant information on legacy and emerging OHCs in Eurasian eagle-owls from Norway. The present study provides much needed OHC concentrations determined for the first time in feathers of eagle-owls. High concentrations were found compared to concentrations from other bird of prey species from the Norwegian environment, which highlights the concern of environmental pollution in this endangered species. We cannot rule out that age may play a role in explaining these high concentrations, since the eagle-owls studied here were adults (of unknown age), while previous studies on other species involved mostly nestlings. Within our four-year framework study, we observed between-year variations in POPs and OPEs but not in PFAS. This latter group of contaminants seemed to vary more strongly depending on geographic area, with owls sampled in southern Norway closer to urban areas and the European continent, showing the highest levels. Overall, our results promote the utility of feathers as a tool to monitor the occurrence and potential risk of legacy and emerging contaminant exposure in wildlife species. Despite feathers are a matrix still under development, they offer a unique opportunity to obtain such information in a non-invasive manner, useful to study protected wildlife species.

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.

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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.2021.112372.

Credit author statement

L.M. Investigation; Data curation; Formal analyses; Writing – original draft; Writing – review & editing. M.E.L. Supervision; Writing – original draft; Writing – review & editing. M.J.D.P. Lab work; Writing – review & editing. I.E. Stable isotope analyses; Writing – review & editing. O.K. Molecular analyses; Writing – review & editing. A.C. POPs & OPEs analyses; Writing – review & editing. J.P.B. PFAS analysis; Data curation; Writing – review & editing. R.A. PFAS analysis; Writing – review & editing. J.P.Z. Stable isotope analyses; Writing – review & editing. R.S. Stable isotope analyses; Writing – review & editing. P.W. Sample collection; Writing – review & editing. O.H. Sample collection; Writing – review & editing. I.J.Ø. Sample collection; Writing – review & editing. M.J.S. Sample collection; Writing – review & editing. V.L.B.J. Supervision; Writing – review & editing. T.N. Conceptualization; Supervision; Writing – review & editing.

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