Science of the Total Environment 903 (2023) 166213

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Per- and poly-fluoroalkyl substances in Tawny Owl (*Strix aluco*) feathers from Trøndelag, Norway

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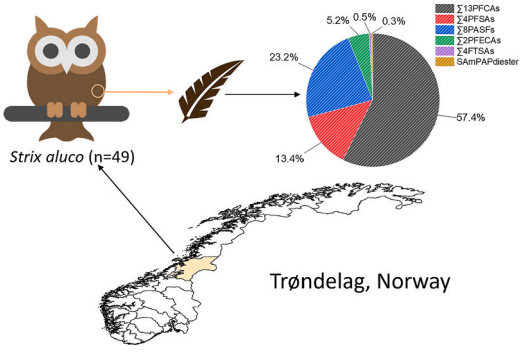
HIGHLIGHTS

- First report on the occurrence of 32 PFASs in Tawny Owl feathers.
- 8 PFASs demonstrated detection rates >50 %, including PFOS.
- PFOA was detected in only 31 % of the feather samples.
- PFHpA was detected in 90 % of the feather samples.
- $\sum_{32}$PFASs concentrations were ranging from 31 to 203 ng/g in the samples.
- No significant differences of PFASs concentrations were observed among the four sampling years (2017–2020).

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Dimitra A Lambropoulou

Keywords:
Wildlife
Tawny Owls
Perfluorochemicals (PFCs)
Polyfluorinated substances
Norway
PFOS

ABSTRACT

Per- and polyfluoroalkyl substances (PFASs) are contaminants of global concern due to their ubiquitous occurrence in the environment, bioaccumulation and the adverse effects on organisms. Tawny Owls (*Strix aluco*) are documented to be exposed to increasing concentrations of perfluoroalkyl carboxylic acids (PFCAs), and have been suggested in literature as a key raptor monitoring species. Therefore, non-destructive biomonitoring efforts are of high interest. Thus far, the use of feathers for biomonitoring PFASs in Tawny Owls has not been investigated. In this study, 32 PFASs were analyzed in 49 Tawny Owl body feather samples collected from 2017 to 2020 in Trøndelag, Norway. There were 30 PFASs detected in at least one feather, with the sum concentrations ranging from 31 to 203 ng/g (w.w.). Perfluoroheptanoic acid (PFHpA) (median: 33 ng/g) and perfluorooctane sulfonamidoacetic acid (FOSAA) (median: 18 ng/g) were the two compounds with the highest concentrations. Perfluorooctane sulfonic acid (PFOS), which is banned for production and use in Norway since 2007, was found in all samples (median: 4.14 ng/g), indicating its high persistence. 8 PFASs were detected in at least 50 % of the samples: FOSAA (11–127 ng/g), PFHpA (<0.04–115 ng/g), perfluorobutanesulfonic acid (PFBS) (<0.28–21 ng/g), PFOS (0.23–13 ng/g), perfluorotridecanoic acid (PFTrDA) (0.24–5.15 ng/g), perfluorododecanoic acid (PFDoDA) (<0.28–4.45 ng/g), perfluoroundecanoic acid (PFUnDA) (<0.28–2.33 ng/g), and 1H,1H,2H,2H-perfluorooctanesulfonic acid (6:2 FTSA) (0.07–1.01 ng/g). No significant differences were found for the

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https://doi.org/10.1016/j.scitotenv.2023.166213
Received 16 May 2023; Received in revised form 5 August 2023; Accepted 8 August 2023
Available online 9 August 2023

concentrations of PFASs between calendar years and locations, but a slight increase could be observed in the sum concentration of PFASs (Σ_{32}PFASs) over the sampling years. As Tawny Owls are residential owls that usually do not cover great distances, their feathers can be used as a potential alternative matrix for future biomonitoring studies. To our knowledge, this is the first study on the occurrence of 32 PFASs investigated in feathers of a Tawny Owl population.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (Wang et al., 2021) and have been produced since the late 1940s (Wang et al., 2017). The high interest on PFASs is due to their ubiquitous occurrence in the environment, bioaccumulation and their adverse effects on organisms (Evich Marina et al., 2022). Feathers as a non-destructive or less destructive matrix have been widely used for monitoring environmental contaminants in birds (Burger and Gochfeld, 1993; Jaspers et al., 2019; Pacyna-Kuchta, 2022; Varagiya et al., 2021). In the recent past, legacy persistent organic pollutant [POPs; e.g., polychlorinated biphenyls and *p*,*p*′-dichlorodiphenyldichloroethylene (*p*,*p*′-DDE)] concentrations in feathers were found to correlate with those in internal tissues (Dauwe et al., 2005; Garcia-Fernandez et al., 2013; Jaspers et al., 2007), while reflecting regional pollution pressures (Jaspers et al., 2009). Nowadays, PFASs as emerging organic pollutants have also been documented in bird feathers (Gomez-Ramirez et al., 2017; Groffen et al., 2020; Jaspers et al., 2013; Meyer et al., 2009; Persson, 2017; Sun et al., 2019). Perfluorooctane sulfonic acid (PFOS) concentrations were reported to be significantly correlated between feathers and livers in Belgian Barn Owls (*Tyti alba*) (Jaspers et al., 2013). A recent study reported that the concentrations of perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoDA) and PFOS were all significantly higher in bird feathers collected from nests near a fluorochemical industry plant, when compared to other locations (Groffen et al., 2020). Although not all PFASs demonstrated significant associations between feathers and internal tissues, feathers can be useful sentinels for PFASs biomonitoring (Groffen et al., 2020).

Several studies have investigated on PFASs occurrence and effects in predatory birds (Briels et al., 2019; Løseth et al., 2019; Sun et al., 2021). A recent study by Sun et al. (2021) reported the relationships between PFASs exposure and thyroid disruption/immune related effects in free-ranging nestling peregrine falcons (*Falco peregrinus*). Other studies on birds indicated significant relationships between concentration profiles of thyroid hormones and PFASs (Løseth et al., 2019; Nøst et al., 2012). The acute and chronic adverse effects of PFASs were systematically studied on mallards (*Anas platyrhynchos*) (Newsted et al., 2006; Newsted et al., 2007b), northern bobwhite (*Colinus virginianus*) (Newsted et al., 2006; Newsted et al., 2007b), chicken (*Gallus gallus*) (Briels et al., 2018) and Japanese quail (*Coturnix japonica*) (Bursian et al., 2021) under controlled exposure scenarios. Egg-injection studies were also

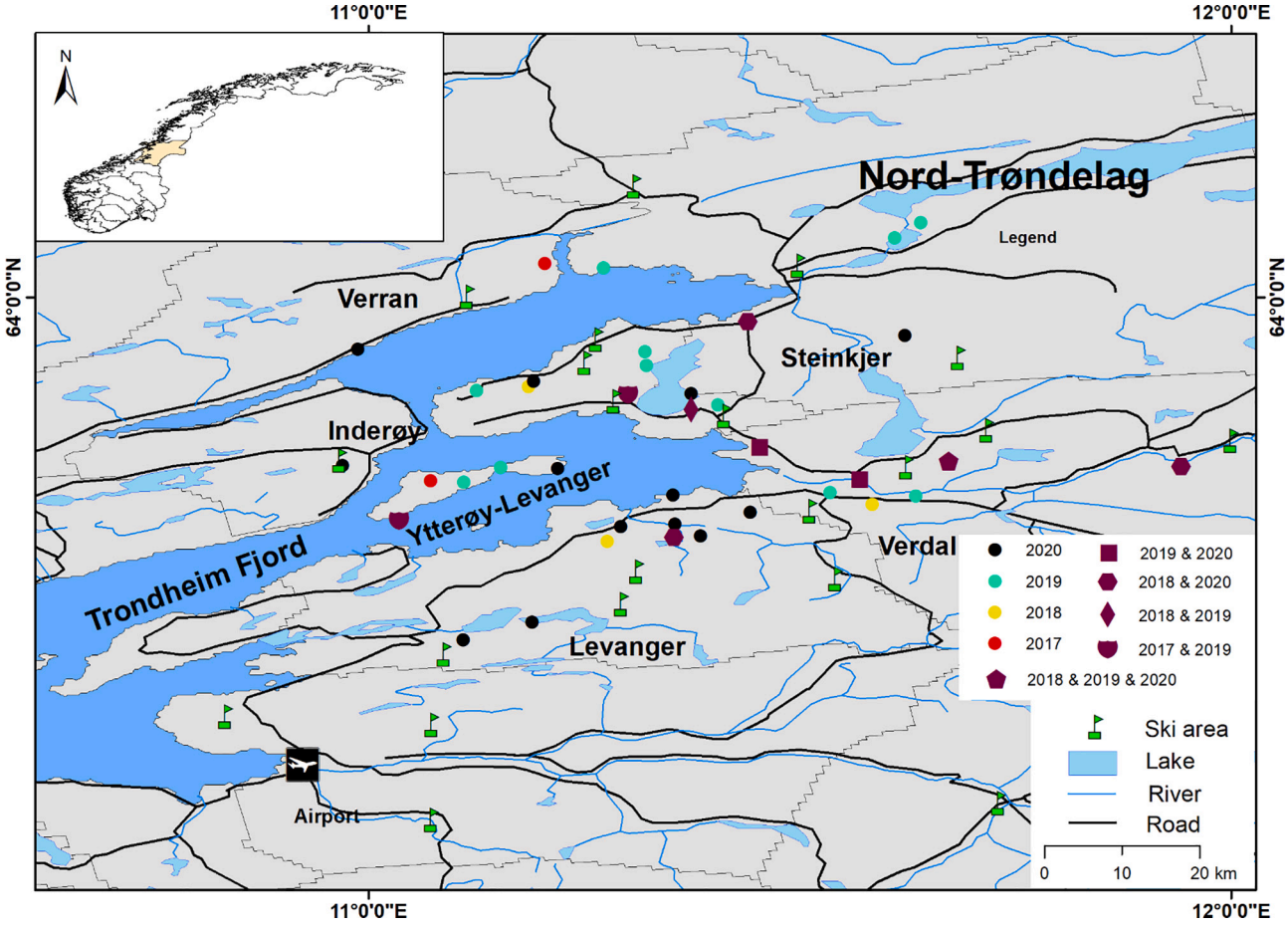

Fig. 1. Locations of Tawny Owl (*Strix aluco*) nest boxes sampled in Trøndelag (Norway) between 2017 and 2020.

conducted with PFASs, assessing the effects on mechanistic and atypical embryonic endpoints in several avian species (Ankley et al., 2021; Briels et al., 2018). Although PFASs data are still considered limited in birds, sulfonate analogues were documented to be more toxic than the carboxylates for similar fluorocarbon-chain lengths, while the 8-carbon chain length PFASs were more toxic than the shorter chain analogues (Ankley et al., 2021).

Recently, the Tawny Owl (*Strix aluco*) was proposed as a key free-ranging species for a harmonized pan-European contaminant monitoring scheme due to its migratory habits, diet, habitat, foraging and positioning in the food web (Badry et al., 2020), In addition, this species is considered residential since it does not cover great distances (König and Weick, 2001), and therefore, it is ideal for reflecting local pollution sources. The Trøndelag county in central Norway represents the northern boundary for the Tawny Owl population in Europe (König and Weick, 2001), while PFASs were previously documented in their eggs (Ahrens et al., 2011; Bustnes et al., 2015; Bustnes et al., 2022; Eriksson et al., 2016), indicating the exposure of Tawny Owls to PFASs.

With this background, the present study aimed to investigate concentrations of 32 PFASs in adult Tawny Owl body feather samples collected in Trøndelag, Norway. The objectives were to: (1) investigate the occurrence of PFASs in the feathers of Tawny Owls; (2) assess the regional patterns of these contaminants along the Trøndelag county; and (3) compare the concentrations found herein to previous studies on PFASs in bird feathers and eggs from Tawny Owls. To our knowledge, this is the first study on the occurrence of 32 PFASs investigated in feathers of a Tawny Owl population.

2. Materials and methods

2.1. Study area and sampling

The sampling of the Tawny Owl feathers used in this study was performed in the northeastern region of the Trondheim fjord (64°N, 11°E). The sampling was performed in the municipalities of Verran (currently part of Steinkjer municipality), Steinkjer, Inderøy, Verdal, and Levanger, and it was part of the BirdLife Norway monitoring program (**Table S1**). Samples were collected from 2017 to 2020 with permission from the Norwegian Food Safety Authority. The nests were located mainly in cultural landscapes including agricultural areas, small towns, industrial areas, roads, and in proximity to ski tracks and a commercial airport, where high concentrations of PFASs were expected. All nest boxes were below 200 m above sea level and are shown in Fig. 1. Feathers were collected from the lower chest of 49 breeding female owls and stored in LDPE zip-lock-bags at −20 °C until analysis. Wing length, body mass, number of eggs and number of nestlings were recorded for all sampled individuals and are presented in **Table S2**. For more information regarding the sampling procedure, see Kroglund (2019).

2.2. Chemicals and materials

Milli-Q ultrapure water was used for cleaning the samples. N-hexane, sodium hydroxide (NaOH, solid), hydrochloric acid (HCl, 37 % v/v) and methanol (MeOH, HPLC grade) used for sample preparation and extraction were purchased from VWR International AS (Oslo, Norway). Acetic acid (glacial, 100 %) and ammonium acetate (NH_4OAc) used for extraction were purchased from Merck (Olso, Norway). Target analyte (TA) standards of PFHxA (C6), PFOA (C8), PFDA (C10), PFDoDA (C12), PFOS (C8), perfluoro-pentanoic acid (PFPeA, C5), -heptanoic acid (PFHpA, C7), -nonanoic acid (PFNA,C9), -undecanoic acid (PFUnDA, C11), -tridecanoic acid (PFTrDA, C13), -tetradecanoic acid (PFTeDA, C14), -butanesulfonic acid (PFBS, C4), -hexanesulfonic acid (PFHxS, C6), and *N*-ethylperfluorooctanesulfonamide (EtFOSA, C10) were purchased from Sigma-Aldrich (Oslo, Norway). The TAs purchased from Chiron AS (Trondheim, Norway) were: perfluorohexadecanoic acid (PFHxDA, C16), perfluorooctane sulfonamide (PFOSA, C8), 2-(*N*-methylperfluorooctanesulfonamido)acetic acid (MeFOSA, C9), *N*-methylperfluorooctanesulfonamidoethanol (MeFOSE, C11), *N*-ethyl-*N*-(2-hydroxyethyl) perfluorooctylsulphonamide (EtFOSE, C12) and 2-(*N*-methylperfluorooctanesulfonamido)acetic acid (MeFOSAA, C11). TAs of sodium 1H,1H,2H,2H-perfluorohexane sulfonate (4:2 FTSA, C6), sodium 1H,1H,2H,2H-perfluorooctane sulfonate (6:2 FTSA, C8), sodium 1H,1H,2H,2H-perfluorodecane sulfonate (8:2 FTSA, C10), and sodium 1H,1H,2H,2H-perfluorododecane sulfonate (10:2 FTSA, C12) were purchased from Cambridge Isotopes Laboratories, Inc. (Tewksbury, USA). The TAs of 7H-Dodecafluoroheptanoic Acid (7HPFHpA, C7) and perfluorooctane sulfonamidoacetic acid (FOSAA, C10) were purchased from Toronto Research Chemicals (Ontario, Canada). The standards of perfluoro-3,7-dimethyloctanoic acid (P37DMOA, C10), and perfluoroheptanesulfonic acid (PFHpS, C7) were purchased from Dr. Ehrenstorfer (Oslo, Norway). 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (Gen-X, C6) was purchased from AccuStandard (New Haven, USA). The TAs of sodium dodecafluoro-3H-4,8-dioxanonanoate (ADONA, C7), potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS, C8), and sodium *N*-ethyl-*N*-[2-(phosphonooxy)ethyl]perfluorooctanesulfonamide (SAmPAPdiester, C12) were purchased from Wellington Laboratories (Jonsered, Sweden). Internal standards (IS) of $^{13}C_8$-perfluorooctanoic acid ($^{13}C_8$-PFOA, 99 %) and $^{13}C_8$-perfluorooctanoic sulfonate sodium salt ($^{13}C_8$-PFOS, 99 %) were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, USA). All standards were stored at −20 °C. ENVI-carb (Superclean SPE bulk pack) used for extraction was purchased from Sigma-Aldrich (Oslo, Norway). 1.7 mL polypropylene (PP) tubes were purchased from Eppendorf Norge A/S (Oslo, Norway). 15 mL PP tubes were purchased from VWR International AS (Oslo, Norway).

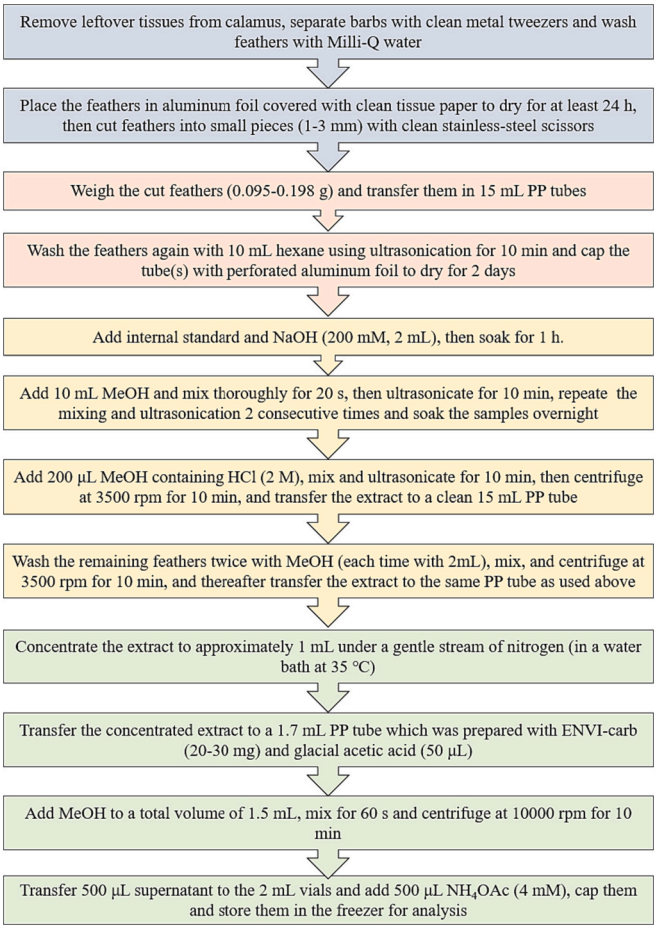

Fig. 2. The workflow of the sample preparation for PFASs in bird feathers.

2.3. Sample preparation

The feather samples for analysis ($n = 49$) were all washed following the same procedure as presented by Jaspers et al. (2007). The feathers were measured (5–15 cm), and washed with water (Milli-Q, ultra-pure) in a large petri dish, while the left-over tissue was removed from the calamus (Fig. 2). Barbs were separated with clean metal tweezers and were washed thoroughly before placing them in aluminum foil trays covered with clean tissue paper to dry. After drying, the feathers were cut into small pieces (1–3 mm) with clean stainless-steel scissors and accurately weighed. All equipment used for washing and cutting feathers were consecutively cleaned with water (Milli-Q, ultra-pure) and MeOH between the processing of each sample.

The extraction protocol was used as previously described with only minor modifications (Jaspers et al., 2013) (Fig. 2). The cut feathers were transferred and weighed (0.095–0.198 g) in 15 mL PP tubes. All samples were washed with 10 mL hexane followed by ultrasonication for 10 min. The hexane was decanted, and the sample was capped with aluminum foil (with punctured small holes) and was left to dry. The mixture of internal standards ($^{13}C_8$–PFOA and $^{13}C_8$–PFOS; 20 μL of 1 μg/mL) was added to each sample followed by the addition of NaOH dissolved in MeOH (200 mM, 2.00 mL), and the samples were thoroughly mixed and left to soak for 1 h. Then 10 mL MeOH were added to each sample, thoroughly mixed for 20 s and ultrasonicated for 10 min. The mixing and ultrasonication was repeated 2 consecutive times before the samples were left to soak overnight. Thereafter, HCl dissolved in MeOH (2 M, 200 μL) was added to each sample after which thorough mixing and ultrasonication for 10 min was performed. Following the ultrasonication, samples were centrifuged at 3500 rpm for 10 min. The extracts were transferred to clean 15 mL PP tubes, while the remaining feather samples were washed twice with MeOH (2 × 2 mL), including vortex mixing and centrifugation (at 3500 rpm for 10 min) in each wash. These extracts (~4 mL) were also collected and combined with those collected earlier (total extraction volume ~14 mL). The extract was evaporated to approximately 1 mL under a gentle stream of nitrogen (in a water bath at 35 °C).

Clean-up was carried out in 1.7 mL PP tubes containing 25 mg of Super clean ENVI-carb and 50 μL of glacial acetic acid. The concentrated extracts were transferred to these tubes and MeOH was added to a total volume of 1.5 mL in every tube before the samples were vortex mixed for 60 s and centrifuged at 10,000 rpm for 10 min. Exactly 500 μL of extract from each sample were transferred to an LC vial, and ammonium acetate solution (4 mM, 0.5 mL) was added before the vials were capped and stored at −20 °C until analysis.

2.4. Instrumental analysis

The separation of the 32 PFASs was performed using a Waters Acquity UHPLC system (Waters, Milford, USA) with a column manager, flow through needle sample manager and binary solvent manager. The chromatographic column used was a Kinetex C18 column (30 × 2.1 mm, 1.3 μm, 100 Å. Phenomenex, Værløse, Denmark) serially connected to a Phenomenex guard column (C18, 10 × 2.1 mm). Water with 2 mM ammonium acetate (A) and MeOH (B) were used as mobile phase. The mobile phase gradient used is described in **Table S3**. The flow rate was 0.25 mL/min and the volume of the injection was 4 μL. PFASs were determined by a triple quadrupole mass spectrometer Xevo-TQS (MS/MS; Waters, Milford, USA). Negative ion multiple reaction monitoring (MRM) mode was used for the identification of the TAs. The capillary potential applied was 2 kV. The cone gas (N_2) was set at a flow rate of 150 L/h. The desolvation temperature was set at 450 °C and the desolvation gas flow rate at 650 L/h. The nebuliser was set at 6 bar and the temperature in the source was maintained at 150 °C.

Table 1
Concentrations of PFASs in feather samples (ng/g, w.w.) from Tawny Owls (*Strix aluco*; $n = 49$) from Trøndelag, Norway.

	Min	Max	Median	Mean	DR[a] (%)
PFPeA	<0.42	44	0.42	3.81	24
PFHxA	<0.42	8.9	0.42	0.97	22
PFHpA	<0.04	115	33	36	90
7HPFHpA	<0.04	2.2	0.04	0.18	8.16
PFOA	<0.28	5.32	0.28	0.96	31
PFNA	<0.04	26	0.04	1.17	8.16
PFDA	<0.04	3.26	0.04	0.4	24
P37DMOA	<0.28	0.52	0.28	0.29	4.08
PFUnDA	<0.28	2.33	0.5	0.66	57
PFDoDA	<0.28	4.45	0.65	0.89	67
PFTrDA	0.24	5.15	2.12	2.27	100
PFTeDA	<0.42	2.71	0.42	0.59	33
PFHxDA	<0.42	1.52	0.42	0.45	4.08
$\sum_{13}$PFCAs	3.37	119	47	48	100
PFBS	<0.28	21	3.2	4.98	69
PFHxS	<0.04	9.98	0.04	1.36	35
PFHpS	<0.04	5.69	0.04	0.55	16
PFOS	0.23	13	4.14	4.48	100
$\sum_4$PFSAs	0.59	28	11	11	100
PFOSA	<0.28	0.8	0.28	0.29	2
MeFOSA	<0.04	0.18	0.04	0.05	16
FOSAA	11	127	18	23	100
EtFOSA	<0.04	0.62	0.04	0.07	20
MeFOSAA	<0.14	0.37	0.14	0.15	10
MeFOSE	<4.2	<4.2	<4.2	<4.2	0
EtFOSE	<0.1	2.9	0.1	0.16	6.12
$\sum_8$PASFs	12	128	19	24	100
6:2 FTSA	0.07	1.01	0.37	0.4	100
8:2 FTSA	<0.04	0.29	0.04	0.05	2.04
4:2 FTSA	<0.04	<0.04	<0.04	<0.04	<0.04
10:2 FTSA	<0.28	<0.28	0	<0.28	0
$\sum_4$FTSAs	0.11	1.05	0.42	0.45	100
GenX	<4.2	49	4.2	7.16	18
ADONA	<0.04	0.47	0.04	0.08	33
$\sum_2$PFECAs	4.24	49	4.24	7.23	39
SAmPAPdiester	<0.28	0.55	0.28	0.29	4.1
9Cl-PF3ONS	<0.04	<0.04	<0.04	<0.04	0

[a] DR = detection rate.

2.5. Quality assurance/quality control

Quality assurance/Quality control (QA/QC) samples were prepared using feathers from 49 samples pooled together and distributed to 13 samples (3 standard pooled samples, 6 pre-extraction spiked samples and 4 post-extraction matrix matched samples), and additionally preparing 3 reagent (solvent) blanks. Most target PFASs presented (relative) recoveries % ranging from 90 to 130 % (**Table S4**). Triplicate analysis of samples fortified at 10 and 20 ng/mL (concentration in the vial prior to analysis) showed the relative standard deviation (RSD%) ranging from 1 to 18 % with an average of 7 % for all TAs, except for 4 TAs, GenX, EtFOSE, SAmPAPdiester and PFHxDA that presented 24, 25, 33 and 33 % RSD, respectively, at the low fortified concentration (**Table S5**). Calibration curves were prepared with the TAs at concentrations ranging from 0.01 to 20 ng/mL (0.01, 0.05, 0.10, 0.20, 0.50, 1.00, 2.00, 5.00, 10.0, 20.0). All PFASs calibration curves showed excellent correlation coefficients (R^2) > 0.98. The limit of quantification (LOQ) was set at the lowest point of the calibration curve, while the limit of detection (LOD) was estimated as LOQ/3. For each PFAS the LOD was higher than 3 times the noise level. The LODs ranged from 0.003 to 0.3 ng/mL (**Table S6**). The method limit of detection (mLOD) and method limit of quantification (mLOQ) for each TA were estimated from the respective instrumental LOD and instrumental LOQ at a nominal sample weight mass of 0.1 g. The mLODs and mLOQs of the PFASs ranged from 0.06 to 6 ng/g and from 0.2 to 18 ng/g (**Table S6**), respectively.

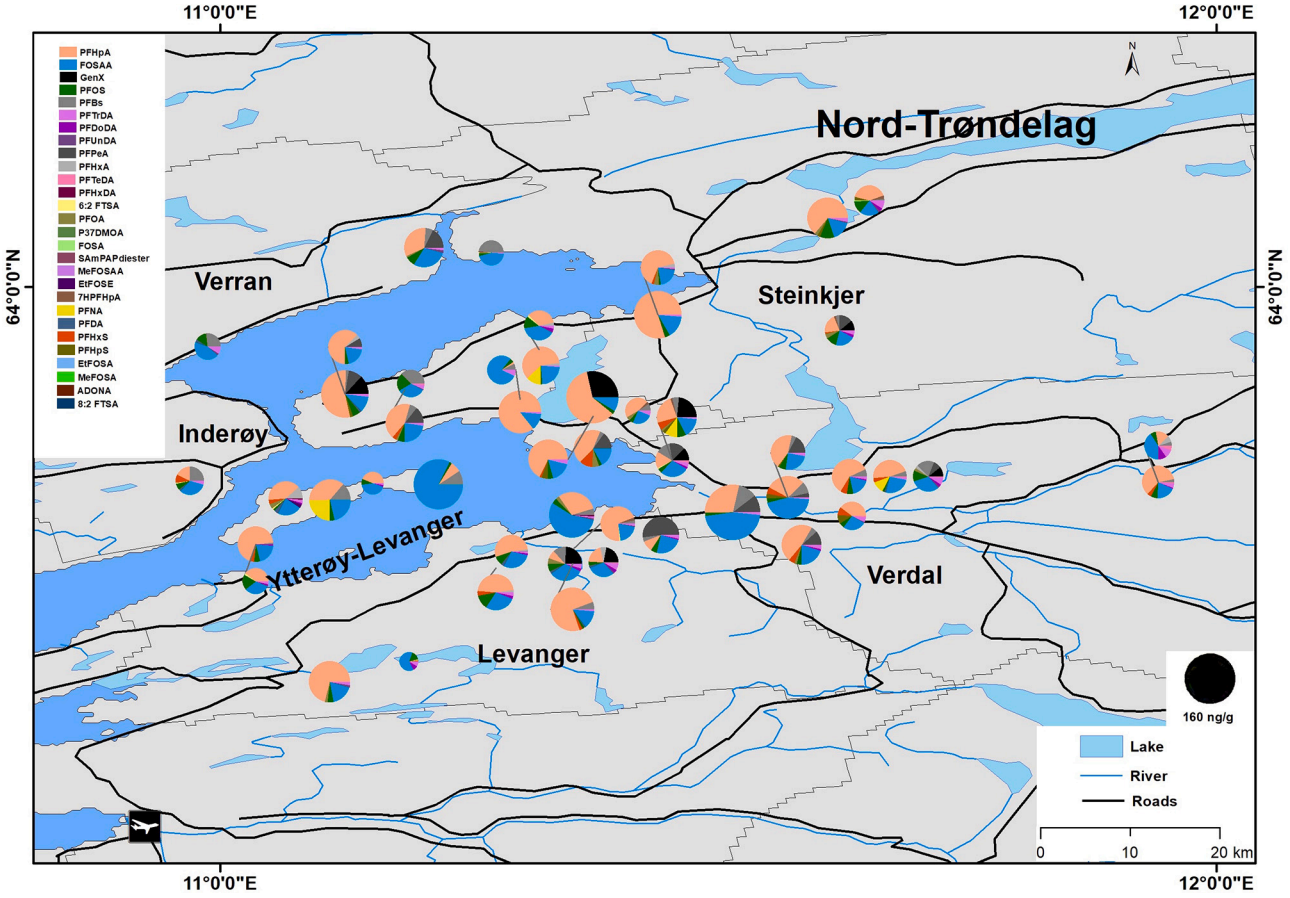

Fig. 3. Occurrence and distribution of PFASs in Tawny Owl (*Strix aluco*) feathers from Trøndelag (Norway) between 2017 and 2020.

2.6. Data treatment

MassLynx v4.1 (Waters, Milford, USA) was used to acquire UHPLC-MS/MS data and TargetLynx was used for integrations. Data was processed with Microsoft Excel 2019 (Washington, USA) and Origin 9.0 (Northampton, USA). Statistical analysis was performed in SPSS 22 (IBM, Armonk, USA). Data < mLOD was substituted with one-half the square root of the mLOD. Concentrations were reported as ng/g wet weight (w.w.) since freeze-drying was not involved in the sample preparation of the feathers. Normality tests were performed with the Shapiro-Wilk test. Data (concentration values) were log-transformed prior to performing principal component analysis (PCA) and correlation analysis. Correlations among PFASs with detection frequency above 50 % were performed using Pearson correlation as well as between PFASs concentrations and the biometrics of the birds.

3. Results and discussion

3.1. PFASs concentrations in Tawny Owl feathers

The sum concentration of PFASs ($\sum_{32}$PFASs) ranged from 31 to 203 ng/g (median: 83 ng/g). All PFASs were determined in at least one feather sample, except for MeFOSE, 9Cl-PF3NOS, 4:2 FTSA and 10:2 FTSA that were not detected (Table 1). Perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs), perfluoroalkane sulfonyl fluorides (PASFs) and fluorotelomer sulfonic acids (FTSAs) were detected in all feather samples, and perfluoroalkyl ether carboxylic acids (PFECAs) were found in 19 feather samples (Table 1 & **Table S7**). PFCAs accounted for 57.4 % of all PFASs based on median concentrations followed by PASFs (23.2 %), PFSAs (13.4 %), PFECAs (5.2 %), FTSAs (0.5 %) and SAmPAPdiester (0.3 %) (**Fig. S1**). 8 PFASs demonstrated detection rates (DRs) above 50 %, including 4 PFCAs (PFHpA, PFTrDA, PFDoDA, PFUnDA), 2 PFSAs (PFOS, PFBS), 1 FTSA (6:2 FTSA), and 1 PASF (FOSAA) (Table 1). PFHpA (median: 33 ng/g) and FOSAA (median: 18 ng/g) were the two PFASs with the highest concentrations. PFHpA is a 7 carbon PFCA which is a metabolite of longer chained PFASs (Johnson et al., 2021), and its concentrations ranged from <0.04 to 115 ng/g. The concentrations of FOSAA ranged from 11 to 127 ng/g. FOSAA is not a standalone commercial product (Zhao et al., 2019), but it derives from EtFOSE (Yeung et al., 2013), which is an important manufacturing intermediate used in fluorosurfactant processes (Westbury, 2014), including the manufacture of EtFOSE-based surfactants that are used in food contact paper (Yeung et al., 2013). PFOS, which has been banned for production and use in Norway since 2007 (Gomez-Ramirez et al., 2017), was found in all samples (0.23–13 ng/g), indicating its high persistence, even though concentrations were reported declining in eggs of Tawny Owls from Norway from 1986 to 2019 (Bustnes et al., 2022). However, it is noteworthy that PFOS was found in all the sampled eggs of Tawny Owls from 2019 (Bustnes et al., 2022). The concentration of PFBS ranged from <0.28 to 21 ng/g. PFBS has been used as a substitute for PFOS since 2003 (Chengelis et al., 2009; Newsted et al., 2007a). The most common chemical used as an alternative to PFOS is 6:2 FTSA, and in recent years it has been increasingly used in electroplating and in the production of firefighting foams (Jin et al., 2017); the concentration of 6:2 FTSA ranged herein from 0.07 to 1.01 ng/g. Regarding PFCAs, PFTrDA, PFDoDA and PFUnDA are all long chain PFCAs, and their concentrations ranged between 0.24–5.15, <0.28–4.45, and <0.28–2.33 ng/g, respectively. In comparison, PFOA, which was added to the Stockholm Convention POP list in 2019 (Convention, 2019), was detected in only 31 % of the samples with concentrations ranging from <0.28 to 5.32 ng/g. PFTrDA concentrations were significantly correlated with those of PFDoDA (r = 0.468, p = 0.001). This can be

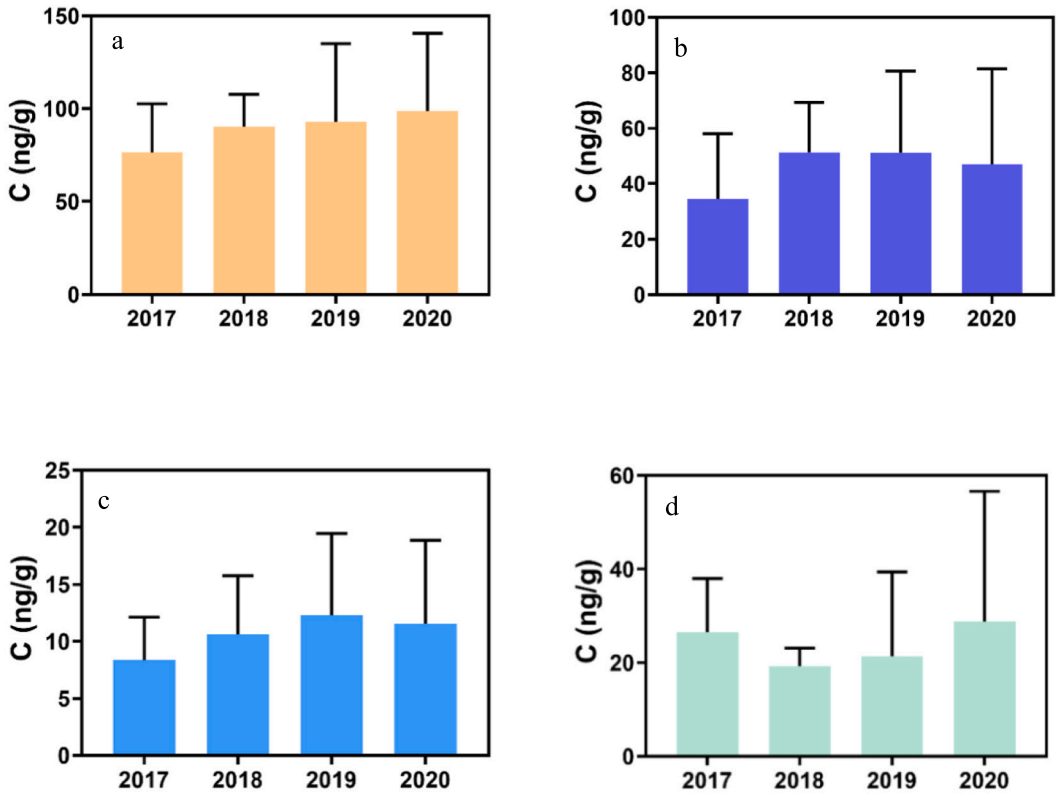

Fig. 4. Mean concentrations with standard errors of PFASs (a), PFCAs (b), PFSAs (c) and PFAFs (d) in Tawny Owl (*Strix aluco n* = 49) feathers from the different sampling years.

attributed to the fact that both PFTrDA and PFDoDA are degradation products of 12:2 fluorotelomer alcohol (Langberg et al., 2022). In addition, positive correlations were also found between the concentration of PFBS and FOSAA ($r = 0.458$, $p = 0.001$), and PFOS and PFTrDA ($r = 0.295$, $p = 0.040$), suggesting concomitant source of exposure for those. PFBS and FOSAA can both be biotransformed from EtFOSA in plants (wheat, soybean and pumpkin) (Zhao et al., 2018).

3.2. PFASs in relation to spatiotemporal and biological factors

The occurrence profile of PFASs at each sampling location (based on median concentrations) is shown in Fig. 3. There is an airport approximately 35 km northwest of the sampling area, which was identified as a potential source of PFASs (Ahrens et al., 2015). The concentrations of Σ_{32}PFASs were 41–166, 32–138, 52–147, 58–208, 53–111 and 50–183 ng/g in feathers collected from Ytterøy-Levanger (35 km), Levanger (35 km), Inderøy (40 km), Verdal (45 km), Verran (54 km) and Steinkjer (66 km from the airport), respectively. There were no significant differences among the feathers from the different locations ($p > 0.05$). Similarly, no differences could be observed in the PCA (**Fig. S2**), which was calculated by the PFASs with DR above 50 %. A possible reason for this is that the sampling areas are all located in a circular area around the fjord (about 7800 km^2 in total area) further away from the airport (Fig. 1). In addition, many ski areas that are scattered around the sampling area, can be considered hot spots for PFASs contamination since these chemicals can be used in ski waxes (Carlson and Tupper, 2020; Fang et al., 2020). The $\sum_{32}$PFASs concentrations were ranging from 52 to 111, 57 to 115, 41 to 208 and from 32 to 183 ng/g in the samples collected in 2017, 2018, 2019, and 2020, respectively (Fig. 4). There were no significant differences of PFASs concentrations among the four sampling years ($p > 0.05$). This finding was similar to the results of Persson (2017) who analyzed PFASs in feathers of Eurasian Eagle-Owls (*Bubo bubo*) collected in 1979, 1989, and 2013–2016 (Persson, 2017). In addition, it is important to consider that the short duration of the sampling period may influence the concentration trend of PFASs. While Sun et al. (2019) observed a clear temporal trend of PFASs in the body feathers of White-Tailed Eagles (*Haliaeetus albicilla*) from Norway between 1968 and 2015, such trend may not be evident in a randomly selected four-year period. However, a slight increase with the sampling year could be found for the concentrations of PFASs (Fig. 4a), PFCAs (Fig. 4b) and PFSAs (Fig. 4c), although not statistically significant. Differences observed between sampling times at the same location can be explained by moulting since the body feathers of the Tawny Owl are moulted annually (Jenni and Winkler, 2020).

No significant correlations were found between the concentrations of PFASs in feathers and the body weight ($r = -0.027$, $p = 0.852$) nor the wing length ($r = 0.098$, $p = 0.503$) of the birds. Some lab studies indicated an adverse effect of PFOS or PFASs on birds' body weight (Dennis et al., 2020; Newsted et al., 2006). For example, the northern bobwhite quail (*Colinus virginianus*) chick was heavier after 21 days of PFOS exposure compared to the control group (Dennis et al., 2020). However, the above-mentioned studies were lab exposure studies which provided the birds with food or water fortified with PFASs, and therefore, direct comparison to the feather concentrations found in the current study is not possible.

3.3. Comparisons with other studies

There were several studies that reported the occurrence of PFASs in bird feathers (Table 2). Some of the results from previous studies were reported on dry weight, after freeze drying the feathers. But the moisture of feathers was approximately 11 % (Merritt, 2016). Hence there is no large difference between wet and dry weight and the concentration results can be compared among studies. PFOS was the compound reported

Table 2
Concentrations of PFASs (ng/g ww) reported in bird feathers in literature and in the present study[a].

	Country	Collecting time	Type	PFOA	PFHpA	PFTrDA	PFDoDA	PFUnDA	PFOS	PFBS	6,2-FTSA	FOSAA	Ref.
Tawny Owl (*Strix aluco*)	Norway	2017–2020	Body	<0.28–5.32	<0.04–115	0.24–5.15	<0.28–4.45	<0.28–2.33	0.23–13	<0.28–21	0.07–1.01	11–127	This study
Eurasian Eagle-Owls (*Bubo bubo*)[b]	Norway	1979,1989, 2013–2016	Wing	<0.20–1.10	<0.20–0.36	<0.20–19.76	<0.10–7.85	<0.10–10.90	3.43–73.9	<0.30–0.76	0.002–1.33	<0.05	Persson, 2017 Monclús et al., 2022
Grey Heron (*Ardea cinerea*)	Belgium	2005	Wing	<7.3	N.A.	N.A.	N.A.	N.A.	250[c]	N.A.	N.A.	N.A.	Meyer et al., 2009
Eurasian Sparrowhawk (*Accipiter nisus*)				<7.3	N.A.	N.A.	N.A.	N.A.	100[c]	N.A.	N.A.	N.A.	
Herring Gull (*Larus argentatus*)				<7.3	N.A.	N.A.	N.A.	N.A.	80[c]	N.A.	N.A.	N.A.	
Eurasian Magpie (*Pica pica*)				<7.3	N.A.	N.A.	N.A.	N.A.	40[c]	N.A.	N.A.	N.A.	
Eurasian Collared Dove (*Streptopelia decaocta*)				<7.3	N.A.	N.A.	N.A.	N.A.	60[c]	N.A.	N.A.	N.A.	
Belgian Barn Owls (*Tyto alba*)	Belgium	2008–2009	Wing	<14.1–670	N.A.	<39.6	<30.4	<20.6	<2.2–56.6	N.A.	N.A.	N.A.	Jaspers et al., 2013
White-tailed Eagle (*Haliaeetus albicilla*)	Norway	2014	Body	0.10–0.61	N.A.	0.71–2.32	0.17–0.40	0.63–1.99	1.89–16.38	N.A.	N.A.	N.A.	Gomez-Ramirez et al., 2017
Acciptier[b]	China	–	Wing	<0.27	<0.21	<0.38	<0.34	<0.27	0.35–9.44	0.35–3.18	N.A.	N.A.	Li et al., 2018
Northern Goshawk (*Accipiter gentilis*)	Norway	2015–2016	Body	0.07–0.83	<0.002	<0.029–4.04	<0.029–2.62	<0.029–1.82	<0.029–9.38	N.A.	N.A.	N.A.	Briels et al., 2019
White-Tailed Eagle (*Haliaeetus albicilla*)	Norway	2015–2016	Body	<0.029–0.91	<0.002	<0.029–2.02	<0.029–0.78	<0.029–1.07	<0.22–90.2	N.A.	N.A.	N.A.	Løseth et al., 2019
White-Tailed Eagle (*Haliaeetus albicilla*)	Norway	1971–2015	Body	<0.006–3.0	N.A.	<0.02–10.7	<0.02–3.2	<0.06–3.2	<0.01–43.4	N.A.	N.A.	N.A.	Sun et al., 2019
	Sweden	1968–2011	Body	<0.006–3.5	N.A.	<0.02–3.1	<0.02–1.3	<0.06–1.3	1.0–38.4	N.A.	N.A.	N.A.	
	Greenland	1984–2013	Body	<0.006–2.1	N.A.	<0.02–9.3	<0.02–1.1	0.6–4.4	1.3–7.9	N.A.	N.A.	N.A.	
Great Tit (*Parus major*)[d]	Belgium	2015–2016	Body	698	<mLOQ	1.7	9.5	26	217737	134	N.A.	N.A.	Groffen et al., 2020
Snowy Sheathbill (*Chionis albus*)[b]	Antarctic Peninsula	2010–2011, 2012–2013	Body	1.11–1.74	N.A.	<0.17	<0.32–0.39	<1.41–1.43	<0.98	N.A.	N.A.	N.A.	Padilha et al., 2022
South Polar Skua (*Stercorarius maccormicki*)[b]				<1.06–1.9	N.A.	<0.17–1.99	<0.32–1.69	<1.41–3.96	<0.98–2.62	N.A.	N.A.	N.A.	
Kelp Gull (*Larus dominicanus*)[b]				<1.06–2.82	N.A.	<0.17	<0.32	<1.41–2.31	<0.98	N.A.	N.A.	N.A.	
Southern Giant Petrel (*Macronectes giganteus*)[b]				<1.06–1.57	N.A.	<0.17	<0.32	<1.41–2.14	<0.98	N.A.	N.A.	N.A.	
Antarctic Tern (*Sterna vittata*)[b]				<1.06–1.68	N.A.	<0.17	<0.32	0.73–2.35	<0.98	N.A.	N.A.	N.A.	
Adélie (*Pygoscelis adeliae*)[b]				0.20–1.94	N.A.	<0.17–0.33	<0.32–0.61	<1.41–1.71	<0.98	N.A.	N.A.	N.A.	
Chinstrap (*P. antarcticus*)[b]				0.20–1.97	N.A.	<0.17	<0.32	<1.41–2.47	<0.98	N.A.	N.A.	N.A.	
Gentoo (*P. papua*)[b]				0.14–1.63	N.A.	<0.17–1.01	<0.32–0.59	<1.41–1.90	<0.98	N.A.	N.A.	N.A.	
Gentoo penguins (*Pygoscelis papua*) and southern giant petrels (Macronectes giganteus)	Antarctic Peninsula	2009	Body	N.D.[e]	1.63–2.85	N.D.	N.D.	N.D.	N.D.-<0.3	N.D.-<0.03	N.A.	N.A.	Alava et al., 2015

[a] Only PFASs with DR >50% in the present study are included. N.A. = not analysed.
[b] Concentrations in ng/g dw (feathers were freeze dried).
[c] Concentrations estimated from a graph in the published study.
[d] Median concentrations.
[e] None detected.

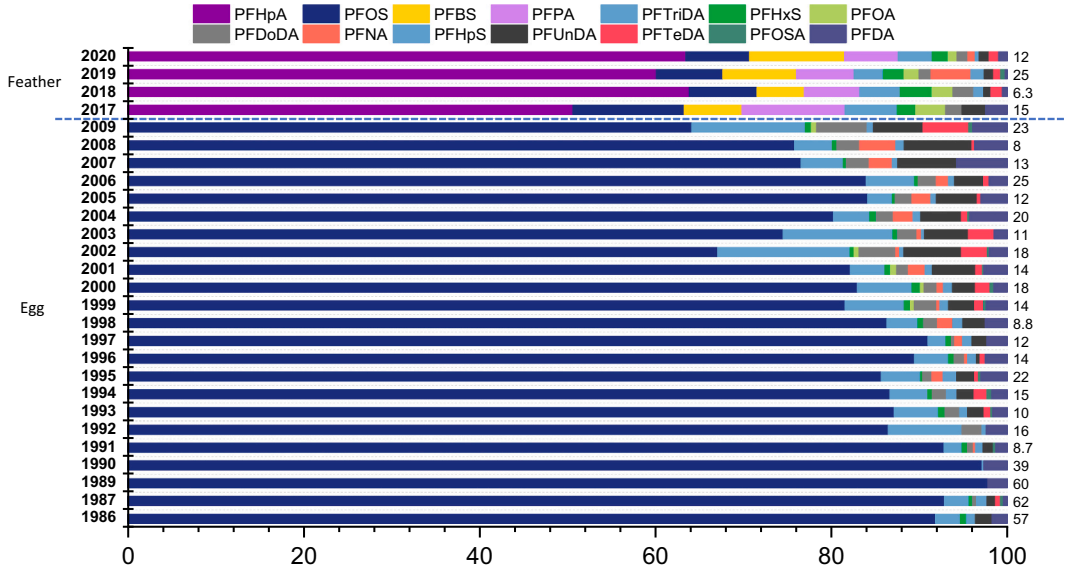

Fig. 5. Distribution of PFASs in feathers (2017–2020; this study) and in eggs (1986–2009; data collected from Ahrens et al., 2011) from Tawny Owl (*Strix aluco*) from Trøndelag (Norway).

most frequently (Table 2). The concentration of PFOS in this study was similar to those in feathers from other predatory birds, such as the Northern Goshawk (*Accipiter gentillis;* from Norway) (Briels et al., 2019), White-tailed Eagle (*Haliaeetus albicilla*; from Norway, Greenland and Sweden) (Sun et al., 2019) and *Accipiter* species (China) (Li et al., 2018). The concentrations were higher than those reported in seabird (*Pygoscelis penguins*, *Stercorarius maccormicki*, and *Macronectes giganteus*) feathers collected from the Antarctic Peninsula (Padilha et al., 2022). However, the concentrations of PFOS were lower than those reported in feathers from a songbird (*Parus major*) in Belgium, which can be attributed to a fluorochemical plant producing PFASs in proximity to the specific sampling site in Belgium (Groffen et al., 2020).

Although there have been no studies reporting the occurrence of PFASs in Tawny Owl feathers, three studies analyzed PFASs in eggs of Tawny Owls collected from Norway between 1986 and 2019 (Ahrens et al., 2011; Bustnes et al., 2015; Bustnes et al., 2022), while one study also analyzed PFASs in eggs of Tawny Owls from Sweden in 2014 (Eriksson et al., 2016). The concentrations in eggs from those studies could be compared to those found in the feathers in this study, as the feathers in the current study were collected solely from breeding female Tawny Owls. The comparison of the concentrations in eggs and feathers is presented in the Supporting Information (**Table S8)**. The concentration of PFOS in eggs (1.93–49.1 ng/g) was higher than those measured in feathers (0.23–13 ng/g), while the concentration of PFOA was higher in feathers (<0.28–5.32 ng/g) than those in eggs (<0.03–0.35 ng/g). Jaspers et al. (2013) reported a similar trend, in which the concentration of PFOA was higher in the tail feathers than that in soft tissues.

The occurrence profile of PFASs in eggs and feathers was also compared (Fig. 5). PFOS was the dominant compound in eggs from 1986 to 2009, and PFHpA, a short chain PFCAs, was the dominant analogue in feathers from 2017 to 2020 (Fig. 5). PFHpA was also detected in five feather samples from Gentoo penguins (*Pygoscelis papua*) and southern giant petrels (*Macronectes giganteus*), with concentrations ranging from 1.63 to 2.85 ng/g (w.w.), while the presence of other PFASs was not detected (Alava et al., 2015). PFHpA was also found in the liver and muscle of Antarctic fur seal (*Arctocephalus gazella*) pups and the eggs of Adélie penguin (*Pygoscelis adéliae*, *Pygoscelis papua*) (Schiavone et al., 2009). Compared to the PFOS concentrations in eggs, the proportion of PFOS was not high in feathers (Fig. 5). PFOS was one of the most used PFASs until it was banned in Norway in 2007 (Gomez-Ramirez et al., 2017). A recent study reported that the transfer to eggs is increasing with PFASs carbon chain length, and therefore, the longer chain PFASs were found in higher abundance in eggs (Jouanneau et al., 2022).

3.4. Implications and limitations

To the best of our knowledge, this is the first study on the occurrence of 32 PFASs in Tawny Owl feathers. As we mentioned before, Tawny Owls are residential owls that usually do not cover great distances. Therefore, the feathers of Tawny Owls could be a suitable biomonitoring matrix for future monitoring studies. In addition, Tawny Owls are found in a wide range of habitats from deciduous and mixed forests with openings to urban areas like parks, large gardens and church towers (Mikkola, 2013), which further entails that the Tawny Owl can be a suitable species to monitor terrestrial regional pollution. Some studies have reported correlations of PFOS in feathers and tissues (Briels et al., 2019; Groffen et al., 2020). However, the correlations of other PFASs in feathers and tissues was previously found challenging (Jaspers et al., 2019; Løseth et al., 2019). In addition, the potential external contamination on the feathers cannot be neglected even though protocols are in place for washing and removing background contamination efficiently (Jaspers et al., 2019; Løseth et al., 2019). Therefore, future studies should be performed on feathers and internal tissues from the same individuals to validate any relations to internal organ(s) contaminant concentrations in the Tawny Owl.

CRediT authorship contribution statement

Junjie Zhang: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. **Veerle L.B. Jaspers:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. **Jonas Røe:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Gabriela Castro:** Methodology, Resources, Writing – review & editing. **Ingvild B. Kroglund:** Investigation, Resources, Writing – review & editing. **Susana Villa Gonzalez:** Resources, Writing – review & editing. **Jan Eivind Østnes:** Investigation, Resources, Writing – review & editing. **Alexandros G. Asimakopoulos:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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

Data will be made available on request.

Acknowledgements

This project was supported financially by the Departments of Chemistry and Biology at the Norwegian University of Science and Technology. Samples were collected with permission from the Norwegian Food Safety Authority (animal ethics approval numbers - FOTS ID - 12024 and 23120). All sample chemical analysis was conducted at the Department of Chemistry, NTNU.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166213.

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