PER- AND POLYFLUOROALKYL SUBSTANCES IN PLASMA AND FEATHERS OF NESTLING BIRDS OF PREY FROM NORTHERN NORWAY

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ABSTRACT

Plasma samples from nestlings of two top predators, White-tailed eagle (*Haliaeetus albicilla*) and Northern goshawk (*Accipiter gentilis*) from northern Norway were analysed for a wide range of PFASs. Body feathers from the White-tailed eagles were also analysed and significant associations between specific PFASs in blood plasma and body feathers were found ($0.36 < R^2 < 0.67$; all $p < 0.05$). This result suggests that analysing body feathers of White-tailed eagle could potentially be a useful non-invasive strategy to monitor PFASs exposure in nestlings of this species. White-tailed eagles showed significantly higher levels of contaminants than Northern goshawks (plasma ∑PFASs Median = 45.83 vs 17.02 ng mL^{-1}, $p < 0.05$). The different exposure between both species seemed to be related to different dietary input, as quantified by stable carbon and nitrogen isotope analysis of body feathers. *A priori*, the bird of prey populations studied are not at risk for PFASs, since the levels in plasma of both species were hundreds to thousand times lower than the toxic reference values reported for predatory birds. However, further studies on larger sample sizes are needed to confirm this hypothesis since toxic thresholds for nestling birds of prey are not established.

KEYWORDS

Predatory birds; PFASs; stable isotopes; plasma; feathers; biomonitoring

1. Introduction

Per- and polyfluoroalkyl substances (PFASs; Buck et al., 2011) are a large group of chemicals commonly used for the past six decades due to their water and oil repellence, thermal stability, and surfactant properties (Lindstrom et al., 2011). Their wide application in industrial and consumer-use products (i.e. non-stick cookware, waterproof and breathable textiles, aqueous film forming foams to fight electrical fires and protective coatings for paper, food packing materials, and carpets) has raised concerns because many of them are persistent, bioaccumulative and toxic (PBT). Moreover, these compounds can be considered ubiquitous, since residues have been found worldwide, even in remote areas as the Arctic, both in biotic and abiotic samples (Lau et al., 2007). Due to their ubiquity and potential to cause adverse effects in animal and human health, the number of studies concerning PFASs has considerably increased in this decade. According to these studies, diet is the main source of exposure to PFASs (Domingo and Nadal, 2017). As a consequence of this concern, some legislative and voluntary actions to reduce emissions have been taken since 2002, such as the inclusion of perfluorooctane sulfonate (PFOS) and its salts in Annex B of the Stockholm Convention on Persistent Organic Pollutants (POPs; UNEP, 2009). However, fluorinated alternatives do not seem to be a safer option due to their high persistence and toxic potential (Wang et al, 2015).

To assess the effectiveness of such restrictions and the current levels of chemicals in the environment, biomonitoring using birds has been very useful (Gómez-Ramírez et al., 2014). Furthermore, when biomonitoring activities include the study of effects, new data can be obtained on adverse effects in a range of sensitive animal species (García-Fernández and María- Mojica, 2000; García-Fernández et al., 2008; National Research Council, 1991). While the toxic effects of “legacy compounds” (i.e. organochlorine pesticides, PCBs or metals) have frequently been studied in birds, the knowledge about the potential effects of emerging compounds such as several PFASs is scarce. However, both field and laboratory studies have shown evidence of alterations at the biochemical level in birds, among which hepatotoxicity, immunotoxicity, endocrine disruption and developmental toxicity (Jones et al., 2003; Lau et al., 2007; Sletten et al., 2016; Sonne et al., 2012; Zhang et al., 2011).

Birds of prey are considered suitable sentinels for biomonitoring of PBT compounds (Sergio et al., 2005). In particular, territorial and non-migratory species are useful for evaluating local contamination status. Such local information is even more accurate when samples are obtained from nestlings, as these are usually fed with prey caught close to the nest (Frank and Lutz, 1999). Moreover, nestlings are easier to capture and sample than adults (Andersen, 2007). Due to ethical and legal reasons, feathers and blood are preferred matrices when monitoring contaminants in birds of prey, as these are considered non-invasive and/or non-destructive samples and adequately reflect the body burden of these compounds, including PFASs (Espín et al., 2012; Espin et al. 2016; Jaspers et al., 2007; 2013; Keller et al., 2004).

Diet is considered the main exposure pathway for most pollutants. Thus, ecotoxicological studies usually focus on the study of dietary habits and how they act as an exposure pathway for pollutants. Conventional approaches (e.g. pellets, regurgitates or stomach contents) often provide only a ‘snapshot’, while stable isotope (SI) values can integrate dietary information

over a longer period of time (Inger and Bearhop, 2008). This technique is based on the fact that the isotopic composition of consumer tissues is the weighted average of the isotopic composition of its food sources, modified by the net isotopic fractionation between diet and animal tissues. Thus, by a careful selection of tissues we can infer an animal's diet or habitat preference at temporal and spatial scale. In this sense, while blood would give recent information about diet or habitat (Pearson et al., 2003), feathers inform about the period when they were grown (Bearhop et al., 2003, Pearson et al., 2003). Recent studies suggest that the variability of isotopic composition of a population or a species (i.e., its isotopic niche) may be used as a proxy to assess the trophic niche of this population or species, and/or the degree of individual specialisation in the population (Bearhop et al., 2004). This isotopic niche concept has also been developed considerably through diverse numerical methods (Jackson et al., 2011; Layman et al., 2007; Matthews and Mazumder, 2004; Newsome et al., 2007). Hence, the first objective of the present study was to investigate whether the contaminant load was related to dietary habits in these species. Thus, the analysis of stable carbon and nitrogen isotope values of their feathers would be useful to infer dietary habits over the larger part of the nestling stage (Eulaers et al., 2013). In addition, the results of isotopic composition will be modelled to assess the trophic niche of each species.

Previous studies in nestlings of White-tailed eagle (*Haliaeetus albicilla*) and Northern goshawk (*Accipiter gentilis*) found significant associations between organohalogen contaminants in plasma and body feathers (Eulaers et al., 2011a, 2011b, 2014), which suggests that feathers can reflect body burdens of these compounds. However, there are no studies assessing the relationship between PFASs in feathers and plasma of birds of prey nestlings. Hence, the sencond objective of the present study was to evaluate the use of feathers as a non-destructive biomonitoring tool for PFASs in nestling birds of prey.

2. Material and methods

2.1. Study area and sampling methods

Samples were obtained in 2014 from nestling Northern goshawks (NG; *n*=11) and White-tailed eagles (WTE; *n*=14) hatched in northern Norway (Nordland- N 68.30 – 68.47º, E 24.54 – 25.27º- and Troms- N 68.77 – 67.39º, E 20.39 – 23.34º- counties, respectively; Fig. 1). A total of 14 different nests (*n*=10 from WTE and *n*=4 from NG) were monitored from late April until June to determine if the birds had laid eggs and nestlings had hatched and were suitable for sampling (when NG were 18-26 days old and WTE aged 35-56 days). The nestlings were lowered from the nest in a nylon bag and we tried to minimize stress by covering the head of the nestlings and being quiet during manipulation. Blood samples (1-4 mL) were obtained taking into account general and specific considerations described in Espín et al (2016), and following the EURAPMON protocol (Espín et al., 2014), by puncturing the brachial vein with a 23G needle and a heparin-coated syringe. Samples were transported refrigerated to the laboratory, where they were centrifuged at 10,000 rpm for 10 min and supernatant plasma was transferred to sterile tubes and frozen at -20 ºC until contaminant analysis. About 10 body feathers (chest and back combined) were gently plucked from goshawks 11 NG and 14 WTE and were kept in plastic bags at room temperature until analysis. Body condition was determined, using the scaled mass index according to the best regression between Ln body mass and Ln of different

measurements (Peig and Green, 2009). In the case of NG body weight was scaled on the beak height while for WTE, body weight was scaled on tarsus depth. The study was approved by the National Animal Research Authority of Norway.

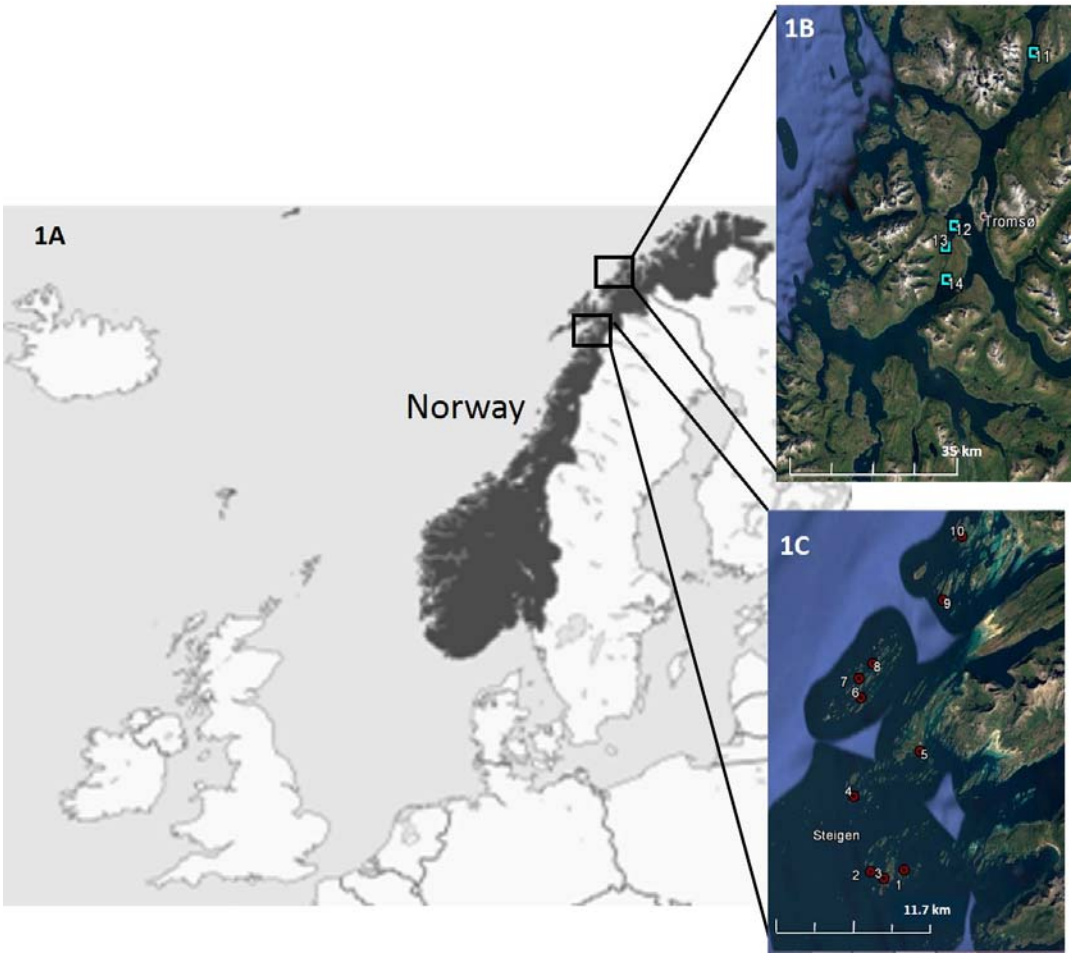

Fig 1. Map showing the study area (1A), in the North of Norway (square in the map). The Northern goshawk nests are located in Troms county (1B) and the White-tailed eagle nests are located in the Nordland county (1C).

2.2. Per- and polyfluoroalkyl substance analysis in plasma and body feathers

A total of 14 and 10 plasma samples from WTE and NG, respectively, were analysed for 17 PFASs, among which perfluorooctane sulfonamide (PFOSA), perfluorobutanoate (PFBA), perfluoropentanoate (PFPA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDcA), perfluoroundecanoate (PFUnA), perfluorododecanoate (PFDoA), perfluorotridecanoate (PFTriA), perfluorotretradecanoate (PFTeA), perfluorobutanesulfonate (PFBS), perfluorohexane sulfonate (PFHxS), linear (lin-PFOS) and branched PFOS (br-PFOS) and perfluorodecane sulfonate (PFDcS), using the method described by Powley et al. (2005). At the bird ecotoxicology lab (NTNU), a volume of 200 µL plasma was spiked with internal standard (0.1 ng μL^{-1} ^{13}C-labeled PFAS mix) and mixed with 1 mL of MeOH by shaking and vortexing in Eppendorf tubes. The samples were extracted in an ultrasonic bath three times for 10 min, with intermittent vortexing. Samples were centrifuged at 10,000 rpm for 10 min and the

supernatant was transferred to Eppendorf tubes with 25 mg ENVI-Carb graphitized carbon absorbent and 50 µL of glacial acetic acid for purification. Finally, the tubes were vortexed and centrifuged at 10,000 rpm for 10 min. An exact amount of 0.5 mL of supernatant was then transferred to glass vials, and a recovery standard solution (3,7-diMe-PFOA, 0.102 ng μL^{-1}) was added. Prior to UHPLC-MS/MS analysis of 100 µL of extract was transferred into an autosampler vial with insert and mixed with the same amount of 2 mM NH_4OAc in water.

Only WTE feathers (*n*=14) were analysed for PFASs, since the amount of NG feathers available was too low for these analyses (<100 mg). Detailed description of PFASs extraction method used at the bird ecotoxicology lab (NTNU) is provided in Appendix.

Quantification of PFASs was performed subsequently at The Norwegian Institute for Air research (NILU) in Tromsø, Norway. Analysis was performed by ultra-high pressure liquid chromatography triple quadrupole mass spectrometry (UHPLC-MS/MS). A detailed description of the analysis is described by Hanssen *et al.* (2013), Herzke et al. (2009) and Berger and Haukås (2005) and is provided in Appendix. Quantification was done using the internal standard method with 13- labelled PFASs (PFHxA, PFOA, PFNA, PFDcA, PFUnA, PFDoA, PFHxS and PFOS) and the LCQuan software from Thermo Scientific (Version 2.6; Thermo Fisher Scientific Inc., Waltham, MA, USA). Concentrations are calculated on a wet weight basis (ww). For every 10 samples, one blank was run to assure the quality of the method. Average recoveries for 13C-labelled compounds ranged between 42 % and 110 % for all PFASs. The blanks were treated identically to the other samples, except that no matrix was added. The limit of detection (LOD) was defined as 3x the signal to noise ratio for the analysed matrix. None of the PFASs were found above LOD in the blanks.

2.3. Stable isotope analysis

Feather barbs of 11 NG and 14 WTE were ground to obtain homogeneous material. Aliquots of the homogenized feathers (0.94 - 4.59 mg) were loaded into tin cups and analysed at the Laboratory of Oceanology at the University of Liège (Belgium) for stable carbon (^{13}C, ^{12}C) and nitrogen isotopes (^{15}N, ^{14}N) via continuous flow - elemental analysis - isotope ratio mass spectrometry (CF-EA-IRMS), using a vario MICRO cube elemental analyser (Elementar Analysensysteme GmBH, Hanau, Germany) coupled to an IsoPrime100 mass spectrometer (Isoprime, Cheadle, United Kingdom). Isotopic ratios were expressed using the international δ notation (Coplen, 2011). Sucrose (IAEA-C6, $\delta^{13}C$ = -10.8 ± 0.5‰, mean ± SD) and ammonium sulphate (IAEA-N2, $\delta^{15}N$ = +20.3 ± 0.2‰, mean ± SD) were used as certified reference materials. Both of these reference materials are calibrated against the international isotopic references, i.e. Vienna Pee Dee Belemnite (VPBD) for carbon and Atmospheric Air for nitrogen. Standard deviations on multi-batch replicate measurements of lab standards (fish tissues) analysed interspersed among the samples (2 lab standards for 15 samples) were 0.1 and 0.3 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Glycine (Merck) was used as elemental standard, and elemental contents were expressed as percentage of dry mass.

2.4. Statistical analysis and Isotopic niche modelling

Data below the limit of detection (LOD) were assigned a value of f × LOD, where f is the proportion of samples above LOD (Voorspoels et al., 2002). PFTeA was not taken into account

for statistical analysis because f was < 0.50 for both species. Differences in PFASs in plasma and SIs between species were studied. For this, normality of each variable was tested using Shapiro-Wilk tests and QQ plots. If a variable was normally distributed, a Student's t-test was used. If not, a Wilcoxon rank test was used. Moreover, metabolization of PFASs may not be independent among nestlings, so to study differences among and within nests we performed an ANOVA test with PAFSs values in plasma, after normalization of PFASs by a Box Cox transformation. To investigate the relation between individual PFASs in feathers and plasma of WTE, we used a linear model, with the previously Box Cox transformed PFASs. We also used a Bonferroni adjustment of studentized residuals for outlier detection. It was not possible to use linear mixed effects to avoid pseudoreplication due to the low sample size. Correlations between $\delta^{13}C$ and $\delta^{15}N$ and each of these with PFASs in WTE feathers were assessed using Pearson (*r*) correlation tests. This correlation test was also used to study the relationship between the body condition index and PFASs in plasma and feathers. Stable isotope niche parameters, i.e. standard ellipse areas for restricted sample sizes, were computed using the SIBER (Stable Isotope Bayesian Ellipses in R; Jackson et al., 2011) package (version 2.0). All data analyses were performed using the R statistical software (R Core team 2017). All tests were two-tailed and the level of significance was set at α=0.05.

3. Results and discussion

3.1. Concentrations of PFASs in plasma and body feathers

The concentrations of PFASs found in plasma of both species are shown in table 1. No quantifiable levels of PFOSA, PFBS, PFDcS, PFBA, PFPA, PFHxA nor PFHpA were found in plasma, while PFTeA was only detected in two samples of NG plasma. Compared to the levels found by Sonne et al. (2012) in NG born between 2008 and 2010 in the same study area (Troms county), the concentrations of ∑PFASs in our samples were lower (∑PFASs mean = 21-151 vs 19.1 ng mL^{-1}). In general, PFASs concentrations in plasma were higher in WTE than in NG (Table 1), which was also generally found by Sonne et al. (2012). WTE and NG are non-migratory, highly territorial species breeding in Norway (BirdLife International, 2014). However, differences regarding contaminant intake through the diet are likely to exist. NG are related to woodland areas while WTE are located near coastal open water (Hardey et al., 2009), feeding predominantly on species from the marine food web (Gjershaug et al., 2008; Herzke et al., 2002, 2005). In fact, fish-eating animals usually tend to accumulate higher loads of these compounds (Lau et al., 2007) and PFASs have been found in lower concentrations in terrestrial species compared to aquatic ones (Borg and Håkansson, 2012). The differences between the species here studied could also be explained by local pollution sources, since the studied areas are more than 250 km apart. However, the levels found by Sletten et al. (2016) in plasma of WTE nestlings hatched in Troms Province in 2011-2012 were similar to the WTE nestlings from Nordland (∑PFASs mean: 49.63 vs 45.4 ng mL^{-1}). Due to the lack of proper reference values, the mean levels of ∑PFASs in Norwegian WTE were compared with those in in Bald eagle (*Haliaeetus leucocephalus*) nestlings from a remote area from the Upper Midwestern United States (Route et al., 2014) and resulted to be four times lower. The higher levels in the United States may be related to a different production and/or use pattern than in Europe.

Only WTE feathers were analysed for PFASs, since the amount of NG feather matrix available was too low for these analyses (<100 mg). The concentrations of PFASs in feathers of WTE are shown in table 1. Similar to plasma samples, PFBS, PFDcS, PFBA, PFPA, PFHxA and PFHpA were not detected in feathers. To our knowledge, only three studies have analysed PFASs in feathers of birds of prey. The levels of PFASs reported here were similar to those found by Herzke et al. (2011) in body feathers from adult WTE from Norway and Greenland (∑PFASs median: 12.28 vs 12.5 ng g^{-1} dw), but lower than those in tail feathers from adult Barn owls (*Tyto alba*) found road-killed in Belgium (median PFOS: 6.18 vs 15.8 ng g^{-1} ww; median PFOA: 0.3 vs 37.1 ng g^{-1} ww; Jaspers et al., 2013) and much lower than those studied by Meyer et al. (2009) in Eurasian sparrowhawks (*Accipiter nisus*) from Antwerp region (Belgium), an area with high background PFASs contamination (mean PFOS: 6.87 vs 100 ng g^{-1} dw; mean PFHxS: 0.63 vs 20 ng g^{-1} dw).

Table 1. Median; mean±SD (minimum-maximum) concentrations of PFASs in plasma and body feathers of Northern goshawk and White tailed eagle nestlings from northern Norway. Within rows, values not sharing the same letter are significantly different. All differences were found significant at α = 0.01. The detection frequency for each compound is given as well.

	Northern goshawk	**White-tailed eagle**	
	Plasma (ng mL^{-1}) (*n*=10)	Plasma (ng mL^{-1}) (*n*=14)	Body feathers (ng g^{-1}) (*n*=14)
PFOSA	<LOD	<LOD	1.36; 1.46±0.54 (0.46-2.61) 100%
PFHxS	0.65; 0.61±0.57 (<LOD-1.33) 70%	0.57; 0.89±0.75 (<LOD-2.37) 93%	0.05; 0.63±0.86 (<LOD-2.23) 36%
brPFOS	0.01;1.15±1.87 A (<LOD-4.58) 30%	9.85; 8.96±6.32 B (<LOD-23.16) 86%	1.50; 1.68±1.79 A (<LOD-5.84) 100%
linPFOS	9.54; 10.89±8.07 A (2.70-24.52) 100%	22.68; 22.55±6.55 B (13.09-37.12) 100%	4.87; 5.19±2.60 A (1.82-10.55) 100%
∑PFOS	9.55; 12.05±9.49 A (2.71-29.10) 100%	32.33; 31.51±12.06 B (16.25-60.28) 100%	6.18; 6.87±4.31 A (1.89-16.38) 100%
PFOA	0.97; 1.08±0.84 A (0.18-2.27) 100%	1.07; 1.05±0.42 A (0.40-1.99) 100%	0.3; 0.3±0.15 B (0.10-0.61) 100%
PFNA	1.40; 1.48±1.40 A (0.07-3.56) 60%	4.05; 4.06±1.46 B (1.64-6.49) 100%	0.76; 0.86±0.85 A (<LOD-2.91) 71%
PFDcA	0.62; 0.56±0.32 A (0.19-1.00) 100%	1.76; 1.93±0.74 B (0.98-3.25) 100%	0.43; 0.48±0.28 A (0.14-1.12) 100%
PFUnA	1.80; 1.72±0.70 A (0.88-2.80) 100%	4.01; 4.18±1.01 B (2.87-6.06) 100%	0.92;1.01±0.36C (0.63-1.99) 100%
PFDoA	0.74; 0.66±0.30 A (0.20-1.07) 100%	0.60; 0.62±0.14 A (0.40-0.80) 100%	0.25;0.26±0.06 B (0.17-0.40) 100%
PFTriA	1.00; 0.88±0.39A (0.28-1.34)	1.19; 1.13±0.25B (0.79-1.59)	1.00; 1.07±0.40 (0.71-2.32)

	100%	100%	100%
PFTeA	0.04; 0.05*A 20%	<LOD A	0.01; 0.04±0.05 B (<LOD-0.19) 29%
∑PFASs	17.02; 19.06±13.70 A (5.01-41.95) 100%	45.83; 45.38±14.84 B (27.63-79.38) 100%	12.28; 12.98±6.14 C (4.54-25.61) 100%

*only detected in two samples

The lower levels of PFASs found in the samples from Norway (both plasma and feathers) may be explained by the fact that these compounds are not produced in this specific country, as well as the relatively remote location in Northern Norway. In addition, the use of PFOS and PFOS related-substances in textiles, impregnating agents and fire-fighting foam has been banned in Norway since 2007 (Seow, 2013). Thus, the PFOS residues found may be due to long-range transport, bioaccumulation in the food chain or the high persistence in the environment. The latter may be especially true in the case of one NG nest (Mean ∑PFOS = 22.2 ng mL^{-1}), which is close to a military airport where aqueous film forming foams were frequently used previously for military training. In fact, the concentrations of all the PFASs except br-PFOS and PFTeA were significantly different mainly due to the higher levels in this nest ($p < 0.01$). Differences among WTE nests were also studied but only PFNA and PFDoA were significantly different ($p = 0.005$ and $p= 0.03$ respectively). These results reinforce the usefulness of nestlings of these species as biomonitors of local pollution. On the other hand, the concentrations of PFASs among WTE siblings could not be properly compared as most nests (n=6) had only one nestling. In the case of NG, the number of nests is low, and the number of siblings in each nest was different.

Linear PFOS was the predominant compound in all samples, both plasma and feathers, in terms of frequency of detection as well as median concentrations. However, the PFASs profile was slightly different among species and between matrices within the same species, being in NG plasma: lin-PFOS>PFuNA>PFNA>PFOA; in WTE plasma: lin-PFOS>br-PFOS>PFNA≈PFUnA>PFDcA and in WTE feathers: lin-PFOS>br-PFOS>PFOSA>PFTriA≈PFUnA. The high frequency and abundancy of PFOS in our samples (both feathers and plasma) is in agreement with the fact that this compound is the predominant PFASs in biota, while PFOA is dominant in environmental abiotic matrices (Senthilkumar, 2005). In addition, the higher frequency and concentrations of linear PFOS compared to branched PFOS has been found both in laboratory and field studies, including polar bear (*Ursus maritimus*), fish, chicken embryo and mice (Benskin et al., 2009; Greaves and Letcher, 2013; Houde et al., 2008; O'Brien et al., 2011). However, the different PFASs profile between species can be attributed to the ingestion of prey with different PFASs patterns (Gebbink and Letcher, 2010).

In WTE nestlings the concentrations of PFASs were higher in plasma compared to feathers on a weight basis (see table 1). However, although PFOSA was not found in the plasma samples, it was detected in all feather samples. This was also observed in WTEs from Norway and Greenland (Herzke et al., 2011). A possible explanation for this could be exposure by air of this neutral compound rather than via the diet/ feed in contrast to the ionic PFASs, indicating the presence of a PFOS precursor in the air masses. In the present study, another compound, i.e. PFTeA, was also found in four feathers but not in any plasma of WTE. This profile could

indicate either feathers to be a route for excretion of PFOSA and PFTeA or possible external exposure on the feather surface (Herzke et al., 2011). Thus, a different washing method should be evaluated in future studies to investigate the contribution of internal versus external PFASs concentrations for feathers. However, in human studies, PFOSA has been shown to have stronger affinity to whole blood than to plasma (Kärrman et al., 2006); i.e. PFOSA was the second most abundant PFASs in the human blood. The analysis of PFASs in whole blood in future studies may also help to elucidate this question.

3.2. Evaluation of the usefulness of feathers for biomonitoring PFASs

One of the aims of the current study, and more general in the field of biomonitoring of contaminants using bird samples, was the evaluation of nestling body feathers as a non-destructive biomonitoring strategy. Ethical and legal reasons, as well as the ease of collection and storage of this matrix, have led to the increased use of feathers. Most studies, especially at the beginning, focused on levels of metals in feathers. More recently, organohalogen compounds have been analysed in feathers of a wide range of species, usually showing good correlations among concentrations in different internal tissues (García-Fernández et al., 2013). More specifically, strong correlations were previously found for POPs in the same species as used in this study, and in the same study area (Eulaers et al., 2011a, 2011b, 2014). With regard to PFASs in feathers, significant strong correlations with internal tissues have previously been found in different species, although the number of studies is low. Meyer et al. (2009) studied PFHxS, PFOS, PFOA and PFNA in liver, spleen and feathers of 5 bird species and they found a significant correlation between feather and liver PFOS (R=0.622, p <0.01) when they grouped the samples of the 5 species (n=50). However, the correlation between the PFOS levels in the feathers and livers was not significant in each individual species (Grey heron *Ardea cinerea*, Herring gull *Larus argentatus*, Eurasian sparrowhawk *Accipiter nisus*, Eurasian magpie *Pica pica* and Eurasian collared dove *Streptopelia decaocta*, n=10 for each species). However, sample size may not be the only explanation for a lack of association as Jaspers et al. (2013) found strong positive correlations between feather and liver PFOS and PFHxS (R=0.78 and 0.86, p<0.01 respectively) in 15 Barn owls. In the current study, significant positive associations between feathers and plasma were found in nestlings for specific PFASs (PFHxS: R^2 = 0.41, p = 0.01; PFDcA: R^2 = 0.35, p = 0.03; PFDoA: R^2 = 0.67, p < 0.01 and PFTriA: R^2 = 0.36, p = 0.03; see Table 2 and Fig 2), but not for PFOS, PFOA and PFNA. An outlier (PFTriA = 2.32 ng g^{-1}; Bonferonni test p = 0.02) was detected among the feather samples and was excluded from the linear model. As mentioned above, to our knowledge, this is the first study of the relation between PFASs in blood and feathers. It is therefore difficult to confirm or rule out the utility of feathers, as in the previous studies the tissue was different and concentrations of PFASs higher. Clearly more research is needed before feathers can be considered as a reliable biomonitor matrix for PFASs, in particular the extent and removal of external contamination requires further investigation.

Table 2. Linear regression between nestling plasma and body feather concentrations of PFASs in White-tailed eagle nestlings. We show intercepts and estimates of regression line, multiple regression coefficients R^2, F statistics and p value. All variables were previously Box-Cox transformed. * p<0.05, **p<0.01.

	Intercept	Estimate	R^2	*F*	*p*	
PFHxS	544	0.56	0.41	8.18	0.01	**
brPFOS	10614	-0.98	0.08	1.02	0.33	
linPFOS	20010	0.49	0.04	0.47	0.51	
∑PFOS	32110	-0.09	0.00	0.01	0.92	
PFOA	1016	0.11	0.00	0.02	0.89	
PFNA	3304	0.88	0.26	4.28	0.06	
PFDcA	1163	1.59	0.35	6.43	0.03	*
PFUnA	3482	0.69	0.06	0.78	0.39	
PFDoA	151	1.83	0.67	24.25	0.00	**
PFTriA	297	0.84	0.37	6.37	0.03	*
∑PFASs	42670	0.21	0.01	0.09	0.77	

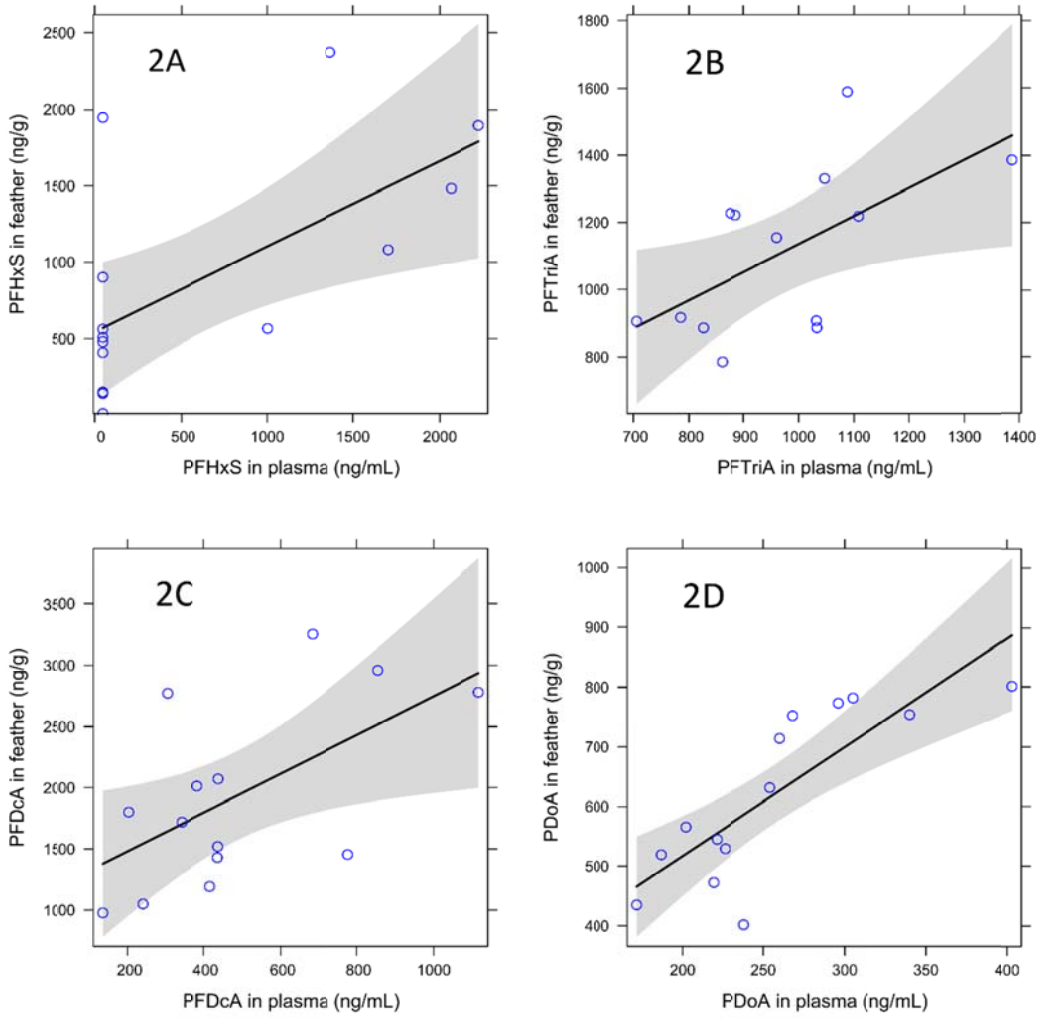

Fig 2. Linear regressions between White-tailed eagle nestling plasma and body feather concentrations of PFHxS- fPFHxS: R^2 = 0.41, p = 0.01 (2A); PFTriA- fPFTriA: R^2 = 0.37, p = 0.03 (2B); PFDcA-f PFDcA: R^2 = 0.35, p = 0.026 (2C) and PFDoA- fPFDoA: R^2 = 0.67, p < 0.01 (2D). Linear regressions were performed by Box-Cox transformations.

3.3. Use of stable isotopes to evaluate inter- and intraspecific contaminant exposure.

The SI values in the feathers did not differ among siblings but differed significantly between the species (δ^{13}C: Z = -4.22, $p < 0.01$; δ^{15}N: Z = -4.22, $p < 0.01$) and are shown in Fig 3. δ^{13}C values were significantly higher in WTE (median; mean ± SD (range): –18.5 ‰; –18.6 ‰ ± 0.7 (–19.9‰ - –17.9‰)) than those in NG feathers (–23.9‰; –23.9 ‰ ± 0.5 (–24.7‰ - –22.7‰)). Similarly, values for δ^{15}N were significantly higher in WTE feathers (median; mean ± SD (range): 14.4‰; 14.3‰ ± 0.4 (13.3 - 14.9‰)) than those of NG (7.5‰; 7.1‰ ± 1.3 (5.1 to 9.1‰)). These results are similar to earlier findings by Eulaers et al. (2013), who also indicated that dietary ecology and regional nest location explained best the differences in POP exposure in WTE, NG and Golden eagle (*Aquila chrysaetos*) nestlings from northern Norway. Hence, according to Kelly (2000), δ^{13}C values in NG feathers were in the range established for terrestrial birds (mean: –22.9 ± 2.6%) and δ^{13}C values in WTE feathers in the range for carnivorous seabirds (mean: –18.8 ± 2.2%). The study of prey items was not included in the current research, and therefore, the trophic level of both species could not be compared. However, the results of SIs are in agreement with the general food habits described for both raptors in previous studies. Moreover, isotopic niches showed no overlap between the two species, confirming the fact that both feed in different food webs and habitats. However, no information about the parents of the nestlings was available for comparison.

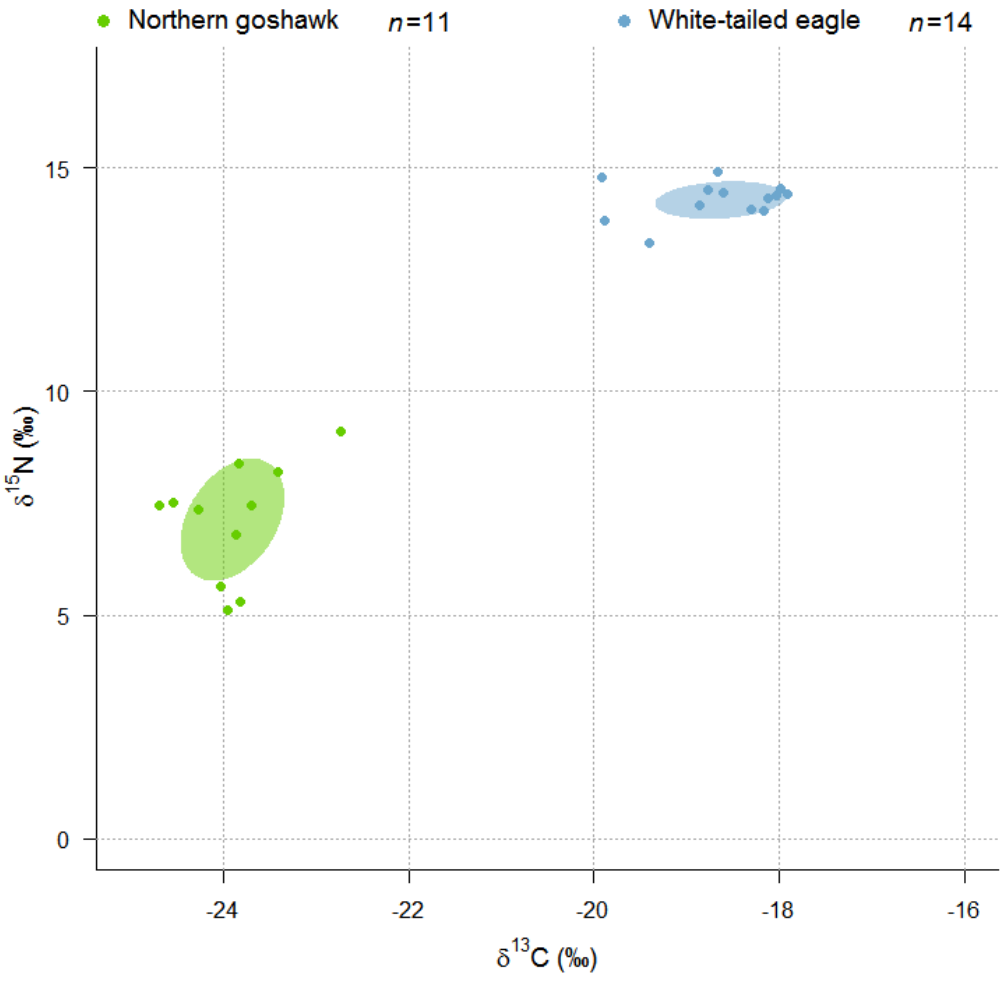

Fig 3. $\delta^{13}C$ and $\delta^{15}N$ values in feathers of White-tailed eagle and Northern goshawk nestlings from northern Norway. The ellipses depict the standard ellipse area, corrected for limited sample sizes, for each species (Jackson et al., 2011)

In the present study, no significant correlations between $\delta^{13}C$ and feather PFASs in WTE were detected, except for PFHxS (r = -0.57, p = 0.03). In spite of the lack of significance, the sign of the correlation is important to interpret the results. Positive correlations of contaminants with $\delta^{13}C$ indicate marine sources while negative ones are related to freshwater or terrestrial food sources (Boutton, 1991). Although only few studies have investigated the relation between stable carbon and nitrogen isotope values and PFASs concentrations, similar correlations were found by Gebbink et al. (2011) in gull (*Larus glaucescens, L. californicus, L. delawarensis and L. argentatus*) eggs laid in freshwater ecosystems from Canada, where adults tended to rely on human refuse. In our case, the negative correlation may suggest that, although PFASs concentrations are lower in terrestrial compared to marine species (Borg and Håkansson, 2012), the proximity to coast and therefore, to anthropogenic sources may be the cause of higher levels of most PFASs, and especially PFHxS in the WTE nestlings.

On the other hand, the correlations between $\delta^{15}N$ and PFASs in feathers of WTE were positive but non-significant (See table A1). The positive correlation between $\delta^{15}N$ and POPs has been related to their biomagnification potential through the food chain (Barón et al., 2015; Bustnes et al., 2013; Jardine et al., 2006; Elliott et al., 2009; Eulaers et al., 2013, 2014). Despite this biomagnification potential, weak relations with $\delta^{15}N$ have also been found in other less lipophilic contaminants such as polybrominated diphenyl ethers (PBDEs) in previous studies in birds of prey (Elliott et al., 2015; Eulaers et al., 2013).

Another explanation for the lack of correlations between SIs and PFASs may be a low variability in the origin of the prey items (carnivorous seabirds). As aforementioned, feathers from NG could not be analysed for PFASs. Further studies including samples from both species with different feeding habits (terrestrial vs marine) would be useful in this sense.

3.4. Risk assessment of PFASs

Information on toxic levels of environmental contaminants in birds of prey is scarce, and the great number of compounds that can be found simultaneously in their tissues complicates risk assessment. In addition, several factors such as age, sex or physiological status should be considered. In the present study, no significant relations between body condition index with level of PFASs in plasma have been found (all Pearson correlation tests $P > 0.05$) neither with PFASs in feathers (all Pearson correlation tests $p > 0.05$) except for fPFUnA. For PFUnA a negative correlation was found ($r = -0.54$, $p = 0.04$) possibly due to low level values recorded.

Some toxicity reference values (TRV) have been established for PFOS in fish-eating birds such as eagles and ospreys (Newsted et al., 2005). According to these TRV values (5,900 and 360 ng mL^{-1} in adult males and females, respectively), the studied individuals would not be considered at risk for exposure to PFOS as their levels were several orders of magnitude lower. The levels of PFOS in serum were also higher in other experimental studies in adult birds. For example, a reduction of survival was found in Northern bobwhite quails (*Colinus virginianus*) with 8,700 ng mL^{-1} of PFOS, while no effects were found in mallards (*Anas platyrhynchos*) with 16,600 ng mL^{-1} PFOS (Newsted et al., 2007). However, differences in sensitivity due to factors such as species, age and sex (in adult individuals) should always be considered. Although, likewise, TRV have been calculated for PFBS in serum of predatory birds (5,700 and 8,700 ng mL^{-1} in males and females, respectively, Giesy et al., 2010), this compound was not detected in our study. On the other hand, some field studies have investigated the relation between levels of PFASs and adverse effects. For example, in the WTE nestlings studied by Sletten et al (2016), no association was found between some biomarkers of health (superoxide dismutase activity, plasma Ig Y levels or telomers length) and higher levels of linear PFOS but similar levels of PFUnA compared to our study. On the contrary, lower levels of PFASs found in black-legged kittiwake (*Rissa tridactyla*) and northern fulmar (*Fulmarus glacialis*) chicks sampled in Svalbard (Norway), showed positive associations with total thyroxin (Nøst et al., 2012).

4. Conclusions

Although no strong significant correlations were found between concentrations in blood and feathers from this study, more research is needed with higher sample sizes; and the issue of potential external contamination on the feathers should be examined to conclude on the

suitability of feathers as a potential biomonitoring matrix for PFASs. Differences in concentrations and profiles of the contaminants studied between the species seem to be ecosystem specific (terrestrial versus aquatic), as indicated by stable isotope analysis of the nestlings' body feathers. Further studies with higher sample sizes are needed to present more conclusive results, but a first comparison to existing threshold levels in the literature suggests that the studied birds are currently not at risk for health effects as a result of exposure to PFASs.

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REFERENCES

Andersen, D.E., 2007. Survey techniques. In: Bird, D.M., Bildstein, K.L., (Eds.), Raptor Research and Management Techniques. Hancock House Publishers Ltd, Canada and Hancock House Publishers, USA, pp 89-100.

Barón, E., Giménez, J., Verborgh, P., Gauffier, P., De Stephanis, R., Eljarrat, E., Barceló, D., 2015. Bioaccumulation and biomagnification of classical flame retardants, related halogenated natural compounds and alternative flame retardants in three delphinids from Southern European waters. Environ. Pollut. 203, 107–115.

Bearhop, S., Furness, R.W., Hilton, G.M., Votier, S.C., Waldron, S., 2003. A forensic approach to understanding diet and habitat use from stable isotope analysis of (avian) claw material. Funct. Ecol. 17, 270–275

Berger, U., Haukas, M., 2005. Validation of a screening method based on liquid chromatography coupled to high-resolution mass spectrometry for analysis of perfluoroalkylated substances in biota. J. Chromatogr. A 1081, 210–217.

BirdLife International, 2014. Species factsheet: *Haliaeetus albicilla*. Downloaded from http://www.birdlife.org on 06/11/2014.

Borg, D., Håkansson, H., 2012. Environmental and health risk assessment of perfluoroalkylated and polyfluoroalkylated substances (PFASs) in Sweden. Swedish Environmental Protection Agency Report 6513

Benskin, J.P., De Silva, A.O., Martin, L.J., Arsenault, G., McCrindle, R., Riddell, N., Mabury, S.A., Martin, J.W., 2009. Disposition of perfluorinated acid isomers in Sprague-Dawley rats; Part 1: Single dose. Environ. Toxicol. Chem. 28, 542–554.

Boutton, T.W., 1991. Stable carbon isotope ratios of natural materials: II. Atmospheric, terrestrial, marine, and freshwater environments. In: Coleman, D.C., Fry, B., (Eds.), Carbon isotope techniques. Academic Press Inc., San Diego, pp. 173–183.

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., Voogt, P. De, Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P.J., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. Integr. Environ. Assess. Manag. 7, 513–541.

Bustnes, J.O., Bårdsen, B.J., Herzke, D., Johnsen, T. V, Eulaers, I., Ballesteros, M., Hanssen, S.A., Covaci, A., Jaspers, V.L.B., Eens, M., Sonne, C., Halley, D., Moum, T., Nøst, T.H., Erikstad, K.E., Ims, R.A., 2013. Plasma concentrations of organohalogenated pollutants in predatory bird nestlings: associations to growth rate and dietary tracers. Environ. Toxicol. Chem. 32, 2520–2527.

Coplen, T., 2011. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Commun. Mass Spectrom. 25, 2538-2560.

Domingo, J. L., Nadal, M., 2017. Per-and polyfluoro alkyl substances (PFASs) in Food and Human Dietary Intake: A Review of the Recent Scientific Literature. J. Agric. Food Chem. 65, 533–543.

Elliott, J.E., Brogan, J., Lee, S.L., Drouillard, K.G., Elliott, K.H., 2015. PBDEs and other POPs in urban birds of prey partly explained by trophic level and carbon source. Sci. Total Environ. 524-525, 157–165.

Elliott, K.H., Cesh, L.S., Dooley, J.A., Letcher, R.J., Elliott, J.E., 2009. PCBs and DDE, but not PBDEs, increase with trophic level and marine input in nestling bald eagles. Sci. Total Environ. 407, 3867–3875.

Espín, S., García-Fernández, A.J, Herzke, D., Shore, R.F., van Hattum, B., Martínez-López, E., Coeurdassier, M., Eulaers, I., Fritsch, C., Gómez-Ramírez, P., Jaspers, V.L.B., Krone, O., Duke, G., Helander, B., Mateo, R., Movalli, P., Sonne, C., van den Brink, N.W., 2014. Sampling and contaminant monitoring protocol for raptors. Research Networking Programme. EURAPMON. www.eurapmon.net

Espín, S., García-Fernández, A.J., Herzke, D., Shore, R.F., Hattum, B. Van, Martínez-López, E., Coeurdassier, M., Eulaers, I. Fritsch, C., Gómez-Ramírez, P., Jaspers, V.L., Krone, O., Duke, G., Helander, B., Mateo, R., Movalli, P., Sonne, C., van den Brink, N.W., 2016. Tracking pan-continental trends in environmental contamination using sentinel raptors-what types of samples should we use? Ecotoxicology 25, 777-801.

Espín, S., Martínez-López, E., Gómez-Ramírez, P., María-Mojica, P., García-Fernández, A.J., 2012. Razorbills (*Alca torda*) as bioindicators of mercury pollution in the southwestern Mediterranean. Mar. Pollut. Bull. 64, 2461–2470.

Eulaers, I., Covaci, A., Herzke, D., Eens, M., Sonne, C., Moum, T., Schnug, L., Hanssen, S.A., Johnsen, T.V., Bustnes, J.O., Jaspers, V.L.B., 2011a. A first evaluation of the usefulness of

feathers of nestling predatory birds for non-destructive biomonitoring of persistent organic pollutants. Environ. Int. 37, 622–630.

Eulaers, I., Covaci, A., Hofman, J., Nygård, T., Halley, D.J., Pinxten, R., Eens, M., Jaspers, V.L.B., 2011b. A comparison of non-destructive sampling strategies to assess the exposure of White-tailed eagle nestlings (*Haliaeetus albicilla*) to persistent organic pollutants. Sci. Total Environ. 410–411, 258–265.

Eulaers, I., Jaspers, V.L.B., Bustnes, J.O., Covaci, A., Johnsen, T. V, Halley, D.J., Moum, T., Ims, R.A., Hanssen, S.A., Erikstad, K.E., Herzke, D., Sonne, C., Ballesteros, M., Pinxten, R., Eens, M., 2013. Ecological and spatial factors drive intra- and interspecific variation in exposure of subarctic predatory bird nestlings to persistent organic pollutants. Environ. Int. 57–58, 25–33.

Eulaers, I., Jaspers, V.L.B., Pinxten, R., Covaci, A., Eens, M., 2014. Legacy and current-use brominated flame retardants in the Barn Owl. Sci. Total Environ. 472, 454–462.

Frank R., Lutz R.S. 1999. Productivity and survival of Great horned owls exposed to dieldrin. Condor 101:331-339.

García-Fernández, A.J., Calvo, J.F., Martínez-López, E., María-Mojica, P., Martínez, J.E., 2008. Raptor ecotoxicology in Spain: a review on persistent environmental contaminants. Ambio 37, 432-439.

García-Fernández, A.J., Espín, S., Martínez-López, E., 2013. Feathers as a biomonitoring tool of polyhalogenated compounds: a review. Environ. Sci. Technol. 47, 3028–3043.

García-Fernández, A.J., María-Mojica, P., 2000. Contaminantes ambientales y su repercusión sobre la fauna silvestre. In: Ministerio de Agricultura, Pesca y Alimentación. (Ed.), Globalización Medioambiental. Perspectivas Agrosanitarias y Urbanas, pp. 215–227.

Gebbink, W.A., Letcher, R.J., Burgess, N.M., Champoux, L., Elliott, J.E., Hebert, C.E., Martin, P., Wayland, M., Weseloh, D.V., Wilson, L., 2011. Perfluoroalkyl carboxylates and sulfonates and precursors in relation to dietary source tracers in the eggs of four species of gulls (*Larids*) from breeding sites spanning Atlantic to Pacific Canada. Environ. Int. 37(7), 1175-1182.

Gebbink, W.A., Letcher, R.J., 2010. Linear and branched perfluorooctane sulfonate isomer patterns in herring gull eggs from colonial sites across the Laurentian Great Lakes. Environ. Sci. Technol. 44, 3739–3745.

Giesy, J.P., Naile, J.E., Khim, J.S., Jones, P.D., Newsted, J.L., 2010. Aquatic toxicology of perfluorinated chemicals. In: Whiteacre, D.M., (Ed.)., Reviews of environmental contamination and toxicology, Springer, New York, pp. 1-52.

Gjershaug, J.O., Kålås, J.A., Nygård, T., Herzke, D., Folkestad, A.O., 2008. Monitoring of raptors and their contamination levels in Norway. Ambio 37:420–424.

Gómez-Ramírez, P., Shore, R.F., van den Brink, N.W., van Hattum, B., Bustnes, J.O., Duke, G., Fritsch, C., García-Fernández, A.J., Helander, B.O., Jaspers, V., Krone, O., Martínez-López, E.,

Mateo, R., Movalli, P., Sonne, C., 2014. An overview of existing raptor contaminant monitoring activities in Europe. Environ. Int. 67, 12–21.

Greaves, A.K., Letcher, R.J., 2013. Linear and branched perfluorooctane sulfonate (PFOS) isomer patterns differ among several tissues and blood of polar bears. Chemosphere 93, 574–580.

Hanssen, L., Dudarev, A.A., Huber, S., Odland, J.Ø., Nieboer, E., Sandanger, T.M., 2013. Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan. Sci. Total Environ. 447, 430-437

Hardey, J., Crick, H., Wernham, C., Riley, H., Etheridge, B., Thompson, D., 2009. Raptors: a field guide for surveys and monitoring (2nd edition). The Stationery Office, Edinburgh.

Herzke, D.,Berger,U., Kallenborn,R., Nygård, T., Vetter,W., 2005. Brominated flame retardants and other organobromines in Norwegian predatory bird eggs. Chemosphere 61, 441–449

Herzke, D., Jaspers, V.L.B., Boertmann, D., Rasmussen, L.M., Sonne, C., Dietz, R., Covaci, A., Eens, M., Bustnes, J.O., 2011. PFCs in feathers of White tailed eagles (*Haliaeetus albicilla*) from Greenland and Norway; useful for non-destructive monitoring? Organohalogen compounds 73,1337-1339

Herzke, D., Kallenborn, R., Nygård, T., 2002.Organochlorines in egg samples from Norwegian birds of prey: congener-, isomer-and enantiomer specific considerations. Sci. Total Environ. 291, 59–71.

Herzke, D., Nygård, T., Berger, U., Huber, S., Rov, N., 2009. Perfluorinated and other persistent halogenated organic compounds in European shag (*Phalacrocorax aristotelis*) and common eider (*Somateria mollissima*) from Norway: A suburban to remote pollutant gradient. Sci. Total Environ. 408, 340–348

Houde, M., Czub, G., Small, J. M., Backus, S.M., Wang, X., Alaee, M., Muir, D.C.G., 2008. Fractionation and bioaccumulation of perfluorooctane sulfonate (PFOS) isomers in a Lake Ontario food web. Environ. Sci. Technol., 42, 9397–9403.

Inger, R., Bearhop, S., 2008. Applications of stable isotope analyses to avian ecology. Ibis 150, 447–461.

Jackson, A.L., Inger, R., Parnell, A.C., Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. J. Anim. Ecol. 80, 595–602

Jardine, T.D., Kidd, K.A., Fisk, A.T., 2006. Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. Environ. Sci. Technol. 40, 7501–7511.

Jaspers, V.L., Voorspoels, S., Covaci, A., Lepoint, G., Eens, M., 2007. Evaluation of the usefulness of bird feathers as a non-destructive biomonitoring tool for organic pollutants: a comparative and meta-analytical approach. Environ. Int. 33, 328–337.

Jaspers, V.L.B., Herzke, D., Eulaers, I., Gillespie, B.W., Eens, M., 2013. Perfluoroalkyl substances in soft tissues and tail feathers of Belgian barn owls (*Tyto alba*) using statistical methods for left-censored data to handle non-detects. Environ. Int. 52, 9–16.

Jones, P.D., Hu, W., De Coen, W., Newsted, J.L., Giesy, J.P., 2003. Binding of perfluorinated fatty acids to serum proteins. Environ. Toxicol. Chem. 22, 2639–2649.

Kärrman, A., van Bavel, B., Järnberg, U., Hardell, L., Lindström, G., 2006. Perfluorinated chemicals in relation to other persistent organic pollutants in human blood. Chemosphere 64, 1582-1591.

Keller, J.M., Kucklick, J.R., Harms, C.A., McClellan-Green, P.D., 2004. Organochlorine contaminants in sea turtles: correlations between whole blood and fat. Environ. Toxicol. Chem. 23, 726-738.

Kelly, J. F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can. J. Zool. 78, 1-27.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99, 366-394.

Layman, C.A., Allgeier, J.E., 2012. Characterizing trophic ecology of generalist consumers: A case study of the invasive lionfish in the Bahamas. Mar. Ecol. Progr. 448, 131-141.

Lindstrom, A.B., Strynar, M.J., Libelo, E.L., 2011. Polyfluorinated compounds: past, present, and future. Environ. Sci. Technol. 45, 7954–7961.

Matthews, B., Mazumder, A., 2004. A critical evaluation of intrapopulation variation of $\delta^{13}C$ and isotopic evidence of individual specialization. Oecologia 140, 361-371.

Meyer, J., Jaspers, V.L., Eens, M., de Coen, W., 2009. The relationship between perfluorinated chemical levels in the feathers and livers of birds from different trophic levels. Sci. Total Env. 407, 5894–5900.

Newsome, S.D., Del Rio, C.M., Bearhop, S., Phillips, D.L., 2007. A niche for isotopic ecology. Front. Ecol. Environ. 5, 429-436.

Newsted, J.L., Jones, P.D., Coady, K., Giesy, J.P., 2005. Avian toxicity reference values for perfluorooctane sulfonate. Environ. Sci. Technol. 39, 9357–9362.

Newsted, J.L., Coady, K.K., Beach, S.A., Butenhoff, J.L., Gallagher, S., Giesy, J.P., 2007. Effects of perfluorooctane sulfonate on mallard and Northern bobwhite quail exposed chronically via the diet. Environ. Toxicol. Pharmacol. 23, 1–9.

Nøst, T.H., Helgason, L.B., Harju, M., Heimstad, E.S., Gabrielsen, G.W., Jenssen, B.M., 2012. Halogenated organic contaminants and their correlations with circulating thyroid hormones in developing Arctic seabirds. Sci. Total Environ. 414, 248-256.

NRC (U.S. National Research Council), 1991. Animals as sentinels of environmental health hazards. National Academy Press, Washington.

O'Brien, J.M., Kennedy, S.W., Chu, S., Letcher, R.J., 2011. Isomer-specific accumulation of perfluorooctane sulfonate in the liver of chicken embryos exposed *in ovo* to a technical mixture. Environ. Toxicol. Chem. 30, 226–231.

Pearson, S.F., Levey, D.J., Greenberg, C.H., del Rio, C.M., 2003. Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. Oecologia 135, 516–523.

Peig, J., Green, A.J., 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118, 1883-1891.

Powley, C.R., George S.W., Ryan, T.W., Buck, R.C., 2005. Matrix effect-free analytical methods for determination of perfluorinated carboxylic acids in environmental matrixes. Analytical Chemistry 77, 6353-6358.

Route, W.T., Russell, R.E., Lindstrom, A.B., Strynar, M.J., Key, R.L., 2014. Spatial and temporal patterns in concentrations of perfluorinated compounds in bald eagle nestlings in the upper Midwestern United States. Environ. Sci. Technol. 48, 6653–6660.

R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Seow, J., 2013. Firefighting foams with perfluorochemicals — environmental review. http://www.hemmingfire.com/news/get_file.php3/id/287/file/Seow_WA-DEC_PFCs_Firefighting_Foam_final_version_7June2013.pdf (2013)

Sergio, F., Newton, I., Marchesi, L., 2005. Conservation: top predators and biodiversity. Nature 436, 192.

Senthilkumar, K., 2005. Fluorinated organic chemicals: a review. Res. J. Chem. Environ. 9:50–79

Sletten, S., Bourgeon, S., Bårdsen, B.-J., Herzke, D., Criscuolo, F., Massemin, S., Zahn, S., Johnsen, T.V., Bustnes, J.O., 2016. Organohalogenated contaminants in White-tailed eagle (*Haliaeetus albicilla*) nestlings: An assessment of relationships to immunoglobulin levels, telomeres and oxidative stress. Sci. Total Environ. 539, 337–349.

Sonne, C., Bustnes, J.O., Herzke, D., Jaspers, V.L.B., Covaci, A., Eulaers, I., Halley, D.J., Moum, T., Ballesteros, M., Eens, M., Ims, R.A., Hanssen, S.A., Erikstad, K.E., Johnsen, T. V, Rigét, F.F., Jensen, A.L., Kjelgaard-Hansen, M., 2012. Blood plasma clinical–chemical parameters as biomarker endpoints for organohalogen contaminant exposure in Norwegian raptor nestlings. Ecotoxicol. Environ. Saf. 80, 76–83.

UNEP (United Nations Environmental Programme). 2009. The Stockholm Convention on Persistent Organic Pollutants. United Nations, adoption of amendments to Annexes A, B and C.

Voorspoels, S., Covaci, A., Maervoet, J., Schepens, P., 2002. Relationship between age and levels of organochlorine contaminants in human serum of a Belgian population. Bull. Environ. Contam. Toxicol. 69, 22–29.

Wang, Z., Cousins, I. T., Scheringer, M., Hungerbuehler, K., 2015. Hazard assessment of fluorinated alternatives to long-chain perfluoroalkyl acids (PFAAs) and their precursors: status quo, ongoing challenges and possible solutions. Environ. Int. 75, 172-179.

Zhang, H., Ding, L., Fang, X., Shi, Z., Zhang, Y., Chen, H., Yan, X., Dai, J., 2011. Biological responses to perfluorododecanoic acid exposure in rat kidneys as determined by integrated proteomic and metabonomic studies. PLoS ONE 6(6): e20862.