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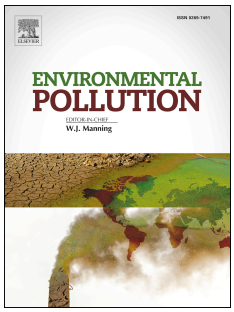

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Plasma concentrations of organohalogenated contaminants in white-tailed eagle nestlings – the role of age and diet

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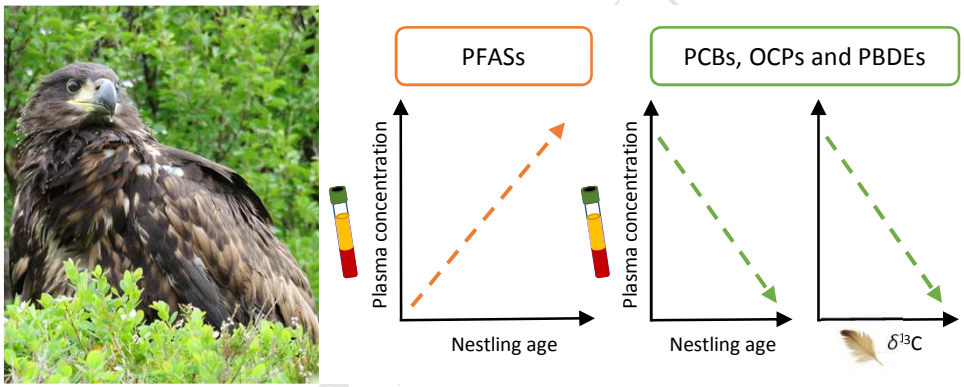

1. Introduction

Organohalogenated contaminants (OHCs) are a diverse group of chemicals that have been used in lubricants, pesticides, flame retardants and surface treatments (Mackay et al., 2006). OHCs include legacy compounds such as polychlorinated biphenyls (PCBs), as well as emerging compounds such as per- and polyfluoroalkyl substances (PFASs). By being resistant to chemical and biological degradation, OHCs persist in the environment (Muir and de Wit, 2010; UNEP, 2009). While most legacy OHCs are lipophilic, the emerging PFASs are amphipathic due to hydrophilic functional groups and different chemical structures (Lau et al., 2007). Even so, the physicochemical properties and persistency of both legacy OHCs and PFASs result in high potentials for bioaccumulation and biomagnification through food chains (Borgå et al., 2004; Kelly et al., 2009). The concentrations of OHCs can show significant temporal and spatial variations both in the environment and wildlife (Faxneld et al., 2016; Helgason et al., 2008; Hung et al., 2016; Wierda et al., 2016). Most of these variations are due to changes in production and use of the compounds (Hung et al., 2016; Wang et al., 2014). However, environmental and biological factors can also contribute significantly to the observed variations (Bourgeon et al., 2013; Bustnes et al., 2015; Leat et al., 2011).

The white-tailed eagle (*Haliaeetus albicilla*) occupies a high trophic level and can accumulate a wide range of OHCs, even at an early age (Bustnes et al., 2013; Eulaers et al., 2014; Løseth et al., 2019; Sletten et al., 2016). Nestlings are exposed to maternally transferred OHCs during development in the egg (Faxneld et al., 2016; Nordlöf et al., 2010; Nygård and Polder, 2012) and the exposure continues after hatching through their dietary intake (Bourgeon et al., 2013). Adult white-tailed eagles are mostly resident within their breeding areas (Willgohs, 1984), thus the contaminant burdens of their eggs and nestlings

reflect contaminant levels in local prey. This makes white-tailed eagle nestlings good sentinels of local environmental pollution (Helander et al., 2008; Olsson et al., 2000).

The diet of the white-tailed eagle consists mainly of terrestrial and marine carrion, fish and seabirds (Koivusaari et al., 1976; Nadjafzadeh et al., 2016; Willgohs, 1984), which may have accumulated high concentrations of OHCs. As the diet is a major source of OHC exposure following hatching, stable isotopes of nitrogen (δ^{15}N) and carbon (δ^{13}C) are often applied as dietary proxies to investigate the nestlings' trophic position and dietary carbon source, respectively (Fry, 2006; Inger and Bearhop, 2008; Nadjafzadeh et al., 2016). The ratio of ^{15}N to ^{14}N increases by about 2-5 ‰ per trophic level as the lighter nitrogen isotopes are excreted through nitrogenous waste products. The ratio of ^{13}C to ^{12}C can also increase with increasing trophic level, though it is mostly used to distinguish between marine and terrestrial dietary carbon sources. Terrestrial primary producers have lower δ^{13}C values compared to marine ones. This is reflected in the tissues of their consumers and persists at higher trophic levels within the food chain (Fry, 2006; Inger and Bearhop, 2008; Kelly, 2000). Keratinized matrices, such as feathers, are metabolically inert after their growth and can preserve the stable isotopes deposited into the matrix during its growth (Inger and Bearhop, 2008). A homogenate of nestling feathers can therefore provide information about their diet during the growth period of the feathers (Bearhop et al., 2002).

As many OHCs have been shown to interfere with physiological processes linked to development and growth (Cassone et al., 2012; Jenssen et al., 2010; Nøst et al., 2012), there is special concern about levels and effects of these compounds in young developing birds. As nestlings develop and grow, their maternally transferred contaminants are significantly diluted by their growth (Bourgeon et al., 2013; Bustnes et al., 2013). However, nestlings are also exposed to OHCs through their diet and plasma concentrations of compounds with high ability for bioaccumulation may increase as the nestlings reach their adult body size at

fledging (Borgå et al., 2004; Bustnes et al., 2013). Previously, only few studies have accounted for age and growth when investigating OHCs in nestlings (Bourgeon et al., 2013; Bustnes et al., 2013; Dauwe et al., 2006; Olsson et al., 2000). In the present study, we aimed to investigate variations of OHC concentrations in plasma from white-tailed eagle nestlings sampled from two locations in two consecutive years. Secondly, we aimed to explore if variation in dietary proxies (δ^{13}C and δ^{15}N) and biological variables (such as body mass or age of the nestlings) could account for parts of the spatial and temporal variation of these OHCs. As the diet is the major source of OHCs, we expected to find a strong influence of the dietary proxies presenting increased plasma OHCs with increasing δ^{15}N (higher trophic position) and increasing δ^{13}C (more marine prey). Thus, we also expected to find some variation in OHCs in nestlings from the two locations as habitat differences may also influence the diversity of prey species at the two locations. No differences were expected between the two sampling years, as to the authors knowledge there are no local sources of OHCs at the two locations. We also expected to find higher concentrations in plasma of older and/or larger nestlings as OHCs have a high potential for bioaccumulation.

2. Materials and methods

The plasma OHC concentrations of the individual OHCs have been published previously (Løseth et al., 2019, supplementary information), in a study where three non-invasive matrices (plasma, feathers and preen oil) from white-tailed eagle nestlings were compared for legacy and emerging contaminants. In the current study, however, we present unpublished data on stable isotopes and age to explain variation in the plasma concentrations of ΣPCBs, ΣOCPs, ΣPBDEs and ΣPFASs.

2.1. Field sampling

The study was conducted on 70 white-tailed eagle nestlings from two archipelagos in Norway, Smøla (63.3-63.5°N; 7.8-8.2°E) and Steigen (67.7-67.9°N; 14.6-14.8°E), during the breeding seasons of 2015 and 2016 (Figure 1). We sampled 35 nestlings both from Smøla (2015: n = 13, 2016: n = 22) and Steigen (2015: n = 14, 2016: n = 21) during June-July of these two years (see supplementary information (SI), Table S1 for details). Sex determination was based upon morphometric measurements (Helander et al., 2007), while the age was estimated from the tail feather length. The tail feather emerges at day 30 and grows with 4.95 ± 0.02 (mean ± SE) mm per day (Pers. comm. Torgeir Nygård). Wing length has previously been used to estimate age in Swedish white-tailed eagle nestlings (Helander et al., 2007) and in our study wing and tail feather length were strongly correlated (r_{70} = 0.94, p < 0.01). All nestlings were sampled for body feathers and blood as described in Løseth et al. (2019). Body feathers were gently pulled from the dorsal region and stored in polyethylene zipper bags (VWR, USA) at -20°C. A blood sample of 8 mL was collected in heparinised vacutainers through brachial venepuncture. The blood samples were centrifuged at 860 g and plasma was transferred into cryogenic tubes (Nalgene®, USA) and stored at -20 °C. The sampling was approved by the Norwegian Food Safety Authority (Mattilsynet; 2015/6432 and 2016/8709) and the handling of the birds were in accordance with the regulations of the Norwegian Animal Welfare Act.

2.2. Stable isotope analyses

We analysed stable isotopes in the body feathers, which were still growing at the time of sampling and thus connected to the blood circulation at the calami. The analysis for bulk feather stable carbon (^{12}C and ^{13}C) and nitrogen isotopes (^{14}N and ^{15}N) was performed at the MARE Centre of the University of Liège, Belgium. Clean stainless steel and glass tools were used to remove the calami and for washing and cutting of the feathers. The tools were thoroughly rinsed with acetone between individuals. Feathers were washed in Milli-Q water

as previously described in Løseth et al. (2019) to remove dust and particles from feathers prior to analysis. A subsample of homogenised cleaned feather material (mean ± SD: 1.55 ± 0.37 mg) was wrapped into a tin combustion cup and analysed for its elemental and isotopic composition using a vario MICRO cube elemental analyser (Elementar Analysen systeme GmBH, Hanau, Germany) coupled to an IsoPrime100 mass spectrometer (Isoprime, Cheadle, United Kingdom). 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., glycine) was measured for every tenth sample and revealed an imprecision (±1 SD) of 0.23 and 0.16 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.3. Chemical analyses

The targeted compounds for the analyses were polychlorinated biphenyls (PCB; IUPAC congeners 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 138, 149, 153, 156, 170, 171, 177, 180, 183, 187, 194, 206 and 209) and organochlorinated pesticides (OCPs; dichlorodiphenyltrichloroethane (*p,p'*-DDT), *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), three isomers of hexachlorocyclohexane (α-, β-, and γ-HCH), chlordanes (*oxy*-chlordane (OxC), *cis*-nonachlor (CN) and *trans*-nonachlor (TN)) and hexachlorobenzene (HCB)). The targeted legacy flame retardants were polybrominated diphenyl ether (PBDE) congeners; BDE 28, 47, 99, 100, 153, 154 and 183. The targeted perfluoroalkyl substances (PFASs) were perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoanoic acid (PFDcA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrA), perfluorotetradecanoic acid (PFTeA), perfluorooctanesulfonamide (PFOSA), perfluorobutane sulfonate (PFBA), perfluoropentane sulfonate (PFPS), perfluorohexane

sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), linear and branched perfluorooctane sulfonate (Lin-PFOS and Br-PFOS) and perfluorononane sulfonate (PFNS).

Procedures used for the extraction and quantification have been described in detail by Løseth et al. (2019). In brief, PCBs, OCPs and PBDEs were extracted from plasma using *n*-hexane:dichloromethane (DCM, 1:1, v:v) and fractionation was performed on Supelclean™ ENVI Florisil cartridges (500 mg, 3 mL, Supelco® Analytical). The compounds were eluted with *n*-hexane:DCM and quantified according to Eulaers et al. (2011a). PFASs were extracted with methanol using the Powley method (Powley et al., 2005) and quantified according to Herzke et al. (2009). Internal standards and their recoveries are listed in SI (Table S2 and S3) and ranged from 30 – 118 % for PCBs, 41 – 90 % for OCPs, 74 – 97 % for PBDEs, and 59 – 101 % for PFASs. For every tenth plasma sample, a procedural blank was analysed to control for background contamination. To control the performance of the analytical method of the PCB, OCP and PBDE extraction, a human plasma sample from the Arctic Monitoring and Assessment Programme interlaboratory exercise was analysed for every 20th sample. For PFAS extractions, a commercially available human plasma sample (NIST SRM 1957, USA) was analysed for every tenth sample. No background contamination was encountered in the blanks for any of the analysed PFASs. For legacy POPs not detectable in the blanks, the limits of quantification (LOQs) were set to ten times the signal-to-noise ratio of sample runs or were calculated as three times the standard deviation of the procedural blanks for each compound. For PFASs, the LOQs were calculated as three times the signal-to-noise ratio of the procedural blanks for each compound. The LOQs for all compounds are available in the SI (Tables S4-S6). Concentrations of all compounds are given on a wet weight basis.

2.4. Statistical analyses

147 The statistical analyses were performed using R (v. 3.4.2, R Development Core Team, 2008).
148 The compounds that could be quantified in more than 50 % of the samples within each year
149 and location were 14 PCB congeners (CB 99, 101, 105, 118, 138, 153, 156, 170, 171, 177,
150 180, 183, 187 and 194), seven OCPs (OxC, TN, CN, *p,p'*-DDE, *p,p'*-DDT, HCB and *β*-
151 HCH), five PBDE congeners (BDE 47, 99, 100, 153 and 154) and eight PFASs (Br-PFOS,
152 Lin-PFOS, PFOA, PFNA, PFDcA, PFUnA, PFDoA and PFTriA) (Table 1 and Table S7).
153 Data below the limit of quantification (LOQ) were substituted with LOQ * detection
154 frequency (Voorspoels et al., 2002) for each compound. Profiles of the compounds included
155 in the statistical analyses are available in Figure S1. Due to the structure of the data, with two
156 to three chicks in some nests, only statistical tests from the *nlme*: Linear and nonlinear mixed
157 effect models package (Pinheiro et al., 2018) were applied and nest identity was always
158 included as a random variable to avoid pseudoreplication of nestlings within nests. Statistical
159 significance was assumed at $\alpha = 0.05$.

160 Due to collinearity between compounds within each contaminant group (Table S8 and S9),
161 compounds were summed (Σ) per group (Σ_{14}PCBs, Σ_{7}OCPs, Σ_{5}PBDEs and Σ_{8}PFASs) for
162 statistical modelling. All variables were investigated for influential outliers, normality and
163 homoscedasticity (Zuur et al., 2010). Variables that were not normally distributed were $\log_e$
164 transformed to meet criteria of parametric statistics. To ensure normality of the residuals of
165 the model, two outliers were removed from the OCP modelling. These outliers were two
166 young individuals sampled in Steigen in 2015 (47.2 and 52.4 days old) which also had the
167 highest plasma concentrations of OCPs (46.3 and 52.2 ng/mL, respectively).

168 Age was included as an explanatory variable, instead of body mass or body condition due to
169 multicollinearity. It is important to note that each nestling was only sampled once and to
170 investigate the true variation with increasing age it is preferred to sample the same
171 individuals repeatedly. A detailed description of the calculation of body condition and

correlations between age, body mass and body condition can be found in the SI. Body mass, size and age are all correlated when the nestlings are growing, but body mass may show large variations between sexes and on an individual level due to different climates, habitats, diets and parental experience. Age presents a more stable variable as it, on an individual level, can only increase, regardless of sex and diet.

Correlations between $\log_e \Sigma$ contaminant groups, age, $\delta^{13}C$ and $\delta^{15}N$ were investigated using Pearson correlation coefficient test. A strong correlation was detected between $\delta^{15}N$ and $\delta^{13}C$ ($r_{70} = 0.76$, $p < 0.01$, Figure S3), but both variables were included in the first model selection as they represent trophic position and dietary source, respectively. To investigate temporal and spatial variation of Σ_{14}PCBs, Σ_7OCPs, Σ_5PBDEs, Σ_8PFASs, age, $\delta^{13}C$ and $\delta^{15}N$, linear mixed effect analyses of variance (Lme-Anovas) were applied with location, year and the interaction between location and year as explanatory variables (Table S10). Tukey's honestly significant difference (HSD) post hoc test was applied to investigate differences in age between locations and years.

To investigate how age and the dietary proxies may contribute to the observed temporal and spatial variation, we performed linear mixed effect models for each compound group. The initial full model included location, year, the interaction between location and year, age, $\delta^{15}N$ and $\delta^{13}C$. The most parsimonious models were selected using Akaikes Information Criterion for small sample sizes (AICc). Each model was analysed for variance inflation factors (VIF) with a threshold of VIF < 3 to identify problems with collinearity among explanatory variables (Zuur et al., 2009, 2010). The model selection showed that the effect of $\delta^{15}N$ was only significant with the presence of $\delta^{13}C$ in the model, and VIF values for $\delta^{15}N$ were over 3 for some of the models. This may be due to the significant correlation detected between the two stable isotopes. For the final model selection, we therefore chose to include only $\delta^{13}C$, age, location, year and the interaction between location and year. Model selection was

197 performed on models fitted with maximum likelihood (ML), while parameters were estimated
198 using restricted maximum likelihood (REML). Models with ΔAICc < 2 are discussed below.
199 In addition to AICc, marginal pseudo-R^2 (R_m^2; explaining the variation of the fixed factors)
200 and conditional pseudo-R^2 (R_c^2; explaining the variation of both fixed and random factors)
201 were extracted according to Nakagawa and Schielzeth (2013).

202 ## 3. Results and discussion

203 ### 3.1. Organohalogenated contaminants

204 The compound groups found with the highest median wet weight concentrations in plasma
205 were PFASs > PCBs > OCPs > PBDEs. Within each compound group, the compounds with
206 the highest concentrations were linear PFOS (3.86 – 31.85 ng/mL), CB 153 (0.21 – 26.27
207 ng/mL), *p,p′*-DDE (0.48 – 47.61 ng/mL) and BDE 47 (0.01 – 1.82 ng/mL), respectively
208 (Table S7). The concentrations of Σ_{14}PCBs, Σ_7OCPs, Σ_5PBDEs and Σ_8PFASs (Table 1,
209 Figure S2A) were lower than or within the same range of those previously reported in plasma
210 from white-tailed eagle nestlings from Norway (Bustnes et al., 2013; Eulaers et al., 2011a,
211 2011b, 2013, 2014; Gómez-Ramírez et al., 2017).

212 ### 3.2. Nestling age and dietary proxies

213 The age span of the nestlings varied significantly between locations and years, although the
214 nestlings were sampled within the same two calendar weeks each year (Table 1, Figure S2B).
215 In 2015, the nestlings from Smøla were on average 79 days old, which was 15 days older
216 than those from Steigen (z = 3.5, $p < 0.01$). The Smøla nestlings sampled in 2015 were also
217 13 days older than those sampled at Smøla and Steigen in 2016 (z = 3.2 – 3.4, $p < 0.01$, Table
218 S10). In 2016, there were no significant age differences between the nestlings sampled at
219 Smøla and Steigen. We also found significantly higher δ^{15}N and δ^{13}C, as well as narrower

dietary niches, in nestlings from 2015 than in nestlings from 2016 ($F_{(1,44)}$ = 8.8 and 4.9, p < 0.01, respectively, Figure S3, Table 1). The results also showed that the nestlings from Steigen fed on a diet more enriched in ^{15}N than those from Smøla ($F_{(1,44)}$ = 15.7, p < 0.01, Figure S3), indicating that the Steigen nestlings may have been feeding on a higher trophic position. The temporal variation found for both stable isotopes may indicate a slight change in prey species between the two years at both locations. Within both years, some birds from Smøla and Steigen had $\delta^{13}C$ values lower than -20 ‰ which can indicate influence of more terrestrial prey in their diet (Fry, 2006). This was coherent with the observed prey remains around their nests, which, besides from fish and seabirds, consisted of terrestrial species such as greylag goose (*Anser anser*), hare (*Lepus timidus*) and hedgehogs (*Erinaceus europaeus*). The interannual dietary changes reported here are not uncommon for opportunistic feeders such as white-tailed eagles (Inger and Bearhop, 2008), as it can correspond to variations in availability of prey species (Nadjafzadeh et al., 2016).

3.3. Model selection to best explain OHC variation

The model selection confirmed age and diet as important predictors for the temporal and spatial variation of legacy OHCs observed in the initial analyses (Table S10) as they were included in all the most parsimonious models for PCBs, OCPs and PBDEs (Table 2, see Table S11 - S13 for all competing models). For PFASs on the other hand, only age was selected as an important predictor for the observed temporal and spatial variation (Table S10) as it was included in all the most parsimonious models for PFASs variation (Table 2, see Table S14 for all competing models). It is important to note that these results are statistical models which estimates the OHC variation and in order to investigate the true OHCs variation with increasing age, repeated sampling is necessary.

3.3.1 Legacy OHC variation

Contrary to our hypothesis, the models for Σ_{14}PCBs, Σ_7OCPs and Σ_5PBDEs indicated significantly lower concentrations of legacy OHCs in older nestlings and in nestlings with a diet more enriched in ^{13}C (i.e. more marine prey; Figure 2). Some of these models also included location, year and the interaction between location and year, which contributed to a better fit of the model. The results of the lme-Anova showed significant temporal and spatial variation in PCB, OCP and PBDE levels (Table S10), however when we accounted for age and diet in the model selection, the temporal and spatial variations for PCBs and PBDEs were not significant anymore (Table 2). It was only for Σ_7OCPs that the estimates indicated significantly higher concentrations in nestlings from Steigen than those from Smøla (p = 0.01), as well as significantly higher concentration in nestlings from Steigen in 2015 than in 2016 (p = 0.03). In contrast to what was observed for Σ_{14}PCBs and Σ_5PBDEs, the effect of age was not statistically significant for Σ_7OCPs (β_1 = 0.012, p = 0.07). However, it is important to mention that for these models two of the youngest and most contaminated individuals were excluded from the analyses to ensure normality of the residuals, and that the inclusion of these outliers resulted in a significant effect of age on Σ_7OCPs (β_1 = 0.018, p = 0.03). This should therefore be considered in the interpretation of the estimates of the Σ_7OCP models.

3.3.1.1 Influence of age

The inverse relationship between plasma legacy OHC concentrations and age at sampling found in the present study was in accordance with previous reports for CB 153 and *p,p'*-DDE in plasma of white-tailed eagle nestlings (Bustnes et al., 2013), plasma levels of PCBs and PBDEs in great tit (*Parus major*) nestlings (Dauwe et al., 2006) and liver concentrations of PCBs, *p,p'*-DDE and HCB in European shag (*Phalacrocorax aristotelis*) nestlings (Jenssen et al., 2010; Murvoll et al., 2006). In contrast, a previous study on white-tailed eagle nestlings did not find decreased PCB or *p,p'*-DDE concentrations in plasma of older nestlings (Olsson

et al., 2000), neither did a study of PBDEs in plasma of bald eagle (*Haliaeetus leucocephalus*) nestlings (Guo et al., 2018). The nestlings from the present study were on average 69 days old (range: 44 – 87 days old), while most of the nestlings from Olsson et al. (2000) were less than 57 days old (range: < 36 – 57 days old). The nestlings investigated in Guo et al. (2018) were on average 46 days old (range: 28 – 56 days old). The significant effect of age in the present study may be due to the greater age span, larger sample size and homogenous age classes of the nestlings. Thus, allowing more time for growth dilution or changes in metabolic capability/excretion in older nestlings and a higher statistical probability to detect such changes.

Even though nestlings are continuously exposed to OHCs through their diet, a study on experimental feeding of great skua chicks (*Stercorarius skua*) found that their contaminant load was more influenced by maternal than trophic transfer regardless of diet (Bourgeon et al., 2013). A study of paired egg and plasma samples of bald eagles from the Great Lakes between 2000 and 2012 found that egg concentrations of PBDEs were over 30 times higher than the plasma concentrations of nestlings from the same nests (Guo et al., 2018). Nygård and Polder (2012) also found very high concentrations of PCBs (mean: 2839 ng/g fresh weight (fw)) and *p,p'*-DDE (mean: 950 ng/g fw) in white-tailed eagle eggs sampled in Norway between 2005 and 2010. Although egg and plasma concentrations cannot be directly compared, these reported concentrations were several folds higher than the plasma concentrations found in the present study. As concentrations in plasma reflect internal concentrations in the nestling, we propose that the decreasing legacy OHC concentrations with increasing age may be due to growth dilution of maternally derived compounds deposited with high concentrations in the eggs.

3.3.1.2 Influence of diet

293 Our results also indicated decreasing Σ_{14}PCBs, Σ_{7}OCPs and Σ_{5}PBDEs concentrations with
294 increasing δ^{13}C, which corresponds with previous reports of decreases in CB 153, *p,p'*-DDE
295 and HCB in white-tailed eagle nestlings with diets more enriched in ^{13}C (Bustnes et al.,
296 2013). Bustnes et al. (2013) explained this relationship by the depleted ^{13}C levels found in
297 lipids compared to proteins (Post et al., 2007) and suggested that the diet of the more
298 contaminated nestlings may have contained more lipid-rich prey, such as gulls (*Laridae*),
299 which may also have contained higher concentrations of biomagnifying OHCs (Bustnes et al.,
300 2013). Surprisingly, the more contaminated nestlings from Smøla were feeding on a lower
301 trophic position (depleted in ^{15}N) and terrestrial prey remains surrounding their nest which
302 were located more inland on the island. The contaminant concentrations in these nestlings
303 may therefore have been highly influenced by maternally derived OHCs (Bourgeon et al.,
304 2013). White-tailed eagles have been reported to change their diet in the winter according to
305 the availability of prey species (Willgohs, 1984). It is therefore possible that the mothers of
306 these nestlings have fed on a diet more enriched in lipids, containing higher concentrations of
307 OHCs, during the winter months and before egg laying. Such seasonal dietary changes of the
308 mothers may influence the concentrations of legacy OHCs in their eggs and subsequently in
309 their nestlings (Bourgeon et al., 2013). In contrast, stable isotopes deposited in the keratin in
310 nestling feathers originate mostly from their diet and not from maternal transfer (Bearhop et
311 al., 2002). Although we cannot be certain whether such a dietary change has taken place, one
312 should always keep in mind that the stable isotopes analysed in feathers only reflect the diet
313 in the period during which they were grown (Bearhop et al., 2002).

314 A study on bald eagle nestlings also found that δ^{13}C was generally a better predictor of legacy
315 OHC concentrations than δ^{15}N in eagles from marine environments, even when the two stable
316 isotope ratios were correlated (Elliott et al., 2015). This was confirmed by the results in the
317 current study as the final model selection did not include δ^{15}N and no significant correlations

were found between $\delta^{15}N$ and the OHC groups. However, significant positive correlations between $\delta^{15}N$ or trophic level and several legacy POPs have been found in previous studies on both white-tailed eagle (Bustnes et al., 2013; Eulaers et al., 2013, 2014) and bald eagle nestlings (Elliott et al., 2015).

3.3.2. PFAS variation

Contrary to the legacy OHCs models, the models for PFASs indicated no significant effect of $\delta^{13}C$ on PFAS concentrations in plasma and the most parsimonious model included age, location and year (Table 2, Figure 3). These results were not unexpected as PFASs, have different physicochemical properties than legacy OHCs and may therefore have different exposure routes and toxicokinetics (Lau et al., 2007).

3.3.2.1 Influence of age

Interestingly, we found opposite age-related effects for PFASs compared to PCBs, OCPs and PBDEs. This confirms our initial hypothesis that older nestlings have higher plasma concentrations than younger nestlings. Similar increases with age have previously been reported for PFOS in white-tailed eagle nestlings (Bustnes et al., 2013) and for PFNA and PFUnA in bald eagle nestlings (Route et al., 2014). In contrast to the legacy OHCs, the PFAS concentrations in the present study were similar to those found in Norwegian white-tailed eagle eggs sampled between 2005 and 2010 (mean: 55.3 ng/g fw; Nygård and Polder, 2012). Concentrations of maternally deposited compounds are diluted in nestlings during growth regardless of their physicochemical properties (Bustnes et al., 2013). Although egg and plasma concentrations cannot be directly compared, these results and the higher PFAS concentrations found in older nestlings suggests continuous dietary intake as an important PFASs source in the present study, rather than maternal transfer.

341 *3.3.2.2 Spatial variation*

342 The model estimates also indicated significantly higher PFAS concentrations in nestlings
343 from Steigen than in those from Smøla (Table 2, $p < 0.01$). At the same time, significantly
344 higher $\delta^{15}N$ were detected in nestlings from Steigen than nestlings from Smøla as well as
345 significant correlations between PFAS concentrations and $\delta^{13}C$ ($r_{70} = 0.25$, $p = 0.03$) and
346 $\delta^{15}N$ ($r_{70} = 0.44$, $p < 0.01$). Thus, we cannot exclude trophic position as an important factor
347 influencing this PFAS variation. Nevertheless, the absence of stable isotopes in the most
348 parsimonious PFAS models corresponds with previous reports in plasma from Norwegian
349 white-tailed eagle nestlings (Bustnes et al., 2013; Gómez-Ramírez et al., 2017) and several
350 seabirds (Gebbink et al., 2011; Haukås et al., 2007; Leat et al., 2013; Miller et al., 2015;
351 Vicente et al., 2015).

352 *3.3.2.3 Temporal variation*

353 The model also indicated significantly higher PFAS concentrations in nestlings sampled in
354 2015 than in 2016, at both locations (Table 2, $p < 0.01$). This interannual variation
355 corresponds with a previous study on white-tailed eagle nestlings from Troms and
356 Vesterålen, Norway in 2011 and 2012 (Sletten et al., 2016). The authors of that study
357 suggested dietary differences as the main reason for that variation (Sletten et al., 2016),
358 which corresponds with the present study as we also detected significant differences in stable
359 isotopes between years. Interestingly, the difference between 2015 and 2016 in PFAS plasma
360 concentrations in the present study also corresponds with reports on PFASs in air, where
361 higher concentrations of several PFASs were found at three monitoring stations in Norway in
362 2015 compared to 2016 (Bohlin-Nizzetto et al., 2017; Bohlin-Nizzetto and Aas, 2016). Thus,
363 yearly differences in long range transport of PFASs and its precursors may play a role, as
364 they can be subsequently taken up into the food web (Houde et al., 2011) and their top

predators (Bustnes et al., 2015). To our knowledge, there are no significant PFAS sources at the two locations that may influence PFASs concentrations in the white-tailed eagle nestlings. However, due to the significantly higher stable isotope values in nestlings from 2015 and correlation between δ^{15}N values and PFAS concentrations, we suggest a combination of PFAS exposure from long range transport and dietary sources as important factors explaining this temporal variation.

4. Conclusions

In the present study, we report age as one of the most important predictors for spatial and temporal variation of OHCs in plasma from white-tailed eagle nestlings from Smøla and Steigen, Norway. It is important to note that the nestlings in the present study were only sampled once, and that the models were based on results from nestlings ranging from 44 to 87 days old. Our results indicated lower plasma concentrations of PCBs, OCPs and PBDEs, and higher concentrations of PFASs in nestlings sampled at an older age. The variations of PCBs, OCPs and PBDEs were also significantly explained by the dietary carbon source (δ^{13}C), indicating that nestlings feeding on diets enriched in ^{13}C, such as marine or lipid rich prey, had lower plasma concentrations of these compounds. The stable isotope ratio of nitrogen (δ^{15}N) indicated that nestlings from Steigen were feeding at a higher trophic position than those from Smøla, although it was of less importance in explaining the OHC variations. We also found higher stable isotope ratios in nestlings sampled in 2015 compared to 2016 which may suggest dietary differences. The present study demonstrates the importance of taking age into consideration when investigating OHC concentrations in bird of prey nestlings, regardless of the sample matrix (as strong correlations were found between concentrations of PCBs, OCPs and PBDEs in feathers, plasma and preen oil; see Løseth et al., 2019). Our results also indicate that diet may contribute to variations in plasma OHC

concentrations, especially for PCBs, OCPs and PBDEs in opportunistic birds such as the white-tailed eagle.

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Table 1: Median, min and max values of stable isotopes from body feathers, age and sum of PCBs, OCPs, PBDEs and PFASs detected in plasma of white-tailed eagle nestlings sampled in Smøla and Steigen (Norway) in 2015 and 2016. A full list of concentration data for the individual compounds can be found in Løseth et al. (2019).

		Smøla						**Steigen**					
		2015 *n* = 13			2016 *n* = 22			2015 *n* = 14			2016 *n* = 21		
	unit	median	min	max	median	Min	max	median	min	max	median	min	max
$\delta^{13}C$	‰	-18.56	-20.82	-17.15	-19.02	-20.79	-17.15	-18.66	-19.24	-17.73	-19.11	-20.34	-18.33
$\delta^{15}N$	‰	+13.82	+12.45	+15.07	+13.39	+11.54	+15.28	+14.54	+13.89	+15.17	+13.96	+13.43	+14.73
Age	days	80.51	64.75	87.37	66.77	52.22	81.92	64.65	44.34	84.75	70.61	50.40	81.92
Σ_{14}PCBs[a]	ng/mL	2.00	0.82	8.47	4.86	1.86	34.52	5.12	2.95	59.05	5.79	1.58	35.92
Σ_{7}OCPs[b]	ng/mL	2.01	0.89	6.28	2.75	1.05	15.33	4.75	2.80	52.19	5.79	1.31	12.96
Σ_{5}PBDEs[c]	ng/mL	0.10	0.06	0.46	0.16	0.05	1.51	0.34	0.1	2.64	0.23	0.03	0.73
Σ_{8}PFASs[d]	ng/mL	25.69	10.29	46.65	9.18	4.58	13.26	31.80	18.36	52.94	12.76	7.21	32.90

[a] Σ_{14}PCBs: CB 99, 101, 105, 118, 138, 153, 156, 170, 171, 177, 180, 183, 187 and 194
[b] Σ_{7}OCPs: OxC, TN, CN, *p,p'*-DDE, *p,p'*-DDT, HCB and β-HCH
[c] Σ_{5}PBDEs: BDE 47, 99, 100, 153 and 154
[d] Σ_{8}PFASs: Br-PFOS, Lin-PFOS, PFOA, PFNA, PFDcA, PFUnA, PFDoA and PFTriA

Table 2: Model estimates from the most parsimonious models (ΔAICc < 2) explaining the variation of Σ_{14}PCBs, Σ_7OCPs, Σ_5PBDEs and Σ_8PFASs in plasma of white-tailed eagle nestlings (n = 70) from Smøla and Steigen. The table includes the model intercept (β_0), model estimates (β_x), significance values (p), and marginal pseudo-R^2 (R^2_m) and conditional pseudo-R^2 (R^2_c). The year variable (Yr) represents 2016 and location variable (Loc) represents Steigen. Beta estimates follow the order of the factors in the models. Statistical significance (α = 0.05) is marked with *.

Compound group	**Explanatory variables**	β_0	β_1	β_2	β_3	β_4	β_5	***p-values***	**ΔAICc**	R^2_m	R^2_c
Σ_{14}PCBs	~ age + δ^{13}C + Loc	-3.07	-0.03	-0.36	0.43			<0.01*; 0.01*; 0.08	0.00	0.28	0.89
	~ age + δ^{13}C	-2.61	-0.03	-0.35				<0.01*; 0.01*	0.81	0.22	0.89
	~ age + δ^{13}C + Loc + Yr + Loc:Yr	-3.66	-0.03	-0.35	1.03	0.57	-0.95	0.01*; 0.02*; 0.01*; 0.12; 0.06	1.03	0.34	0.89
Σ_7OCPs[a]	~ age + δ^{13}C + Loc + Yr + Loc:Yr	-5.00	-0.01	-0.36	0.91	0.13	-0.80	0.07; <0.01*; <0.01*; 0.62: 0.03*	0.00	0.37	0.91
	~ δ^{13}C + Loc + Yr + Loc:Yr	-5.71	-0.35	1.07	0.28	-0.98		<0.01*; <0.01*; 0.23; <0.01*	0.15	0.37	0.88
Σ_5PBDEs	~ age + δ^{13}C	-6.71	-0.03	-0.38				<0.01*; <0.01*	0.00	0.22	0.86
	~ age + δ^{13}C + Loc + Yr + Loc:Yr	-8.39	-0.02	-0.43	0.87	0.14	-0.86	0.02*; <0.01*; 0.03*; 0.70; 0.08	0.46	0.32	0.86
	~ age + δ^{13}C + Loc	-7.07	-0.03	-0.38	0.31			<0.01*; <0.01*; 0.19	0.54	0.25	0.86
	~ age + δ^{13}C + Yr	-7.28	-0.03	-0.43	-0.31			<0.01*; <0.01*; 0.23	0.83	0.23	0.86
	~ age + δ^{13}C + Loc + Yr	-7.65	-0.03	-0.43	0.31	-0.31		<0.01*; <0.01*; 0.19; 0.22	1.34	0.27	0.86
Σ_8PFASs	~ age + Loc + Yr	1.66	0.02	0.54	-0.80			<0.01*; <0.01*; <0.01*	0.00	0.73	0.93

[a] Two outliers were removed from these models, n = 68.

List of figures

Figure 1

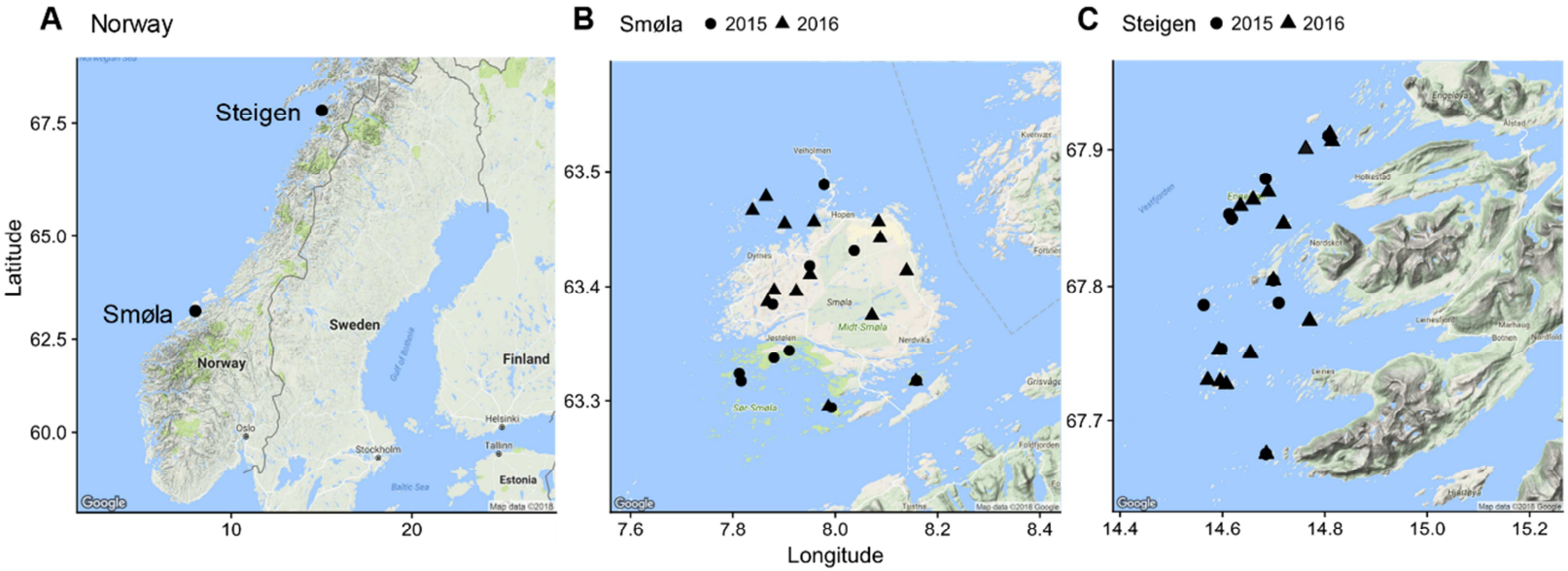
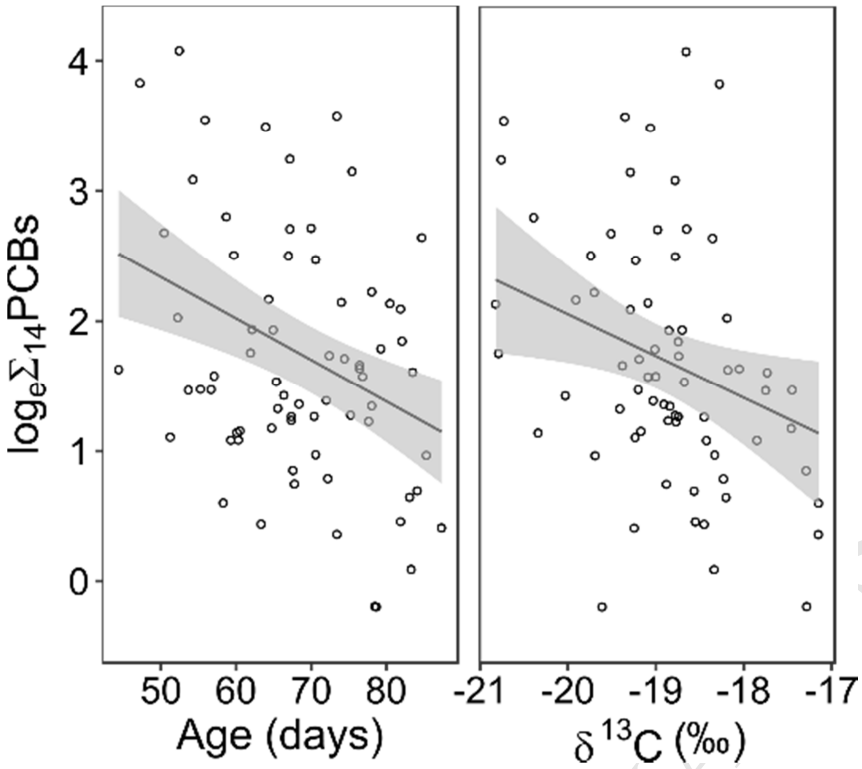
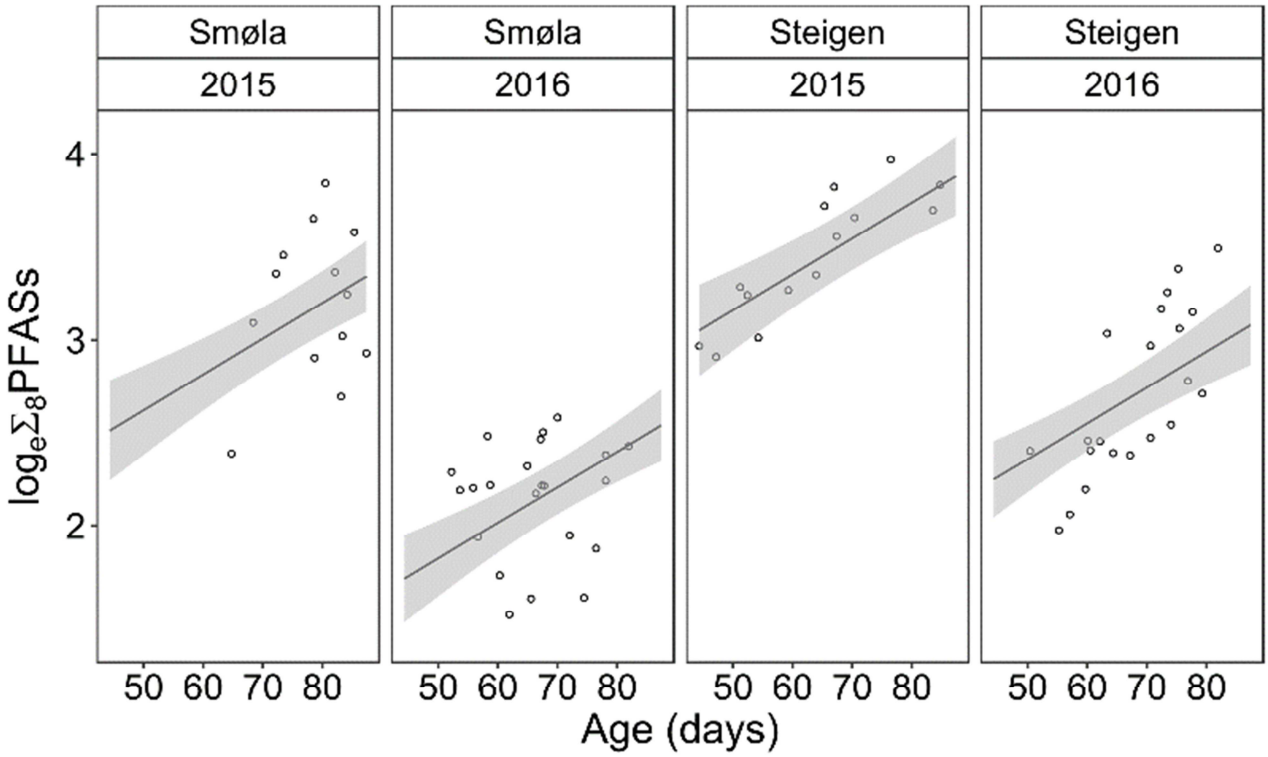


Figure 2



23 **Figure 3**



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Highlights:

- Significant temporal and spatial variations were found for all compound groups
- Age was the most important predictor for contaminant variation in nestling plasma
- Concentrations of legacy PCBs, OCPs and PBDEs decreased with age
- Concentrations of PFASs increased with age
- $\delta^{13}C$ significantly predicted the variation of legacy PCBs, OCPs and PBDEs