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# Feathers as an integrated measure of organohalogen contamination, its dietary sources and corticosterone in nestlings of a terrestrial bird of prey, the northern Goshawk (*Accipiter gentilis*)

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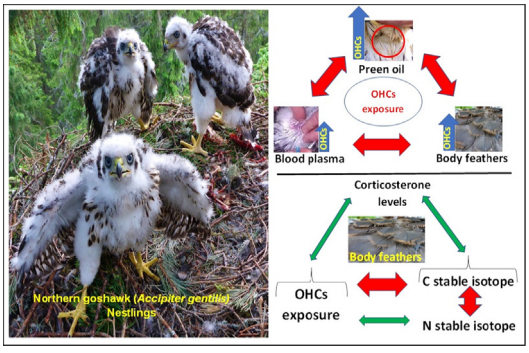
## HIGHLIGHTS

- OHC levels were significantly correlated between body feathers, plasma and preen oil in nestling northern goshawks.
- OHCs, stable C and N isotopes, as well as corticosterone, were all quantified in nestling body feathers.
- $\delta^{13}C$ was identified as the most suitable predictor for variation in feather OHC concentrations.
- A relationship among OHC levels, $\delta^{13}C$ and $\delta^{15}N$ values and corticosterone levels was not found.
- Body feathers can be used as a tool to assess nestling ecophysiology and ecotoxicology.

## GRAPHICAL ABSTRACT

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## ABSTRACT

In this study, we evaluated the suitability of body feathers, preen oil and plasma for estimation of organohalogen compound (OHC) exposure in northern goshawk *Accipiter gentilis* nestlings ($n$ = 37; 14 nests). In addition, body feathers received further examination concerning their potential to provide an integrated assessment of (1) OHC exposure, (2) its dietary sources (carbon sources and trophic position) and (3) adrenal gland response (corticosterone). While tetrabromobisphenol A was not detected in any sample, the presence of polychlorinated biphenyls, organochlorine pesticides, polybrominated diphenyl ethers and hexabromocyclododecane in body feathers (median: 23, 19, 1.6 and 3.5 ng $g^{-1}$ respectively), plasma (median: 7.5, 6.2, 0.50 and 1.0 ng $g^{-1}$ ww, respectively) and preen oil (median: 750, 600, 18 and 9.57 ng $g^{-1}$ ww, respectively) suggests analytical suitability for biomonitoring of major OHCs in the three matrices. Furthermore, strong and significant associations ($0.20 \leq R^2 \leq 0.98$; all $P < 0.05$) among the OHC concentrations in all three tissues showed that body feathers and preen oil reliably reflect circulating plasma OHC levels. Of the dietary proxies, $\delta^{13}C$ (carbon source) was the most suitable predictor for variation in feather OHCs concentrations, while no significant relationships between body feather OHCs and $\delta^{15}N$ (trophic position)

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Plasma
Stable isotopes

were found. Finally, body feather corticosterone concentrations were not related to variation in OHC concentrations. This is the first study to evaluate feathers of a terrestrial bird of prey as an integrated non-destructive tool to jointly assess nestling ecophysiology and ecotoxicology.

## 1. Introduction

Biomonitoring organohalogen compound (OHC) exposure and associated health effects in avian wildlife is of great concern given earlier observations on the potential of these contaminants to elicit drastic population declines (Bourgeon et al., 2012; Letcher et al., 2010). OHCs undergo long-range transport (Rigét et al., 2010) and can elicit toxic effects in biota at areas remote from sources (de Wit et al., 2010; Letcher et al., 2010). High persistence and high lipophilicity of major OHCs, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs), result typically in biomagnification and thus render especially apex predators vulnerable to their toxicity. At the same time, these characteristics make apex predators, such as birds of prey, excellent sentinel species for monitoring of environmental OHC contamination (Abbasi et al., 2017a, 2017b; Garcia-Fernandez et al., 2008).

Currently, feathers and blood are recognized as non-destructive matrices to quantify OHC exposure in birds of prey (Espín et al., 2016). While predominantly adult feathers have been validated and used (Jaspers et al., 2006a, 2006b, 2007a, 2007b), nestling feathers have more recently been suggested as a promising for biomonitoring (Briels et al., 2019; Løseth et al., 2019a; Monclús et al., 2019). Feathers are overall an attractive OHC biomonitoring matrix as they simultaneously archive the exposure to OHCs and its dietary origin (Eulaers et al., 2013, 2014). To interpret the latter, measurements of stable carbon and nitrogen isotopes have become the analytical backbone to unravel a bird's feeding habitat and trophic level, respectively (Bustnes et al., 2013; Ramos and González-Solís, 2012). Then again, preen oil, another promising biomonitoring matrix, has received much less attention (Eulaers et al., 2011b; Løseth et al., 2019b; Jaspers et al., 2008; Yamashita, 2007). Preen oil is a lipid-rich substance, secreted through the preen gland located at the base of the tail feathers, picked up by the beak and smeared on the feathers of birds to protect them from parasites and make them water-repellant (Yamashita, 2007; Yamashita et al., 2018). Previously preen oil has been successfully utilized for quantification of persistent organic pollutants in common magpie, *Pica pica* (Jaspers et al., 2008), nestlings of white-tailed eagle *Haliaeetus albicilla* (Eulaers et al., 2011b; Løseth et al., 2019b) and seabirds (Yamashita, 2007; Yamashita et al., 2018). The use of feathers and preen oil is increasingly promising when collected at the nestling stage, as confounding by biological variables, such as sex and physiology, e.g. metabolic capacity, moult and reproductive status, is minimised (Eulaers et al., 2011a, 2013; Jaspers et al., 2007b). Finally, sampling nestlings is highly relevant, as compromised health during this stage of development may have essential survival consequences later on.

Certain OHCs structurally resemble steroid hormones and may interfere with an organism's homeostasis, development, reproduction, behaviour and survival, as endocrine disruptors (Schoech et al., 2011; Letcher et al., 2010). Of particular concern is the potential of OHCs to disrupt glucocortocoidogenesis (Bourgeon et al., 2012; Love et al., 2003b; Nordstad et al., 2012). Quantification of glucocorticoids is a robust integrator of the capacity to cope with environmental stressors. Still, it has been mostly restricted to the measurement of blood corticosterone (CORT), reflecting short-term stress responses only (Bortolotti et al., 2008). In contrast, measurement of CORT deposited in growing feathers was suggested to allow for long-term integrated measurement of stress exposure (Bortolotti et al., 2008; Fairhurst et al., 2013, 2015). Previously, feathers CORT levels in red kite (*Milvus milvus*) nestlings were found to be associated with the most persistent OHCs but not to emerging organic compounds (Monclús et al., 2019). In contrast, a negative relationship with feather's CORT level and corresponding perfluoroalkyl substances (PFASs) was reported in white-tailed eagle nestlings (Løseth et al., 2019a). While CORT levels in feathers of birds of prey nestlings have been widely accepted as a potential indicator to measure stress in relation to environmental contaminant exposure (Ganz et al., 2018). Although a study has recently been reported (Løseth et al., 2019a) on marine birds of prey, white-tailed eagle *Haliaeetus albicilla*, to the best of our knowledge an integrated assessment of OHCs, dietary sources and CORT levels in feathers of a terrestrial bird of prey has not been performed yet. Therefore, the current study is designed to (1) investigate the analytical suitability of minimally invasive biomonitoring approaches such as body feathers, preen oil and plasma for monitoring OHCs and (2) assess the relationship between OHCs exposure, dietary tracers and corticosterone in body feathers of northern goshawk nestlings.

## 2. Materials and methods

### 2.1. Field sampling

The study area (63.02–64.28°N, 10.03–12.32°E) was located in the county of Trøndelag in Norway. The breeding activity of northern goshawk (hereafter NG) pairs was checked during May 2014, and nests with observed breeding activity were revisited for sampling shortly before the expected fledgling age in late June/early July 2014. The nestlings were shortly removed from the nest for morphometric measurement and minimally invasive sampling. Sampling was conducted on nestlings between 3 and 6 weeks old. The sex was determined based on tarsus width (Helander et al., 2007), for which females show a distinctly larger tarsus (>6.8 mm) than males do (<6.8 mm), while age was determined following Kenward (2010) using the wing length. Scaled mass index (SMI) was calculated to quantify the body mass of the birds relative to their body size, following the equation provided by Peig and Green (2009). A blood sample (<2.0 mL) was taken from the brachial vein with a heparinized syringe and centrifuged the same day at 6000 rpm for 6–7 min. A total of 37 nestlings from 14 nests were successfully sampled for 4–7 recently grown body feathers (from the interscapular region) and blood (<2.0 mL), while from eight nestlings from four different nests, an additional preen oil sample was also obtained. Plastic spoons were used to squeeze out oil from the preen gland. All samples were stored in a cool and dark environment during transportation and kept frozen at −20 °C before chemical analysis. The study was conducted with permission from the Ethical board of the Norwegian Food Safety Authority (FOTS ID 6430).

### 2.2. Analysis for organohalogen compounds

Sample preparation and quantification of targeted OHCs in feathers, plasma and preen oil were carried out at the Toxicological Centre of the University of Antwerp (Belgium) following analytical protocols earlier reported by Eulaers et al. (2011b, 2014), with minor adjustments. Briefly, one body feather was kept for CORT analysis while the other body feathers of the same individual were homogenised to be used for OHC and stable isotope analysis. Prior to feather homogenisation, the calamus was removed and each feather's mass and length were recorded. Feathers were washed with distilled water and dried at room temperature. Each sample (mean ± S.D) of feather 0.12 ± 0.04 g, preen oil 0.03 ± 0.01 g and 0.75 ± 0.17 mL plasma was spiked with an internal standard mixture comprising CB 143, $\varepsilon$-hexachlorohexane (HCH), BDE 77, $^{13}$C-isomers ($\alpha$, $\beta$ and $\gamma$) of hexabromocyclododecane (HBCD) and $^{13}$C-tetrabromobisphenol A (TBBPA) before extraction. The samples were extracted using matrix-specific protocols as described by Eulaers et al. (2011b, 2014).

All samples were analysed for 27 PCB congeners (CB 28, 49, 52, 74, 95, 99, 101, 105, 118, 128, 132, 138, 146, 149, 156, 170, 171, 174, 177, 180,

183, 187, 194, 196/203, 199, 206 and 209), 7 PBDE congeners (BDE 28, 47, 99, 100, 153, 154 and 183), hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (*p,p'*-DDT), dichlorodiphenyldichloroethylene (*p,p'*-DDE), chlordanes (CHLs) such as *cis*-nonachlor (CN), *trans*-nonachlor (TN) and oxychlordane (OxC), HBCD diastereoisomers ($\alpha$-, $\beta$-, and $\gamma$-isomers), and TBBPA. All OHCs were quantified using a gas chromatograph (Agilent GC 6890, Palo Alto, CA, USA) coupled to a mass spectrometer (Agilent MS 5973), except for HBCDs and TBBPA, which were quantified using a liquid chromatograph (Agilent 1100) coupled to a triple quadrupole mass spectrometer (Agilent 6410). Further details are reported earlier by Eulaers et al. (2011b, 2014). Mean (± SD) recovery of the internal standards PCB 143 and BDE 77 were 86 ± 6% and 93 ± 10%, respectively. Concentrations were corrected by average procedural blank values, and 3 x SD of the procedural blank value represented the limit of quantification (LOQ). LOQs for compounds not detectable in blanks were set at a 10:1 signal to noise ratio (Table SI-1). The concentrations of all OHCs are expressed as ng $g^{-1}$ wet weight (ww) for plasma and preen oil and ng $g^{-1}$ feather. In previous studies the concentrations in feathers are reported as ng $g^{-1}$ dry weight (dw) but drying at room temperature may still contain some moisture contents as indicated by freeze drying hence, we avoid using dry weight for feathers.

### 2.3. Analysis for corticosterone

The analysis for feather CORT concentrations was performed by enzyme-linked immunosorbent assay (ELISA; Corticosterone Enzyme Immunoassay kit ADI-900-097; Enzo® Life Sciences, New York, USA) at the Department of Biology at the Norwegian University of Science and Technology. Prior to homogenisation, one body feather was cleaned with distilled water, its calamus was removed and its length (mean ± S.D; 218.5 ± 31.6 mm) and mass (mean ± S·D; 9.26 ± 2.15 mg) were recorded. A methanol-based extraction technique was used as described in detail by Bortolotti et al. (2008). Evaporated extracts were reconstituted in 0.5 mL 1:10 phosphate buffer saline (PBS, pH 7.4), based on previous in-house analyses of NG nestling feathers. The optical density was measured on a Synergy HT microplate reader (Bio-Tek Instruments, Winooski, USA). A detailed description of the ELISA procedure can be found in the kit's manual (EnzoLifeSciences, ADI-900-097). Our analysis revealed a mean intra-assay coefficient of variation (± SD) of 15 ± 0.36%, and blank samples (extraction and assay blanks), total activity, non-specific binding and maximum binding activity were controlled for. Finally, the producer reported the kit to provide high sensitivity (27 pg $mL^{-1}$) and low cross-reactivity for other steroids (EnzoLifeSciences, ADI-900-097). All CORT data are registered as a mean of duplicate analyses, both as pg $g^{-1}$ and as pg $mm^{-1}$.

### 2.4. Analysis for stable isotopes

The analysis for stable carbon ($^{12}C$ and $^{13}C$) and nitrogen ($^{14}N$ and $^{15}N$) isotopes (SIs) in the nestlings' body feathers was performed at the Oceanology laboratory of the University of Liège (Belgium). A subsample of homogenised cleaned feather material (mean ± SD: 1.50 ± 0.29 mg) was wrapped into a tin combustion cup and was analysed for its elemental and isotopic composition using a Vario MICRO cube elemental analyser (Elementar Analysensysteme GmBH, Hanau, Germany) coupled to an IsoPrime100 mass spectrometer (Isoprime, Cheadle, United Kingdom). The reported stable carbon ($\delta^{13}C$) and nitrogen isotope values ($\delta^{15}N$) are expressed as $\delta$ (‰) relative to the international reference standards Vienna PeeDee Belemnite and atmospheric nitrogen, respectively. IAEA-CH-6 (sucrose) and IAEA-N-2 (ammonium sulphate) were used as CRMs for carbon for nitrogen, respectively. Glycine (Merck, Darmstadt, Germany; $\delta^{13}C = -47.5 \pm 0.3$‰; $\delta^{15}N = 2.3 \pm 0.3$‰) was interspersed among samples and used as a secondary analytical standard. One sample was randomly selected for replication and measured every 15 analyses. Standard deviation on this replicated sample was 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$.

### 2.5. Data treatment and statistical analysis

The statistical analysis was carried out using R version 3.1.2 (R Core Team, 2014). Data below the LOQ was substituted with DF x LOQ, where DF is the detection frequency of individual samples above the LOQ, and compounds DF < 0.50 were excluded from further data analyses. Data exploration for outliers, normality and homoscedasticity were performed following recommendations by Zuur et al. (2010). As such, all OHC data required ln transformation. The statistical significance was set to $\alpha = 0.05$, while $0.05 \leq P < 0.10$ was considered a trend given the limited sample size of the present study.

To compare the suitability of body feathers and preen oil to reflect internal plasma OHC concentrations and profiles, congener profiles were created from the relative proportion of the specific congener to its higher OHC class (i.e. PCBs, PBDEs, OCPs) or for the OHC class to the total OHC load. Proportion data was arcsine-transformed, and analysis of variance (ANOVA) was used to detect differences among groups. Tukey's Honesty Significant Differences Test were used to make post-hoc comparisons for which $P$ values were adjusted ($P_{adj}$). Associations between plasma OHC concentrations and those in body feathers or preen oil were performed by linear regression. We report regression slopes ($\beta_0$) and intercepts ($\beta_1$), adjusted $R^2$ and $P$ values. For this analysis, the total sum of an OHC class was calculated from only those compounds detected in all matrices so that a direct comparison between the matrices could be made.

To investigate the associations among feather OHC concentrations, SI values and CORT concentrations, we used linear mixed effect models (LMEM), including the random variable nest, to avoid pseudoreplication (*nmle* package; Pinheiro et al., 2015). No interactions were included in order to maximize degrees of freedom for the main effect variables due to the limited sample sizes. The relationships to sex and scaled mass index (SMI) were tested before model selection. No significant relationships were found between the variables and sex ($P = 0.27$, $R^2 = 0.83$) nor SMI ($P = 0.71$; $R^2 = 0.82$), so neither were included as predictors in the model selection procedure. Two individuals (KO2 and HÅ1) were found influential outliers as they changed the relationship between pollutants and SIs. The standardized residuals of KO2 exceeded 2.5 (not detectable for HÅ1 as it was the only individual in the nest), and they were both considered high leverage points, clearly visualized in Figs. SI-1 and SI-2. Therefore, they were both excluded from LMEM analysis. The most parsimonious models describing the variation in CORT levels were chosen according to Akaike's Information Criteria corrected for small sample sizes ($AIC_c$). Models with a $\Delta AIC_c < 2.00$ were considered as plausible candidate models. Finally, ANOVA was performed for the best models to evaluate the variables explaining feathers OHCs level.

## 3. Results

### 3.1. Comparison of concentrations and profiles among matrices

A holistic summary for all individual OHCs detected in at least one of the sampling matrices from the northern goshawk nestlings is presented in Table 1. A list of all analysed OHCs with their limit of quantification and detection frequency (DF; Table SI-1), the concentrations and ranges of OHCs per lipid weight in preen oil (Table SI-2) are presented in supporting information. In general, PCBs, DDTs, CHLs, HCB, PBDEs and HBCDs were found in detectable ranges in at least one matrix. In contrast, individual congeners such as CB 28, 49, 52, 74, 132, BDE 28 and TBBPA were not detected in any sample hence not presented in Table 1. Individual compounds detected in less than 50% of the samples (DF < 0.50) in any tissues were not included for further statistical analysis. In general, detection frequencies of all the individual compounds were highest in preen oil, followed by body feathers and plasma. The maximum concentrations of all the compounds were recorded in preen oil, followed by body feathers and plasma. HBCDs ($\alpha$-, $\beta$- or $\gamma$-stereoisomers), although detected in goshawk nestling feathers, blood plasma and preen oil, were found in less

**Table 1**
Median and range of OHC concentrations (ng $g^{-1}$ ww for plasma and preen oil; ng $g^{-1}$ feather[a]) in nestling northern goshawk body feathers, plasma and preen oil. Compounds detected in <50% of individual tissue samples are indicated with an asterisk. Limits of detections can be found in supporting info (Table SI-1). Summation of each compound is shown in bold.

| | Body feathers $n = 33$ | Plasma $n = 37$ | Preen oil $n = 8$ |
|---|---|---|---|
| | ng $g^{-1}$ feather[a] | ng $g^{-1}$ ww | ng $g^{-1}$ ww |
| CB 95 | 0.25 (0.13–0.89) | 0.05[b] (<LOD[c] – 0.11) | <LOD[b] |
| CB 99 | 0.81 (0.15–8.39) | 0.11 (<LOD – 0.59) | 8.7 (2.73–43) |
| CB 101 | 0.60 (0.16–2.56) | 0.24[b] (<LOD – 0.28) | 1.5 (0.38–7.0) |
| CB 105 | 0.46[b] (<LOD – 2.82) | 0.24[b] (<LOD – 0.23) | 3.7 (0.75–30) |
| CB 118 | 0.81 (0.21–10) | 0.21 (0.08–1.5) | 20 (4.78–220) |
| CB 128 | 0.49 (0.19–14) | 0.13 (0.07–1.3) | 6.7 (1.96–58) |
| CB 138 | 3.73 (1.5–76) | 0.66 (0.14–8.4) | 43 (13.8–480) |
| CB 146 | 0.91 (0.22–22) | 0.34 (0.09–4.9) | 28 (8.39–270) |
| CB 149 | 0.16[b] (<LOD – 2.3) | 0.11[b] (<LOD – 0.37) | 3.2 (2.46–8.9) |
| CB 153 | 5.6 (1.4–120) | 2.1 (0.31–28) | 200 (53–2500) |
| CB 156 | 0.21 (0.11–6.3) | 0.13 (0.07–2.7) | 9.7 (2.4–120) |
| CB 170 | 1.4 (0.36–59) | 0.63 (0.09–13) | 43 (12–550) |
| CB 171 | 0.41[b] (<LOD – 8.1) | 0.35[b] (<LOD – 1.4) | 5.5 (1.5–56) |
| CB 174 | 0.16[b] (<LOD – 0.95) | 0.22[b] (<LOD – 0.21) | 1.5 (0.76–8.0) |
| CB 177 | 0.26 (0.12–19) | 0.07 (0.05–2.5) | 6.1 (3.6–55) |
| CB 180 | 2.2 (0.63–64) | 1.8 (0.23–30) | 160 (44–2300) |
| CB 183 | 0.59 (0.19–23) | 0.18 (0.09–4.1) | 24 (6.6–310) |
| CB 187 | 2.4 (1.0–58) | 0.89 (0.21–11) | 110 (57–910) |
| CB 194 | 0.24[b] (<LOD – 2.6) | 0.22[b] (<LOD – 0.54) | 4.4 (1.6–59) |
| CB 196/203 | 0.31 (0.16–7.0) | 0.13 (0.06–2.0) | 22 (6.1–310) |
| CB 199 | 0.25 (0.13–4.6) | 0.13 (0.07–2.0) | 22 (9.4–260) |
| CB 206 | 0.08[b] (<LOD – 0.72) | 0.16[b] (<LOD – 0.29) | 4.9 (2.0–55) |
| CB 209 | 0.05[b] (<LOD – 0.24) | 0.03[b] (<LOD – 0.11) | 3.2 (2.2–20) |
| **ΣPCBs** | **22 (7.8–480)** | **7.6 (1.7–100)** | **750 (240–8600)** |
| OxC | 0.30 (0.12–2.3) | 0.18 (0.08–0.92) | 10 (4.3–21) |
| TN | 0.43[b] (<LOD – 1.8) | 0.24 (0.07–0.63) | 9.7 (2.9–35) |
| CN | 0.11[b] (<LOD – 0.44) | 0.14[b] (<LOD – 0.13) | 1.0 (0.47–7.4) |
| **ΣCHLs** | **0.30 (0.12–2.3)** | **0.45 (0.16–1.4)** | **19 (7.6–63)** |
| **HCB** | **0.75 (0.18–5.3)** | **0.11 (0.06–0.69)** | **18 (11 – 33)** |
| *p,p′*-DDE | 19 (8.1–210) | 5.5 (2.2–48) | 560 (400–2400) |
| *p,p′*-DDT | 0.25 (0.13–0.62) | 0.05[b] (<LOD – 0.18) | 1.8 (1.7–3.5) |
| **ΣDDTs** | **19 (8.2–210)** | **5.5 (2.2–48)** | **560 (290–2400)** |
| BDE 47 | 0.49 (0.12–5.5) | 0.07 (0.03–0.71) | 3.3 (1.6–25) |
| BDE 99 | 0.25 (0.08–2.6) | 0.07 (0.04–0.79) | 3.9 (2.5–38) |
| BDE 100 | 0.66 (0.20–3.9) | 0.30 (0.04–2.2) | 5.0 (4.7–59) |
| BDE 153 | 0.27 (0.15–1.4) | 0.46[b] (<LOD – 1.2) | 1.4 (0.73–5.9) |
| BDE 154 | 0.30[b] (<LOD – 0.70) | 0.27[b] (<LOD – 0.33) | 3.9 (2.3–53) |
| BDE 183 | 0.03[b] (<LOD – 0.01) | 0.32[b] (<LOD – 0.94) | 1.0 (0.55–12) |
| **ΣPBDEs** | **1.6 (0.62–12)** | **0.50 (0.11–3.5)** | **18 (12–190)** |
| *α*-HBCD | 0.16[b] (<LOD – 16) | 0.08[b] (<LOD – 4.3) | <LOD[b] |
| *β*-HBCD | 0.14[b] (<LOD – 2.1) | 0.03[b] (<LOD – 0.50) | 0.13[b] (<LOD – 1.1) |
| *γ*-HBCD | 0.11[b] (<LOD – 2.3) | 0.03[b] (<LOD – 0.17) | 0.25[b] (<LOD – 12) |
| **ΣHBCDs** | 0.41[b] (<LOD – **16**) | 0.14[b] (<LOD – **4.3**) | **0.38**[b] (<LOD – **12**) |

[a] Feather concentrations in previous studies have been reported as dry weight (dw), but feathers dried at room temperature may still contain moisture as indicated by freeze drying. Therefore, we avoid using dw in this study.
[b] DF is <0.50 i.e. values below LOD in more than 50% of the samples.
[c] <LOD represents that the values are below detection limits.

than 50% of samples for all the tissues (Table 1). Further, none of the individuals had detectable levels of HBCDs in more than one tissue.

The dominating OHC classes were ΣPCBs and ΣDDTs, in similar proportions, together accounting for over 95% of the total tissue OHC load (Fig. 1). The profile of the remaining classes differed slightly among matrices (Fig. 1). Preen oil displayed a profile of HCB > ΣPBDEs > OxC. The PCB profile was dominated by CB 153 in all tissues and CB 138, 153, 170, 180 and 187 were consistently the remaining five dominant congeners, constituting up to 75% of the total PCB load (Fig. SI-3). Body feathers contained proportionally more of the lower chlorinated congeners ($5.74 \leq F \leq 38.99$ and all $P < 0.01$for CB 99, 101, 118, 128 and 138), a higher proportion of CB 138, and a lower proportion of CB 180 ($F = 90.96$; $P < 0.01$) compared to plasma and preen oil. Then again, the higher chlorinated CB 187, 196/203 and 199 were more abundant in preen oil ($20.27 \leq F \leq 61.6$; all $P < 0.01$). The PBDE profile differed considerably among the matrices (Fig. SI-4). BDE 99 was the main congener in body feathers (mean 40%), plasma (mean 57%) and preen oil (mean 29%), though with very variable contributions depending on the tissue ($F = 24.95$; all $P < 0.05$). Furthermore, BDE 47 and 153 were significantly higher in contribution to the total feather PBDE load ($17.68 \leq F \leq 8.74$; all $P < 0.01$).

The statistical output for the linear regressions is summarized per compound in Table 2. Most compounds that were detected in body feathers, plasma and preen oil associated significantly among all matrices ($0.20 \leq R^2 \leq 0.98$; all $P < 0.05$). The associations were strong and significant, despite low sample sizes, except for HCB and OxC.

### 3.2. Associations among OHCs, SIs and CORT

$\delta^{13}C$ and $\delta^{15}N$ were measured in body feathers with a median (min-max) of −23.3‰ (−23.9 to −22.4‰) and +8.2‰ (+5.7 to +10.1‰), respectively. The two stable isotopes had a significant ($P < 0.01$, $R^2 = 0.98$) relationship to each other (Fig. SI-5). The most parsimonious models explaining the relationship between OHC concentrations and SIs are presented in Table 3. Variation in concentrations of individual OHC compounds or OHC classes were best explained by models including $\delta^{13}C$, $\delta^{15}N$ or $\delta^{13}C + \delta^{15}N$, although not significantly (Table 3). $\delta^{13}C$ was identified as the most suitable predictor for variation in feather concentrations of PCBs, HCB, *p,p′*-DDE and PBDEs, although the negative associations were not significant ($0.87 \leq R^2 \leq 0.96$; $0.29 \leq P \leq 0.93$). Further, the association of $\delta^{15}N$ was found the most suitable predictor for variation in feather concentrations of OxC, only showing a positive but not significant association ($R^2 = 0.85$; $P = 0.12$).

CORT was detected in body feathers with a median (min-max) of 3.58 pg $mm^{-1}$ (1.72–13.0 pg $mm^{-1}$). The values of CORT, expressed in a mass-dependent unit, were recorded with a median (min-max) of 8.51 pg $mg^{-1}$ (3.82–29.6 pg $mg^{-1}$). Table 4 summarizes the most parsimonious models to explain the variation in body feather CORT levels, given the possible predictors $\delta^{13}C$, $\delta^{15}N$, age or individual OHC or OHC class concentration. None of the predictors significantly explained the observed variation in CORT levels (all $P > 0.05$). However, the $AIC_c$-based model selection indicated that variation in CORT concentrations was best explained by OHC exposure or age.

## 4. Discussion

### 4.1. Comparison of concentrations and profiles among tissues

The current study's first aim was to investigate different minimally invasive sampling methods to assess exposure to OHCs in nestling northern goshawks. In many previous studies blood plasma has been used for determining OHCs exposure in bird of prey nestlings as well as adults (Abbasi et al., 2017b; Espín et al., 2016; Eulaers et al., 2011b). However, using blood has received a lot of criticism because of its semi-invasiveness approach. Sampling blood, particularly from nestlings, is difficult, tricky and may cause some serious health effects, including survival challenges (Bustnes et al., 2013). On the other hand, the use of feathers (Dauwe et al., 2005; Garcia-Fernandez et al., 2013; Jaspers et al., 2019) and preen oil (Yamashita et al., 2018; Eulaers et al., 2011b; Løseth et al., 2019b; Jaspers et al., 2008) for OHCs monitoring has received a lot of attention in the past decade because of their minimally invasive approach. Feathers are an interesting biomonitoring tool because they serve as an archive for contaminants in relation to the blood during their growth (Abbasi et al., 2017b; Eulaers et al., 2011a, 2011b). On the other hand, preen oil is primarily influenced by diet, location, and exogenous contamination of OHCs (Yamashita et al., 2018; Eulaers et al., 2011b; Jaspers et al., 2008). The current study investigated feathers as a unique biomonitoring matrix to analyse exposure to OHCs, dietary habits, and corticosterone levels in a terrestrial bird of prey. We found higher concentrations of OHCs in preen oil compared to body feathers and plasma. This is due to the high lipid content of preen oil which in turn depicts greater affinity for compounds with a high octanol-water partition coefficient, $K_{ow}$ (Yamashita, 2007). The high

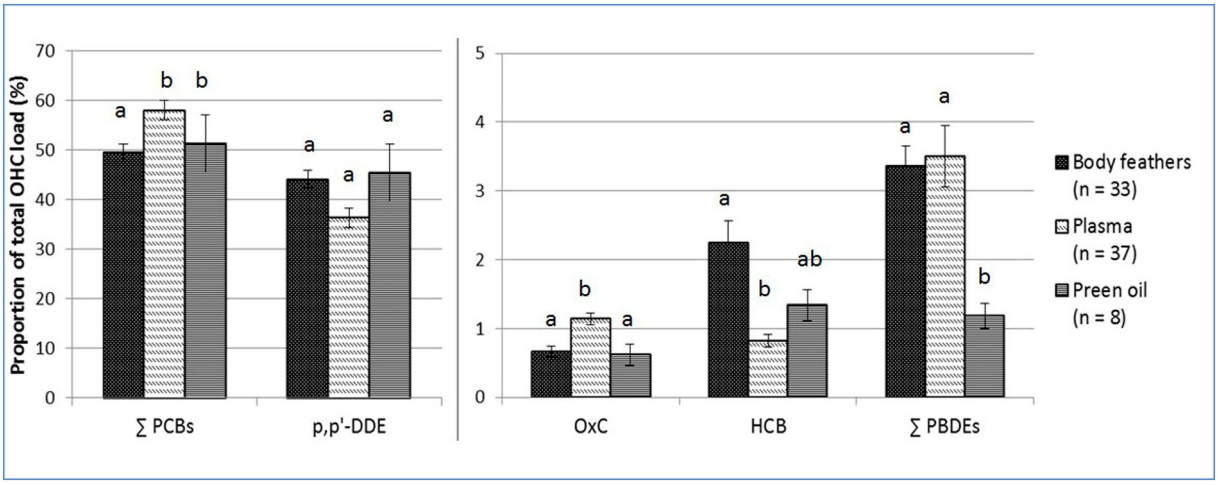


**Fig. 1.** OHC class profile (mean % ± SE) for body feathers, plasma and preen oil of northern goshawk (*Accipiter gentilis*) nestlings. Different letters (a–b) denote significantly different means between tissues.

lipophilicity makes preen oil a potential and interesting matrix for investigating new and (re)emerging lipophilic OHCs. Preen oil was evaluated earlier as a valuable biomonitoring matrix for OHCs in white-tailed eagles, and its further use in biomonitoring activities may therefore allow for comparisons of contaminant exposure of birds in marine and terrestrial environments (Eulaers et al., 2011b; Løseth et al., 2019b). This would increase our limited knowledge of the distribution of new and (re)emerging contaminants in wildlife.

In living organisms, pollutant concentrations in the blood directly reflect their recent dietary exposure (Abbasi et al., 2017b). At the same time, pollutants are redistributed from the blood to other tissues depending upon the tissue-specific perfusion rates and the affinity of the compounds to the different tissues (Drouillard et al., 2003). This explains the significantly higher proportion of the most lipophilic PCBs (octa-, nona- and deca-PCBs) in preen oil compared to body feathers. Although the preen oil has a high ratio of the most lipophilic compounds, the pentachlorinated PCBs were also found. Significant higher proportions of the less persistent penta- and hexa-chlorinated PCBs were found in body feathers compared to plasma and preen oil. Our findings are in line with multi-tissue comparisons of previous studies on nestlings of white tailed eagle and northern goshawk (Briels et al., 2019; Løseth et al., 2019b; Eulaers et al., 2011a). In general, the highest concentrations of PCBs followed by organochlorine pesticides (OCs) and PBDEs are in line with recent and past studies on Norwegian birds of prey (Briels et al., 2019; Løseth et al., 2019b; Sonne et al., 2012;

**Table 2**
Regression coefficients ($\beta_0$ and $\beta_1$), coefficient of determination ($R^2$), and significance ($P$) of the association between ln-transformed OHC concentrations in body feathers, plasma and preen oil of northern goshawk nestlings. Significance levels: *$P < 0.05$, **$P < 0.01$. T indicates a trend towards significance: $0.05 \leq P < 0.10$. "nd" indicates this compound not being detected in both tissues and thus not allowing for regression testing. Summation of each compund is shown in bold.

| | Plasma ~ body feathers | | | | Plasma ~ preen oil | | | | Preen oil ~ body feathers | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $n = 14$ | | | | $n = 8$ | | | | $n = 8$ | | | |
| | $\beta_0$ | $\beta_1$ | $P$ | $R^2$ | $\beta_0$ | $\beta_1$ | $P$ | $R^2$ | $\beta_0$ | $\beta_1$ | $P$ | $R^2$ |
| CB 99 | −0.87 | 0.33 | <0.01** | 0.54 | −1.45 | 0.50 | <0.01** | 0.66 | 1.21 | 0.53 | <0.05* | 0.62 |
| CB 101 | nd | nd | nd | nd | nd | nd | nd | nd | 0.18 | 0.15 | 0.82 | 0.01 |
| CB 118 | −1.56 | 0.69 | <0.01** | 0.76 | −1.75 | 0.80 | <0.01** | 0.88 | 1.47 | 0.86 | <0.01** | 0.84 |
| CB 128 | −0.73 | 0.70 | <0.01** | 0.84 | −1.54 | 0.72 | <0.01** | 0.72 | 1.11 | 0.72 | <0.01** | 0.75 |
| CB 138 | −0.46 | 0.39 | <0.01** | 0.20 | −1.56 | 0.81 | <0.01** | 0.78 | 1.08 | 0.94 | <0.01** | 0.75 |
| CB 146 | −0.45 | 0.75 | <0.01** | 0.81 | −1.80 | 0.89 | <0.01** | 0.83 | 1.57 | 0.80 | <0.01** | 0.81 |
| CB 153 | −0.36 | 0.83 | <0.01** | 0.80 | −2.18 | 1.02 | <0.01** | 0.86 | 1.77 | 0.84 | <0.01** | 0.82 |
| CB 156 | −0.40 | 0.75 | <0.01** | 0.79 | −1.65 | 0.70 | <0.01** | 0.79 | 1.62 | 0.88 | <0.01** | 0.75 |
| CB 170 | −0.48 | 0.89 | <0.01** | 0.82 | −2.14 | 1.03 | <0.01** | 0.90 | 1.57 | 0.82 | <0.01** | 0.80 |
| CB 177 | −0.67 | 0.76 | <0.01** | 0.84 | −1.93 | 0.85 | <0.01** | 0.89 | 1.38 | 0.72 | <0.01** | 0.95 |
| CB 180 | −0.22 | 0.94 | <0.01** | 0.83 | −2.42 | 1.10 | <0.01** | 0.90 | 1.90 | 0.86 | <0.01** | 0.88 |
| CB 183 | −0.56 | 0.80 | <0.01** | 0.87 | −1.89 | 0.80 | <0.01** | 0.86 | 1.62 | 0.83 | <0.01** | 0.82 |
| CB 187 | −0.50 | 0.86 | <0.01** | 0.82 | −2.44 | 1.07 | <0.01** | 0.77 | 1.83 | 0.73 | <0.01** | 0.86 |
| CB 196/203 | −0.51 | 0.92 | <0.01** | 0.84 | −2.11 | 0.84 | <0.01** | 0.87 | 1.77 | 0.90 | <0.01** | 0.90 |
| CB 199 | −0.35 | 0.87 | <0.01** | 0.84 | −2.18 | 0.90 | <0.01** | 0.84 | 1.87 | 0.80 | <0.01** | 0.91 |
| **ΣPCBs** | **−0.27** | **0.82** | **<0.01**** | **0.81** | **−2.03** | **0.97** | **<0.01**** | **0.88** | **1.79** | **0.86** | **<0.01**** | **0.84** |
| OxC | −0.71 | 0.15 | 0.18 | 0.06 | −0.79 | −0.14 | 0.34 | 0.15 | 1.06 | 0.32 | 0.51 | 0.08 |
| TN | nd | nd | nd | nd | −1.65 | 0.71 | <0.01** | 0.71 | nd | nd | nd | nd |
| **ΣCHLs** | **−0.71** | **0.15** | **0.18** | **0.06** | **−1.26** | **0.51** | **<0.01**** | **0.41** | **1.06** | **0.32** | **0.51** | **0.08** |
| **HCB** | **−0.97** | **−0.22** | **0.06$^T$** | **0.11** | **−1.49** | **0.28** | **0.58** | **0.05** | **1.18** | **−0.42** | **<0.05*** | **0.50** |
| *p,p′*-DDE | −0.08 | 0.58 | <0.01** | 0.44 | −1.08 | 0.65 | <0.05* | 0.53 | 2.00 | 0.63 | 0.06$^T$ | 0.47 |
| *p,p′*-DDT | nd | nd | nd | nd | nd | nd | nd | nd | −1.98 | −2.51 | 0.12 | 0.35 |
| **ΣDDTs** | **−0.08** | **0.58** | **<0.01**** | **0.44** | **−1.08** | **0.65** | **<0.05*** | **0.53** | **1.99** | **0.64** | **0.06 $^T$** | **0.46** |
| BDE 47 | −1.04 | 0.77 | <0.01** | 0.78 | −1.68 | 0.62 | <0.01** | 0.77 | 0.99 | 0.96 | <0.01** | 0.91 |
| BDE 99 | −0.61 | 0.91 | <0.01** | 0.53 | −1.86 | 0.87 | <0.01** | 0.79 | 1.20 | 0.98 | <0.01** | 0.97 |
| BDE 100 | −0.66 | 0.71 | <0.01** | 0.74 | −1.73 | 0.71 | <0.01** | 0.98 | 1.36 | 0.86 | <0.01** | 0.94 |
| BDE 153 | nd | nd | nd | nd | nd | nd | nd | nd | 1.55 | 1.25 | <0.01** | 0.96 |
| **ΣPBDEs** | **−0.64** | **0.86** | **<0.01**** | **0.65** | **−1.72** | **0.81** | **<0.01**** | **0.93** | **1.22** | **0.96** | **<0.01**** | **0.97** |

**Table 3**
Variation in OHC levels in body feathers (ln-transformed; $pg\,mm^{-1}$) of northern goshawk, explained by $\delta^{13}C$ and $\delta^{15}N$.

| | | Estimate | SE | t | P | Inter-nest SD / Intra-nest SD | $R^2$ |
|---|---|---|---|---|---|---|---|
| CB 146 | Intercept | −4.55 | 10.55 | −0.43 | 0.67 | 0.80 | 0.96 |
| | $\delta^{13}C$ | −0.19 | 0.45 | −0.43 | 0.68 | 0.33 | |
| CB 153 | Intercept | −0.12 | 9.88 | −0.01 | 0.99 | 0.77 | 0.96 |
| | $\delta^{13}C$ | −0.08 | 0.42 | −0.19 | 0.85 | 0.30 | |
| CB 187 | Intercept | −3.16 | 8.46 | −0.37 | 0.71 | 0.61 | 0.95 |
| | $\delta^{13}C$ | −0.18 | 0.36 | −0.49 | 0.63 | 0.28 | |
| ΣPCBs[a] | Intercept | 1.22 | 9.31 | 0.13 | 0.90 | 0.70 | 0.96 |
| | $\delta^{13}C$ | −0.08 | 0.40 | −0.21 | 0.84 | 0.29 | |
| OxC | Intercept | −3.48 | 1.30 | −2.68 | 0.02 | 0.52 | 0.85 |
| | $\delta^{15}N$ | 0.25 | 0.15 | 1.64 | 0.12 | 0.54 | |
| HCB | Intercept | 8.55 | 12.53 | −0.68 | 0.50 | 0.70 | 0.87 |
| | $\delta^{13}C$ | −0.36 | 0.54 | −0.66 | 0.52 | 0.59 | |
| *p,p′*-DDE | Intercept | −4.81 | 7.24 | −0.67 | 0.51 | 0.45 | 0.92 |
| | $\delta^{13}C$ | −0.34 | 0.31 | −1.09 | 0.29 | 0.29 | |
| BDE 47 | Intercept | −8.61 | 10.07 | −0.86 | 0.40 | 0.74 | 0.96 |
| | $\delta^{13}C$ | −0.34 | 0.43 | −0.78 | 0.44 | 0.33 | |
| BDE 99 | Intercept | −1.20 | 9.57 | −0.13 | 0.90 | 0.69 | 0.95 |
| | $\delta^{13}C$ | −0.04 | 0.41 | −0.09 | 0.93 | 0.31 | |
| ΣPBDEs[a] | Intercept | −4.50 | 9.21 | −0.49 | 0.63 | 0.65 | 0.95 |
| | $\delta^{13}C$ | −0.22 | 0.40 | −0.55 | 0.59 | 0.31 | |
| ΣOHCs[b] | Intercept | −0.65 | 8.16 | −0.08 | 0.94 | 0.56 | 0.94 |
| | $\delta^{13}C$ | −0.20 | 0.35 | −0.56 | 0.58 | 0.28 | |

[a] ΣPCBs and ΣPBDEs are the sum of all the PCB or PBDE congeners detected in feathers.
[b] ΣOHCs is the sum of ΣPCBs, ΣDDTs, ΣCHLs, HCB and ΣPBDEs detected in feathers.

Eulaers et al., 2011a). As these compounds are no longer used in Norway nor the rest of Europe (regulated by the Stockholm Convention), the pattern of PCBs, OCs and PBDEs probably reflects the historical presence in the environment including the concentrations emitted during demolition of old buildings or leaching from landfilled waste and long-range atmospheric transport. On the other hand, the detection of HBCDs at relatively low concentrations in very few samples and no detection of TBBPA reflects their low presence in the Norwegian environment.

The most abundant PCB congeners in all matrices were CB 153, CB 180 and CB 187, and the PBDE profile was dominated by BDE 99 in all matrices. This pattern is previously reported in terrestrial species (Eulaers et al., 2011a; Jaspers et al., 2006a, 2006b, 2007a, 2007b). White-tailed eagle nestlings from Trøndelag had slightly higher concentrations of most OHCs than these goshawks from the same region, except for *p,p′*-DDE (in all matrices) and HCB (only in body feathers) (Eulaers et al., 2011b). A similar comparison between the species was made in the northern area of Tromsø, indicating higher exposure to certain OCPs in the terrestrial environment where these compounds were used (Eulaers et al., 2011a). The PCB and PBDE profiles between the white-tailed eagle and goshawk nestlings from Trøndelag suggest that goshawks have the highest accumulation of the more halogenated PCBs and PBDEs, similar to what is observed between the species in Tromsø (Eulaers et al., 2011a, 2011b). These contaminants have a low solubility in water, but higher partitioning to particles, suggesting higher concentrations in the terrestrial environment (Noyes et al., 2009). Altogether, the OHC profiles in body feathers, plasma and preen oil were generally comparable and indicate that feathers and preen oil reflect the internal plasma levels of OHCs. A wider range of OHCs was detected in body feathers and preen oil than in plasma. Using samples from body feathers and preen oil rather than or in addition to plasma can therefore increase our knowledge of the exposure and bioaccumulation of OHCs normally not detected in plasma. The stronger associations between feathers and preen oil may be explained by their lower turnover rate than the plasma, and thus representing exposure over a longer period.

The relationships between OHCs in nestling feathers and plasma from the northern goshawks were generally higher than those previously found for white-tailed eagle nestlings from Trøndelag (Eulaers et al., 2011b). Variation in preening activity, age, feather atrophy and metabolic capacity are suggested as potential confounding factors that may affect the relationships (Eulaers et al., 2011b; Jaspers et al., 2008). Results from predatory birds in Tromsø were comparable with those reported here, although the authors reported slightly higher and more significant correlation coefficients (Eulaers et al., 2011a). Altogether, the associations for OHC concentrations between body feathers and plasma of NG nestlings were highly substantial with strong coefficients, indicating that nestling NG feathers reflect the internal levels for most OHCs. Both nestling body feathers and preen oil seem to be promising minimally invasive tissues for OHC exposure in nestlings of terrestrial bird of prey species.

**Table 4**
Variation in CORT levels (ln-transformed; $pg\,mm^{-1}$) in body feathers of northern goshawks, explained by the accumulation of a specific OHC, by $\delta^{13}C$ or $\delta^{15}N$, and/or age.

| | | Estimate | SE | t | P | Inter-nest SD / Intra-nest SD | $R^2$ |
|---|---|---|---|---|---|---|---|
| CB 146 | Intercept | 1.24 | 0.10 | 12.86 | 0.00 | 0.27 | 0.82 |
| | ln s (CB 146) | 0.10 | 0.10 | 0.97 | 0.35 | 0.30 | |
| CB 153 | Intercept | 1.05 | 0.22 | 4.81 | 0.00 | 0.27 | 0.82 |
| | ln s (CB 153) | 0.10 | 0.11 | 0.92 | 0.37 | 0.30 | |
| CB 187 | Intercept | 1.11 | 0.16 | 6.74 | <0.01 | 0.27 | 0.82 |
| | ln s (CB 187) | 0.12 | 0.13 | 0.94 | 0.36 | 0.30 | |
| ΣPCBs[a] | Intercept | 0.87 | 0.38 | 2.29 | <0.05 | 0.27 | 0.83 |
| | ln s (ΣPCBs) | 0.11 | 0.12 | 0.98 | 0.34 | 0.30 | |
| OxC | Intercept | 0.80 | 0.65 | 1.24 | 0.23 | 0.26 | 0.81 |
| | Age | 0.01 | 0.02 | 0.68 | 0.51 | 0.30 | |
| HCB | (Intercept) | 1.21 | 0.09 | 13.17 | <0.01 | 0.23 | 0.79 |
| | ln s (HCB) | −0.10 | 0.08 | −1.22 | 0.24 | 0.31 | |
| *p,p′*-DDE | (Intercept) | 0.80 | 0.65 | 1.24 | 0.23 | 0.26 | 0.81 |
| | Age | 0.01 | 0.02 | 0.68 | 0.51 | 0.30 | |
| BDE 47 | (Intercept) | 0.80 | 0.65 | 1.24 | 0.23 | 0.26 | 0.81 |
| | Age | 0.01 | 0.02 | 0.68 | 0.51 | 0.30 | |
| BDE 99 | (Intercept) | 0.80 | 0.65 | 1.24 | 0.23 | 0.26 | 0.81 |
| | Age | 0.01 | 0.02 | 0.68 | 0.51 | 0.30 | |
| ΣPBDEs[a] | (Intercept) | 0.80 | 0.65 | 1.24 | 0.23 | 0.26 | 0.81 |
| | Age | 0.01 | 0.02 | 0.68 | 0.51 | 0.30 | |
| ΣOHCs[b] | (Intercept) | 0.83 | 0.55 | 1.51 | 0.15 | 0.27 | 0.83 |
| | ln s (ΣOHCs) | 0.10 | 0.14 | 0.75 | 0.46 | 0.30 | |

[a] ΣPCBs and ΣBDEs are the sum of all the PCB or PBDE congeners detected in feathers.
[b] ΣOHCs is the sum of ΣPCBs, ΣDDTs, ΣCHLs, HCB and ΣPBDEs detected in feathers.

### 4.2. The relationship between OHCs exposure, dietary tracers and corticosterone in feathers

The current study intended to elucidate the associations of OHCs with SIs and CORT, all analysed in feathers. At first, the individual values and associations between $\delta^{13}C$ and $\delta^{15}N$ values detected in this study were comparable or slightly lower than those previously reported for golden eagle (*Aquila chrysaetos*) and northern goshawk nestlings from Tromsø (Eulaers et al., 2013, 2014). Relatively lower $\delta^{13}C/\delta^{15}N$ levels confirmed that the goshawk preferentially feeds in the terrestrial environment since the terrestrial and freshwater environment is generally depleted in $^{13}C$ compared to the marine environment (Kelly, 2000). Secondly, the current study illustrated $\delta^{13}C$ as the best predictor for most OHCs in feathers, whereas $\delta^{15}N$ showed weak and non-significant association with OHCs. Our results corroborated the previous findings of feeding habitat and ecology as primary determinants compared to trophic positions for concentrations of organic pollutants in different species of birds of prey (Eulaers et al., 2014; Abbasi et al., 2017a, 2017b). Weak but positive associations between OHCs and $\delta^{15}N$ reflects relatively lower bioaccumulation potentials of these compounds in NG nestlings. The relatively small sample size and reduced OHCs exposure in NG nestlings might be factors contributing to the lower bioaccumulation potential (Abbasi et al., 2017a, 2017b; Yu et al., 2011).

Interestingly, ΣPCBs levels reflected a weak but negative association when regressed with $\delta^{15}N$ values. Previously, Elliott et al. (2009) reported

similar trends for PBDEs in bald eagle (*Haliaeetus leucocephalus*) nestlings. The weak negative association can be explained through decreasing exposure of PCBs in the European environment as a result of the ban on open use of PCBs since the 1970s, variation in feeding habitat, ecology, relatively smaller sample size and uneven distributions of individual PCBs congeners (Eulaers et al., 2014; Elliott et al., 2009). In general, weaker positive associations between OHCs and $\delta^{15}N$ values suggest that reduced exposure in the environment entails less bioaccumulation and subsequent biomagnification trends. The inclusion of spatial variables such as the stable isotopes of sulphur ($\delta^{34}S$) could be an advantage in future studies. It may describe the variation in habitat occupancy in more detail (Eulaers et al., 2014; Morrissey et al., 2013).

CORT was successfully measured in body feathers of the NG nestlings, and were found within the range of those of recently reported for nestlings of white-tailed eagle (Løseth et al., 2019a), red kite (Monclús et al., 2019) and common buzzard (*Buteo buteo*) (Martínez-Padilla et al., 2013). Further, the levels of CORT in feathers of the current study were either comparable or slightly lower than previously reported for Eurasian sparrowhawk (*Accipiter nisus*), common kestrel (*Falco tinnunculus*), barn owl (*Tyto alba*), and tawny owl (*Strix aluco*) (Strong et al., 2015). Stress effects in birds have been reported to be associated with OHCs exposure (Tartu et al., 2014). The concentrations of OHCs were found to influence blood plasma clinical-chemical parameters in nestlings of Norwegian birds of prey (Sonne et al., 2012). And recently, Løseth et al. (2019a) have reported a negative relationship with feather's CORT level and corresponding PFASs concentrations in white-tailed eagle nestlings. Monclús et al. (2019) reported that concentrations of most persistent organic pollutants such as PCBs, PBDEs, HCH and DDTs, but not organophosphate esters (OPEs) flame retardants, were associated with high concentrations of CORT in down feathers of cinereous vulture (*Aegypius monachus*) nestlings. In the present study, no significant correlations were detected between CORT and specific OHCs in feathers as presented in Table 4. Thus, our results suggest that there are other factors which may explain the variation of CORT in NG nestling feathers. We assume that CORT levels in the goshawk nestlings of the current study are driven by other biological and/or ecological factors rather than moderate exposure to OHCs, which is in line by what was observed by Løseth et al. (2019a) in Norwegian white-tailed eagle nestlings.

Despite this novel approach of investigating dietary pathways, stress and OHC exposure together in body feathers of northern goshawks, no significant associations were found between diet, stress or OHC levels. As such, the present study finds no direct influence of OHC exposure or feeding habits on baseline CORT levels in the nestling goshawks. Besides dietary pathways, factors such as maternal transfer and growth dilution, body condition, sex, sibling order and local sources may also be good predictors of nestling goshawk OHC exposure (Bustnes et al., 2013; Harms et al., 2010; Love et al., 2003a). On the other hand, CORT levels are likely to reflect a general stress response and respond to many other factors besides pollution, including disturbance, predation pressure, sibling competition, disease, etc. (Schoech et al., 2011). Seeing that the goshawks in the current study did not have exceptionally high OHC concentrations, these were therefore likely not a significant contributor to the corticosterone levels in the birds. However, the combined feather-based measures of OHC concentrations, SI values and CORT levels show the potential to provide a new understanding of how chronic stress may be influenced by the long-term exposure to contaminants and/or by dietary habits, which may be more pronounced in other populations of birds of prey that are more exposed to pollution.

## 5. Conclusions

The present study presented a side-by-side evaluation of the suitability of nestling body feathers and preen oil as minimally invasive alternatives for the use of blood when biomonitoring OHC exposure in a terrestrial bird of prey species. Confirming earlier findings on marine bird of prey nestlings, the OHC profiles were generally comparable and significantly related, indicating that OHC concentrations in body feathers and preen oil reflect internal body burdens. The present study is the first to quantify OHC exposure, its dietary sources (SI values) and adrenal gland response (CORT levels) simultaneously in nestling feathers from a terrestrial bird of prey. However, the obtained CORT levels were not significantly associated with concentrations of individual OHCs or OHC classes, nor changes in feeding habits, nor their combination. Regardless, the present study shows the analytical feasibility for jointly assessing for ecophysiological and toxicological measures in nestling bird of prey body feathers, though underscores the need for further investigation of causal integration of their outcome.

## CRediT authorship contribution statement

The contribution of all the authors is given as follows:

| | |
|---|---|
| Sina T. Randulff | Studentship in this project, project design, Field sampling, lab analysis, manuscript, preparation, referencing |
| Naeem Akhtar Abbasi | Major editing in manuscript, statistical analysis, data interpretation, language improvement |
| Igor Eulaers | Field sampling, Lab analysis, statistical analysis, data interpretation, manuscript, preparation and Editing, language improvement |
| Torgeir Nygård | Project design, Field sampling, lab analysis, Editing, manuscript improvement |
| Adrian Covaci | Analysis on GC-MS, data analysis, editing, language improvement |
| Marcel Eens | Data analysis, language improvement, manuscript editing |
| Govindan Malarvannan | Data analysis on GC-MS, editing, manuscript improvement |
| Gilles Lepoint | Data analysis on stable isotopes, editing, manuscript improvement |
| Mari E. Løseth | Data analysis, data interpretation, manuscript improvement |
| Veerle L. B. Jaspers | Project leader, supervision, Project design, Field sampling, data analysis, manuscript preparation, Manuscript improvement |

## Declaration of competing interest

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

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## Appendix A. Supplementary data

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