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Feathers as an integrated measure of organohalogen contamination, dietary tracers and stress in Goshawk (*Accipiter gentilis*) nestlings from Trøndelag, Norway

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Photo: Sina Thu Randulff

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Abstract

Because of their high sensitivity and wide distribution, predatory birds have proven useful as sentinel species for monitoring exposure of organohalogen contaminants (OHCs). Nestling feathers have been evaluated as valid non-destructive sample matrices for OHCs, and are also believed to be useful biomatrices for the detection of effect parameters of OHC exposure. In this thesis, we investigated the prevalence of OHCs in body feathers, blood plasma and preen oil of Goshawk nestlings (*Accipiter gentilis*) from Trøndelag, Norway. Additionally, the suitability of using feathers as an integrated biomarker for OHC exposure and effects was assessed by simultaneously detecting stable isotopes (SIs; $\delta^{13}C$ and $\delta^{15}N$) and corticosterone (CORT) in feathers. Polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) were detected in body feathers (median: 22.3, 19.2 and 1.63 ng g^{-1} dw, respectively), blood plasma (median: 7.55, 6.23 and 0.50 ng g^{-1} ww, respectively) and preen oil (median: 748, 606 and 18.4 ng g^{-1} ww, respectively). Strong, significant correlations between the OHC concentrations in the three matrices indicated that feathers and preen oil reflect the internal circulating blood levels of most OHCs. $\delta^{13}C$ was found to be the best predictor of OHC accumulation in feathers, indicating that the Goshawks' dietary carbon source is of higher importance in predicting OHC exposure than trophic level, although not significant. Variation in CORT was best explained by a positive relationship to PCBs and age (not significant). Overall, this thesis presents, for the first time, an integrated measure of OHCs, SIs and CORT in Goshawk feathers. This methodological approach looks promising, and may provide increased understanding to how ecological, toxicological and physiological factors are interrelated during the whole nestling stage.

Keywords

Corticosterone
Goshawk
Nestling body feathers
Non-destructive sampling
Organohalogen compounds
Preen oil
Stable isotopes
Stress

Samandrag

På grunn av deira sensitivitet og breie distribusjon, har rovfuglar vist seg å vera nyttige som overvåkingsartar for eksponering av organohalogenerte miljøgifter (OHCs). Fjær frå fugleungar (pullus) har blitt evaluert som gyldige ikkje-destruktive prøvematriser for OHCs, og er også anteke å vera nyttige biomatriser for påvising av effektparametrar av OHC-eksponering. I denne oppgåva undersøkte me utbreiinga av OHCs i kroppsfjær, blodplasma og uropygialolje av hønsehaukungar (*Accipiter gentilis*) frå Trøndelag, Noreg. I tillegg vart dei ønskjelege eigenskapane ved bruk av fjær som ein integrert biomarkør for OHC-eksponering og -effektar vurdert ved å samstundes detektera stabile isotopar (SIs: $\delta^{13}C$ og $\delta^{15}N$) og kortikosteron (CORT) i fjær. Polyklorerte bifenylar (PCBs), organoklorinerte pesticider (OCPs) og polybromerte difenyleterar (PBDEs) vart detektert i kroppsfjær (median: 22,3, 19,2 og 1,63 ng g^{-1} tørrvekt, respektivt), blodplasma (median: 7,55, 6,23 og 0,50 ng g^{-1} våtvekt, respektivt) og uropygialolje (median: 748, 606 og 18,4 ng g^{-1} våtvekt, respektivt). Sterke, signifikante samanhengar mellom OHC-konsentrasjonar i dei tre matrisene indikerte at fjær og uropygialolje reflekterer de interne sirkulerande blodnivåa til dei fleste OHCs. $\delta^{13}C$ sto fram som den beste prediktoren for OHC-akkumulering i fjær, noko som indikerer at hønsehauk si karbonkjelde frå kosten er av større betyding for å føreseia OHC-eksponering enn trofisk nivå, sjølv om det ikkje er signifikant. Variasjonen i CORT vart best forklart ved eit positivt forhold til PCB og alder (ikkje signifikant). Samla sett presenterer denne oppgåva, for fyste gong, eit integrert mål på OHCs, SIs og CORT i hønsehaukfjær. Denne metodiske tilnærminga ser lovande ut, og kan gi auka forståing for korleis økologiske, toksikologiske og fysiologiske faktorar heng saman under utviklingsperioden til fugleungane.

Nøkkelord

Fjær frå fugleungar
Hønsehauk
Ikkje-destruktive målingar
Kortikosteron
Organohalogenerte forbindelsar
Stabile isotopar
Stress
Uropygialolje

Table of content

Abbreviations

%	Percent
$(NH_4)_2SO_4$	Ammonium sulphate
˚C	Degrees Celsius
ΔAIC_c	Delta Aikaike's Information Criteria corrected for small sample sizes
µl	Microliters
‰	Per mille
^{13}C	^{13}Carbon: a stable isotope of carbon
^{15}N	^{15}Nitrogen: a stable isotope of nitrogen
ANOVA	Analysis of Variance
CHL	Chlordane
CN	*Cis*-nonachlor
CO_2	Carbon dioxide
CORT	Corticosterone
CV	Coefficient of variation
DCM	Dichloromethane
DF	Degrees of Freedom
dw	Dry weight
ELISA	Enzyme-linked immunosorbent assay
F	F-value
fwo	FWO research project
g	Gram
GCs	Glucocorticoids
HBCD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HCH	Hexaclorohexane
HCl	Hydrochloric acid
Hex	Hexane
Hex:DCM	Hexane:dichloromethane
IUPAC	International Union of Pure and Applied Chemistry
K_{ow}	Octanol-Water Partition Coefficient
lipid %	Lipid percent
LMEM	Linear Mixed Effect Model
ln-transformation	Transformation using the natural logarithm
log_{10}-transformation	Transformation using log10
LOQ	Limit of quantification
lw	Lipid weight
M	Molar (moles/litre)
MeOH	Methanol
mg	Milligram
mL	Millilitre
mm	Millimetres
n	Number of observations
N_2	Nitrogen gas
nd	Not detected

ng	Nanogram
ng g^{-1}	Nanograms per gram
NINA	Norsk Institutt for Naturforskning
nm	Nanometer
NO_2	Nitrogen dioxide
NTNU	Norwegian University of Science and Technology
OCPs	Organochlorinated pesticides
OHCs	Organohalogen Compounds
OxC	Oxychlordane
p	Probability of rejecting the hypothesis
p,p'-DDE	*p,p'*-dichlorodiphenyldichloroethylene
p,p'-DDT	*p,p'*-dichlorodiphenyltrichloroethane
PBDE	Polybrominated diphenyl ethers
PCB	Polychlorinated biphenyl
pg	Picogram
pg mm^{-1}	Picogram per millimetre
POP	Persistent organic pollutant
ppm	Parts per million
R^2	R squared
rpm	Rounds per minute
R_{sample}	Feather samples
$R_{standard}$	International reference standards
SD	Standard Deviation
SE	Standard Error
SIs	Stable Isotopes
SMI	Scaled Mass Index
t	t-value
TBBPA	Tetrabromobisphenol A
TN	*trans*-nonachlor
Tukeys HSD	Tukeys Honesty Significance Difference Test
v/v	Volume/Volume
W_2O_3	Tungsten(III)Oxide
ww	Wet weight
α	The probability of rejecting the null hypothesis
β_0	Intercept (Regression coefficient)
β_1	Slope (Regression coefficient)
$\delta^{13}C$	Delta C-13: a ratio of the stable isotopes ^{13}C:^{12}C
$\delta^{15}N$	Delta N-15: a ratio of the stable isotopes ^{15}N:^{14}N

1. Introduction

The adverse effects of organohalogen compounds (OHCs) on wildlife and the natural environment are of increasing concern, partly due to the ability of OHCs to undergo long range atmospheric, hydrologic or biotic transport to areas remote from sources (de Wit et al., 2010, Letcher et al., 2010, Macdonald et al., 2005). These anthropogenic contaminants include industrial chemicals such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) as well as unintentional by-products of industrial processes (dioxins or furans) and organochlorine pesticides (OCPs) (de Wit et al., 2010, Law et al., 2006, Letcher et al., 2010). Their persistence and high lipophilicity make them subject to bioaccumulation and biomagnification, where they potentially exert toxic effects in individuals and populations (de Wit et al., 2010, Letcher et al., 2010). As a result, population declines and severe effects were seen in predatory birds (Blus et al., 1995, Furness et al., 1993, Ratcliffe, 1967). Many traditional OHCs are regulated as persistent organic pollutants (POPs), with generally slowly decreasing concentrations in the environment (Rigét et al., 2010). Despite this, they are frequently detected in wildlife, because predatory birds feed at a high trophic level and are sensitive to environmental changes (Bustnes et al., 2013, Chen and Hale, 2010, Eulaers et al., 2013, 2014b, Furness, 1993, Garcia-Fernandez et al., 2008, Jaspers et al., 2013). These characteristics makes them relevant biomonitor species for the identification of OHC related health risks (Chen and Hale, 2010, Furness, 1993, Gjershaug et al., 2008, Jaspers et al., 2011,).

The chemical and physical properties of OHCs resemble endogenous compounds, and may explain their possible interference with an organism's homeostasis of endogenous hormones, metabolism, development, immune system, reproduction, behavior and eventually their survival (Letcher et al., 2010). Of special concern are chemicals causing endocrine disruption, partly due to the effects on steroidogenesis (Bourgeon et al., 2012, Love et al., 2003b, Nordstad et al., 2012). Steroid hormones such as glucocorticoids (GCs) are responsible for the regulation of stress, an important physiological response known as the "fight or flight" response (Angelier and Wingfield, 2013, Sapolsky et al., 2000). Animals that live on the edge of their physiological tolerance have to cope with a variety of natural stressors such as food

scarcity, predation and diseases. However, anthropogenic stressors such as contamination may alter the GC synthesis (Love et al., 2003b, Nordstad et al., 2012). Elevated levels of GCs over time are detrimental for physiological responses, health and fitness, while short term increases in GC levels can have advantageous effects (Blas et al., 2007, Bortolotti et al., 2008, Butler et al., 2010, Sapolsky et al., 2000, Schoech et al., 2011).

Quantification of GCs is a powerful integrator of environmental stressors, but has been restricted to blood measurements of the GC corticosterone (CORT), reflecting only short-term stress responses (Bortolotti et al., 2008, Fairhurst et al., 2011). Measurement of CORT levels in feathers on the other hand, allows for a long-term integrated measurement of stress (days to weeks) during the period of feather growth (Bortolotti et al., 2008, Fairhurst et al., 2013a). Analysing keratinous matrices such as feathers, hair or nails has unique advantages; it is non-destructive, easy to sample and store, is stable over time and integrates a variety of biochemical compounds (Bortolotti et al., 2009, 2010, Fairhurst et al., 2013a). Only during growth, the highly vascularized cells in a feather are connected to the blood stream (Bortolotti et al., 2009). Circulating functional and non-functional compounds and elements (e.g. stable isotopes, hormones, contaminants and trace elements) will be incorporated in the feather (Bortolotti, 2010). The lack of metabolism in the feather preserves these compounds, and enables us to use feathers to measure several biochemical compounds over the period of feather growth (Fairhurst et al., 2013b, Jardine et al., 2006).

The assessment of OHCs in feathers is today regarded as a valuable technique (Jaspers et al., 2006b, 2007b). Feather levels reflect the internal concentrations of OHCs, allowing us to interpret the exposure and accumulation of OHCs in wildlife (Jaspers et al., 2007b, Van den Steen et al., 2007). Since diet is the main source of OHC accumulation in predatory birds, analysis for stable isotopes (SIs) has become a primary tool to link dietary habits to OHC accumulation (Ramos and González-Solís, 2012, Ruus et al., 2002, van Drooge et al., 2008). Also for this, feathers have been shown to be a convenient matrix (Bustnes et al., 2013, Fairhurst et al., 2013a). Ratios of the stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) have been shown useful to explain differences in diet composition, trophic position and habitat

(Bustnes et al., 2013, Caut et al., 2009, Fairhurst et al., 2013b, Jardine et al., 2006). When combining and interpreting results of several variables in feathers, it is important to emphasize that they reflect the same time frame of incorporation (Bortolotti, 2010, Eulaers et al., 2011b, Ramos and González-Solís, 2012). Functional elements are incorporated into the feather matrix in a mass dependent way, contrary to non-functional compounds that are rather incorporated as a function of time. Nestling feathers are advantageous over adult feathers in the sense that they allow for controlling biological variables such as gender and age, and avoid confounding factors such as migratory activity, metabolizing capacity, moult and reproductive status (Eulaers et al., 2011a, 2013, Jaspers et al., 2007b). The restricted mobility of nestlings due to incomplete feather growth also allows for easier sampling, as they stay in the nest until fledging.

Studies on predatory nestlings in Norway have identified ecological and spatial factors that help explain the variation in exposure to contaminants (Eulaers et al., 2013, 2014b, Nygård and Gjershaug, 2001). Factors that have been suggested to influence OHC accumulation include trophic level, local habitat and regional nest location (Eulaers et al., 2013, Nygård and Gjershaug, 2001). Levels of OHCs in Goshawk nestlings (*Accipiter gentilis*) have been shown to equal those in other birds of prey for which negative health effects were reported (Sonne et al., 2010, 2012). The detected levels were prone to impact blood plasma biochemistry as well as liver, kidney, bone and metabolism (Sonne et al., 2010, 2012). However, the information on health effects of OHCs is scarce for the terrestrial environment compared to the marine. The Goshawk may be a highly suitable bioindicator for OHCs in the terrestrial environment (Eulaers et al., 2011a, 2013). It is a relatively long lived bird with a widespread distribution over the Northern hemisphere, and with a well-known ecology (Kenward, 2010). The Goshawk is positioned at the top of its food chain, and has a diverse diet consisting of different insectivorous, herbivorous and omnivorous prey species (Grønnesby and Nygard, 2000, Kenward, 2010).

1.1. Objectives

To the best of our knowledge, no studies have yet performed an integrated assessment of diet ingestion, OHC accumulation and CORT levels in feathers of a predatory species: In this thesis we aim to investigate this using Goshawk nestlings from Sør- and Nord-Trøndelag (Norway). The objectives of the thesis were to 1) measure the prevalence of OHCs in body feathers and blood plasma (and preen oil) in Goshawk nestlings from Sør- and Nord-Trøndelag, 2) investigate the suitability of feathers as an integrated biomarker for exposure and effects, by measuring OHCs, SIs and CORT simultaneously in body feathers, and 3) investigate the influence of OHCs and SIs on CORT levels in the feathers. The analysis of preen oil was included as a pilot study to investigate an additional non-destructive biomonitoring tool for OHC exposure. Prey items collected at the nest were included to complement the results of the dietary tracers. We hypothesized that integration of all results could contribute to an increased understanding of how OHC accumulation, dietary tracers and stress interact.

2. Materials and methods

2.1. Field sampling

The study area (63.02-64.28 ^{0}N, 10.03-12.32 ^{0}E) was located in the counties Nord- and Sør-Trøndelag in Norway (Figure 1). The breeding activity of Goshawk pairs was checked in May 2014, and nests with observed breeding activity were revisited for sampling shortly before the expected fledgling age in late June/early July 2014. Sampling was conducted on nestlings between 3 to 5 weeks old. The nestlings were removed from the nest, and morphometric measurements, blood and feather samples were taken (Table A1, Appendix 7.1). Sex was determined based on tarsus width, with distinctly larger tarsus for females than males. A blood sample (< 2.0 mL) was taken from the brachial vein with a heparinized syringe, transferred into Eppendorf vials and centrifuged the same day (6000 rpm, 6-7 min). From each nestling, 4-7 body feathers were pulled from the back, pooled and stored in a polyethylene bag. By the very end of the field work preen oil samples were successfully sampled from 8 nestlings. Plastic spoons were used to squeeze out oil from the preen gland. The oil stuck to the surrounding down feathers, and these were cut off and put in Eppendorf tubes. Prey items were collected at the nest site or within 200 metres of the nest, and stored in separate bags. The final sample size used for the analysis of body feathers and blood plasma was 37 individuals from 14 nests.

All samples were stored in a cool and dark environment during transportation, and kept frozen at -20 ^{0}C prior to analysis. The study was conducted with permission from the Ethical board of the Norwegian Food Safety Authority. The local police was informed of the sampling campaign.

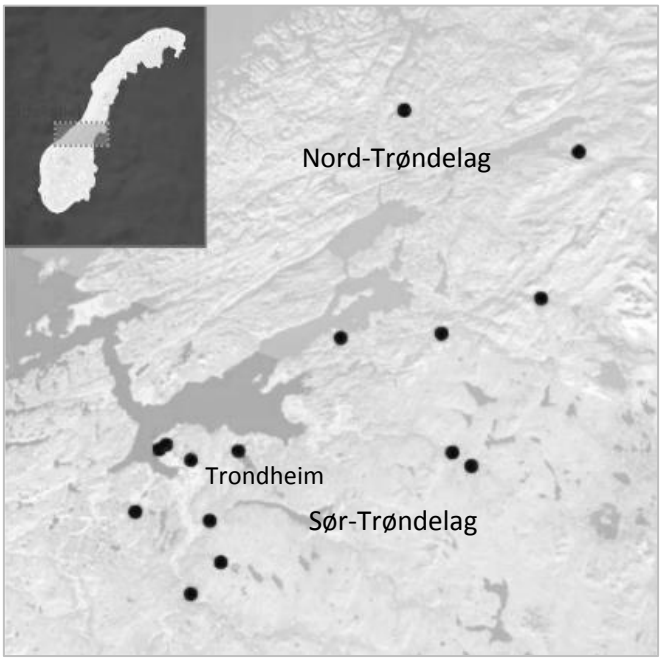

Figure 1: Goshawk nestlings were sampled in the counties of Nord- and Sør-Trøndelag in Norway (63.02-64.28 ^{0}N, 10.03-12.32 ^{0}E) in June/July, 2014.

2.2. Analysis for organohalogen compounds

The sample preparation and detection of OHCs in feathers, blood plasma and preen oil were carried out at the Toxicological Centre of the University of Antwerp (Belgium).

2.2.1. Body feather sample preparation

To remove external contamination via dust, soil and other particles from the surface of the feathers, we followed a documented washing procedure (Jaspers et al., 2008a). Washing with an organic solvent such as hexane (Hex) is known to remove possible external contamination (Jaspers et al., 2008a). Organic solvents can also remove preen oil from the feather surface and from the keratine structure of the feathers, which has previously been correlated with the internal OHC concentrations (Jaspers et al., 2008a). Therefore, in order to prevent a significant alteration of the POP levels and profiles, the feathers were washed with deionized water, and dried overnight at ambient temperature with a cover of standard laboratory paper. The calamus of each feather was cut off, and the remaining main part was weighed on an analytical balance (mean 0.034 g, range 0.006 – 0.081 g) and the length was measured (mean 70.24 mm, range 35 - 101 mm). One to two body feathers were used for detection of the amount of CORT (0.011 - 0.053 g), while the remaining feathers were used for analysis of OHCs and SIs. These were cut in < 5 mm fragments into self-made aluminium trays, and mixed thoroughly to approach homogeneity. An aliquot of the mixture (1.5 mg) was reserved for the SI analysis, and the remaining (0.027 – 0.200 g feathers) were used for OHC analysis.

The homogenized feathers were weighed and spiked with internal standards (CB 143, *ε*- hexaclorohexane (HCH), BDE 77, ^{13}C-*α*-hexabromocyclododecane (HBCD), ^{13}C-*β*-HBCD, ^{13}C-*γ*-HBCD and ^{13}C-TBBPA), followed by overnight incubation at 45 ^{0}C in 7 mL of hydrochloric acid (HCl, 4 M) and 7 mL of hexane:dichloromethane mixture (Hex:DCM; 4:1, v/v) in analytical glass recipients. The organic layer was cleaned-up onto 6 mL cartridges, prewashed with Hex:DCM (4:1, v/v) and containing acid silica (6 mL, 44% H_2SO_4) topped with 0.5 g anhydrous sodium sulphate. Prior to transfer, 5 mL of Hex:DCM (4:1, v/v) was added to the pear-shaped glass containers and vortexing was applied in order to optimise partitioning of OHCs to the organic layer. This step was once more repeated prior to concentrating the obtained extract

to 500 µL under a gentle nitrogen stream, in order to allow for elution of the columns with Hex:DCM in the same glass tubes. After that, the extracts were again up-concentrated to 100 µL, and Hex (0.5 mL) was added prior to fractionation on silica SPE columns (3 mL/500 mg; Agilent Bond Elut SI), conditioned with Hex. After transfer of the extract onto the SPE columns, a first fraction was eluted with Hex in order to obtain PCBs, PBDEs and OCPs. A second fraction containing HBCDs and TBBPA was collected in fresh tubes by elution of the same columns with DCM. Both fraction 1 and 2 were evaporated to dryness and reconstituted in 80 µL *iso*-octane and 75 µL MeOH, respectively.

2.2.2. Blood plasma sample preparation

Each individual blood plasma sample (median of 800 µL, range 200-1000 µL) was spiked with internal standards (see 2.2.3. *Body feather sample preparation*), and 100 µL of acetone, 1000 µL of MilliQ water and 200 µL of formic acid were added prior to ultrasonication in a water bath for 20 minutes. Intermittent vortexing was performed between each of the additions above. The clean-up was carried out on 3 mL OASIS HLP cartridges prewashed and conditioned consecutively with DCM, MeOH and MilliQ water. Plasma samples were applied to the cartridges, washed with water, and eluted with DCM. Afterwards, the cleaned extracts were evaporated to dryness under a gentle N_2 flow and reconstituted in 0.5 mL of Hex. The concentrated extracts were then transferred to Hex-washed silica SPE cartridges (3 mL Agilent Bond Elut SI) topped with 200 mg of 44 % acid silica. The fractionation and reconstitution was conducted as described for the feather samples.

2.2.3. Preen oil sample preparation

Prior to analysis the preen oil sample was accurately weighed and dissolved in 2 mL of Hex. The organic layer was separated from the down feathers with glass Pasteur pipettes, and transferred to pre-weighed glass tubes. This step was repeated once more to assure that all lipids were transferred. The known amount of Hex was subtracted from the total weight in the tubes to get the lipid mass, which was used to estimate the lipid content. Since it was necessary to separate the preen oil from the down feathers this was regarded the most convenient approach. The extraction, clean-up and fractionation steps were followed as described above for the blood plasma.

2.2.4. Detection and quantification of OHCs

All feather, blood plasma and preen oil samples were analysed for 27 PCB congeners (IUPAC: 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), seven PBDE congeners (IUPAC: BDE 28, 47, 99, 100, 153, 154 and 183), *p,p'*-dichlorodiphenyltrichloroethane (*p,p'*-DDT) with metabolite *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), hexachlorobenzene (HCB), chlordanes (CHLs) such as *cis*-nonachlor (CN), *trans*-nonachlor (TN) and oxychlordane (OxC), HBCD diastereoisomers *α*-HBCD, *β*-HBCD, and *γ*-HBCD, and tetrabromobisphenol A (TBBPA). All OHCs except for HBCD and TBBPA were quantified using a gas chromatograph (Agilent GC 6890, Palo Alto, CA, USA) connected to a mass spectrometer (Agilent MS 5973, Palo Alto, CA, USA). PCBs and DDTs were separated on a HT-8 capillary column (30 m x 0.22 mm x 0.25 µm; SGE Analytical Science, Zulte, Belgium) in electron impact ionization mode, while HCBs, CHLs and PBDEs were analysed with a DB-5 capillary column (30 m x 0.25 mm x 0.25 µm; J&W Scientific, Folsom, CA, USA) in electron capture negative ionization mode. The separation of TBBPA, *α*-, *β*-, and *γ*- HBCD was achieved using a dual pump Agilent 1100 Series liquid chromatograph equipped with autosampler and an Agilent Zorbax Extended-C18 reversed phase analytical column (50 mm x 2.1 mm i.d., 3.5 µm particle size). Mass spectrometric analysis was performed using an Agilent 6410 triple quadrupole mass spectrometer operated in negative electrospray ionization mode.

2.2.5. Quality assurance and quality control

The internal standards were CB 143, *ε*-HCH and BDE 77, all dissolved in iso-octane, and ^{13}C-*α*-HBCD, ^{13}C-*β*-HBCD, ^{13}C-*γ*-HBCD and ^{13}C-TBBPA, all dissolved in methanol. MeOH were used throughout the entire analytical procedure, and were obtained from Accustandard (New Haven, CT, USA), Wellington Laboratories, and Dr. Ehrenstorfer laboratories. Mean ± SD recoveries of the internal standards PCB 143 and BDE 77 were 86 ± 6 % and 93 ± 10 %, respectively. The quality control was further performed by analysing procedural blanks every 13th sample for all feathers and blood plasma, and every 7th preen oil sample. Concentrations of compounds were corrected by subtraction of the average procedural blank values, and 3 x SD of

the procedural blank concentration was used to set the limit of quantification (LOQ). LOQs for compounds not detectable in blanks were set at a 10:1 signal to noise ratio. LOQs for all analysed compounds ranged from 0.10 - 0.50 ng g^{-1} dry weight (dw) for body feathers, 0.05 - 0.30 ng g^{-1} wet weight (ww) for blood plasma, 0.20 - 1.00 ng g^{-1} ww (0.40 – 2.00 ng g^{-1} lipid weight (lw)) for preen oil (Table A2, Appendix 7.2). Solvents of pesticide grade (Merck, Darmstadt, Germany) were applied throughout the analytical process.

2.3. Analysis of feathers for corticosterone

2.3.1. Hormone analysis with enzyme immunoassay

The analysis for CORT in the feather samples was performed by a conventional enzyme-linked immunosorbent assay (ELISA; Corticosterone Enzyme Immunoassay kit, ADI-900-097, Enzo® Life Sciences, New York, NY, USA) at the Department of Biology at the Norwegian University of Science and Technology. Feathers were washed, the calamus was removed and the length and mass were measured, prior to cutting the feathers into pieces <5 mm with scissors. A methanol based extraction technique was used, described in detail in Bortolotti et al. (2008). MeOH (5 mL) was added prior to 30 min sonication in room tempered water. After overnight incubation in a shaking water bath the extract was separated from the remaining feather matrix through filtration on 6 mL filtration tubes with a synthetic polyester plug. The cartridges were then washed with MeOH, which was added to the extract. The extractes were evaporated to dryness under a gentle air-flow, and reconstituted in 0.5 mL 1:10 phosphate buffer saline (PBS, pH 7.4). The dilution factor was based on previous in-house analyses of nestling feathers of Goshawk and White-tailed Eagle (*Haliaeetus albicilla*). The reconstituted extracts were kept at -20 ^{0}C until CORT analysis on ELISA. A detailed description of the ELISA procedure is found in the kits manual (EnzoLifeSciences, 2010). Briefly, polyclonal antibodies where added to 96-wells plate and incubated for 3 hours. Excess reagents were washed away and substrate added to produce a yellow colour. The optical density was measured on a Bio-Tek Synergy HT microplate reader (Bio-Tek instruments, Winooski, VT, USA) at 405 nm.

2.3.2. Quality assurance and quality control

The MeOH extraction was performed simultaneously for all samples, and the same reagents and chemicals (Merck, Darmstadt, Germany, analysis grade) were used throughout the analysis. Samples were analysed in duplicate in a single batch. Additional duplicates were analysed for three samples in order to determine the intra-assay coefficient of variation (mean CV = 15.26 ± 0.36 %). Blank samples (extraction and assay blanks), total activity, non-specific binding and maximum binding activity were controlled for. The kit had a high sensitivity (26.99 pg mL^{-1}), and the cross-reactivity for other steroids was reported to be low (CORT: 100 %; deoxycorticosterone: 28.6 %; progesterone: 1.7 %; tetrahydrocorticosterone: 0.28 %; aldosterone: 0.18 %; testosterone: 0.13 %; EnzoLifeSciences, 2010). A standard curve was produced from 5 standard dilutions on the plate, and used to calculate the concentration of CORT on the plate. Hormone data is presented as a mean of the duplicates, as both pg CORT g^{-1} feather, and as pg CORT mm^{-1} feather.

2.4. Analysis of stable isotopes

2.4.1. Sample preparation and detection

Aliquots of the homogenized feather mixtures (1.12 – 2.19 mg) were accurately weighed at the group Systemic Physiological and Ecotoxicological Research at the University of Antwerp, and loaded into tin cups along with tungsten(III)oxide (W_2O_3), in order to facilitate combustion. The samples were analysed for stable carbon (^{12}C, ^{13}C) and nitrogen isotopes (^{14}N, ^{15}N), at the Laboratory of Oceanology at the University of Liège (Belgium), using an element analyser (Vario microtube, Elementar, Germany) coupled to an isotope mass spectrometer (Isoprime 100, Isoprime, UK). Variations in the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the feather samples (R_{sample}) were normalised against those in international reference standards ($R_{standard}$), i.e. Vienna PeeDee Belemnite and atmospheric nitrogen, respectively. The resulting SI values are expressed in standard delta notation as parts per thousands (‰) (Equation 2.4.1.).

$$\delta X\ (‰) = \left(\frac{R_{sample}}{R_{standard}}\right) \qquad \text{(Equation 2.4.1.)}$$

2.4.2. Quality assurance and quality control

Certified reference materials, i.e. sucrose (IAEA-C6; $\delta^{13}C$ = −10.8 ± 0.5 ‰) and $(NH_4)_2SO_4$ (IAEA-N2; $\delta^{15}N$ = 20.3 ± 0.2 ‰), were purchased from the International Atomic Energy Agency (IAEA, Vienna, Austria) and were used to calibrate the carrier gasses CO_2 and NO_2. The analysis was validated by routinely running procedural blanks, reference samples, and duplicates. Sulfanilic acid was used for elemental data calculation (48.90 ± 0.38 % C and 2.86 ± 0.04 % N). The precision of routine measurements was 0.1 ‰ for $\delta^{13}C$ and 0.3 ‰ for $\delta^{15}N$, and the analytical precision did not exceed 0.1 ‰ SD.

2.5. Prey item determination

The collected prey items were identified at the Norwegian Institute for Nature Research (NINA) in Trondheim, based on morphological features. Size, shape, colour and texture were used to determine the feathers, bones (humerus and sternum) or body parts to the lowest taxonomic level possible (Haftorn, 1971, Selstam and Selstam, 1982). Due to the Goshawks' plucking behaviour several preys lacked feathers or fur, and were therefore completely or partly unidentifiable. These were categorized to genus or family if possible, based on the skeletal remains (Table A3, Appendix 7.3). Since Goshawks are monogamous and territorial, the pairs mostly return to the same nest the following years. Therefore, certain bone items found in or around the nets could originate from an earlier year.

2.6. Data treatment and statistical analysis

The statistical analysis was carried out using R-3.1.2 (R Core Team, 2014). Data below the LOQ was substituted with DF x LOQ, where DF equals the detection frequency of individual samples above LOQ. Eleven compounds had a DF below 0.5 in all sample matrixes and were therefore excluded from further statistical analysis (CB 28, CB 49, CB 52, CB 74, CB 132, BDE 28, *α*-HBCD, *β*-HBCD, *γ*-HBCD and TBBPA). Data exploration was performed on all data, and outliers, normality and homoscedasticity were carefully investigated (Zuur et al., 2010). Shapiro Wilk's test for normality and QQ-plots showed that OHC data required log transformation in order to meet normality. The statistical significance was set to α = 0.05 for all

analysis, while $0.05 \leq P < 0.10$ was considered a trend. Concentrations of OHCs are given in ng g^{-1} dw for feathers, ng g^{-1} ww for blood plasma and preen oil samples. Preen oil data is also presented as ng g^{-1} lw in Table A2.2, Appendix 7.2.

Linear regression was used to relate feather mass to length. Congener profiles were created from the relative proportion of the congener to its higher OHC class or the OHC class to the total OHC load. For the congener profiles the sum of an OHC class represented each congener detected in that matrix, and thereby represents the compounds' specific accumulation in the different matrices. Proportion data was Arcsine-transformed, and analysis of variance (Anova) was used to detect differences between groups. Post-hoc comparisons were made by use of adjusted *P* values from Tukey's Honesty Significant Differences Test (Tukey's HSD). Associations of OHC concentrations among body feathers, blood plasma and preen oil were performed by linear regression analysis, and reported with regression slopes (β_0) and intercepts (β_1), adjusted R^2 values and *P* values. For this analysis the total sum of an OHC class was calculated from only those compounds that were detected in feathers, blood plasma as well as in preen oil, so that a direct comparison between the matrixes could be made.

Linear mixed effect models (LMEM) with nest ID as random variable were used to correct for pseudoreplication of nestlings from a single brood, calculated from the LME function in R (*nmle* package; (Pinheiro J. et al., 2015)). R^2 values were obtained by squaring the correlation coefficient of the correlation between the fitted values of the model and the actual observations. No interactions were included in the LMEMs as it would minimize degrees of freedom needed for the main effects, especially undesirable for the limited sample sizes in this thesis. Two individuals (KO2 and HÅ1) were deemed influential outliers and were therefore excluded from analyses involving LMEM: they had very high feather concentrations combined with low levels of the 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 Figure A4.1 and Figure A4.2, Appendix 7.4. The identification of variables that best explained the observed variation in OHC exposure included $\delta^{13}C$ and $\delta^{15}N$ as predictors, with a total of three models. Associations between CORT levels and sex/scaled mass index (SMI) was tested with LMEM prior to selection of variables included in the LMEM selection

process. SMI was calculated by scaling the individuals' wing length against its mass (Peig and Green, 2009), and Kenwards' formula of feathers growth rate was used to determine age (Kenward, 2010). Variation in CORT levels were investigated by LMEMs with the inclusion of individual congeners or OHC classes as well as $\delta^{13}C$, $\delta^{15}N$ and age as predictors. Combinations of the four predictors made a total of 14 models (without interactions). The most parsimonious models describing the variation in OHC or CORT levels were chosen according to Akaike's Information Criteria corrected for small sample sizes (AIC_c). Models with a $\Delta AIC_c < 2$ were considered as plausible candidate models.

3. Results

3.1. Levels of organohalogen compounds, stable isotope values and corticosterone

Table 1 gives the median levels and ranges of the individual compounds detected in body feathers, blood plasma and preen oil, as well as levels of stable isotopes and corticosterone. The five OHC classes PCBs, DDTs, CHLs, HCB and PBDEs were detected in all matrices, but varied in number of detected individual compounds in each matrix. Preen oil clearly appeared as the matrix with highest concentrations and number of detected OHC compounds (22 PCBs, 3 CHLs, HCB, *p,p'*-DDE and DDT, and 6 PBDEs). Blood plasma had the lowest concentrations and DF of the analysed compounds (14 PCBs, 2 CHLs, HCB, *p,p*'-DDE, and 3 PBDEs). LOQs and DFs of all compounds are given in Table A2.1, Appendix 7.2.

HBCDs (*α*-, *β*- or *γ*-stereoisomers) were detected in Goshawk nestling feathers, blood plasma and preen oil, but below the set DF limit of 0.5, Table 1. One fourth of the birds had detectable HBCD levels in feathers, in a range of 0.4 – 15.5 ng g^{-1} dw for *α*-HBCD, 0.5 – 2.1 ng g^{-1} dw for *β*-HBCD and 0.5 – 2.3 ng g^{-1} dw for *γ*-HBCD. Three birds had internal blood plasma levels of HBCD (*α*-HBCD; 0.3 – 4.3 ng g^{-1} ww), of which one bird had detectable concentrations of the *β* - and *γ*-isomer (0.5 and 0.2 ng g^{-1} ww, respectively). The detected levels of the *β*- and *γ*-isomers in two of eight preen oil samples were higher than what was measured in blood plasma, but lower than in feathers (*β*-HBCD: 1.1 ng g^{-1} ww and 5.4 - 11.6 ng g^{-1} ww of the *γ*-isomer). None of the individuals had detectable levels of HBCDs in more than one matrix. TBBPA was not detected in any of the three matrices.

Table 1: Median and ranges of OHC concentrations (ng g^{-1}), stable isotope values (‰) and corticosterone levels (pg mm^{-1} and pg mg^{-1}) in nestling Goshawk body feathers, blood plasma and preen oil.

	Body feathers *n = 33*	**Blood plasma** *n = 37*	**Preen oil** *n = 8*
OHC concentrations	ng g^{-1} dw	ng g^{-1} ww	ng g^{-1} ww lipid % = 70.7
CB 95	0.25 (0.13 - 0.89)	 (< 0.10 - 0.11)	 (< 0.00)
CB 99	0.81 (0.15 - 8.39)	0.11 (0.07 - 0.59)	8.73 (2.73 - 42.8)
CB 101	0.60 (0.16 - 2.56)	 (< 0.10 - 0.28)	1.45 (0.38 - 7.03)
CB 105	 (< 0.20 - 2.82)	 (< 0.10 - 0.23	3.70 (0.75 - 29.9)
CB 118	0.81 (0.21 - 10.1)	0.21 (0.08 - 1.51)	20.2 (4.78 - 224)
CB 128	0.49 (0.19 - 14.1)	0.13 (0.07 - 1.29)	6.69 (1.96 - 58.2)
CB 138	3.73 (1.46 - 76.1)	0.66 (0.14 - 8.35)	43.4 (13.8 - 479)
CB 146	0.91 (0.22 - 21.7)	0.34 (0.09 - 4.86)	27.7 (8.39 - 274)
CB 149	 (< 0.2 - 2.34)	 (< 0.10 - 0.37	3.17 (2.46 - 8.94)
CB 153	5.63 (1.41 - 116)	2.1 (0.31 - 27.6)	202 (53.0 - 2490)
CB 156	0.21 (0.11 - 6.26)	0.13 (0.07 - 2.65)	9.69 (2.36 - 117)
CB 170	1.42 (0.36 - 58.2)	0.63 (0.09 - 13.1)	43.2 (11.8 - 546)
CB 171	 (< 0.2 - 8.12)	 (< 0.10 - 1.43)	5.45 (1.49 - 56.4)
CB 174	 (< 0.2 - 0.95)	 (< 0.10 - 0.21)	1.47 (0.76 - 7.98)
CB 177	0.26 (0.12 - 18.6)	0.07 (0.05 - 2.54)	6.05 (3.58 - 55.4)
CB 180	2.21 (0.63 - 63.8)	1.76 (0.23 - 30.2)	159 (44.1 - 2300)
CB 183	0.59 (0.19 - 22.6)	0.18 (0.09 - 4.05)	24.0 (6.57 - 308)
CB 187	2.42 (1.00 - 57.9)	0.89 (0.21 - 11.1)	112 (57.0 - 906)
CB 194	 (< 0.2 - 2.60)	 (< 0.10 - 0.54)	4.43 (1.64 - 58.8)
CB 196/203	0.31 (0.16 – 7.00)	0.13 (0.06 - 1.97)	21.6 (6.07 - 306)
CB 199	0.25 (0.13 - 4.59)	0.13 (0.07 - 1.96)	21.9 (9.40 - 261)
CB 206	 (< 0.2 - 0.72)	 (< 0.10 - 0.29)	4.86 (2.01 - 55.3)
CB 209	 (< 0.2 - 0.24)	 (< 0.10 - 0.11)	3.18 (2.16 - 19.9)
∑PCBs[a]	**22.3** **(7.77 – 484)**	**7.55** **(1.68 - 106)**	**748** **(237 - 8620)**

OxC	0.30 (0.12 - 2.31)	0.18 (0.08 - 0.92)	10.3 (4.25 - 21.0)
TN	 (< 0.2 - 1.81)	0.24 (0.07 - 0.63)	9.69 (2.85 - 34.5)
CN	 (< 0.2 - 0.44)	 (< 0.10 - 0.13)	1.03 (0.47 - 7.41)
∑CHLs[b]	**0.30** **(0.12 - 2.31)**	**0.45** **(0.16 - 1.39)**	**18.6** **(7.57 - 62.9)**
HCB	**0.75** **(0.18 - 5.26)**	**0.11** **(0.06 - 0.69)**	**18.0** **(11.1 - 32.5)**
p,p'-DDE	19.3 (8.10 - 212)	5.45 (2.19 - 47.6)	559 (403 - 2350)
p,p'-DDT	0.25 (0.13 - 0.62)	 (< 0.10 - 0.18)	1.80 (1.74 - 3.48)
∑DDTs[c]	**19.4** **(8.22 - 213)**	**5.45** **(2.19 - 47.6)**	**561** **(288 - 2350)**
BDE 47	0.49 (0.12 - 5.46)	0.07 (0.03 - 0.71)	3.27 (1.58 - 24.6)
BDE 100	0.66 (0.20 - 3.89)	0.30 (0.04 - 2.19)	4.96 (4.65 - 58.5)
BDE 99	0.25 (0.08 - 2.56)	0.07 (0.04 - 0.79)	3.94 (2.50 - 38.3)
BDE 153	0.27 (0.15 - 1.39)	 (< 0.10 - 1.19)	1.43 (0.73 - 5.93)
BDE 154	 (< 0.10 - 0.70)	 (< 0.10 - 0.33)	3.85 (2.26 - 53.3)
BDE 183	 (< 0.10 - 0.01)	 (< 0.10 - 0.94)	1.02 (0.55 - 11.4)
∑PBDEs[d]	**1.63** **(0.62 - 12.3)**	**0.50** **(0.11 - 3.47)**	**18.4** **(12.3 - 192)**
α-HBCD	 (< 0.20 – 15.5)	 (< 0.10 – 4.28)	 (< 0.00)
β-HBCD	 (< 0.20 – 2.14)	 (< 0.10 – 0.50)	 (< 0.50 - 1.05)
γ-HBCD	 (< 0.20 – 2.29)	 (< 0.10 – 0.17)	 (< 0.50 - 11.6)
∑HBCDs	 (**< 0.20 - 15.5**)	 (**< 0.10 - 4.30**)	 **(< 0.25)**
TBBPA	 (**< 0.00**)	 (**< 0.00**)	 **(< 0.00)**
Stress hormone levels **CORT (pg mm^{-1})**	**3.58** **(1.72 - 13.0)**	na	na
CORT (pg mg^{-1})	**8.51** **(3.82 - 29.6)**	na	na
Stable isotope values **δ^{13}C (‰)**	**-23.3** **(-23.9 − -22.4)**	na	na
δ^{15}N (‰)	**8.20** **(5.67 - 10.1)**	na	na

[a] ∑PCBs is the sum of all the 23 PCB congeners, [b] ∑CHLs is the sum of OxC, TN and CN, [c] ∑DDTs is the sum of *p,p'*-DDT and the metabolite *p,p'*-DDE, [d] ∑PBDEs is the sum of all the 6 BDE congeners. The value following "<" gives range from the LOQ of the compound to the max concentration, and marks which compounds that were not detected with DF > 0.5. "na" indicates not analysed.

CORT was detected in body feathers with a median of 3.58 pg mm^{-1} (1.72 - 13.0 pg mm^{-1}). When expressed in a mass-dependant unit the median concentration was 8.51 pg mg^{-1} (3.82 - 29.6 pg mg^{-1}) (Table 1). A significant correlation between feather mass and feather length was found ($P < 0.01$, $R^2 = 0.79$, $DF = 171$; Figure 1).

The two stable isotopes had a significant and negative relationship to each other ($P < 0.01$, $R^2 = 0.98$). From Figure 2 it can be observed that the intra-nest variation ($SD = 0.10$) was lower than the inter-nest variation ($SD = 0.21$), and emphasizes the need for LMEM to control for pseudoreplication within nests.

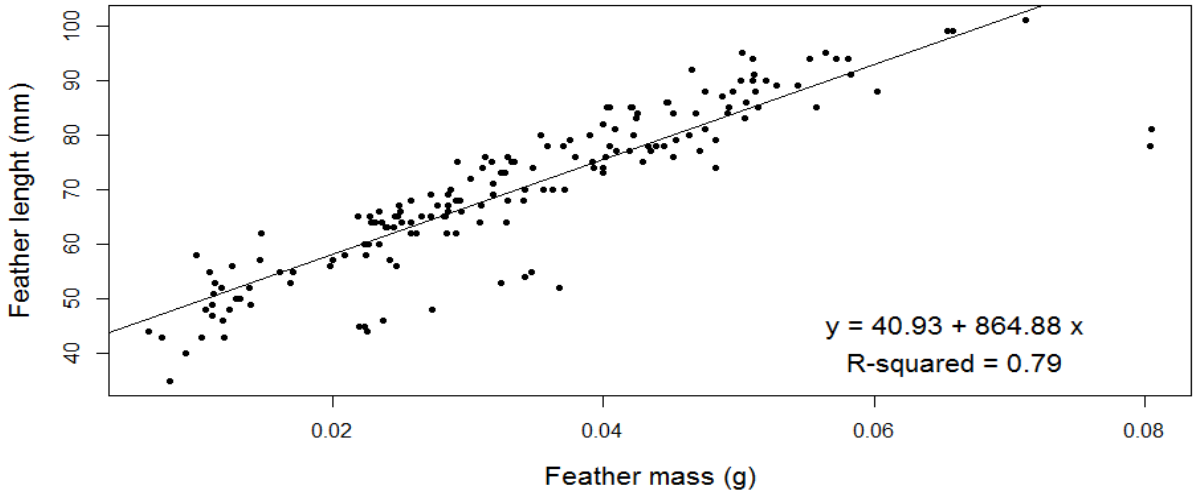

Figure 1: Mass (g) correlated significantly with length (mm) for Goshawk nestling feathers.

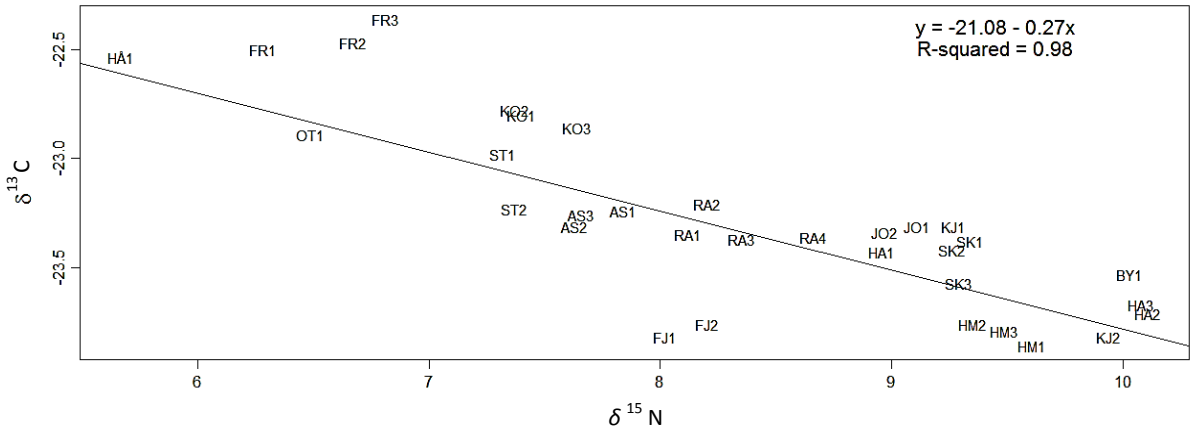

Figure 2: A significant and negative relationship was found between the stable isotopes $\delta^{13}C$ and $\delta^{15}N$. Each point denotes an individual nestling, for which the first two letters marks the nest ID.

3.2. Profile of organohalogen compounds

Figure 3 presents the OHC profiles of ∑PCBs, *p,p'*-DDE, OxC, HCB and ∑PBDEs. The sum of each OHC class incorporated only common compounds that were detected in body feathers, blood plasma and preen oil, since it was believed to optimize the comparison of OHC classes among matrices. ∑PCBs in the OHC profile is therefore the sum of 14 PCBs (CB 99, 118, 128, 138, 146, 153, 156, 170, 177, 180, 183, 187, 196/203 and 199) and ∑PBDEs the sum of BDE 47, 99 and 100. The most dominating OHC classes were ∑PCBs and ∑DDTs, accounting for over 95 % of the total OHC load in all matrices. The profile differed between the matrices, with the proportion of ∑PCBs > *p,p'*-DDE > ∑PBDEs > HCB > OxC for body feathers. Blood plasma differed with a higher contribution of OxC than HCB. Preen oil showed that ∑PCBs > *p,p'*-DDE > HCB > ∑PBDEs > OxC.

The proportion of ∑PCBs and OxC were significantly higher in blood plasma than in body feathers ($P < 0.01$, $5.65 \leq F \leq 8.06$; Table A5, Appendix 7.5). A similar trend for ∑PCBs and OxC was found for blood plasma and preen oil, while significant for OxC only ($P = 0.05$, $F = 8.06$). The proportion of *p,p'*-DDE in blood plasma was below that of feathers and preen oil (not significant). HCB showed the highest proportion in feathers, significantly different to blood plasma ($P < 0.01$, $F = 9.37$). ∑PBDEs had a significantly lower percentage in preen oil than in body feathers ($P < 0.01$, $F = 5.53$).

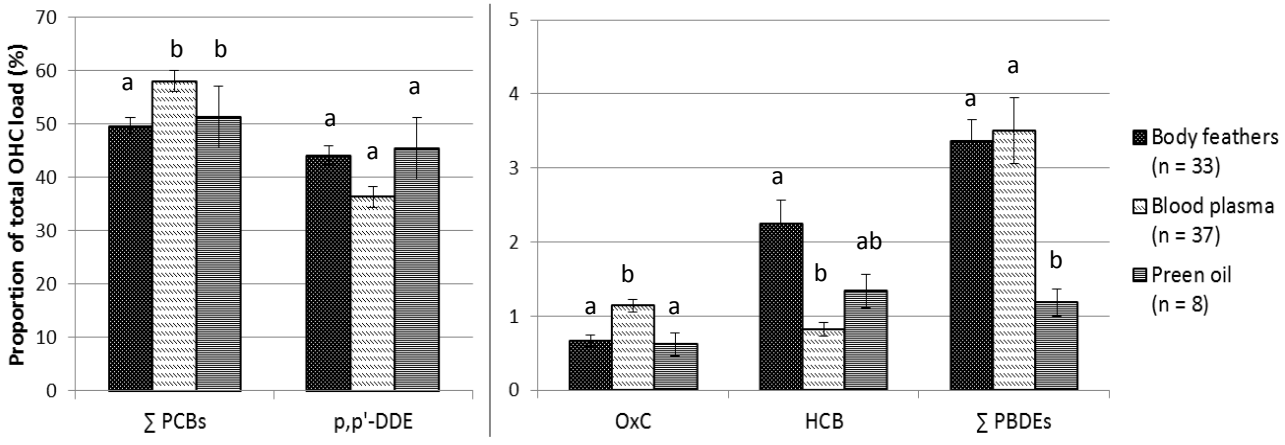

Figure 3: OHC profiles (mean % ± SE) for matrices of Goshawk nestlings. Different letters (a-b) denote significantly different means between matrices.

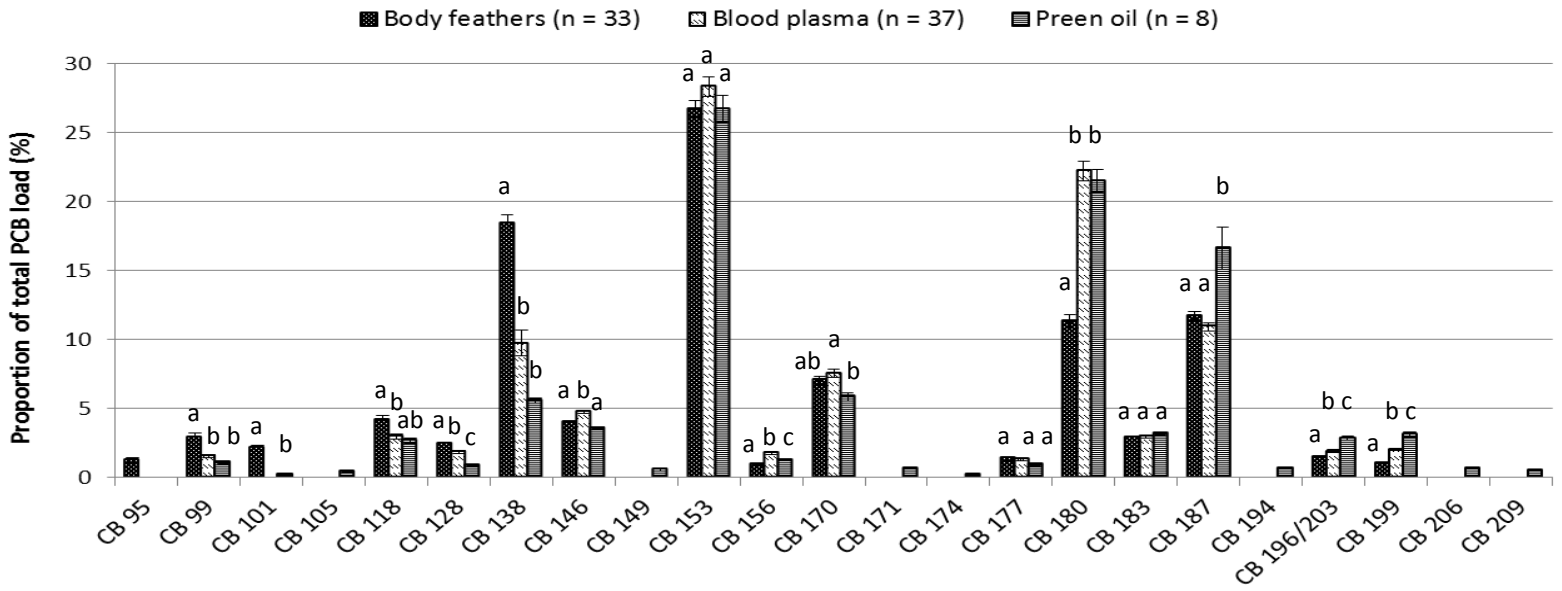

Figure 4: PCB profile (mean % ± SE) for matrices of Goshawk nestlings. Different letters (a-c) denote significantly different means between matrices.

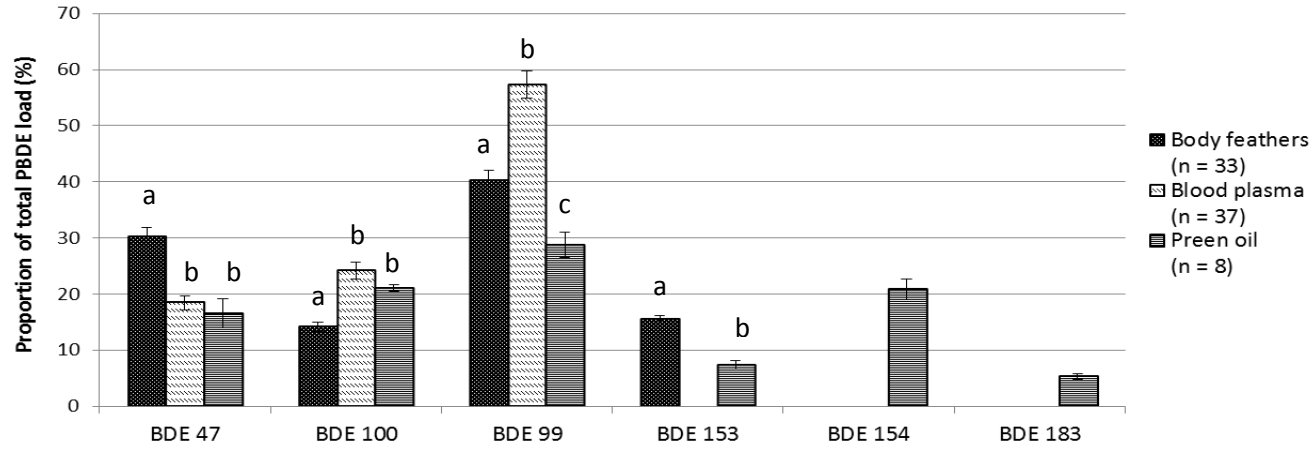

Figure 5: PBDE profile (mean % ± SE) for matrices of Goshawk nestlings. Different letters (a-c) denote significantly different means between matrices.

The profile of PCB congeners is shown in Figure 4, and was dominated by CB 153 (27-29 %) in all matrices. The 5 main congeners CB 138, 153, 170, 180 and 187 contributed with over 75 % to the total PCB load. Body feathers had a significantly higher proportion of the lower chlorinated PCB congeners compared to blood plasma and preen oil ($P < 0.01$, $5.74 \leq F \leq 38.99$ for CB 99, 101, 118, 128 and 138). The proportion of CB 138 was twice as high in feathers as the other matrices, while the opposite difference was observed for the heavier CB 180 ($P < 0.001$, $F = 90.96$). Among the matrices, the relative concentration of the heavier PCBs (CB 187, 196/203 and CB 199) was significantly higher in preen oil ($P < 0.01$, $20.27 \leq F \leq 61.6$), and lower in feathers for the two latter compounds ($P < 0.01$, $20.27 \leq F \leq 61.6$). The contribution of CB 146, 156 and 170 was significantly higher in blood plasma than in feathers and preen oil ($P < 0.05$, $4.08 \leq F \leq 37.57$) (except for CB 170 between body feathers and blood plasma).

As shown in Figure 5 and Table A5 (Appendix 7.5), the profile of PBDEs differed among the matrices. The main congener in body feathers, blood plasma and preen oil was BDE 99 (40.27, 57.34 and 28.78 %, respectively), but the proportions differed significantly among all matrices ($P < 0.05$, $F = 24.95$). BDE 47 and 153 had a significantly higher contribution to the total PBDE load in feathers ($P < 0.01$, $17.68 \leq F \leq 8.74$), in contrast to the lower contribution of BDE 100 in that matrix ($P < 0.05$, $F = 16.56$). Preen oil was the only matrix were the most brominated PBDE compounds were detected.

3.3. Associations of organohalogen compounds among matrices

The regression coefficients from the linear regression of all $\log_{10}$-transformed compounds are given in Table 2. Most compounds that were detected in body feathers, blood plasma and preen oil associated significantly among all matrices ($P < 0.05$, $0.20 \leq R^2 \leq 0.98$). The associations were strong, in spite of low sample sizes for preen oil specifically ($n = 8$), and all matrices in general (Figure 6). Most significant associations existed between preen oil and blood plasma.

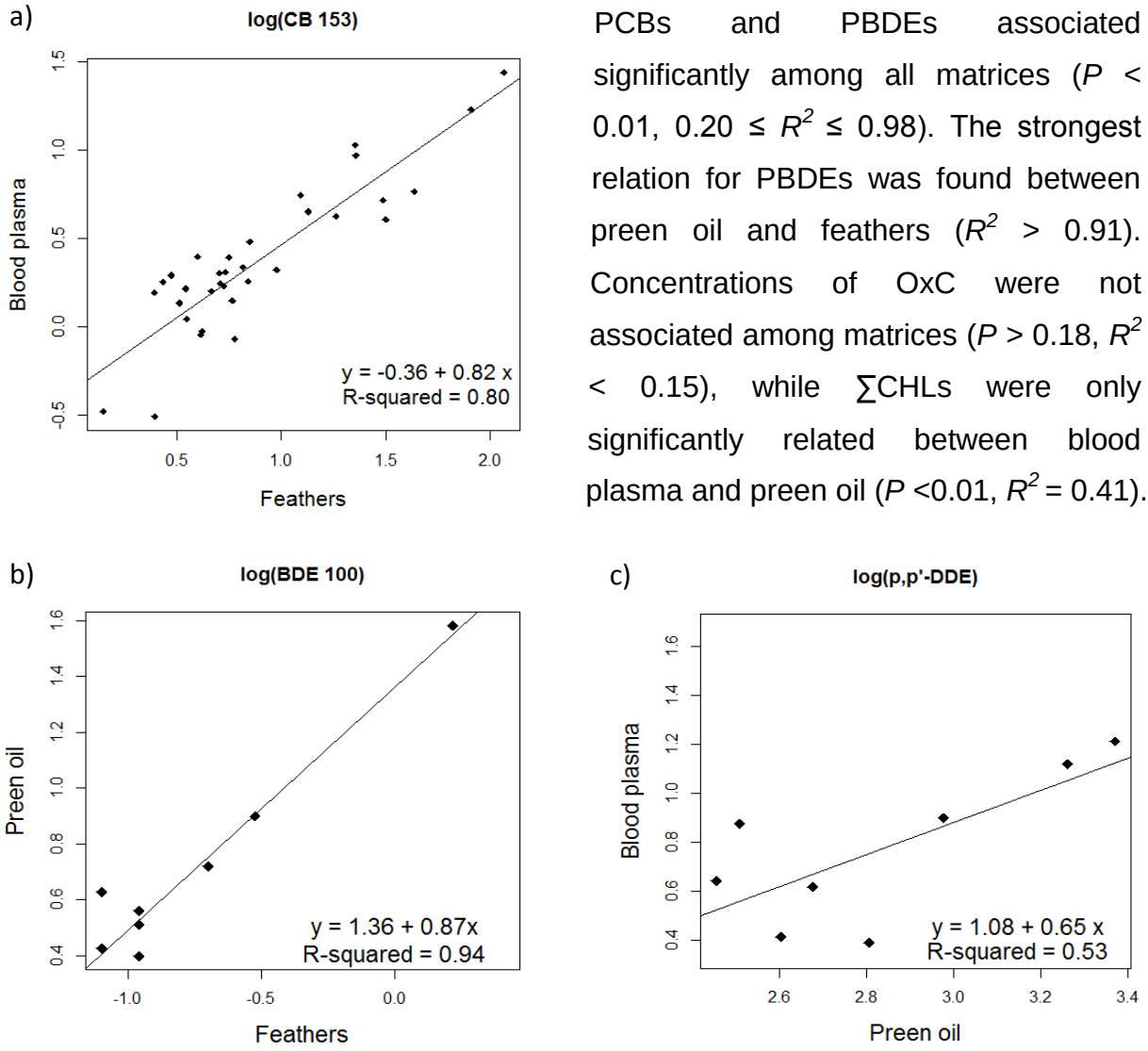

Figure 6: The majority of OHCs detected in body feathers, blood plasma and preen oil from Goshawk nestlings associated significantly among the different matrices. The relationship between (a) CB 153 in body feathers and blood plasma (n = 33), (b) *p,p'*-DDE in preen oil and blood plasma (n = 8), and (c) BDE 100 in body feathers and preen oil (n= 8).

PCBs and PBDEs associated significantly among all matrices (P < 0.01, 0.20 ≤ R^2 ≤ 0.98). The strongest relation for PBDEs was found between preen oil and feathers (R^2 > 0.91). Concentrations of OxC were not associated among matrices (P > 0.18, R^2 < 0.15), while ∑CHLs were only significantly related between blood plasma and preen oil (P <0.01, R^2 = 0.41).

∑DDTs showed a tendency towards significance in preen oil and feather (P = 0.06, R^2 = 0.46), but had stronger coefficients when feathers and preen oil where associated with blood plasma (P < 0.05, 0.44 ≤ R^2 ≤ 0.53). HCB levels only showed a significant relationship between preen oil and body feathers (P < 0.05, R^2 = 0.53). The lack of significant relationships between body feathers and preen oil was also found for CB 101 (P < 0.82, R^2 = 0.01).

Table 2: Coefficient of determination (R^2), significance values (P) and regression coefficients (β_0 and β_1) of the association between $\log_{10}$-transformed OHC concentrations in body feathers, blood plasma and preen oil of Goshawk nestlings from Trøndelag (Norway). Only congeners found in all 3 matrices are included in the table.

	Blood plasma ~ body feather N = 14				**Blood plasma ~ preen oil** N = 8				**Preen oil ~ body feathers** N = 8			
	β_0	β_1	P	R^2	β_0	β_1	P	R^2	β_0	β_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
∑PCBs[a]	**-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
∑CHLs[b]	**-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	<0.001***	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
∑DDTs[b]	**-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 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 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 153	nd	nd	nd	nd	nd	nd	nd	nd	1.55	1.25	<0.01**	0.96
∑PBDEs[c]	**-0.64**	**0.86**	**<0.01****	**0.65**	**-1.72**	**0.81**	**<0.01****	**0.93**	**1.22**	**0.96**	**<0.01****	**0.97**

Significant at *P < 0.05, **P < 0.01. [T] indicates close to significance: 0.10 > P ≤ 0.05. "nd" indicates not detected. [a] ∑PCBs is the sum of the 14 PCB congeners detected in all three matrices, [b] ∑CHLs is the sum of OxC and TN, [c] ∑DDTs is the sum of *p,p'*-DDE and *p,p'*-DDT and [d]∑PBDEs is the sum of the 3 BDE congeners detected in all three matrices.

3.4. Determinants of organohalogen compound concentrations

The variation in the individual OHC compounds and OHC classes were best predicted by models including $\delta^{13}C$, $\delta^{15}N$ and $\delta^{13}C + \delta^{15}N$ (Table 3). $\delta^{13}C$ explained the variation in PCBs, HCB, *p,p'*-DDE and PBDEs the best, although the negative relationships were insignificant ($-0.36 \leq \beta_1 \leq -0.08$, $0.29 \leq P \leq 0.93$, $0.87 \leq R^2 \leq 0.96$). $\delta^{15}N$ was included in the most parsimonious model explaining variation in OxC, positively but not significantly related ($\beta_1 = 0.25$, $P = 0.12$, $R^2 = 0.85$). The candidate models with $\Delta AIC_c < 2$ did not show any distinct and significant relationship to the different OHCs (Table A6, Appendix 7.6). The slopes of the candidate models with $\delta^{15}N$ as predictor were insignificant, close to 0 and not indicative of any trends even within the different OHC classes.

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

		Estimate	*SE*	*DF*	*t*	*P*	Inter-nest SD Intra-nest SD	R^2
CB 146	Intercept	-4.55	10.55	17	-0.43	0.67	0.80	0.96
	$\delta^{13}C$	-0.19	0.45	17	-0.43	0.68	0.33	
CB 153	Intercept	-0.12	9.88	17	-0.01	0.99	0.77	0.96
	$\delta^{13}C$	-0.08	0.42	17	-0.19	0.85	0.30	
CB 187	Intercept	-3.16	8.46	17	-0.37	0.71	0.61	0.95
	$\delta^{13}C$	-0.18	0.36	17	-0.49	0.63	0.28	
∑PCBs[a]	Intercept	1.22	9.31	17	0.13	0.90	0.70	0.96
	$\delta^{13}C$	-0.08	0.40	17	-0.21	0.84	0.29	
OxC	Intercept	-3.48	1.30	17	-2.68	0.02	0.52	0.85
	$\delta^{15}N$	0.25	0.15	17	1.64	0.12	0.54	
HCB	Intercept	8.55	12.53	17	-0.68	0.50	0.70	0.87
	$\delta^{13}C$	-0.36	0.54	17	-0.66	0.52	0.59	
***p,p'*-DDE**	Intercept	-4.81	7.24	17	-0.67	0.51	0.45	0.92
	$\delta^{13}C$	-0.34	0.31	17	-1.09	0.29	0.29	
BDE 47	Intercept	-8.61	10.07	17	-0.86	0.40	0.74	0.96
	$\delta^{13}C$	-0.34	0.43	17	-0.78	0.44	0.33	
BDE 99	Intercept	-1.20	9.57	17	-0.13	0.90	0.69	0.95
	$\delta^{13}C$	-0.04	0.41	17	-0.09	0.93	0.31	
∑PBDEs[a]	Intercept	-4.50	9.21	17	-0.49	0.63	0.65	0.95
	$\delta^{13}C$	-0.22	0.40	17	-0.55	0.59	0.31	
∑OHCs[b]	Intercept	-0.65	8.16	17	-0.08	0.94	0.56	0.94
	$\delta^{13}C$	-0.20	0.35	17	-0.56	0.58	0.28	

[a] ∑PCBs and ∑PBDEs are the sum of all the PCB or PBDE congeners detected in feathers. [b] ∑OHCs is the sum of ∑PCBs, ∑DDTs, ∑CHLs, HCB and ∑PBDEs detected in feathers.

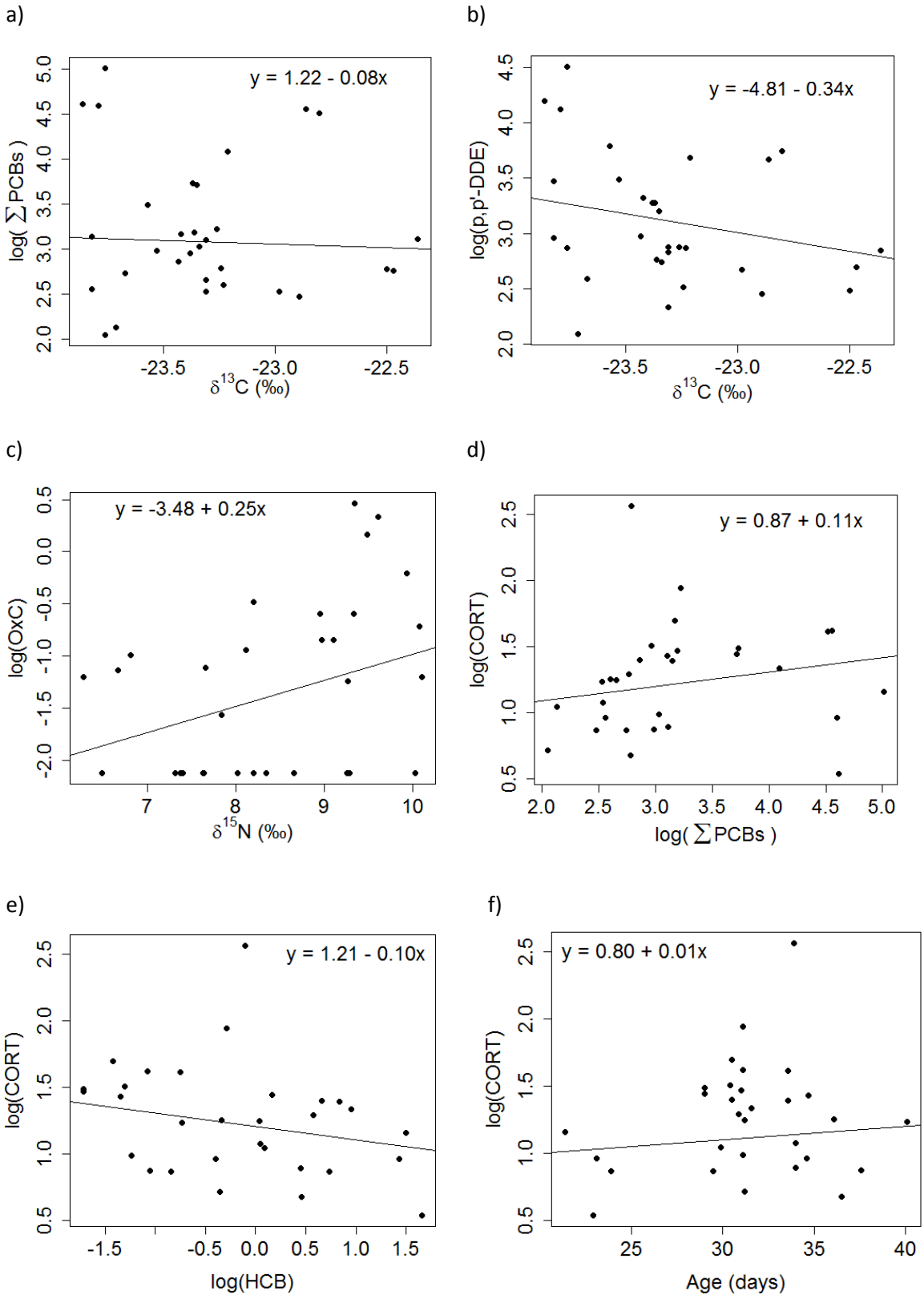

Figure 7: The relationship between different OHCs (ln-transformed, ng mm^{-1}) and SI values in body feathers, shown as (a) ∑PCBs and $\delta^{13}C$, (b) *p,p'*-DDE and $\delta^{13}C$, (c) OxC and $\delta^{15}N$. (d-f) The variation in feather CORT levels (ln-transformed, pg mm^{-1}) were best explained by OHCs or age: (d) CORT and ∑PCBs, (e) CORT and HCB and (f) CORT and age (days). Regression lines were obtained from LME models corrected for pseudoreplication between nests. None of the relationships were significant ($P > 0.5$).

3.5. Determinants of corticosterone levels

Table 4 summarizes the most parsimonious models to explain the variation in CORT levels, possibly by the predictors $\delta^{13}C$, $\delta^{15}N$, age or OHC concentration (individual compounds and OHC classes). The relationships to sex and scaled mass index (SMI) were tested prior to model selection (Figure 8). No significant relationships were found between the variables and sex (β_1= - 0.14, P = 0.27, R^2 = 0.83, n_{nests} = 14, n_{birds} = 32) nor SMI (β_1 = 0.00, P = 0.71, R^2 = 0.82, n_{nests} = 12, n_{birds} = 28), so neither were included as predictors in the model selection.

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

		Estimate	***SE***	***DF***	***t***	***P***	**Inter-nest SD / Intra-nest SD**	***R^2***
CB 146	Intercept	1.24	0.10	16	12.86	0.00	0.27	0.82
	log(CB 146)	0.10	0.10	16	0.97	0.35	0.30	
CB 153	Intercept	1.05	0.22	16	4.81	0.00	0.27	0.82
	log(CB 153)	0.10	0.11	16	0.92	0.37	0.30	
CB 187	Intercept	1.11	0.16	16	6.74	<0.01	0.27	0.82
	log(CB 187)	0.12	0.13	16	0.94	0.36	0.30	
∑PCBs[a]	Intercept	0.87	0.38	16	2.29	<0.05	0.27	0.83
	log(∑PCBs)	0.11	0.12	16	0.98	0.34	0.30	
OxC	Intercept	0.80	0.65	16	1.24	0.23	0.26	0.81
	age	0.01	0.02	16	0.68	0.51	0.30	
HCB	(Intercept)	1.21	0.09	16	13.17	<0.01	0.23	0.79
	log(HCB)	-0.10	0.08	16	-1.22	0.24	0.31	
***p,p'*-DDE**	(Intercept)	0.80	0.65	16	1.24	0.23	0.26	0.81
	age	0.01	0.02	16	0.68	0.51	0.30	
BDE 47	(Intercept)	0.80	0.65	16	1.24	0.23	0.26	0.81
	age	0.01	0.02	16	0.68	0.51	0.30	
BDE 99	(Intercept)	0.80	0.65	16	1.24	0.23	0.26	0.81
	age	0.01	0.02	16	0.68	0.51	0.30	
∑PBDEs[a]	(Intercept)	0.80	0.65	16	1.24	0.23	0.26	0.81
	age	0.01	0.02	16	0.68	0.51	0.30	
∑OHCs[b]	(Intercept)	0.83	0.55	16	1.51	0.15	0.27	0.83
	log(∑OHCs)	0.10	0.14	16	0.75	0.46	0.30	

[a] ∑PCBs and ∑BDEs are the sum of all the PCB or PBDE congeners detected in feathers.
[b] ∑OHCs is the sum of ∑PCBs, ∑DDTs, ∑CHLs, HCB and ∑PBDEs detected in feathers.

All predictors failed to significantly explain the observed variation in CORT levels (P > 0.05). The variation was nonetheless best explained by the individual OHC congeners/class or age when the models were run against compounds representing the five OHC classes (sum of PCBs, CHLs, HCB, DDTs and PBDEs). Three individual PCBs (CB 146, 153 and 187) and two PBDE congeners (BDE 47 and 99) were also tested, and showed the same trend as the OHC class they belong to (∑PCBs and ∑PBDEs). When CORT levels were related to PCBs and HCB, the congeners alone appeared to be the most parsimonious models explaining variation in CORT. However, the relationships were different for the two, where PCBs (∑PCBs, CB 146, 153 and 187) exhibited a positive slope (β_1 = 0.11), contrary to HCBs negative relationship to CORT (β_1 = -0.10; Figure 7e/f). OxC, *p,p'*-DDE and the PBDEs were not the best predictors for CORT variation, but age appeared as the most parsimonious model here (β_1 = 0.01, Figure 7f). The overall response in CORT to OHC was as described with PCBs (Figure 7d), where the concentration in CORT was correlating positively with OHCs (β_1 = 0.10).

Of the candidate models with ΔAIC_c < 2 age and $\delta^{13}C$ appeared as second best predictors of the variation in CORT (Table A7, Appendix 7.7). Age was more important to describe the variation in PCB and HCB levels, while $\delta^{13}C$ was included in candidate models for OxC, *p,p'*-DDE and PBDEs. The relations to age and $\delta^{13}C$ were insignificant (P > 0.05), but the slopes were positive for both predictors (β_1 = 0.01 and β_1 = 0.11, respectively).

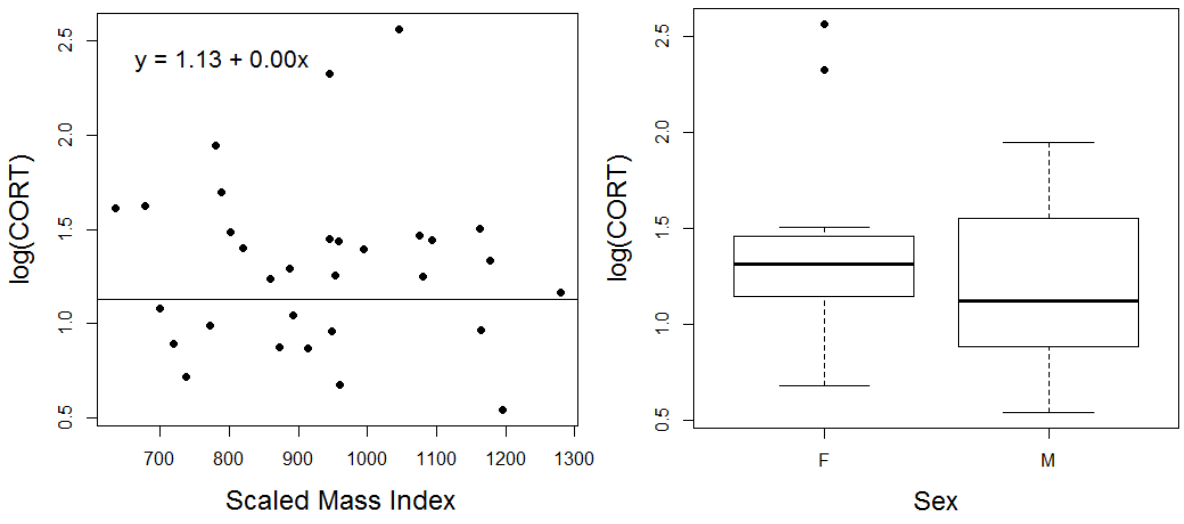

Figure 8: CORT (ln-transformed, pg mm^{-1}) was neither related to body condition (given as scaled mass index) nor sex of Goshawk nestlings (P > 0.5). The relationships were analysed with LMEM, corrected for pseudoreplication between nests.

4. Discussion

4.1. Organohalogen compound exposure

4.1.1. Accumulation levels and profiles of OHCs

High levels of OHCs were found in preen oil compared to body feathers and blood plasma. Preen oil had up to two magnitudes higher PCB and DDT concentrations than blood plasma, and 30 times higher levels than those in body feathers. The difference in concentrations between matrices was largest for HCB, with 160 and 7 times higher levels in preen oil than in blood plasma and body feathers, respectively. CHLs and PBDEs measured in preen oil were 40 times the blood plasma concentration. The high lipid content of preen oil and the increases the affinity for compounds with high K_{ow} (Brink, 1997, Yamashita, 2007). Compounds will be redistributed from the blood to other matrices dependent on the matrices perfusion rate and the affinity of the compounds relative to the blood (Drouillard et al., 2003). Significantly higher proportions of the less persistent penta- and hexa-chlorinated PCBs were found in feather matrix compared to blood plasma and preen oil. Higher proportions of penta PCBs in feathers than in muscle or adipose has been documented for several bird species (Dauwe et al., 2005, Jaspers et al., 2007b), but it is in contrast to other studies including feathers and blood plasma/serum (Eulaers et al., 2011a, Jaspers et al., 2008a). Contrary to feathers, preen oil showed significantly higher percentages of the heavier octa-, nona- and deca-PCBs. Such a pattern is not surprising, as the high K_{ow} of these compounds makes the lipid rich preen oil a sink for higher halogenated OHCs (Brink, 1997, Yamashita, 2007). Although the preen oil has a higher abundance of the most lipophilic compounds, the pentachlorinated compounds were also present. This may indicate the higher degradation in blood plasma compared to preen oil. The most abundant PCB congeners in all matrices were CB 153, CB 180 and CB 187, a pattern generally found in terrestrial species (Eulaers et al., 2011a, 2007b, Jaspers et al., 2006a, 2007a, 2007b). However, body feathers had a significantly higher proportion of CB 138 when compared to blood plasma and preen oil, possibly due to external contamination from the air. The adhesive properties of particles makes pollutants such as PCBs attach to particulate matter that can deposit onto the feather surface (Noyes et al., 2009). The elevated levels of HCB in body feathers may originate from HCB volatilization from secondary sources. The octanol-air and octanol-water

coefficient of HCB is lower than for many other OHCs, indicating a higher chance of revolatilization (Barber et al., 2005). Only a small amount of re-emission is required to maintain the air concentrations of HCB, despite lower HCB concentrations in contemporary soil (Barber et al., 2005). However, other possibilities explaining the higher levels in feathers may be the washing procedure with distilled water, or due to higher affinity of certain compounds for the feather matrix. The higher proportion of HCB in feathers than in blood has also been detected in several other predatory birds (Eulaers et al., 2011a, 2011b). External contamination may be indicative of the surrounding habitat of the bird, but can be misleading for the understanding of the actual bioavailability and bioaccumulation of contaminants to the birds.

The PBDE profile was dominated by BDE 99 in all matrices, as observed in other terrestrial species (Jaspers et al., 2006b, 2007b, Law et al., 2003). Matrix-specific differences were similar as described for PCBs; a higher contribution of the lighter BDE 47 in feathers, and detection of the higher brominated BDE 154 and 183 in preen oil. Elevated levels of BDE 47 in feathers have been shown to occur also in Goshawks, Sparrowhawks (*Accipiter nisus*) and Eagles (Eulaers et al., 2011a, Jaspers et al., 2006a). A possible higher affinity for the feather matrix may explain this observed pattern. The significantly elevated levels of BDE 99 in blood plasma over body feathers and preen oil is different from what has previously been measured in Goshawks (Eulaers et al., 2011a). Based on the detection of BDE 153 in body feathers and preen oil but not in blood plasma, one could argue that the high levels of BDE 99 in the latter may result from the metabolic capacity of blood. Indeed, the prevalence of lighter congeners in blood plasma may reflect metabolic degradation of heavier BDEs, as the presence of BDE 154 and 183 in preen oil indicates that the birds are exposed to these compounds. Biotransformation of the heavier PBDEs that constitutes the commercial OctaBDE mixture was suggested as an explanation for the PBDE pattern in Common Magpie (*Pica pica*) as well (Jaspers et al., 2009). The inclusion of BDE 49 in the analysis would help to provide an answer to whether such biotransformation happens in blood plasma of Goshawk nestlings.

Since the analytical detection and quantification of the different compounds was based on samples that varied in mass and amount, some samples may have been too small for the detection of compounds found in low concentrations. Certain

compounds that were not detected in all matrices (CB 95, 101, 105, 149, 171, 174, 194, 206, 209, *p,p'*-DDT, TN, CN, BDE 153, 154 and 183), or compounds with a DF < 0.5 (CB 28, 49, 52, 74, 132, BDE 28, HBCDs and TBBPA) could be affected by this. Of special interest are the more recent flame retardants HBCD (*α*-, *β*- or *γ*-stereoisomers) and TBBPA. HBCD was detected in Goshawk nestling feathers, blood plasma and preen oil, but below LOQ for over half of the sample size. TBBPA was, on the other hand, not detected in any matrices. The lack of TBBPA and low detection of HBCD could suggest that the environmental levels in the habitat of the Goshawks are low. The concentrations were similar to the low levels found in Common Eider eggs (*Somateria mollissima*), but lower than in Great Black-backed Gull eggs (*Larus marinus*) from Åsefjorden, Norway (Haukås et al., 2009). HBCD concentrations in feathers and preen oil of juvenile and adult Barn Owls (*Tyto alba*) from Belgium and France were also higher than reported here (Eulaers et al., 2014a). Goshawk feathers had higher concentration of *α*-HBCD than blood plasma and preen oil, while the *β*- or *γ*-stereoisomer was detected in highest concentration in preen oil. Preen oil did not contain *α*-HBCD. The relatively high log K_{ow} of HBCD (5.6) and the higher metabolism in blood plasma than in body feathers and preen oil may explain why the concentrations were lower in plasma (MacGregor and Nixon, 1997). None of the individuals with detectable levels in body feathers had detectable levels in blood plasma or preen oil. The presence of the HCBD stereoisomers in blood plasma or preen oil do however support that the compounds, to some extent, bioaccumulate (Haukås et al., 2010).

The concentrations of the detected legacy OHCs in this thesis were generally lower, although comparable to those previously reported for Goshawk and White-tailed Eagle nestlings in Troms/Tromsø (Eulaers et al., 2011a). Goshawk nestlings from Trøndelag (63°N) had somewhat higher contribution of PCBs but less DDTs and PBDEs in body feathers and blood plasma than reported for Goshawks from Tromsø (69 - 71°N) (Eulaers et al., 2011a). The slightly lower concentrations of OHCs in birds from Trøndelag compared to Troms corresponds to the suggested pollution gradient along the Norwegian coast (Eulaers et al., 2011b, Olsson et al., 2000, Steffen et al., 2006). Great Black Backed Gulls and Glaucous Gulls (*Larus hyperboreus*) from northern (Bjørnøya 74°N) and southern colonies (North Sea 55°N) had higher levels of higher chlorinated PCBs than the colonies along the

Norwegian Coast (Norwegian sea 66°N, Barents Sea east and west 70°N). A simple comparison of median OHC concentrations in Goshawk feathers and blood plasma in Sør- and Nord-Trøndelag showed that Goshawks from Nord-Trøndelag had slightly higher levels of OHCs (PCBs and OCPs) than birds from the southern sampling areas. Higher PBDE levels were found in goshawks breeding close to the city of Trondheim. An interesting aspect would be to further investigate whether such an OHC gradient from south in Trondheim and northward exist, or whether the concentrations are due to regional differences. White-tailed Eagle nestlings from Trøndelag had slightly higher levels of most OHCs than 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 done in Tromsø, indicating higher exposure to certain OCPs in the terrestrial environment where these compounds were used (Eulaers et al., 2011a). Goshawks may forage closer to rural areas with agricultural activity, which probably makes them more exposed to OCPs. The body feathers of Goshawk nestlings are less contaminated than birds in southern parts of Europe (Garcia-Fernandez et al., 2013, Jaspers et al., 2007b).

Comparison of the PCB and PBDE profiles between White-tailed Eagle and Goshawk nestlings from Trøndelag suggests that Goshawks have the highest contribution of the more halogenated PCBs and PBDEs, similarly to what is observed between the species in Tromsø (Eulaers et al., 2011a). These contaminants have a low solubility in water, but higher partitioning to particles, therefore suggesting higher concentrations in the terrestrial environment (Noyes et al., 2009). Even though longer food chains in the marine environment increase the biomagnification potential, this comes along with additional steps of biological degradation of the heavier compounds (Burger, 1997). Several factors can affect the detectability and the accumulation levels, such as physiological differences in metabolism or age, and ecological differences in diet and habitat residency. Altogether, the OHC profiles in body feathers, blood plasma and preen oil were generally comparable, and indicates that feathers and preen oil reflect the internal plasma levels of OHCs. A wider range of OHCs were detected in body feathers and preen oil than in blood plasma. Using samples from body feathers and preen oil

rather than or in addition to blood plasma can therefore increase our knowledge of the exposure and bioaccumulation of OHCs normally not detected in blood plasma.

4.1.2. Associations in accumulation among different matrices

Nestling Goshawk body feathers are not yet an established biomonitoring matrix for OHC contamination, and a thorough evaluation of the associations with blood plasma levels were therefore necessary. Moreover, the evaluation of the usefulness of nestling preen oil as a monitoring matrix is even less investigated, and also requires good understanding of the relationships with body feathers and blood plasma. The inclusion of preen oil in this thesis should however be considered a pilot study, as the sampling of preen oil started at the very end of the sample period and therefore resulted in a very limited sample size (n = 8).

High and significant associations were found among all matrices, where only a few compounds failed to show strong relationships (CB 101, DDTs, OxC and HCB). The missing relation for CB 101 between feathers and preen oil is obvious from the different PCB profiles. External contamination on the feather surface or differential matrix affinities may explain the lack of significant relation between compounds detected in different matrices, reported previously for DDTs, OxC and HCB as well (Eulaers et al., 2011a, 2011b, Jaspers et al., 2008b). The sequestration of compounds into the matrices may explain the strong association between feathers and preen oil, since these matrices have a lower turnover rate than blood plasma and represent exposure over a longer time period. The high lipophilicity makes preen oil a potential and interesting matrix for investigating new and (re)emerging contaminants. Preen oil was evaluated earlier as a valuable biomonitoring matrix for OHCs in White-tailed Eagles (Eulaers et al., 2011b), and its further use in biomonitoring activities may therefore allow for comparisons of contaminant exposure of birds in marine and terrestrial environments. This would increase our limited knowledge of the distribution of new and (re)emerging contaminants in wildlife. Future studies with higher sample sizes should therefore further investigate the suitability of preen oil as a biomonitoring tool. The relationships between OHCs in nestling feathers and blood plasma were generally higher than those found for White-tailed Eagle nestlings from Trøndelag (Eulaers et al., 2011b). Variation in

preening activity, feather atrophy and metabolic capacity were suggested as potential confounding factors in those nestlings that could affect the relationships (Eulaers et al., 2011b). Also adult Common Magpies had slightly lower correlations between the two matrices, possibly caused by the effect of age and preening activity (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 blood plasma of Goshawk nestlings were highly significant with strong coefficients, indicating that nestling Goshawk feathers reflect the internal levels for most OHCs. Both nestling body feathers and preen oil are suggested as promising non-destructive biomarkers for OHCs.

4.2. Levels of corticosterone

CORT was successfully measured in feathers of Goshawk nestlings, and fell within the range of recently reported feather CORT levels in Sparrowhawk, Common Kestrel (*Falco tinnunculus*), Common Buzzard (*Buteo buteo*), Barn Owl, and Tawny Owl (*Strix aluco;* mean: 2 - 5 pg mm^{-1}) (Strong et al., 2015). The CORT levels reported previously for Sparrowhawk were somewhat below those of the Goshawk nestlings in this thesis (Strong et al., 2015). Differences in age, season and habitat hampers meaningful comparisons. However, the CORT levels of Goshawk nestlings were similar to those in Buzzard nestlings (Martínez-Padilla et al., 2013). The limited data on feather CORT in Accipitriformes species (Common Buzzard, Sparrowhawk and Goshawk) indicate that there is more variation among these species than that observed among Barn Owl, Common Kestrel and Tawny Owl (Strong et al., 2015). In comparison to other species, the concentrations of CORT in Goshawk nestlings were close to those reported for nestling Tree Swallow (*Tachycineta bicolor*), adult Leach's Storm-Petrels (*Oceanodroma leucorhoa*) and Common Eider (Fairhurst et al., 2013a, 2015, Harms et al., 2015). Colonial Rhinoceros Auklet nestlings (*Cerorhinca monocerata*) and adult Cory's Shearwaters (*Calonectris borealis*) had CORT levels above those of Goshawks (Fairhurst et al., 2012, Will et al., 2014).

CORT release is dependent on several factors, such as species, climate, life history stage, brood competition, food availability, concentrations and type of contaminants studied. This thesis can only take a few factors into account, and the importance of other stressors therefore remains unknown. Statistically, the low sample size restricts the number of factors that could be included in the model selection. Even though body condition (SMI) and sex showed no significant relationship to CORT, their importance relative to OHCs, age and SI values is unresolved. Similarly, Strong et al. (2015) did not find any significant effect of body condition on feather CORT levels (when based on body mass) for the majority of the adult predatory birds included in their study. However, body condition appeared significant for Common Kestrel and Tawny Owl when it was defined as pectoral muscle index/residual, and shows how different approaches in the calculation of body condition index can affect the results (Strong et al., 2015). The lack of relation to sex is supported by the study of Strong et al. (2015) on predatory birds, as well as studies on House Sparrows (*Passer domesticus*) and Red-legged Partridge (*Alectoris rufa)* (Bortolotti et al., 2008, Koren et al., 2012).

4.3. The use of stable isotope values

Low $\delta^{13}C$ levels confirmed that the Goshawk clearly feeds in the terrestrial environment, since the terrestrial and freshwater environment is generally depleted in ^{13}C compared to the marine environment (Kelly, 2000). A lower $\delta^{13}C$ level and higher $\delta^{15}N$ level was found in the Goshawks nestlings from the current study compared to Goshawk and Golden Eagle nestlings (*Aquila chrysaetos*) from Tromsø (Eulaers et al., 2011a). Compared to White-tailed Eagle nestlings the Goshawks exhibited a lower $\delta^{15}N$ range. The levels may be explained by differences in trophic levels, nest location and habitat, or diet composition with highly prey-specific variation in metabolic degradation (Eulaers et al., 2013).

Associations between OHCs and $\delta^{15}N$ and or $\delta^{13}C$ values of body feathers from Goshawk nestlings did not show significant relationships ($P < 0.5$). The low sample size ($n = 13$) reduces the statistical power and thus the likelihood of detecting significant relationships. However, the best models that explained the variation in the exposure to individual OHC congeners and OHC classes had high R^2 values.

Although no significant relationships were detected, the trends are visible. When pseudoreplication between nestlings has to be corrected for, a larger sample size is strongly advised before any conclusions can be made. The overall trend implied that $\delta^{13}C$ is of the two stable isotope tracers the most important driver behind the variation in OHC exposure. The negative association of OHCs (except for OxC) to $\delta^{13}C$ is consistent with earlier findings for CB 153 and *p,p'*-DDE in Goshawk nestlings (Bustnes et al., 2013), but opposite of what has been reported for both White-tailed Eagle nestlings and Bald Eagles (*Haliaeetus leucocephalus*) (Bustnes et al., 2013, Elliott et al., 2009). The relationships were, in contrast to that of Goshawk, found positive for the two marine eagles. Elevated $\delta^{13}C$ values in the marine environment can be used as a proxy for the fjord habitat (Eulaers et al., 2014b). White-tailed Eagles that were breeding by the fjords fed at higher trophic levels and exhibited higher contaminant loads than the ones nesting at islands or in more coastal areas. The different drivers of OHC exposure in marine and terrestrial species may be explained by different food sources and the nutrient content of the prey.

A strong association was found between $\delta^{13}C$ and $\delta^{15}N$, which do suggests that individuals with more negative $\delta^{13}C$ values feed on a higher trophic levels. Higher trophic level often imply higher exposure, a trend only found for OxC in this case. The overall relationship of OHCs to $\delta^{15}N$ was vague and ambiguous and indicated no clear effect on the OHC load. The most parsimonious model describing the variation in the OHC exposure was $\delta^{13}C$, where a negative association was found. Lipids are depleted in ^{13}C compared to carbohydrates and proteins, and variation in lipid content among organisms can change the $\delta^{13}C$ (Post et al., 2007, Sotiropoulos et al., 2004). Since contaminants are lipophilic, it may be that Goshawks that feed on lipid rich prey have low $\delta^{13}C$ levels and elevated OHC levels. More information about lipid-specific differences in the Goshawks prey is needed to confirm whether the association between OHCs and $\delta^{13}C$ is driven by lipid content. However, the negative association between $\delta^{13}C$ and OHCs may also be explained by the canopy effect: $\delta^{13}C$ values are slightly lower in closed forests than in open clearings due to different assimilation from CO_2 and carbon fractionation at decreased light availability (France, 1996). This may imply that Goshawks that feed mainly in the forest are more exposed to contaminants. The very diverse diet of individual

Goshawks may also complicate the interpretation of which factors are driving the OHC exposure. A variety of prey items were found in and around the nest, with dominance of Thrushes (*Turdus sp.*), Ptarmigans (*Lagopus sp.*) and Crows (*Corvus sp.*) (Table A3, Appendix 7.3). Few waders or marine species were found, which indicated high affiliation to the terrestrial environment. However, the high variation made it hard to draw any conclusions from these observations. The narrow range of $\delta^{13}C$ may imply that the birds occupy rather similar habitats, and more specific information about the habitat of the birds would therefore be helpful.

Only one Goshawk nest represented a habitat clearly different to the others, in proximity to the mountains. The respective bird (HÅ1) had aberrant levels of $\delta^{15}N$, $\delta^{13}C$ and OHC levels, and was treated as an outlier in the nodal selection. A study of a bird species that have a well-defined diet on a specific site could possibly give clearer indications of what drives the OHC exposure. Factors like growth dilution, body condition, sex, sibling order and SI values from prey items may be even better predictors of nestling Goshawk OHC exposure (Bustnes et al., 2013, Harms et al., 2010, Love et al., 2003a). In order to explain the variation in OHC exposure from SI values in Goshawk nestlings more sampling is required. Future studies would benefit of the inclusion of spatial variables such as the stable isotopes of sulphur ($\delta^{34}S$) that may describe the variation in habitat occupancy in more detail (Eulaers et al., 2014b, Morrissey et al., 2013).

4.4. Can contaminant exposure, age and dietary tracers explain corticosterone levels?

The relationships between CORT, OHCs, SI values and age were investigated, and identified two factors that explained the variation in CORT in the best possible way, i.e. OHC exposure and age. The R^2 values indicated that around 80 % of the variation in CORT was explained by these predictors, but no relationships were found to be significant. Nevertheless, the trends are discussed. A sensible and careful interpretation of the results is therefore a necessity. Among the four factors included in the model selection, PCB and HCB exposure as well as age appeared to be the most important drivers of CORT exposure. A positive slope between PCBs and CORT indicates that PCB accumulation somehow induces CORT release, as

documented in several other bird species (Peakall et al., 1981, Verboven et al., 2010). Tartu et al. (2015) reported that baseline CORT levels (representing prestressed blood concentrations) were positively related to PCBs in both Black-legged Kittiwakes (*Rissa tridactyla*) and wandering albatrosses (*Diomedea exulans*). Stress-induced CORT levels (blood) were also affected by PCB exposure in Kittiwakes, Snow petrels (*Pagodroma nivea*) and Glaucous Gull (Tartu et al., 2015). However the relationships were dependent on both gender and PCB concentrations, with males being the most susceptible, but also more contaminated. Interestingly, highly contaminated Glaucous Gulls had a negative relationship between the two parameters (Tartu et al., 2015). The relationship can be hard to predict, and although several studies have reported lower CORT levels with elevated OHC concentrations (Love et al., 2003b, Mayne et al., 2004), a moderate PCB load is indicative of increased CORT levels (Tartu et al., 2015). Decreased stress-induced CORT levels with elevated PCB levels may be a physiological defence mechanism that allows birds to cope with high contamination. The PCB concentrations of the Goshawks nestlings were well below those reported for all the adult seabird species, and may explain why no significant results were detected in this thesis (Tartu et al., 2015). Since lipophilic contaminants are inter-correlated, and the concentration and effects of each compound are small, one could argue that perhaps ΣOHCs gives the most realistic picture of how contaminants affect CORT. This however prevents detection of compounds that have a different effect, e.g. the negative relation found for HCB. Few studies document a negative association between HCBs and CORT levels in wildlife, and more studies are needed to confirm whether HCB indeed impedes CORT release similarly to PCBs do in high concentrations. In this thesis, the total OHC exposure was positively associated with CORT and indicated a possible stimulatory effect on the CORT release. Certain contaminants have shown to inhibit/stimulate enzymes such as cytochrome P450 which is involved in the steroidogenesis pathway. Inhibition of CORT to aldosterone or stimulation of CORT synthesis has been shown to both elevate CORT levels (Xu et al., 2006).

The relationship between CORT and age was slightly positive. At the time of nest departure Goshawk nestlings go through a quick transition from reduced activity to full flight. Corticosterone may be of particular importance as a physiological facilitator of locomotor activity and foraging behaviour (Varland et al., 1991). The relationship

will likely depend on the characteristics of the nest, where cavity nesters like the American Kestrel (*Falco sparverius*) are expected to have a higher response than birds that nest in trees or on the ground (Heath, 1997). The Goshawk gradually prepares for nest departure with wing flapping and branch movement, which could cause a moderate increase of CORT levels with age (Boal, 1994). However, Blas et al. (2005) reported higher acute CORT levels in older nestlings, but did not find any age-related difference in baseline levels (Blas et al., 2005). Competition for resources in the nest involves begging behaviour and possibly aggression, and may also stimulate release of CORT (MartÍnez-Padilla et al., 2004). The lack of significance in this thesis may also be due to the comparison of variables representing different time frames; age represents the time of sampling while OHCs and CORT represent the growth period of the feather, discussed more in 4.7 Future perspectives.

4.5. Feathers provide an integrated assessment of OHC exposure, diet and stress

OHC levels, SI values and CORT levels were successfully detected in body feathers of Goshawk nestlings. The OHC profiles and association analysis between matrices indicated that body feathers reflect the internal body burdens of most contaminants. The relationship between feather mass and length was tested, to determine whether comparisons across measurement units were valid. CORT and OHCs are assumed to be incorporated into the feather in a time dependent manner, equivalent to feather length (Bortolotti, 2010). Therefore, the most appropriate unit of measure of these compounds should be per growth rate (Bortolotti, 2010). This unit is commonly used to report CORT levels, while OHCs are commonly given per unit of mass. A highly significant and strong correlation between feather length and mass allowed for comparisons of OHC and CORT levels across units, and thereby also eased comparisons of levels with other studies. CORT was therefore given as pg mm^{-1} and OHCs as pg mg^{-1}. SI values on the other hand, are basic elements that are naturally incorporated into the feather matrix. Since they are reported as ratios the problem with abslute mass is automatically resolved (Bortolotti, 2010).

The interpretation of the relationship between OHC and CORT levels and SI values was limited by the sample size (n = 13), as the intra-nest variation had to be taken into account. The results from the LMEMs are truly depending on an increased sample size before conclusions should be made, but they still provide good indications of trends. By relating OHCs to SI values it became evident that $\delta^{13}C$ was the best predictor for OHC accumulation. Elevation in OHC exposure was associated with enrichment in ^{13}C rather than trophic level, contrary to the expectation. This may indicate that spatial differences or the lipid content of the Goshawks prey is of importance for the variation in OHC. Goshawks shielded from the marine environment may be more prone to exposure of the contaminants, without necessarily feeding at a higher trophic levels. Integration of OHC exposure, SI values and CORT levels can be useful to better characterize the local environment, and to better understand interactions among these variables. By doing so in this thesis, it became clear that SI values alone were not sufficiently explanatory for the observed variation in CORT levels. PCB accumulation and age were identified to be the more valuable predictors, and were positively associated with CORT levels. Based on the observed trends one could suggest that the birds' dietary $\delta^{13}C$ affects the accumulation of PCBs, which again elevate CORT levels.

This study has, for the first time, measured OHC concentrations, SI values and CORT levels simultaneously in nestling body feathers. Nestling feathers appear to be a useful matrix that provides a long-term and synchronous picture of contaminant exposure, dietary tracers and chronic stress. The integration of several ecological and physiological measures that all reflect the same time period can provide new understanding in ecotoxicology and stress physiology. Further studies with larger sample sizes are however needed to investigate the relationships in more detail.

4.6. Future perspectives

Feathers are a unique sample matrix as their sampling is non-destructive and easy to perform, their storage and shipping does not require complicated logistics, and finally they offer the possibility to study time trends using museum collections (Bortolotti et al., 2008, Dauwe et al., 2005). However, OHC and CORT levels in feathers solely represent the period of feather growth, and may not be directly

comparable against variables that represent a snapshot in time (age, body condition). These comparisons would probably take advantage if the feather was cut and analysed in several pieces, representing different life stages, or by measuring baseline CORT levels instead of feather CORT. Such approaches could perhaps elucidate the slight increase in CORT levels of older Goshawk nestlings. However, CORT levels in blood increase within few minutes after stress handling, meaning that blood sampling of birds has to happen shortly after stress is induced (Romero and Reed, 2005). The time demanding process of reaching the Goshawk nest resulted in highly varying sampling times, and would therefore result in unattainable baseline CORT levels. In such cases, the use of feathers as sample matrix is especially advantageous.

The combined feather-based measures of OHC concentrations, SI values and CORT levels has potential to provide new understanding of how the chronic stress response may be influenced of the long-term exposure of contaminants or of dietary habits. However, this also requires knowledge of other influencing factors, such as species, age, body condition, moult, seasonality, migration and dispersal. The use of nestling feathers enables us to control for several confounding factors. Said so, sampling of nestlings can be challenging to time. Some individuals are below the age of feather growth and preen gland development. Moreover some nestlings are ready to fledge and leave the nest when sampling is attempted. Because Goshawks are territorial birds that nest in large trees, the sampling involves tree climbing and travelling between Goshawk territories. This puts restrictions on the sample size and therefore also the ability to draw biologically informative conclusions. A suggestion to this is to focus on predatory birds such as the White-tailed Eagle or owls, which can be more easily sampled in certain habitats. Also, a higher feather mass would be obtainable with a larger bird, allowing for more precise detections of the compounds of interest.

Further studies could look at the relation between CORT in feather per measure of length, and if it is correlated with the feather mass. Strong et al. (2015) documented that feather CORT levels were dependent on feather mass in Barn Owls, although this was not evident in feathers of Sparrowhawk and Tawny Owl. This does suggest that expressing feather CORT content per length does not fully compensate for

differences in feather length and mass for all species (Lattin et al., 2011, Strong et al., 2015). Their results do not disprove the valuable information feathers can provide, but show that the deposition of CORT in feathers requires closer investigation.

The extent of preening may also be of importance when using feathers to measure OHCs and CORT (Eulaers et al., 2014a). Adult birds apply their lipid rich oil containing OHCs onto the feather surface to make them water repellent. If an adult feather is washed without an organic solvent, the analysis of OHCs in the feather will reflect the internal feather concentration, but also the external OHCs originating from preen oil or from airborne particles (Jaspers et al., 2008a). To our knowledge, CORT has not yet been detected in preen oil, although CORT from preen oil is suggested to be applied to the feather surface. Regardless, nestlings do probably not preen their feathers to the same extent as adult birds, and the Goshawks that were sampled for preen oil did not indicate that preening was occurring (Boal, 1994). Therefore, the concentrations measured in these nestling feathers are assumed to reflect the internal feather concentrations of OHCs and CORT.

The measurement of SI values in feathers is advantageous as it provides a more holistic picture of the diet, and diminishes the likelihood of making unfortunate misinterpretations based on dietary observations. Prey remains of a Merlin (*Falco columbarius*) were found at the nest of one Goshawk nestling (HÅ1). Since Merlin is a predatory bird that also belongs to a high trophic level one could assume that the Goshawk nestling had been fed the Merlin, and therefore ought to have a δ^{15}N-rich diet. Anyhow, the measurement of δ^{15}N from body feathers showed that this bird belonged to the lowest trophic level of all, and emphasizes the benefit of the long-term measurement that feathers can provide. The nestling could have eaten this prey after the feather had been formed, or the parents could have consumed the prey. Video recordings have documented that small prey items are not necessarily shared between siblings, and that such remains leave little traces in the nest (Grønnesby and Nygard, 2000). Inclusion of variables that describe the nutritional quality of the diet, the SI values of prey species, sibling order, growth dilution and the habitat of the birds could provide a more complete understanding of which factors affect the general OHC accumulation in Goshawk nestlings.

Predatory birds are in various parts of Europe monitored for contaminants, both because of their importance as sentinel species at a high trophic levels, or simply to evaluate the risk of species of concern (Gómez-Ramírez et al., 2014). Increased understanding of the driving factors of OHC exposure together with effect parameters such as CORT can provide useful knowledge for such monitoring.

5. Conclusion

OHC profiles in body feathers, blood plasma and preen oil of Goshawk nestlings were generally comparable and related, indicating that OHC concentrations in body feathers and preen oil reflect the internal concentrations. However, external contamination, metabolic activity and matrix-specific affinities can explain some of the observed variation between the matrices. A wider range of OHCs were detected in body feathers and preen oil than in blood plasma, which emphasizes that these are promising non-destructive biomarkers of OHC accumulation in the terrestrial environment. The relationship between feather length and mass was significant, and allowed for comparison across different measurement units of OHCs (pg mg^{-1}) and CORT (pg mm^{-1}). $\delta^{13}C$ and $\delta^{15}N$ were negatively correlated, and showed lower variability within nests compared to among nests. OHC concentrations, SI values and CORT levels were measured in feathers, and variation in OHC exposure was better predicted by $\delta^{13}C$ than $\delta^{15}N$, however not significantly. CORT levels related positively but insignificantly to both PCB exposure and age. Nestling feathers appear to be a useful matrix providing a synchronous picture of the chronic stress response, the long-term exposure to OHCs and dietary tracers from the period of feather growth. Future studies are needed to confirm the underlying causalities of OHC exposure and CORT expression, essentially so with larger sample sizes. The integration of several ecological and physiological measures in feathers show however promising venues to provide new fundamental understandings in ecotoxicology and stress physiology.

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7. Appendix

7.1. Biometric information

Table A1: Biometric measurements (median and range) of size and mass, as well as estimated scaled mass index, age and sex of Goshawk nestlings.

Biometric measurement	Median	Min	Max
Beak length (mm)	20.1	17.4	23.8
Beak height (mm)	15.6	12.9	18.9
Tail length (mm)	114	33	180
Wing length (mm)	219	142	292
Tarsus width (mm)	6.7	5.7	8.8
Tarsus depth (mm)	8.6	7.0	11.1
Hallux length (mm)	25.0	19.0	29.7
Mass (g)	845	655	1350
Scaled Mass Index	929.2	635.1	1278.7
Age (days)	30	21	37
Laying date (days)	68	59	75
Sex		18 females and 19 males	

7.2. Quantification of OHCs

Table A2.1: Limit of quantification (LOQ) and detection frequency (DF) for targeted compounds in body feathers, blood plasma and preen oil of Goshawk nestlings.

	Body feathers		**Blood plasma**		**Preen oil**	
	LOQ (*ng g^{-1} dw*)	DF	LOQ (*ng g^{-1} ww*)	DF	LOQ (*ng g^{-1} ww)*	DF
CB 28	0.5	0.24	0.3	0.11	1.0	0.00
CB 49	0.5	0.06	0.3	0.00	1.0	0.00
CB 52	0.5	0.12	0.3	0.00	1.0	0.00
CB 74	0.3	0.39	0.2	0.27	1.0	0.00
CB 95	0.2	0.67	0.1	0.05	0.5	0.00
CB 99	0.2	0.73	0.1	0.68	0.5	1.00
CB 101	0.2	0.79	0.1	0.24	0.5	0.75
CB 105	0.2	0.42	0.1	0.24	0.5	1.00
CB 118	0.2	1.00	0.1	0.84	0.5	1.00
CB 128	0.2	0.94	0.1	0.70	0.5	1.00
CB 138	0.2	1.00	0.1	1.00	0.5	1.00
CB 146	0.2	1.00	0.1	0.95	0.5	1.00
CB 149	0.2	0.12	0.1	0.11	0.5	1.00
CB 151	0.2	0.00	0.1	0.00	0.5	0.00
CB 153	0.2	1.00	0.1	1.00	0.5	1.00
CB 156	0.2	0.55	0.1	0.68	0.5	1.00
CB 170	0.2	1.00	0.1	0.95	0.5	1.00
CB 171	0.2	0.42	0.1	0.35	0.5	1.00
CB 174	0.2	0.12	0.1	0.22	0.5	1.00
CB 177	0.2	0.61	0.1	0.54	0.5	1.00
CB 180	0.2	1.00	0.1	1.00	0.5	1.00
CB 183	0.2	0.94	0.1	0.86	0.5	1.00
CB 187	0.2	1.00	0.1	1.00	0.5	1.00
CB 194	0.2	0.24	0.1	0.22	0.5	1.00
CB 196/203	0.2	0.85	0.1	0.65	0.5	1.00
CB 199	0.2	0.64	0.1	0.73	0.5	1.00
CB 206	0.2	0.06	0.1	0.16	0.5	1.00
CB 209	0.2	0.03	0.1	0.03	0.5	1.00
OxC	0.2	0.61	0.1	0.84	0.5	0.88
TN	0.2	0.45	0.1	0.73	0.5	1.00
CN	0.2	0.12	0.1	0.14	0.5	0.88
HCB	0.2	0.91	0.1	0.57	0.5	1.00
p,p'-DDE	0.4	1.00	0.2	1.00	1.0	1.00
p,p'-DDT	0.2	0.64	0.1	0.05	0.5	0.75
BDE 28	0.1	0.00	0.1	0.00	0.2	0.00
BDE 47	0.1	1.00	0.1	0.57	0.2	1.00
BDE 100	0.1	0.79	0.1	0.73	0.2	1.00
BDE 99	0.1	1.00	0.1	0.89	0.2	1.00

BDE 154	0.2	0.30	0.1	0.27	0.4	1.00
BDE 153	0.2	0.73	0.1	0.46	0.4	1.00
BDE 183	0.2	0.03	0.1	0.32	0.4	1.00
α-HBCD	0.2	0.16	0.1	0.08	0.5	0.00
β-HBCD	0.2	0.14	0.1	0.03	0.5	0.13
γ-HBCD	0.2	0.11	0.1	0.03	0.5	0.25
TBBPA	0.4	0.00	0.2	0.00	0.5	0.00

Table A2.2: Median concentrations and ranges of OHCs (*ng g^{-1} lw*) in preen oil of Goshawk nestlings.

Preen oil			
OHC exposure	*ng g^{-1} lw*	***OHC exposure***	*ng g^{-1} lw*
CB 95	(< 0.00)	CB 206	6.85 (2.85 - 79.4)
CB 99	12.64 (3.88 - 61.5)	CB 209	4.31 (3.07 - 28.6)
CB 101	2.51 (1.79 - 10.1)	**∑PCBs[a]**	**1100** **336 - 12.000)**
CB 105	5.37 (1.06 - 42-9)	OxC	15.1 (6.03 - 30.2)
CB 118	29.4 (6.78 - 322)	TN	13.4 (4.04 - 49.5)
CB 128	9.69 (2.79 - 83.5)	CN	1.53 (0.67 - 10.6)
CB 138	61.7 (19.5 - 689)	**∑CHLs[b]**	**25.4** **(10.7 - 90.4)**
CB 146	39.5 (11.9 - 394)	**HCB**	**26.0** **15.7 - 46.7)**
CB 149	4.23 (2.85 - 12.6)	p,p'-DDE	689 (405 - 3400)
CB 153	290 (75.2 - 3600)	p,p'-DDT	3.62 (1.24 - 4.88)
CB 156	13.9 (3.35 - 168)	**∑DDTs[c]**	**692** **(408 - 3400)**
CB 170	64.0 (2.12 - 81.7)	BDE 47	4.27 (1.86 - 35.4)
CB 171	7.8 (2.12 - 81.1)	BDE 100	5.15 (3.55 - 54.9)
CB 174	2.12 (1.08 - 11.5)	BDE 99	7.14 (5.54 - 84.0)
CB 177	8.41 (5.07 - 79.6)	BDE 153	5.34 (3.21 - 76.5)
CB 180	228 (69.6 - 3300)	BDE 154	1.94 (1.04 - 8.52)
CB 183	34.3 (9.32 - 443)	BDE 183	1.41 (0.77 - 16.4)
CB 187	167 (80.8 - 1300)	**∑PBDEs[d]**	**23.6** **(17.4 - 276)**
CB 194	6.61 (2.33 - 84.5)	**∑HBCDs**	**(<0.00 - 16.7)**
CB 196/203	32.1 (8.61 - 439)	**TBBPA**	**(<0.00)**
CB 199	32.5 (13.3 - 375)	**Lipid percent**	**70.7** **(62.9 - 91.0)**

[a] ∑PCBs is the sum of all the 23 PCB congeners, [b] ∑CHLs is the sum of OxC, TN and CN, [c] ∑DDTs is the sum of *p,p'*-DDT and the metabolite *p,p'*-DDE, [d] ∑PBDEs is the sum of all the 6 PBDE congeners. The value following "<" gives range from the LOQ of the compound to the max concentration, and marks which compounds that were not detected with DF > 0.5.

7.3. Prey items

Table A3: Prey items found in the nest or within 200 metres from it. A total of 59 items could be identified to species or genus based on feather, fur or bone characteristics.

Prey items			
Birds	**Latin name**	**N**	**Percentage**
Black Grouse	*Tetrao tetrix*	1	1.7
Eurasian Jay	*Garrulus glandarius*	6	10.2
Eurasian Magpie	*Pica pica*	3	5.1
Eurasian Jay/Eurasian Magpie	*Corvidae*	2	3.4
Eurasian Woodcock	*Scolopax rusticola*	2	3.4
Fieldfare	*Turdus pilaris*	5	8.5
Great Spotted Woodpecker	*Dendrocopos major*	2	3.4
Hooded Crow	*Corvus cornix*	5	8.5
Common Kestrel	*Falco tinnunculus*	1	1.7
Merlin	*Falco columbarius*	1	1.7
Mistle Thrush	*Turdus viscovorus*	1	1.7
Ptarmigan	*Lagopus sp.*	6	10.2
Redwing/Song Thrush	*Turdus iliacus /Turdus philomelos*	2	3.4
Rook	*Corvus frugilegus*	3	5.1
Siberian Jay	*Perisoreus infaustus*	1	1.7
Song Thrush	*Turdus philomelos*	2	3.4
Song Thrush/Fieldfare	*Turdus philomelos /Turdus pilaris*	1	1.7
Thrushes	*Turdus sp.*	6	10.2
Wader	*Charadrii*	2	3.4
Wood Grouse	*Tetrao urogallus*	1	1.7
Wood Pigeon	*Columba palumbus*	3	5.1
Mammals			
Red Squirrel	*Sciurus vulgaris*	3	5.1
Total		59	100

7.4. Evaluation of outliers affecting the relationship between OHCs, SIs and CORT

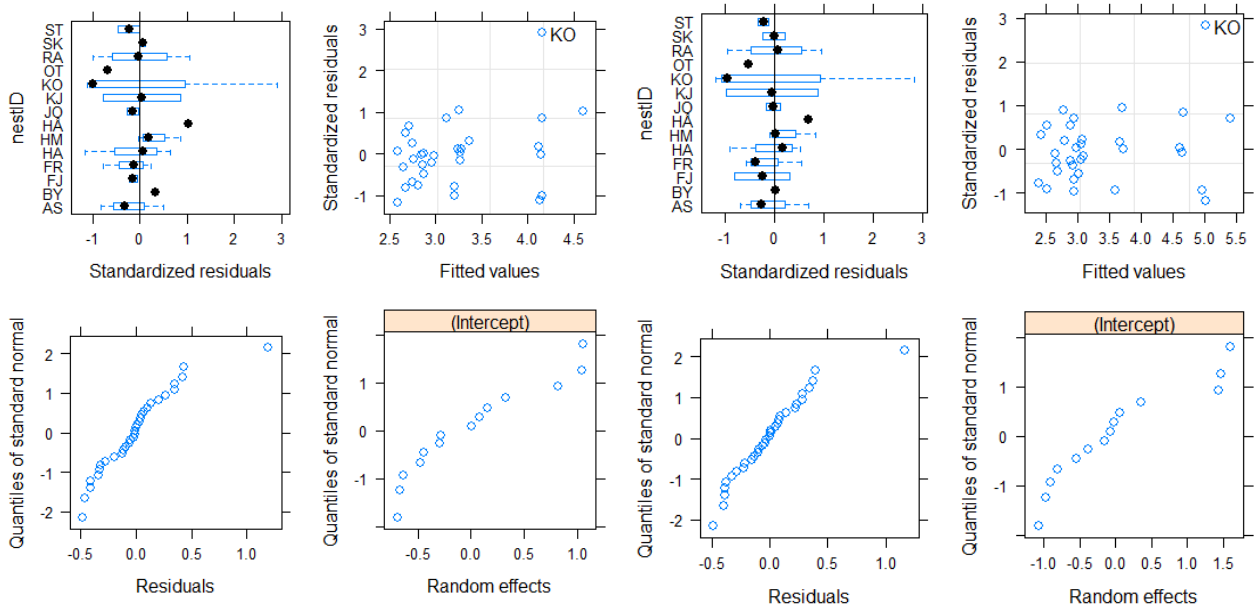

Figure A4.1: Analysis of outliers for (a) ∑PCBs and (b) *p,p'*-DDE. KO2 from the nest KO had standardized residuals exceeding 2.5, and was together with HÅ1 obscuring the other individuals. The method evaluates the variance within each nest and was therefore not able to indicate whether HÅ1 was be an outlier, as it was the only bird from that nest.

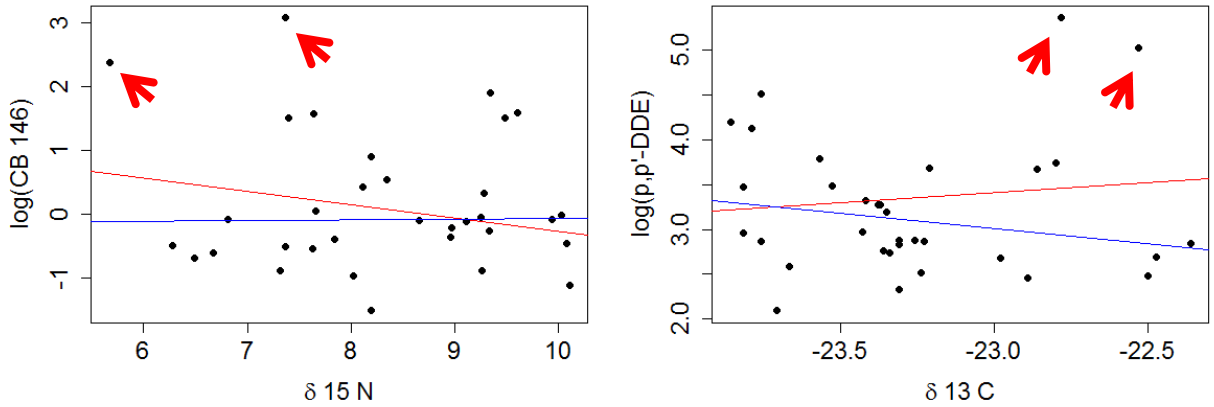

Figure A4.2: Two individuals (red arrows; KO2 and HÅ1) were considered outliers and were excluded from the analysis as they changed the realistic relationship with pollutants and SIs. Red line: Outliers included. Blue line: Outliers excluded.

7.5. Determination of matrix-specific differences in congener profiles

Table A5: Differences in OHC profiles between body feathers, blood plasma and preen oil from Goshawk nestlings.

	Anova		Tukey's HSD	
	F	**P**	**Matrix comparisons**	**P (adjusted)**
ΣPCBs [a]	5.65	< 0.01 **	Plasma - feather	< 0.01 **
			Preen - feather	0.847
			Preen - plasma	0.305
p,p'-DDE	2.58	0.083 T	Plasma - feather	0.137
			Preen - feather	0.866
			Preen - plasma	0.211
OxC	8.06	< 0.001 ***	Plasma - feather	< 0.01 **
			Preen - feather	0.841
			Preen - plasma	< 0.05 *
HCB	9.37	< 0.001 ***	Plasma - feather	< 0.001 ***
			Preen - feather	0.436
			Preen - plasma	0.342
ΣPBDEs [b]	5.53	0.00577 **	Plasma - feather	0.983
			Preen - feather	< 0.01 **
			Preen - plasma	< 0.01 **
CB 99	13.33	< 0.001 ***	Plasma - feather	< 0.001 ***
			Preen - feather	< 0.001 ***
			Preen - plasma	0.495
CB 101	23.84	< 0.001 ***	Preen - feather	< 0.001 ***
CB 118	5.74	< 0.01 **	Plasma - feather	< 0.01 **
			Preen - feather	0.077 T
			Preen - plasma	0.952
CB 128	33.42	< 0.001 ***	Plasma - feather	< 0.001 ***
			Preen - feather	< 0.001 ***
			Preen - plasma	< 0.001 ***
CB 138	38.99	< 0.001 ***	Plasma - feather	< 0.001 ***
			Preen - feather	< 0.001 ***
			Preen - plasma	0.086 T
CB 146	16.97	< 0.001 ***	Plasma - feather	< 0.001 ***
			Preen - feather	0.169
			Preen - plasma	< 0.001 ***
CB 153	1.67	0.194	Plasma - feather	0.199
			Preen - feather	1.000
			Preen - plasma	0.543
CB 156	37.57	< 0.001 ***	Plasma - feather	< 0.001 ***
			Preen - feather	< 0.05 *
			Preen - plasma	< 0.05 *
CB 170	4.08	0.0207	Plasma - feather	0.359
			Preen - feather	0.137

			Preen - plasma	< 0.05 *
CB 177	1.40	0.253	Plasma - feather	0.986
			Preen - feather	0.240
			Preen - plasma	0.275
CB 180	90.96	< 0.001 ***	Plasma - feather	< 0.001 ***
			Preen - feather	< 0.001 ***
			Preen - plasma	0.905
CB 183	0.91	0.409	Plasma - feather	0.930
			Preen - feather	0.376
			Preen - plasma	0.496
CB 187	20.41	< 0.001 ***	Plasma - feather	0.298
			Preen - feather	< 0.001 ***
			Preen - plasma	< 0.001 ***
CB 196203	20.27	< 0.001 ***	Plasma - feather	< 0.01 **
			Preen - feather	< 0.001 ***
			Preen - plasma	< 0.001 ***
CB 199	61.60	< 0.001 ***	Plasma - feather	< 0.001 ***
			Preen - feather	< 0.001 ***
			Preen - plasma	< 0.001 ***
BDE47	17.68	< 0.001 ***	Plasma - feather	< 0.001 ***
			Preen - feather	< 0.001 ***
			Preen - plasma	0.847
BDE 100	16.56	< 0.001 ***	Plasma - feather	< 0.001 ***
			Preen - feather	< 0.05 *
			Preen - plasma	0.688
BDE 99	24.95	< 0.001 ***	Plasma - feather	< 0.001 ***
			Preen - feather	< 0.05 *
			Preen - plasma	< 0.001 ***
BDE 153	8.74	< 0.01 **	Preen - feather	< 0.01 **

Significant at *P < 0.05, **P < 0.01, ***P < 0.001. T indicates close to significance: 0.10 > P ≤ 0.05. .
[a] ∑PCBs is the sum of the 14 PCB congeners detected in all three matrices, [b] ∑PBDEs is the sum of the 3 BDE congeners detected in all three matrices.

7.6. Candidate models to explain organohalogen compound exposure

Table A6: Candidate models including $\delta^{13}C$ and $\delta^{15}N$ to explain the variation in OHC exposure in body feathers (ln-transformed; pg mm^{-1}) (LMEM, $\Delta AIC_c < 2$). Observations were made for 30 Goshawk nestlings from 13 nests, so intra-nest pseudoreplication was corrected for. 2 outliers (KO2 and HÅ1) were excluded from the analysis.

	Model	Parameters	*DF*	AIC_c	ΔAIC_c
CB 146	A	$\delta^{13}C$	4	61.57	0.00
	B	$\delta^{15}N$	4	61.75	0.18
	C	$\delta^{13}C + \delta^{15}N$	5	63.41	1.85
CB 153	A	$\delta^{13}C$	4	57.16	0.00
	B	$\delta^{15}N$	4	57.20	0.04
	C	$\delta^{13}C + \delta^{15}N$	5	59.07	1.92
CB 187	A	$\delta^{13}C$	4	48.59	0.00
	B	$\delta^{15}N$	4	48.82	0.23
	C	$\delta^{13}C + \delta^{15}N$	5	50.51	1.92
∑PCBs[a]	A	$\delta^{13}C$	4	53.84	0.00
	B	$\delta^{15}N$	4	53.86	0.02
	C	$\delta^{13}C + \delta^{15}N$	5	55.63	1.79
OxC	B	$\delta^{15}N$	4	72.40	0.00
	A	$\delta^{13}C$	4	73.92	1.52
	C	$\delta^{13}C + \delta^{15}N$	5	74.28	1.89
HCB	A	$\delta^{13}C$	4	82.02	0.00
	B	$\delta^{15}N$	4	82.47	0.45
	C	$\delta^{13}C + \delta^{15}N$	5	83.57	1.55
p,p'-DDE	A	$\delta^{13}C$	4	43.31	0.00
	B	$\delta^{15}N$	4	43.67	0.36
	C	$\delta^{13}C + \delta^{15}N$	5	45.28	1.97
BDE 47	A	$\delta^{13}C$	4	59.13	0.00
	B	$\delta^{15}N$	4	59.45	0.32
	C	$\delta^{13}C + \delta^{15}N$	5	61.13	2.00
BDE 99	A	$\delta^{13}C$	4	56.29	0.00
	B	$\delta^{15}N$	4	56.29	0.01
	C	$\delta^{13}C + \delta^{15}N$	5	58.26	1.98
∑PBDEs[a]	A	$\delta^{13}C$	4	54.31	0.00
	B	$\delta^{15}N$	4	54.35	0.04
	C	$\delta^{13}C + \delta^{15}N$	5	56.28	1.97
∑OHCs[b]	A	$\delta^{13}C$	4	47.32	0.00
	B	$\delta^{15}N$	4	47.55	0.23
	C	$\delta^{13}C + \delta^{15}N$	5	49.30	1.97

[a]∑PCBs and ∑BDEs are the sum of all the PCB or PBDE congeners detected in feathers. [b]∑OHCs is the sum of ∑PCBs, ∑DDTs, ∑CHLs, HCB and ∑PBDEs detected in feathers.

7.7. Candidate models to explain corticosterone levels

Table A7: Candidate models to explain variation in CORT levels in body feathers (ln-transformed; pg mm^{-1}) ranked by the AIC_c value. Accumulations of a specific OHC, $\delta^{13}C$, $\delta^{15}N$ and/or age were included as predictors in the LMEM. Observations were made for 30 Goshawk nestlings from 13 nests, so intra-nest pseudoreplication was corrected for. 2 outliers (KO2 and HÅ1) were excluded from the analysis.

	Model	Parameters	*DF*	AIC_c	ΔAIC_c
CB 146	1	log(CB 146)	4	33.57	0.00
	5	age	4	34.07	0.50
	11	$\delta^{13}C$	4	34.11	0.54
	4	age + log(CB 146)	5	34.24	0.68
	14	$\delta^{15}N$	4	34.44	0.87
	10	$\delta^{13}C$ + log(CB 146)	5	35.09	1.52
CB 153	1	log(CB 153)	4	33.68	0.00
	5	age	4	34.07	0.39
	11	$\delta^{13}C$	4	34.11	0.44
	4	age + log(CB 153)	5	34.27	0.59
	14	$\delta^{15}N$	4	34.44	0.77
	10	$\delta^{13}C$ + log(CB 153)	5	35.18	1.51
	2	$\delta^{15}N$ + log(CB 153)	5	35.44	1.77
CB 187	1	log(CB 187)	4	33.63	0.00
	5	age	4	34.07	0.44
	11	$\delta^{13}C$	4	34.11	0.48
	4	age + log(CB 187)	5	34.27	0.64
	14	$\delta^{15}N$	4	34.44	0.81
	10	$\delta^{13}C$ + log(CB 187)	5	35.05	1.42
	2	$\delta^{15}N$ + log(CB 187)	5	35.40	1.77
∑PCBs[a]	1	log(∑PCBs)	4	33.56	0.00
	5	age	4	34.07	0.51
	11	$\delta^{13}C$	4	34.11	0.55
	4	age + log(∑PCBs)	5	34.23	0.67
	14	$\delta^{15}N$	4	34.44	0.88
	10	$\delta^{13}C$ + log(∑PCBs)	5	35.08	1.52
	2	$\delta^{15}N$ + log(∑PCBs)	5	35.38	1.81
OxC	5	age	4	34.07	0.00
	11	$\delta^{13}C$	4	34.11	0.04
	14	$\delta^{15}N$	4	34.44	0.37
	1	log(OxC)	4	34.52	0.45
	6	age + $\delta^{15}N$	5	36.00	1.93
	12	$\delta^{13}C$ + $\delta^{15}N$	5	36.03	1.96
	4	age + log(OxC)	5	36.07	2.00
HCB	1	log(HCB)	4	33.12	0.00
	5	age	4	34.07	0.95
	11	$\delta^{13}C$	4	34.11	0.99
	14	$\delta^{15}N$	4	34.44	1.32
	10	$\delta^{13}C$ + log(HCB)	5	34.86	1.73

	4	age + log(HCB)	5	34.96	1.83
	2	δ^{15}N + log(HCB)	5	35.03	1.91
***p,p'*-DDE**	5	age	4	34.07	0.00
	11	δ^{13}C	4	34.11	0.04
	1	log(*p,p'*-DDE)	4	34.25	0.19
	14	δ^{15}N	4	34.44	0.37
	4	age + log(*p,p'*-DDE)	5	35.48	1.41
	10	δ^{13}C + log(*p,p'*-DDE)	5	35.58	1.51
	6	age + δ^{15}N	5	36.00	1.93
	2	δ^{15}N + log(*p,p'*-DDE)	5	36.01	1.94
	12	δ^{13}C + δ^{15}N	5	36.03	1.96
BDE 47	5	age	4	34.07	0.00
	11	δ^{13}C	4	34.11	0.04
	14	δ^{15}N	4	34.44	0.37
	1	log(BDE 47)	4	34.50	0.43
	4	age + log(BDE 47)	5	35.69	1.63
	10	δ^{13}C + log(BDE 47)	5	35.95	1.88
	6	age + δ^{15}N	5	36.00	1.93
	12	δ^{13}C + δ^{15}N	5	36.03	1.96
BDE 99	5	age	4	34.07	0.00
	11	δ^{13}C	4	34.11	0.04
	14	δ^{15}N	4	34.44	0.37
	1	log(BDE 99)	4	34.55	0.48
	6	age + δ^{15}N	5	36.00	1.93
	4	age + log(BDE 99)	5	36.02	1.95
	12	δ^{13}C + δ^{15}N	5	36.03	1.96
∑PBDEs[a]	5	age	4	34.07	0.00
	11	δ^{13}C	4	34.11	0.04
	14	δ^{15}N	4	34.44	0.37
	1	log(∑*P*BDEs)	4	34.55	0.48
	4	age + log(∑*P*BDEs)	5	35.86	1.80
	6	age + δ^{15}N	5	36.00	1.93
	12	δ^{13}C + δ^{15}N	5	36.03	1.96
∑OHCs[b]	1	log(∑OHCs)	4	33.98	0.00
	5	age	4	34.07	0.08
	11	δ^{13}C	4	34.11	0.13
	14	δ^{15}N	4	34.44	0.46
	4	age + log(∑OHCs)	5	34.93	0.94
	10	δ^{13}C + log(∑OHCs)	5	35.39	1.41
	2	δ^{15}N + log(∑OHCs)	5	35.76	1.77

[a] ∑ PCBs and ∑BDEs are the sum of all the PCB or PBDE congeners detected in feathers. [b] ∑OHCs is the sum of ∑PCBs, ∑DDTs, ∑CHLs, HCB and ∑PBDEs detected in feathers.