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Levels of emerging and traditional organic Pollutants in Northern Goshawk (*Accipiter gentilis*) from Trøndelag. Potential Effects on oxidative Stress.

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SAMMENDRAG

Gjennom Stockholm-konvensjonen har det blitt pålagt forbud mot flere organiske forbindelser grunnet deres persistente og toksiske egenskaper. De forbudte tradisjonelle bromerte flammehemmerne har blitt erstattet med nye bromerte og fosfororganiske flammehemmere. Per dags dato er det liten kunnskap om forekomsten og effektene av disse forbindelsene i miljøet, og det er et økende behov for studier som undersøker dette. I dette studiet ble det derfor tatt prøver av 20 hønsehauk-unger (*Accipiter gentilis*) fra ulike reir i Nord- og Sør-Trøndelag. Målet var å undersøke forekomsten av tradisjonelle persistente forbindelser, samt nye bromerte og fosfororganiske flammehemmere, i fjær, blod og gumpolje. Det var forventet at konsentrasjoner i de tre ulike prøve-typene ville korrelere og dermed bekrefte at fjær er et nyttig verktøy for å måle organisk forurensning i fuglene. For å undersøke effekten av forbindelsene på oksidativt stress ble aktiviteten av de enzymatiske antioksidantene katalase, glutation peroxidase og glutation reduktase målt i røde blodceller. Det ble antatt at økende mengder organiske forbindelser i blodprøvene ville resultere i en observerbar endring i enzymaktivitet. Resultatene viste at fosfororganiske flammehemmere kun ble funnet i fjær, og at de muligens stammer fra atmosfærisk avsetning. De nye bromerte flammehemmerne ble generelt ikke detektert, noe som indikerte at hauken er lite eksponert for disse forbindelsene. En rekke tradisjonelle persistente forbindelser ble derimot detektert i ungene, hvor polyklorerte bifenyler (PCB) [plasma: 7.23 ± 1.19 ng mL^{-1}, fjær: 9.90 ± 1.21 ng g^{-1}, gumpolje: 395 ± 62.2 ng g^{-1} lipider] og diklordifenyldikloretylen (*p,p´*-DDE) [plasma: 5.02 ± 0.82 ng mL^{-1}, fjær: 31.8 ± 3.54 ng g^{-1}, gumpolje: 1021 ± 165 ng g^{-1} lipider] utgjorde til sammen 90 % av den totale mengden tradisjonelle forbindelser i alle prøve-typer, fulgt av polybrominerte difenyletere (PBDE) [plasma: 0.27 ± 0.04 ng mL^{-1} , fjær: 1.68 ± 0.33 ng g^{-1}, gumpolje: 38.9 ± 5.44 ng g^{-1} lipider] som utgjorde 2.4 – 4.0 %. ΣPCB, ΣPBDE og *p,p´*-DDE var alle signifikant og positivt korrelert mellom fjær, blod og gumpolje ($0.55 < r_s < 0.93$). Det ble funnet at oksyklordan og PCB kongeneren CB 153 hadde en signifikant effekt på katalase med økende enzymaktivitet ved økende konsentrasjoner. Det ble også funnet at konsentrasjonen av de samme to forbindelsene minket med ungenes økende alder, noe som indikerte at forbindelsene ble overført fra mor via egget. Kort oppsummert viste dette studiet at hønsehauk-unger fra Trøndelag er eksponert for en rekke tradisjonelle persistente forbindelser, og at enkelte av disse kan indusere oksidativt stress. Dette indikerte at de tradisjonelle forbindelsene foreløpig utgjør en større trussel for hønsehauken enn de alternative stoffene.

ABSTRACT

Due to the implemented restrictions and bans of many persistent organic pollutants (POPs), the production and use of alternative chemicals are increasing. The lack of knowledge regarding the environmental occurrence, persistence and potential toxic effects of these unrestricted substitutes causes concern. In the present study, levels of legacy POPs and emerging organic contaminants, being novel brominated flame retardants (nBFRs) and organophosphate flame retardants (OPFRs), were investigated in a terrestrial, avian top predator in Norway. Nestlings of the northern goshawk (*Accipiter gentilis*) from 20 nests in Nord- and Sør-Trøndelag were sampled for body feathers, blood and preen oil. Contaminant levels were measured in all three non-destructive sampling matrices. It was hypothesized that contaminant levels in feathers and plasma would be positively correlated, confirming the usefulness of feathers in biomonitoring of organic pollutants. Further, to investigate the potential effect of the organic contaminants, the activity of three enzymatic antioxidants was measured in red blood cells to assess oxidative stress. It was hypothesized that increasing contaminant levels in plasma would be associated with alterations in enzyme activity. The results showed that nBFRs were generally not detected, most likely due to their low production and usage volumes. OPFRs were only detected in feathers, indicating atmospheric deposition as the source of these contaminants. Most of the targeted legacy POPs were detected in the nestlings, where ΣPCBs [plasma: 7.23 ± 1.19 ng mL^{-1}, feather: 9.90 ± 1.21 ng g^{-1}, preen oil: 395 ± 62.2 ng g^{-1} lipids] and *p,p´*-DDE [plasma: 5.02 ± 0.82 ng mL^{-1} , feather: 31.8 ± 3.54 ng g^{-1}, preen oil: 1021 ± 165 ng g^{-1} lipids] were the most dominant POPs (90 % of the POP load in all matrices) followed by ΣPBDEs [plasma: 0.27 ± 0.04 ng mL^{-1} , feather: 1.68 ± 0.33 ng g^{-1}, preen oil: 38.9 ± 5.44 ng g^{-1} lipids], contributing 2.4 – 4.0 % of total POP load. The ΣPCBs, *p,p´*-DDE and ΣPBDEs were positively correlated ($0.55 < r_s < 0.93$) between all matrices, reflecting the connection of growing feathers with the bloodstream, as predicted. Plasma levels of CB 153 and OxC decreased with increasing nestling age, indicating growth dilution and the maternal, rather than dietary, origin of these POPs. Catalase activity increased with increasing plasma concentrations of CB 153 and OxC, suggesting that these contaminants induced oxidative stress. Overall, the present study showed that northern goshawk nestlings from Trøndelag are exposed to a wide range of legacy POPs, which currently seem to be a more prominent threat than emerging contaminants, especially since CB 153 and OxC might induce oxidative stress in the raptors.

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1. INTRODUCTION

1. 1. Persistent organic pollutants

Persistent organic pollutants (POPs) are defined as halogen containing organic chemicals, possessing specific characteristics that make them of special concern, such as their persistence in the environment, bioaccumulative and toxic properties. POPs are anthropogenic chemicals that have been produced for decades, such as organochlorine (OC) pesticides and halogenated flame retardants, many POPs are also produced non-intentionally in the form of industrial by-products and chemical metabolites (Jones and de Voogt, 1999). POPs are persistent in the environment, where they become widely distributed via natural processes such as transport via the atmosphere and oceanic currents. Through long range transport (LRT) consisting of repeated volatilization and deposition, volatile POPs end up in environments far from sources of production and use, such as the Arctic (AMAP, 1998). POPs also accumulate in the fatty tissues of organisms over time, due to their lipophilic properties. This bioaccumulation is mainly due to uptake from the diet (Thomann, 1989). The concentration of POPs can be further magnified upwards in the food chains, resulting in especially high concentrations in top predators, such as birds of prey (Voorspoels et al., 2007, Yu et al., 2013, Jaspers et al., 2005). This process of trophic magnification is called biomagnification, and results from the chemical absorption from the diet being higher than the elimination rate in the organism (Borga et al., 2012). POPs have been found to cause a wide range of toxic effects in living organisms, ranging from negative effects on reproduction and the immune system, as well as potentially being neurotoxic and carcinogenic (UNEP, 2008c). Some POPs also exert negative effects by the mechanism of endocrine disruption, where contaminants mimic natural hormones by structural similarities, thereby interfering with the endogenous hormone regulation. This disturbance can potentially affect hormone-regulated processes such as growth and development, causing irreversible harm to the organism (Jones and de Voogt, 1999, Kelce et al., 1995)

1. 2. The Stockholm convention

As it became clear that POPs pose a significant global threat to both wildlife and human health, the need for taking measures to prevent and reduce their occurrence in the environment has become evident. International cooperation has therefore been initiated to

define and implement such measures. The Stockholm convention is a global treaty, which was adopted on the 22nd of May 2001, and on the 17th of May 2004 the convention officially entered into force (UNEP, 2008a). The overall aim of the treaty is to protect human and environmental health through evaluation, restrictions and phase out of specific POPs. Through the convention, 12 initial POPs were marked for phase out, including polychlorinated biphenyls (PCBs), the OC pesticide dichlorodiphenyltrichloroethane (DDT) and its metabolite dichlorodiphenyldichloroethylene (DDE) (UNEP, 2008b). Exposure to DDT has been shown to cause eggshell thinning in raptors (Ratcliff, 1967), by DDE disturbing the calcium transport to the eggshell, and DDT exposure was found to be the cause of great population declines of the peregrine falcon (*Falco peregrinus*) in Europe (Wegner et al., 2005). Both PCBs and DDT have still been detected in relatively high levels in five species of terrestrial birds of prey from Belgium after the implementation of the Stockholm Convention, confirming their continuous presence in predator birds despite the chemical restrictions (Jaspers et al., 2006a). DDT and DDE have been detected in Norwegian birds of prey as well, where DDE levels were correlated with eggshell thinning (Nygaard and Gjershaug, 2001). Plasma levels of PCBs in northern goshawk (*Accipiter gentilis*) have been found to be lower than levels in marine birds of prey, but PCBs are still found in potentially harmful concentrations (Sonne et al., 2010). This indicates that the exposure to OCs is of concern also for the terrestrial raptors in Norway (Sonne et al., 2010). However, levels of DDT, DDE and PCBs seem to be decreasing recently in Norwegian raptors (Gjershaug et al., 2008).

1. 3. Flame retardants

Flame retardants are chemical compounds that are applied in a wide range of products to improve fire safety by preventing or delaying fire; such products include textiles, building and insulation materials, electronic wires and plastics. Flame retardants are intensively used in general industry and household products, and the total production of such compounds has increased in the last decades (de Wit, 2002). In 2005, the total global consumption of flame retardants was estimated to be 1 481 000 metric tons. Brominated and organophosphate flame retardants constituted respectively 311 000 and 205 000 metric tons of this consumption (Harju M. et al., 2009). Flame retardants are either categorized as additive, where they are implemented but not chemically bound to the product, or as reactive flame retardants, which are chemically bound to the polymer in the product. It has been discovered that certain types of flame retardants possess properties that place them in the category of POPs, such as

environmental persistence, bioaccumulation and toxicity (AMAP, 2009). Therefore, an increasing concern regarding their use and disposal into the global environment has arisen.

1. 3. 1. Brominated flame retardants

Brominated flame retardants (BFRs) are a group of synthesized halogenated flame retardants that exert a preventing effect on fire spreading by containing bromine that captures free radicals produced during combustion processes. This trapping of free radicals makes the oxidizing agents unavailable to react in the combustion process and prevents the fire from spreading (Alaee et al., 2003). Specific commercial BFR mixtures have been detected in the environment since the 1970s (Law et al., 2006). The chemical properties of these compounds are adding to the concern as they are in general chemically stable and lipid soluble, they are able to bioaccumulate and are found in environmental compartments ranging from water and sediments to air, soil and biota (de Wit, 2002). There is also additional concern regarding the metabolites formed in degradation and debromination of BFRs, as such metabolites are potentially bioavailable and toxic to living organisms (Letcher et al., 2009). Environmental release of BFRs occurs throughout the compound's lifetime, from production and use to disposal and degradation, and they also undergo LRT (de Wit et al., 2010).

1. 3. 2. Polybrominated diphenyl ethers

Polybrominated diphenyl ethers (PBDEs) constitute a group of additive legacy BFRs. The two commercial mixtures of Octa-BDE and Penta-BDE, which were previously produced and employed in large volumes across the world, were designated for elimination through addition into Annex A in the Stockholm convention in 2004 (Directive, 2003/11/EC). These chemicals and their degradation products have been detected in air, sewage sludge, soil and sediments across Europe despite the decision to eliminate the chemicals. The reason why they are still detected in environmental compartments is thought to be slow breakdown and chemical persistence, resulting in the build-up of PBDE depots in sediments and soil, as well as continuous release from existing products with PBDE content (Law et al., 2006). PBDEs have been detected in marine fish (Bustnes et al., 2012), arctic seal and polar bear (*Ursus maritimus*) (Dietz et al., 2013), birds of prey (Chen et al., 2007, Jaspers et al., 2006a) and raptor eggs (Lindberg et al., 2004, Herzke et al., 2009, Jaspers et al., 2005). It has also been shown that terrestrial birds of prey might be more exposed to higher brominated congeners of PBDEs than marine birds of prey (Jaspers et al., 2006a).

1. 4. A new challenge

Through the work of the Stockholm convention, several organic contaminants have been added to the list of POPs in recent years, among them the technical Penta- and Octa-BDE mixtures, as well as hexabromocyclododecane (HBCD), which all are brominated flame retardants (UNEP, 2008b). In order to replace the banned POPs, new chemicals have been developed, and previously used compounds are also being re-introduced. This development has resulted in a new problem; many of these emerging chemicals have similar structures and appliances as the banned POPs, but their occurrence and faith in the environment is still relatively unknown (Covaci et al., 2011, Abbasi et al., 2016). Results of recent studies on European barn owl (*Tyto alba)* from Belgium and France suggest that levels of PBDEs are declining, but also that the availability and uptake of other chemicals substituting the banned PBDEs, are increasing (Eulaers et al., 2014b). Examples of such substitutes are novel brominated flame retardants (nBFRs) and organophosphate flame retardants (OPFRs) (Vorkamp and Riget, 2014, van der Veen and de Boer, 2012). Covaci et al. (2011) defined the term novel BFRs as compounds "relating to BFRs which are new to the market or newly/recently discovered in the environment". Both nBFRs and OPFRs have been detected in feathers and blood of Norwegian (*Haliaeetus albicilla*) eagle nestlings, proving their presence in birds of prey (Eulaers et al., 2014a).

1. 4. 1. Novel brominated flame retardants

In a report by the Norwegian Environment Agency (2009), a rough estimate of the total global production of 21 investigated nBFRs was made, predicting a total production of 100 000 metric tons nBFRs per year. As the sources and structures of nBFRs are generally quite similar to the legacy BFRs, their chemical behavior and properties are suspected to be of resemblance (Harju M. et al., 2009). 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB), bis(2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate (TBPH) and 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE) are some of the most prominent emerging BFRs, where TBB and TBPH both are components of the flame retardant mixture Firemaster 550, which has been used as replacement of the Penta-BDE mixture (Covaci et al., 2011). Both chemicals have been listed as high production chemicals by the United States Environmental Protection Agency (US EPA) (Saunders et al., 2015).

At present time, there is still a lack of information regarding the occurrence and effects of nBFRs. Both TBB and TBPH have been detected in Arctic biota, indicating that they undergo

LRT. TBB has also been found to biomagnify, raising concern for the potential TBB-levels that may accumulate in predators high up in the food chain, such as common eider (*Somateria mollissima)*, kittiwakes (*Rissa tridactyla*), Arctic fox (*Vulpes lagopus*) and polar bear (Sagerup et al., 2010). BTBPE was shown to bioaccumulate in glaucous gulls (*Larus hyperboreus*) and also to be maternally transferred to its eggs. The compound was generally detected in low levels, but considering that BFRs are found in mixtures in the environment the possible summed effects could be of concern (Verreault et al., 2007). Both TBB and TBPH have been shown to exert estrogenic effects, indicating their potential as endocrine disrupters (Saunders et al., 2015).

1. 4. 2. Organophosphate flame retardants

Organophosphate flame retardants (OPFRs) have been in use for decades, but are now increasingly used as substitutes for the banned BFRs, OPFRs are also referred to as re-emerging contaminants (Reemtsma et al., 2008). The occurrence of OPFRs is in general more investigated in abiotic compartments than in biota. They have been detected in sediments (up to 24 mg kg^{-1}), air (up to 47 µg m^{-3}) and surface water (up to 379 ng L^{-1}) (van der Veen and de Boer, 2012). A general trend of OPFR concentrations being higher than the BFR levels in the same matrices has been shown, for example in atmospheric samples from the Great Lakes in the United States (US) (Salamova et al., 2014). The compounds have been produced simultaneously as legacy BFRs, and OPFRs have been detected in the environment since the 1970s (Sheldon and Hites, 1978). The future global OPFR production and consumption is expected to increase due to the need for substitutes of the now banned BFRs; such an increase has already been observed in Western Europe (Reemtsma et al., 2008). OPFRs constituted approximately 20 % of the total consumption of FRs in Europe in 2006, whereas BFRs constituted 10 % of the total consumption, and the main part of the remaining consumption (54 %) was by chlorinated paraffins (van der Veen and de Boer, 2012).

Early investigations of the compounds' environmental persistence indicated that OPFRs most likely were readily degraded (Muir and Grift, 1981). Later research has on the other hand shown that certain OPFRs are environmentally persistent, one example being tris-2-chloropropyl phosphate (TCPP) (Kawagoshi et al., 2002). It has also been shown that OPFRs undergo LRT, and the contaminants have been detected in arctic atmosphere and organisms, where they to some degree might actually bioaccumulate (Moller et al., 2011, Evenset et al., 2009, Sheldon and Hites, 1978, Hallanger et al., 2015). Certain OPFRs have been shown to

biomagnify to some extent in benthic food webs, one example being tris(2-chloroethyl) phosphate (TCEP) (Brandsma et al., 2015). TCEP has also been shown to be environmentally persistent and carcinogenic, which in fact is a potential toxic effect of all the chlorinated OPFRs (Reemtsma et al., 2008). Other potential toxic effects of OPFRs are neurotoxicity and reproductive toxicity (van der Veen and de Boer, 2012). Tris(1-chloro-2-propyl) phosphate (TCIPP), tris(2-butoxyethyl) phosphate (TBEP) and TCEP have all been detected in eggs of herring gull (*Larus argentatus*), indicating a bioaccumulation potential (Chen et al., 2012). Bioaccumulation of OPFRs does not seem to be connected to lipid content, as levels of contaminants have been found to poorly correlate with lipid content in samples. In this respect, OPFRs differ from the BFRs (Malarvannan et al., 2015). TCIPP and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) have been shown to affect the thyroid hormone homeostasis, as well as the expression of xenobiotic metabolizing enzymes after *in ovo* exposure in chickens (Farhat et al., 2013). TDCIPP also induced enzymes involved in regulation of oxidative stress, and affected the expression of genes involved in the immune system in exposed chicken (*Gallus gallus domesticus*) embryos (Farhat et al., 2014). OPFRs have been detected in both plasma and feathers of Norwegian white-tailed eagle (*Haliaeetus albicilla*) nestlings, showing that these contaminants are present in Norwegian birds of prey (Eulaers et al., 2014a). Clearly more knowledge about the environmental occurrence, as well as uptake and effects of OPFRs in wildlife, is of urgent need.

1. 5. Avian biomonitoring

Birds have been used successfully for a long time in environmental monitoring of pollution. They inhabit both the terrestrial and marine environment, and are situated in several different levels of the food chain. Exploiting the fact that some birds are migratory, whilst others are resident species, also enables us to study geographical and temporal differences in pollution (Gomez-Ramirez et al., 2014). Bird ecology and physiology is generally well studied, and many species are commonly found across the continents, offering the opportunity to compare pollution between different geographical regions (Eulaers et al., 2014b). Considering all of these aspects it is clear that birds are suitable organisms to use in biomonitoring of environmental pollution. Predatory birds are likely to accumulate higher levels of organic contaminants than organisms with a lower trophic position, and have therefore been used in biomonitoring of organic pollutants in Norway for several decades (Gjershaug et al., 2008).

1. 5. 1. Non-invasive sampling

In biomonitoring, non-invasive sampling methods have become tools of great importance for both ethical and practical reasons. Using blood, eggs or keratinous tissues such as hair or feathers enables relatively quick and easy sampling, where no living organisms are sacrificed. In avian biomonitoring of POPs, both blood (Glauert et al., 2008, Kocagoz et al., 2014, Sonne et al., 2012) and eggs (Van den Steen et al., 2009, Herzke et al., 2005) have been shown to be useful tools, as well as preen oil (Yamashita et al., 2007). Preen oil has been evaluated as a relatively non-invasive and useful tool in biomonitoring, especially due to the high lipid content which enables good detections in small volumes (Eulaers et al., 2011b). Preen oil reflects absorbed contaminants in the body (Espín et al., 2016). Preen oil has been shown to reflect POP levels in internal tissues in other predatory birds well, such as muscle and liver in white-tailed eagles (Jaspers et al., 2013). Other studies have however showed that this is not always the case, as POP levels detected in preen oil from seabirds were not found to correlate with muscle and liver levels (Kocagoz et al., 2014).

Feathers have long been used for detecting and monitoring metals in birds (Goede and Debruin, 1984, Janssens et al., 2001, Bustnes et al., 2013a). However, feathers can also be used to monitor organic pollutants (Jaspers et al. 2006b). During growth, the feathers are coupled to the bloodstream and contaminants in the blood are deposited in the feather structure. It has been shown that the POP concentrations in the feathers correspond to the concentrations in the blood during feather growth, reflecting the exposure over time and not just recent exposure, as is the case with a blood sample (Dauwe et al., 2005). Strong and significant correlations have been found for PCB concentrations between newly grown feathers and internal tissues such as liver, brain, muscle and blood (Van den Steen et al., 2007). External deposition of organic contaminants onto feathers is of less confounding concern than for metals, further supporting the benefit of using feathers as indicators of organic contamination in avian species (Jaspers et al., 2007a). The advantages of using feathers in analysis of pollutant exposure are numerous. First of all, easy and effective sampling of feathers cause less stress to the birds than other, more invasive sampling methods. There is no need to sacrifice the birds for sampling, and the ethical conflicts regarding studies on free-living species can thereby be of less prominence when using feathers as biomarkers (Eulaers et al., 2011b). Previously, eggs have been used to assess both organic and inorganic contamination in birds (Nygaard and Gjershaug, 2001, Herzke et al., 2005). Feathers might offer a broader and better range of use as both male and females from

the whole population can be sampled, including individuals that are not sexually mature. Also, feather sampling is not restricted to a limited period of time and less harm will be caused to the population, compared to using eggs for analysis (Dauwe et al., 2005). Feathers are also considered as useful tools for studying geographical differences in contamination (Jaspers et al., 2009). Other advantages are easy storage and the existing collections of feathers, which can be used to investigate time-trends in contamination (Dietz et al., 2006).

The type of feathers used for analysis is of importance, as it has been found that the concentrations of contaminants differ between feather types. Body feathers, when compared to tail and primary wing feathers, are the most convenient to use because they contain relatively high concentrations of pollutants. An additional advantage is that body feathers are considered relatively easy and less harmful to sample, compared to tail and wing feathers (Jaspers et al., 2011). Raptor feathers are well suited for measuring organic contaminants, reflecting the internal concentrations of pollutants (Jaspers et al., 2007b, Jaspers et al., 2006b). Sampling larger and fewer feathers from raptors will also be less harmful than sampling from smaller birds like passerines, where a larger amount of feather material is needed for detection (Dauwe et al., 2005).

1. 5. 2. Using nestlings in biomonitoring

In nestlings, feathers are still growing and the connection between the blood circulation and the feathers has not yet been broken. Nestling feathers are well suited as tools in biomonitoring of organic contaminants, as stronger correlations between feather and blood levels of POPs have been found in nestlings compared to adult birds (Eulaers et al., 2011a). The general levels of POPs have been found to be lower in nestlings than in adult birds, but this was expected as nestlings have been exposed for a shorter period of time. Nestling feathers have been used in studies of both legacy and emerging organic contaminants in birds of prey (Eulaers et al., 2014a). Other advantages of using nestlings are that they have not yet been subjected to as many ecological and physiological confounding factors as adult birds, such as reproduction, migration and molting. Additionally, nestlings stay put in the nest and are easier to capture than adult birds (Eulaers et al., 2011a). There are of course challenges to using nestlings, such as timing of sampling. Age differences will not just affect the contaminant levels due to differences in exposure time, but as nestlings approach the age of fledging it is thought that also physiological changes affect the contaminant levels in the blood (Eulaers et al., 2011b).

1. 6. The northern goshawk

The northern goshawk (*Accipiter gentilis*) is a raptor, living in the terrestrial environment in the northern hemisphere. The goshawk is sedentary in Norway, and is mostly found in old coniferous forests, nesting in mainly spruce and pine trees. It can however also nest in deciduous forests, especially in northern areas of the country (Grønlien, 2004, Jacobsson and Sandvik, 2014). The goshawks diet mainly consists of medium-sized birds, such as pigeons and thrush. The male and female goshawks differ in size, with females being larger than males. This sexual dimorphism is useful for determining the sex of an individual, where the tarsus width is a much-used measurement (Kenward, 2010). The goshawk lies on average 2-4 eggs (Jacobsson and Sandvik, 2014). Incubation time is normally around 38 days, and the nestlings will leave the nest when they are approximately six weeks old (Kenward, 2010). It has been estimated that there are between 1375 and 1880 pairs of northern goshawks in Norway (Grønlien, 2004). Due to several factors, the Norwegian goshawk population has been declining in the last century; recent numbers describing the national status of goshawk stocks indicate that the situation is not improving and that the population is still declining (Shimmings and Øien, 2015). Because of this, the species is protected in Norway and is currently listed as 'near threatened' (Henriksen and Hilmo, 2015). Recent estimates set the NG populations in the two counties Nord- and Sør-Trøndelag at respectively 50-100 and 90-130 pairs (Shimmings and Øien, 2015). The population of northern goshawk in Nord-Trøndelag has been monitored since 1994, and the mean annual reproduction rate for NG in the period 1994-2004 was estimated to be 1.43. This might not be sufficient to maintain the population (Nygård, 2005).

The factors causing the decline in the northern goshawk population range from hunting and persecution to pesticides, lack of food resources and habitat destruction (Shimmings and Øien, 2015). Forestry is thought to have a severe negative effect on available habitat for the Norwegian goshawk, by fragmenting and removing old coniferous forest, which is the raptors preferred habitat (Jacobsson and Sandvik, 2014). Pollution is also considered a threat to the goshawk, and several POPs have been detected in their eggs, feathers and plasma (Herzke et al., 2005, Sonne et al., 2012, Eulaers et al., 2013). This clearly highlights the potential and importance of this species in the monitoring of POPs in the terrestrial environment. Further

investigation of the occurrence and effects of organic pollutants in the northern goshawk has been recommended, as well as the use of nestlings (Eulaers et al., 2011a).

1. 7. Oxidative stress

Reactive oxygen species (ROS) are defined as reactive chemical species that are produced both from normal, endogenous cellular reactions, as well as being triggered by exogenous sources such as xenobiotics (Finkel and Holbrook, 2000). ROS are radical or non-radical oxygen species, such as the hydroxyl radical (•OH) and hydrogen peroxide (H_2O_2), which can interact with and damage molecules such as nucleic acids, lipids and proteins, ultimately leading to cell damage, apoptosis and diseases (Ray et al., 2012). Enzymatic and non-enzymatic antioxidant defense systems exist with the purpose of breaking down ROS and preventing oxidative damage. If this system is not able to balance out ROS, oxidative stress can occur in the biological system (Ray et al., 2012). Catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) are enzymatic antioxidants, whilst glutathione (GSH) is an example of a non-enzymatic antioxidant (Finkel and Holbrook, 2000).

GPx catalyzes the reduction of hydroperoxides such as tert-butyl hydroperoxide and H_2O_2 to water, by the oxidation of reduced glutathione (Equation 1). This reaction is coupled with the reaction catalyzed by GR, where the oxidized glutathione (GSSG) is reduced back to GSH, which again is available for further decomposition of hydroperoxides (Equation 2). The latter reaction depends on the simultaneous oxidation of NADPH to $NADP^+$ (Mannervik, 1987). This oxidation of NADPH causes a decrease in absorbance at 340 nm, which is measurable by spectrophotometry, and directly proportional to the GR-activity in the sample. This also enables the indirect measure of GPx activity, by measuring the decrease in absorbance at 340 nm as NAPDH is oxidized to $NADP^+$ by GR (Weydert and Cullen, 2010).

$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG \qquad \text{(Equation 1)}$$

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+ \qquad \text{(Equation 2)}$$

Catalase is another enzymatic antioxidant, which catalyzes the decomposition of H_2O_2 into water and oxygen (Equation 3). A hydrogen donor is needed for this reaction to take place. By using methanol for this hydrogen donation, the binding of the resulting formaldehyde to a chromogen, such as 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald), enables the spectrophotometrically measurement of catalase activity (Johansson and Borg, 1988).

$2H_2O_2 \rightarrow 2H_2O + O_2$ (Equation 3)

Environmental toxins such as organic contaminants are exogenous sources that potentially affect the level of ROS in living organisms, and induce oxidative stress upon exposure (Finkel and Holbrook, 2000, Bruchajzer et al., 2014). Exposure to organic pollutants has been shown to increase the levels of oxidative stress in birds, and affecting the levels and activity of the enzymatic defense against oxidative stress (Costantini et al., 2014, Hegseth et al., 2011a, Fernie et al., 2005). In nestlings of Norwegian white-tailed eagle, organochlorine levels correlated with depression of enzyme activity even at low concentrations (Sletten et al., 2016). Exposure to PCBs have been shown to induce depression of CAT-activity in herring gull chicks, and might also negatively affect the activity of GPx (Hegseth et al., 2011a).

1. 8. Aims of the present study

In my master project, I did a field study investigating the occurrence of organic pollutants and emerging contaminants in birds of prey. More specifically, I aimed to investigate the occurrence of legacy POPs as well as emerging contaminants, being nBFRs and OPFRs, in plasma, preen oil and feathers of nestlings of the terrestrial raptor species northern goshawk (*Accipiter gentilis*). Nest sites were geographically situated in the two Norwegian counties Nord- and Sør-Trøndelag (Figure 1). I also aimed to investigate the potential effect of the contaminants by analyzing the nestling blood for effect parameters indicating oxidative stress. The hypothesis is that levels of POPs in the blood-, preen oil and feather samples from the same individual will be correlated, thus reflecting the present connection between blood and feathers, confirming the suitability of nestling feathers as tools in biomonitoring. It is also hypothesized that elevated levels of POPs in the NG nestlings will cause oxidative stress as a biological response, indicated by alterations in the blood levels of oxidative stress parameters.

2. METHODS

2. 1. Fieldwork and sampling

Fieldwork was carried out between 16.06.2015 and 03.07.2015 in Northern- and Southern-Trøndelag, where blood, preen oil and body feathers were sampled from nestlings of the northern goshawk (*Accipiter gentilis*) from a total of 20 nests. Sampling locations were chosen based on observed activity in the nests earlier in the breeding season (see Figure 1).

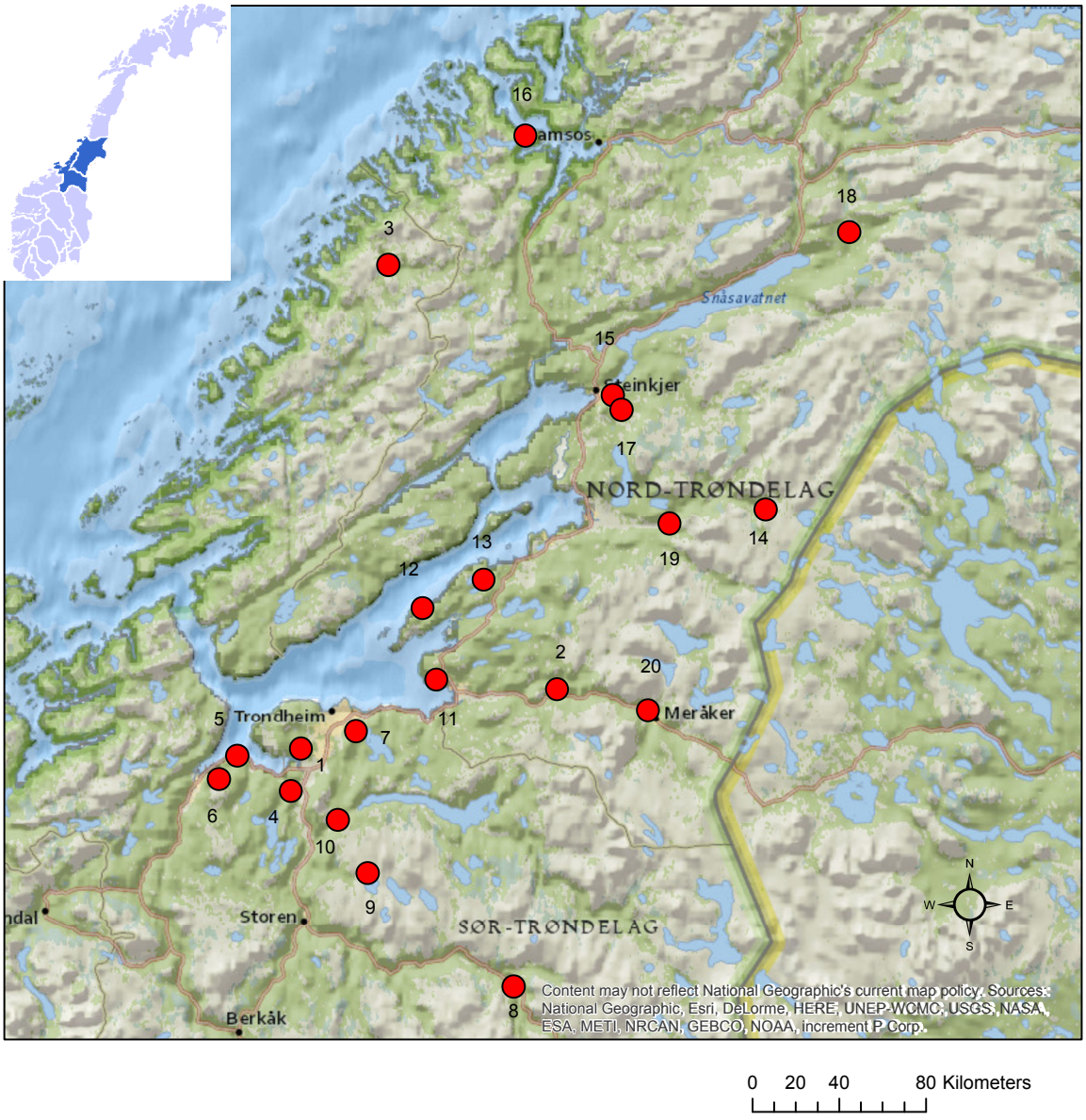

Figure 1. Map showing the sampling locations (n=20) in Nord- and Sør-Trøndelag.

2. 1. 1. Sampling

All sampling was conducted during daytime. Nestlings were taken from the nest and lowered to the ground in a plastic container kept in a nylon bag. All measurements and sampling took place on the ground (see Table 1 for overview of sample types and measurements). Preen oil and blood samples were taken only from the largest chick in the nest. Blood was sampled from the brachial vein, using a heparinized syringe, and was then transferred to sterile 1.5 mL cryogenic tubes and stored in cooler bags during transport. Blood samples were typically 3 - 4 mL per bird. Feathers were pulled from the nestling's back and stored per individual in a sealed plastic bag. Preen oil samples were obtained by gently pressing the preen gland with a glass rod that was pre-cleaned with acetone. Oil-covered feathers surrounding the preen gland were cut of using stainless steel scissors. All preen oil samples were stored in sterile 1.5 mL Eppendorf® tubes at -20 °C the same day as sampling.

Blood samples were processed at the lab and were stored, whenever possible, within 6 hours after sampling. Whole blood samples were centrifuged for 10 min at 1000g separating the plasma from the red blood cells (RBCs). The plasma was then transferred to new, marked cryotubes and stored at -20 °C. The RBCs were washed by adding approximately 0.5 mL sodium chloride isotonic solution (NaCl), followed by centrifugation at 1000g for 5 min. The NaCl solution was iso-osmotic to the blood, and was used for washing to remove white blood cells and plasma remnants, avoiding cell-damage due to differences in osmotic pressure. The supernatant was discarded. This washing procedure was repeated twice before RBC samples were stored at -80 °C for analysis of oxidative stress parameters.

2. 1. 2. Biometric measurements

Bill length and height, hallux length and tarsus width and depth were measured in millimeters (mm) using an electronic slide caliper. Wing length and tail feather length were measured in centimeters (cm) using a metal ruler. In the field, sex was estimated by the tarsus width, where a bird with a tarsus broader than 6.5 mm was determined as a female (Kenward, 2010). Weight was measured in grams (g) using a Pesola® scale. Crop content ranged from 1 (no content) to 3 (full) and roughly estimated in grams if present (Table 1).

2. 1. 3. Behavioral parameters

Behavior of the nestlings was addressed by assigning an activity score and a score of alarming intensity to each bird (Table 1). The scores ranged from 1 (low activity/intensity) to 5 (high

activity/intensity). A stress test was also performed, where a breath count was performed right before and after blood sampling. Breath count corresponded to the number of breaths within 60 seconds. The bird was turned on its back with its head covered during counting.

Table 1. Schematic overview of sample types and measurements of northern goshawk nestlings from 20 nests in Nord- and Sør-Trøndelag, taken in June and July 2015.

Type of sample/measurement	Unit of measurement	Number of individuals
Stress-test	Number of breaths in 60 seconds	20
Blood sample	Milliliters	20
Stress-test	Number of breaths in 60 seconds	20
Biometric measurements: **Bill length** **Bill height** **Wing length** **Tail feather length** **Hallux length** **Tarsus width** **Tarsus depth** **Tarsus length** **Weight**	 Millimeters Millimeters Centimeters Centimeters Millimeters Millimeters Millimeters Millimeters Grams	20
Crop content	Grade 1-3 (in grams if possible)	20
Feather sample	5 -10 feathers	20
Behavioral observations • **Activity score** • **Alarming intensity**	 Grade: 1 (Low) – 5 (High) Grade: 1 (Low) – 5 (High)	 20 20

2. 2. Molecular sex determination

Molecular sexing was carried out as described by Griffiths et al. (1998), where the two genes CHD-Z and CHD-W were amplified by PCR and detected by gel electrophoresis. CHD-W is unique for females, while CHD-Z is found in both sexes. DNA was extracted from 2-4 µL RBCs in 200 µL of a 5 % chelex solution (5 grams of Chelex 100 [BioRad] in 100 mL destilled water) as described by Walsh et al. (1991). In each tube, 2 µL supernatant was mixed with 8 µL of stock solution (0.05 µL Taq, 1.95 µL H2O (autoclaved), 0.40 µL Mix (dNTP), 0.60 µL MgCl, 1.00 µL 10X, 1.00 µL Primer 2718 (10 µM), 1.00 µL Primer 2550 (10 µM), 2.00 µL Q). PCR was run in a GeneAmp PCR system 9700 with the following program: 94 °C for 3 min, then 35 cycles with 30 seconds at 94 °C (denaturation), 45 seconds at 46 °C (annealing) and 45 seconds at 70 °C (elongation). PCR finished off with 10 min at 70 °C.

Gel electrophoresis was run in a 1 % agarose gel (1.2 mL 50xTAE buffer, 59 mL distilled water, 0.6 g agarose)(BioRad Subcell) with 6 µL of SYBR® Safe added as gel stain. 700 mL running buffer (686 mL water, 14 mL 50x TAE buffer) (BioRad Subcell) was added, and DNA extracts were loaded onto the gel. The electrophoresis was run at 75 V for 45 min.

2. 3. Analysis of organic pollutants

Chemical analysis of plasma, body feathers and preen oil from the 20 NG individuals was performed in August 2015 at the Toxicological Centre at the University of Antwerp, Belgium. The legacy organic compounds that were targeted were congeners of polychlorinated biphenyls (PCBs) (IUPAC numbers: 28, 49, 52, 74, 95, 99, 101, 105, 118, 138, 153, 156, 170, 171, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209), dichlorodiphenyltrichloroethane (*p,p'*-DDT) and its metabolite dichlorodiphenyl-dichloroethylene (*p,p'*-DDE), three isomers of hexachlorocyclo-hexane (α-, β-, and γ-HCH), chlordane metabolites (oxychlordane (OxC), cis-nonachlor (CN) and trans-nonachlor (TN)), hexachlorobenzene (HCB) and polybrominated biphenyl ether (PBDE) congeners: BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 183. Also investigated were the anti- and syn- isomers of Dechlorane Plus (a-DP and s-DP). Analysed nBFRs were bis-tribromophenoxy ethane (BTBPE), 2-ethylhexyl tetrabromobenzoate (TBB) and bis(2-ethylhexyl)-tetrabromophthalate (TBPH) targeted. Targeted OPFRs were tris(2-chloroethyl) phosphate (TCEP), tris(2-butoxyethyl) phosphate (TBEP), triphenylphosphate (TPhP), 2-ethylhexyldiphenyl phosphate (EHDPHP) and two isomers of tris(1-chloro-2-propyl) phosphate (TCIPP). The solvents that were used for the analysis (acetone, n-hexane, methanol, dichlorometane, iso-octane) were of SupraSolv grade (Merck, Darmstadt, Germany).

2. 3. 1. Preparation of samples

Clean stainless steel tools were used for washing and cutting of the feathers; tools were thoroughly washed with acetone between individuals. Feathers were thoroughly washed in distilled water as previously recommended (Jaspers et al., 2008, Jaspers et al., 2007a), where washing with distilled water was found to be the best method for removing dust and particles from feathers prior to analysis. Two pairs of tweezers were used to separate the barbs during washing by pulling the barbs downwards and away from each other. Feathers were then dried over night at room temperature, before they were cut into approximately 1-2 mm pieces and accurately weighed. Each sample was then spiked with two internal standards, where internal standard 1 (IS1) contained CB 143 (200 pg/uL), BDE 77 (25 pg/uL) and ε-HCH (25 pg/µL).

Internal standard 2 (IS2) consisted of ^{13}C-TBPH (100 pg/µL), ^{13}C-syn-DP (50 pg/µL), ^{13}C-anti-DP (50 pg/µL), TPHP-d15 (1 ng/µL), TCEP-d12 (1 ng/µL), TDCIPP-d15 (1 ng/µL), TAP (1 ng/µL) and TBOEP-d6 (2 ng/µL). 100 µL of IS1 and 50 µL of IS2 were added to each sample. Then 3 mL of hydrochloric acid (HCl, 4M) and 5 mL of the extraction solvent *n*-hexane/DCM mixture (4:1, v/v) were added, before the samples were incubated at 45 °C overnight in closed pear-shaped flasks. The following day, the incubated sample solution was vortexed for 1 minute to extract the organic compounds into the organic layer. This layer was collected and transferred to a clean glass tube. This liquid-liquid extraction was repeated with 5 mL hexane-DCM mixture (1:4, v/v). The organic layers were combined and evaporated to near dryness by a gentle nitrogen stream at 32 °C and re-solubilized in 100 µLiso-octane followed by 1 min vortex. Further clean-up procedures are described below.

The plasma samples were prepared for chemical analysis by liquid-liquid extraction, starting with adding internal standards IS1 (100 µL) and IS2 (50 µL) to each sample, the sample volume ranged from approximately 750 to 1000 µL. This was followed by the addition of 1000 µL of Milli-Q water, 200 µL of formic acid (98 %) and subsequently 4 mL of the extraction solvent hexane:DCM (4:1, v/v) with intermittent vortexing. The solution was centrifuged at 2000 g for 5 min before the organic layer was transferred to a clean glass tube. This extraction was repeated once more and the organic layers were combined and evaporated to near dryness and re-dissolved by the same method as the feathers.

Preen oil samples were prepared for chemical analysis by the initial addition of the organic solvent dichloromethane (DCM) to dissolve the organic compounds. 0.5 mL DCM was added to each individual tube. The tubes were centrifuged (2000g, 5 min) before the extract was collected and transferred to a clean, pre-weighed glass tube by pipetting, leaving only the feather remnants in the tube. This extraction was repeated once, and the extracts were combined followed by evaporation until dryness by a gentle nitrogen steam at 32 °C. Only the lipids were now left in the tubes. The glass tubes were then weighed accurately to determine the lipid weight. The samples were further dissolved in 1 mL of hexane, internal standards IS1 (100 µL) and IS2 (50 µL) were added, and samples were vortexed for 1 min.

2. 3. 2. Clean-up of samples

Further procedures for clean-up and fractionation of the concentrated extracts were the same for plasma, feathers and preen oil. Fractionation was performed on Supelclean™ ENVI™-

Florisil cartridges (500 mg, 3mL, Supelco® Analytical). Anhydrous Na_2SO_4 powder was added to the cartridge before cleaning with first 8 mL ethyl acetate, then 6 mL hexane. The sample extract was transferred to the clean cartridge. The sample tube was washed twice with 0.5 mL of hexane. The extracts were eluted in two fractions: the first fraction (F1), containing PCBs, PBDEs, DPs, nBFRs, and OCPs was eluted with 12 mL hexane:DCM (1:1, v/v). A second fraction (F2) was collected in new tubes, containing the OPFRs and TBPH. F2 was eluted from the same columns as F1 by 10 mL ethyl acetate. Both fractions were evaporated to near dryness (≈ 500 µL) by a gentle nitrogen steam at 32 °C. F1 was re-solubilized in 2x500 µL of hexane, followed by a second elution of F1 on acidified silica (5 %) in a 3 mL cartridge, pre-cleaned with 3x2 mL hexane. The tube was washed twice with 0.5 mL of hexane and vortexed. Elution was carried out with 10 mL hexane, added 2 mL at the time. For preen oil this second extraction was repeated once, as the extract was not clear enough. F1 was again concentrated to near dryness by a gentle nitrogen stream at 32 °C. Both F1 and F2 were re-solubilized in 50 µL of iso-octane and 50 µL recovery standard (CB 207, 50 pg/µL in iso-octane-toluene 9:1, v/v) and vortexed for 30 seconds. Extracts were transferred to injections vials for GC-MS analysis.

2. 3. 3. Chemical analysis

For measuring PBDEs, chlordanes, HCH isomers, DPs and emerging BFRs, an Agilent 6890-5973 gas chromatograph coupled to a mass spectrometer system (GC-MS) was employed. A 30 meter x 0.25 mm x 0.25 µm DB-5ms capillary column (J&W Scientific, Folsom, CA, USA) was utilized, and the MS was operated in electron capture negative ioinisaion (ECNI) mode. The ion source, quadrupole and interface temperatures were set at respectively 170, 150, and 300 °C, and the reagent gas was methane. In the MS, the selected ion-monitoring (SIM) mode with ions m/z = 79 and 81 was used (acquired during the whole run for PBDEs, and with the two most intense characteristic ions for PCB or each pesticide). Dwell times were set at 30 ms. Of the cleaned extract, one µL was injected in solvent vent mode. The injection temperature was set at initially 90 °C, which was held for 0.05 min, then with 700 °C/min to 300 °C for 25 min. The vent flow was set at 75 mL/min, and the purge vent opened at 1.5 min. The carrier gas was helium, which was set at constant flow (1.0 mL/min). The temperature of the DB-5ms column was kept at 90 °C for 1.50 min, before it was increased to 300 °C at a rate of 10 °C/min, and kept for 20 min.

PCBs, *p,p´*-DDT, *p,p´*-DDE and HCB were measured by an Agilent 6890 GC-5073 MS system, which was operated in electron ionisation (EI) mode. A 25 m x 0.22 mm x 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium) was employed, with the ion source, quadrupole and interface temperatures set at respectively 230, 150 and 300 °C. Of the cleaned extract, one µL was injected in cold-pulsed splitless mode. The injection temperature was set at 90°C, for 0.03 min, then rising to 300 °C with 700 °C/min. Pressure pulse was set at 25 psi, the pulse time and the splitless time were both set at 1.50 min. The carrier gas was helium, which was set at constant flow (1.0 mL/min). The HT-8 column temperature was kept at 90 °C for 1.50 min, before it was increased to 180 °C at 15 °C/min for 2.0 min, then further increased to 280 °C at 5 °C/min before finally it was raised to 300 °C at 40 °C/min, where it was held for 20 min. Selected ion-monitoring (SIM) mode was set for the MS, with 2 ions monitored for each PCB homologue group or individual OCP. The dwell times were set to 30 ms.

OPFRs were measured with an Agilent 6890 GC couples to an Agilent 5973 MS, operated in electron impact ionization (EI) mode. The GC system was equipped with PTV and electronic pressure control. A deactivated retention gap from Agilent (1.0 m × 0.22 mm) was installed before the HT-8 column (25 m × 0.22 mm × 0.25 µm). Of the final extract, one µL was injected using cold splitless injection. The GC temperature program was 90°C, hold 1.25 min, ramp 10°C/min to 240°C, ramp 20°C/min to 310 °C, hold 16 min. The carrier gas was helium, set at constant flow rate of 1.0 mL/minute. The MS was run in selected ion-monitoring (SIM) mode for the analysis of TEHP, TNBP, TCEP, the two isomers of TCIPP, EHDPHP, TPHP, TDCIPP and the four isomers of TMPP. The dwell times were set at 30 ms. Quantification was based on five-point calibration curves. The peaks of the curves were positively identified as the target compounds if: (1) the retention time matched that of the standard compound within ± 0.1 minute and (2) the signal-to-noise ratio (S/N) was higher than 3:1. To check for potential contamination or disturbance in the analysis, procedural blanks were analyzed for every seven sample that was analyzed. Mean blank value was subtracted from each sample. The limit of quantification (LOQ) was calculated as 3 times the standard deviation of the mean blanks. For chemicals that were not detected in procedural blanks, LOQs were calculated for a ratio of signal/noise equal to 10. The ranges of the LOQs were: plasma: 200-400 pg mL^{-1}; feathers: 0.10-4.0 ng g^{-1}; preen oil: 0.4-50 ng g^{-1}.

2. 4. Biochemical analysis of parameters for oxidative stress

RBC pellets were initially diluted 1:10 in distilled water and stored in vials at -80 °C. In all assays, samples, blanks and standards were added in triplicates, replicates were made within and between plates for quality assurance. A CYTATION 5 Imaging Reader (Biotek) was used for reading the absorbance, which was viewed with Gen 5 2.0 All-In-One Microplate reader software (Biotek). All chemicals were purchased at Sigma-Aldrich.

2. 4. 1. Bradford assay

Total protein content in the RBC samples was determined by a Bradford assay. RBC samples were diluted 1:50 in Tris-HCL buffer (0.02 M, pH 7.4: 2.644 g Tris-HCl, 0.3880 g Tris-base adjusted to 1 L in total with milli-Q water). 5 µL diluted sample was added to each sample well in 96-well microtiter plates. 5 µL bovine serum albumin (BSA) protein standards (range 0-1.4 mg/mL) were added to standard wells, the standards were diluted in Tris-HCL buffer. 250 µL Bradford reagent was added to each well, followed by 30 second mixing with a shake at 600 rpm. The plate was covered and incubated for 5 minutes, before the absorbance was read at 595 nm. The sample protein concentrations (mg protein/mL) were determined by plotting the net-absorbance against the concentration for each standard, utilizing the slope and intercept of this graph in the calculations.

2. 4. 2. Glutathione reductase activity

The assay was performed in a 96-well microplate. The absorbance was read at 340 nm at 6 time points with 1-minute intervals. RBC samples were diluted 1:5 in sample buffer (50 mM Potassium phosphate, 1 mM EDTA, pH 7.5, 1 mg/mL BSA). 100 µL of assay buffer (50 mM Potassium phosphate, 1 mM EDTA pH 7.5) was added to each sample well, 120 µL to background wells. 20 µL of diluted sample was added to each sample well. 20 µL of GSSG (9.5 mM) was added to all wells, the reaction was initiated by adding 50 µL of NADPH (1.5 mM) to all wells. Absorbance values were plotted as a function of time, and the change in absorbance (ΔA_{340}) was calculated from two points in the linear range of the curve. ΔA_{340} for the background wells was subtracted from all samples. GR activity was calculated by equation 4 and expressed as nmol/min/mg protein.

GR activity=(ΔA340min/0.003730µM)∗{(0.19/0.02) mL∗sample dilution) (Equation 4)

2. 4. 3. Glutathione peroxidase activity

The assay was performed in a 96-well microtiter plate. RBC samples were diluted 1:5 in sample buffer (50 nM Tris-HCl, 5mM EDTA pH7.6, 1 mg/mL BSA). 120 µL assay buffer (50 nM Tris-HCl, 5mM EDTA pH 7.6) was added to the background wells. To the sample wells, 100 µL assay buffer and 20 µL diluted sample was added. Then, 20 µL of GSH (10 nM) followed by 20 µL GSSG reductase (2.4 U/mL) was added, followed by 20 µL NADPH (1.5 mM) and finally 20 µL *tert*-Butyl hydroperoxide solution (12 mM), initiating the reaction. The time from reaction initiation to the first reading was measured. Absorbance values were plotted as a function of time, and the change in absorbance (ΔA_{340}) was calculated from two points in the linear range of the curve. ΔA_{340} for the background wells was subtracted from all samples. GPx activity was calculated by equation 5 and expressed as nmol/min/mg protein.

GPx activity=(ΔA340min/0.003730µM)∗{[0.20/0.02) mL∗sample dilution) (Equation 5)

2. 4. 4. Catalase activity

Peroxidative activity of catalase (CAT) was measured in RBC samples which were diluted 1:15 in sample buffer (25 nM KH_2PO_4, 1 mM EDTA, 1 % BSA, pH 7.5). 100 µL assay buffer (KH_2 100 mM, pH 7.0) was added to all wells, followed by 20 µL diluted sample/20 µL formaldehyde standard. The standard ranged from 0 to 75 µM formaldehyde. 30 µL methanol was added to all wells, followed by 20 µL diluted hydrogen peroxide (H_2O_2, 35mM) initiating the reaction. The plate was incubated for 30 minutes while shaking at 600 rpm. Further, 30 µL potassium hydroxide (KOH, 10M) was added to all wells to stop the reaction, then 30 µL purpald (34.2 mM) was added to start coloration. The plate was covered and incubated for 10 min while shaking at 600 rpm. 10 µL potassium periodate (KIO_4, 0.5M) was added to stop the coloring reaction and the plate was read at 540 nm. CAT-activity was calculated from linear regression of the standard curve corrected for blank values, and expressed as nmol/min/mg protein.

2. 5. Statistics

For statistical analysis, R version 3.2.2. (©R Core Team, 2014) and R-studio version 0.98.490 (©RStudio, Inc.) were used. Calculations and graphics were made in R and Microsoft Excel version 14. 5. 5. (© 2010 Microsoft Corporation). Principal component analysis (PCA) was performed in SIMCA (®Umetrics). Level of significance was set at 0.05.

2. 5. 1. Age

Age in number of days was estimated by Equation 6 and 7, as described by Kenward (2010) where sex of the nestling is included to account for sexual dimorphism:

Age_{Male} = 0.125*wing length (mm) + 3.60 (Equation 6)

Age_{Female} = 0.111* wing length (mm) + 4.94 (Equation 7)

2. 5. 2. Condition index

Scaled mass index was estimated by standardized major axis regression (SMA), and used as condition index (CI) of the nestlings (Peig and Green, 2009). Of linear body measurements, log transformed bill height showed the strongest correlations with body mass transformed by natural logarithm (ln), correlation was found by Pearson correlation test. The slope of the SMA regression on transformed variables (ln(body mass)~ln(bill height)) was used in calculations of the CI by equation 8:

$$CI = M_i * [L_0/L_i]^{bSMA} \quad \text{(Equation 8)}$$

where M_i is the body mass (g) of the individual, L_0 is the mean bill height (mm) of the n=20 observations, L_i is the bill height (mm) of the individual and b_{SMA} is the estimated slope of the SMA regression. To test for the potential relationship of age and sex with CI, multiple linear regression models were used, with CI as response variable and age and sex as explanatory variables, including the interaction of sex and age.

2. 5. 3. Contaminant concentrations and profiles

Contaminants below the limit of quantification (LOQ) in all samples are listed in Table SI-1 in Appendix B. Compounds with a detection frequency (DF, the proportion of samples above LOQ) below 0.50 were considered to be insufficiently represented and were omitted from further statistical analysis (see Table SI-2 in Appendix B). For compounds with a DF ≥ 0.50, values below LOQ were substituted with DF × LOQ (Voorspoels et al., 2002). All compounds detected in more than 50 % of the samples are listed with mean concentration and standard error in Table 1. For comparison between matrices, the method recommended by Eulaers et al. (2011b) was used, where only commonly detected compounds were included in the total sums of the POP classes of ΣPCBs, ΣPBDEs and ΣPOPs. The mean percentage contribution of congeners to ΣPCBs and ΣPBDEs in the three matrices was calculated. Contaminant-profiles were made, showing the mean percentage contribution of each common group of POP to the total mean ΣPOPs load for plasma, feather and preen oil separately. The sum of TCIPP consists of two isomers, TCIPP1 and TCIPP2.

2. 5. 4. Checking normality assumptions

Contaminants were tested for normal distribution by Shapiro-Wilk tests, where the null hypothesis of normal distribution was rejected at $p > 0.05$. Non-normally distributed contaminants were transformed by natural logarithms (ln) to obtain normal distribution, which was confirmed by both Shapiro-Wilk tests and quantile-quantile (QQ) plots.

2. 5. 5. Comparison of matrices

Due to non-normality of some contaminant variables, despite transformation, non-parametric Spearman rank correlations were used to investigate relationships of contaminants between matrices. Differences in congener concentrations and contribution between matrices were initially investigated by linear regression models, but as the assumptions of heteroscedasticity and normally distributed residuals were not met, the non-parametric Wilcoxon signed rank test on un-transformed variables was used instead.

2. 5. 6. Principal component analysis

Principal component analysis (PCA) was used to visualize and investigate the relationship between all detected contaminants in plasma with CI, age, sex, breath counts as well as the enzymes catalase and GPx. Before the PCA was run, skewed variables were log transformed, and all variables were scaled by being divided by the standard deviation and mean centered by subtraction of the arithmetic mean. Two principal components (PC), PC1 and PC2, explained respectively 60.6 and 8.8 % of the variation in the original variables. To further investigate the effect of contaminants on oxidative stress, a new PCA was run without the enzymes. The scores of PC1, which explained most of the variation (63.7 %), were run in a linear regression model with the individual enzymes as response variables.

2. 5. 7. Regression of selected contaminants on enzyme activity

Due to the contaminants in plasma being highly correlated, five different compounds were selected for further investigation of the effect of contaminants on enzyme levels. In this way, the number of contaminant variables was also reduced. The five main compounds were selected based on their physiochemical properties and abundance, as they were all detected in every plasma sample ($n = 20$). These variables also had some of the highest loading values in the PCA without the enzymes. This method of selecting representative contaminants has previously been used in other studies on raptor nestlings for assessing contaminant associations with physiological processes, such as growth and oxidative stress (Bustnes et al., 2013b, Sletten et al., 2016). The five contaminants were CB 153, *p,p´*-DDE, OxC, HCB and

BDE 99. Correlation tests were performed to investigate the relationship between contaminants with age and CI, where Spearman rank correlation was used, as age and CI were not normally distributed. The potential effects of CI, age and sex on contaminant levels were investigated by multiple linear regressions with individual ln transformed contaminants as response variables.

Multiple linear regression models were also used to investigate the relationship between enzymes levels in red blood cells and POPs in plasma. Models were run with enzyme level as response variable and the ln transformed CB 153, *p,p´*-DDE, OxC, HCB and BDE 99 as explanatory variables. Since the contaminants were correlated, separate models were run for each contaminant. Sex, CI and breath counts (registered right before blood sampling) were also included as explanatory variables to control for potential effects of these factors on oxidative stress. Interactions between some of the variables were also investigated, namely interactions of the contaminant with age, sex, breath counts and CI, and also sex with CI. Number of nestlings in the nest was not included as a variable in the assessment of enzyme activity, as all except for two nests contained two or three nestlings.

Model selection was performed by employing Akaikes Information Criteria adjusted for small sample size (AICc), this was run for all models. This method takes both the complexity (number of parameters) and fit (R^2) of the model into consideration. The best model was defined as the model with the lowest AICc value when all models were ranked by increasing AICc, preferably with a ΔAICc > 2.0 than the second best model (Crawley, 2012). Complete lists of all models tested ranked by corresponding AICc values, ΔAICc and AICc weights are listed in Table SI-3, SI-4 and SI-5 in Appendix B. For all regression models in the current study, model residuals were checked for constant variance and normal distribution by quantile-quantile (Q-Q) plots and residuals plotted against fitted values, to ensure that model assumptions were met. Adjusted R squared (R^2) are reported for the proportion of variability explained by the models, as this effect size takes sample size into consideration (Fritz et al., 2012).

3. RESULTS

3. 1. Organic pollutants

3. 1. 1. Emerging contaminants

3. 1. 1. 1. Novel brominated flame retardants

TBB was not detected in any of the three matrices, BTBPE was only detected in one preen oil sample (1.8 ng g^{-1} lipid) and TBPH was only detected in feathers (0.69-8.77 ng g^{-1}, DF=0.40). Due to this low detection frequency, nBFRs were not used in further statistical analysis.

3. 1. 1 .2. Organophosphate flame retardants

In both plasma and preen oil, the targeted OPFRs were found in few samples, if detected at all [plasma: 0.102 - 1.27 ng mL^{-1}, DF: 0.05 - 0.10, preen oil: 13.3 - 50 ng g^{-1} lipids, DF: 0.05 - 0.20]. For this reason, only feather levels of OPFRs were further included in the present study. One individual, TRD 1 sampled from location Skardberget (Location 1, Figure 1) situated close to Trondheim, had extremely high feather levels of TPhP [168.5 ng g^{-1}], which was approximately 26 times higher than the average concentration of the rest of the detections [6.25 ± 1.2 ng g^{-1}]. TRD1 was therefore removed as an outlier from the OPFR results. TBEP was not detected in feathers. TDCIPP was the only OPFR detected in less than 50 % of the feather samples, leaving four compounds that were included in the results, see Figure 2. TCIPP was the compound detected in the highest mean concentration. The mean ΣOPFRs exceeded the mean feather concentrations of ΣPBDEs, ΣPCBs and *p,p´*-DDE, being approximately 29, 5 and 1.5 times higher, respectively.

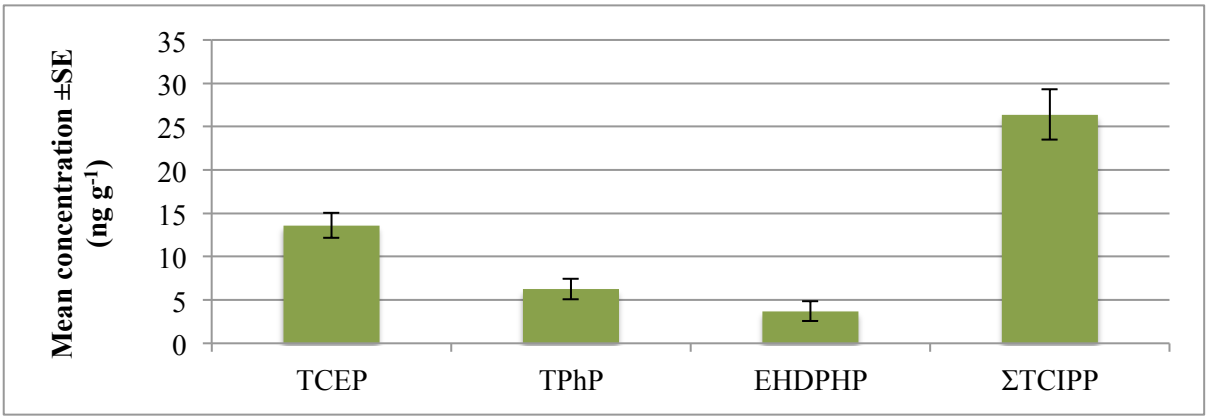

Figure 2. Mean concentration ± standard error (SE) (ng g^{-1}) for OPFRs with DF ≥ 0.50 in feathers of northern goshawk nestlings (n=19) from Trøndelag. ΣTCIPP is summed mean value of isomers TCIPP1 and TCIPP2. One individual, TRD1, was removed due to extreme levels of TPhP.

3. 1. 2. Legacy POPs and contaminant profiles

Mean concentrations ± standard error (SE) of POPs with DF ≥ 0.50 are presented in Table 2. Further comparison of concentrations between matrices only included compounds that were detected in more than 50 % of the samples (DF < 0.50) in all three matrices. Non-detected compounds and compounds with DF < 0.50 are listed in respectively Table SI-1 and SI-2 in Appendix B. Median values with maximum and minimum concentrations are listed in Table SI-6 in Appendix B.

Table 2. Mean concentrations ± SE of POPs detected in more than 50 % of plasma (ng mL^{-1}), feather (ng g^{-1}) and preen oil (ng g^{-1} lipids) samples from northern goshawk nestlings (n=20) from Trøndelag. Nd denotes not detected in more than 50 % of the samples.

	Plasma			Feather			Preen oil		
	ng mL^{-1}			ng g^{-1}			ng g^{-1} lipids		
	Mean		SE	Mean		SE	Mean		SE
CB 101	0.06	±	0.01	0.54	±	0.07	8.05	±	1.49
CB 99	0.11	±	0.02	1.21	±	0.23	19.03	±	3.29
CB 105	0.05	±	0.01	0.28	±	0.05	5.34	±	1.13
CB 118	0.24	±	0.04	0.82	±	0.14	22.44	±	4.59
CB 153	2.12	±	0.36	3.31	±	0.42	149	±	26.7
CB 138	0.99	±	0.16	1.84	±	0.28	45.6	±	6.20
CB 187	0.74	±	0.11	0.72	±	0.07	32.9	±	3.96
CB 183	0.19	±	0.03	0.19	±	0.02	8.99	±	1.59
CB 177	0.07	±	0.01	0.11	±	0.01	3.47	±	0.45
CB 180	1.42	±	0.01	0.50	±	0.06	3.10	±	0.49
CB 170	0.50	±	0.02	0.38	±	0.05	4.01	±	0.66
CB 171	0.06	±	0.27	Nd			50.6	±	8.53
CB 156	0.10	±	0.09	Nd			18.8	±	3.34
CB 199	0.18	±	0.03	Nd			6.13	±	0.74
CB 196/203	0.16	±	0.03	Nd			6.17	±	0.97
CB 194	0.18	±	0.03	Nd			7.11	±	1.16
CB 206	0.04	±	0.01	Nd			2.19	±	0.58
CB 209	0.03	±	0.004	Nd			2.03	±	0.41
ΣPCBs	7.23	±	1.19	9.90	±	1.21	395	±	62.2
OxC	0.17	±	0.03	0.16	±	0.02	6.69	±	0.98
TN	0.16	±	0.03	Nd			9.02	±	1.89
CN	0.04	±	0.01	Nd			Nd		
HCB	0.18	±	0.05	0.23	±	0.05	11.7	±	1.81
***p,p´*-DDE**	5.02	±	0.82	31.87	±	3.54	1021	±	165
***p,p´*-DDT**	0.06	±	0.01	Nd			Nd		
***β*-HCH**	0.03	±	0.002	0.34	±	0.06	4.47	±	0.57
***γ*-HCH**	Nd			0.21	±	0.05	6.35	±	3.04
BDE 47	0.07	±	0.01	0.63	±	0.14	9.87	±	2.20
BDE 100	0.06	±	0.01	0.25	±	0.05	8.65	±	1.12
BDE 99	0.09	±	0.02	0.62	±	0.15	12.9	±	2.02
BDE 153	0.04	±	0.01	0.18	±	0.02	3.48	±	0.27
BDE 183	0.01	±	0.001	Nd			5.74	±	0.56
ΣPBDEs	0.27	±	0.04	1.68	±	0.33	38.9	±	5.44
a-DP	Nd			Nd			1.58	±	0.31

3. 1 .2. 1. Chlorinated contaminants and PCB profiles

The five lowest chlorinated PCB congeners targeted for detection (CB 28, CB 49, CB 52, CB 74 and CB 95) were not detected above the LOQ in blood, preen oil, nor feathers. For feathers, seven of the higher chlorinated congeners (CB 156, CB 171, CB 194, CB 196/203, CB 206 and CB 209) were detected in less than 50 % of the samples ($0.05 < DF < 0.30$). These congeners were therefore not included in the ΣPCBs for comparison between matrices. The eleven remaining PCBs were detected in > 50 % of the samples, in all matrices and their individual mean percentage contributions to mean ΣPCBs are shown in Figure 3.

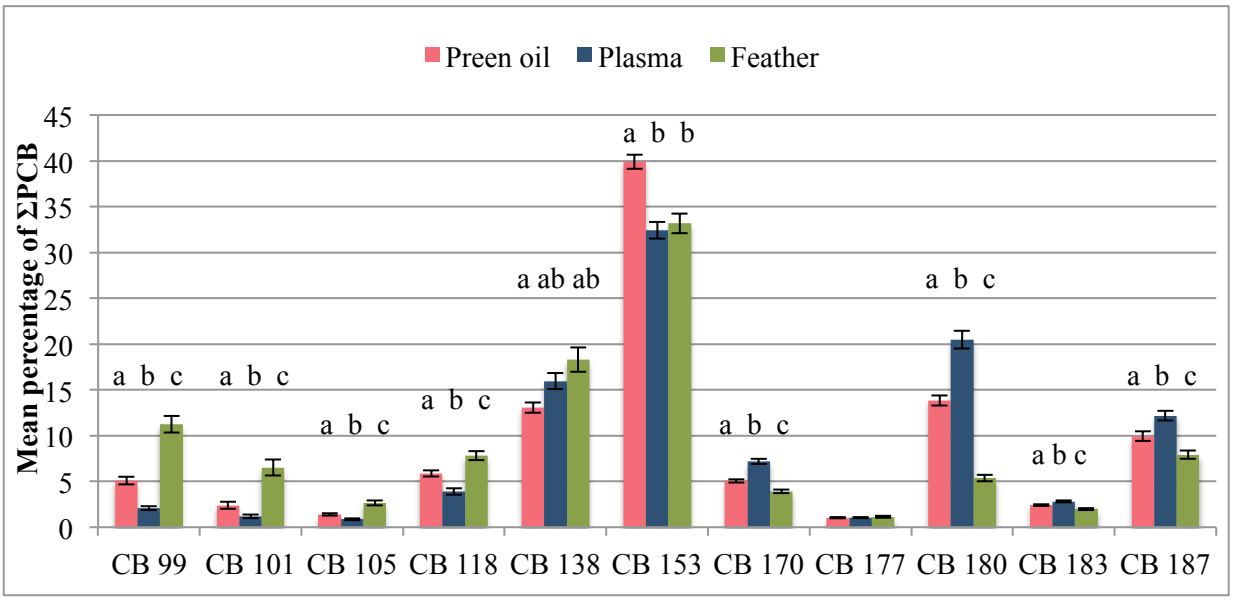

Figure 3. Mean percentage contribution ± SE of PCB congeners to mean ΣPCBs in preen oil, plasma and feathers from northern goshawk nestlings (n = 20). Mean sum is based on the eleven PCB congeners that were common for all three matrices. Significant difference ($p < 0.05$) between matrices denoted by different letters: a, b and c.

Of the PCBs, CB 153 was the most prominent congener, possessing the highest mean concentration in all matrices [plasma: 2.1 ± 0.35 ng mL^{-1}, feather: 3.3 ± 0.4 ng g^{-1}, preen oil: 149.6 ± 26.7 ng g^{-1} lipids], and constituting approximately 32-39 % of the mean ΣPCBs. Feathers tended to have a larger content of lower chlorinated congeners, while plasma samples had the largest percentage contribution of the higher chlorinated congeners, followed by preen oil. Preen oil also showed a higher contribution of lower halogenated PCBs compared to plasma. Testing for differences in matrix concentrations showed that most PCB-congeners had a higher concentration in feathers than in plasma, except for CB 170 ($V = 84$, $p = 0.45$), CB 183 ($V = 114$, $p = 0.75$) and CB 187 ($V = 0.76$, $p = 0.29$), which did not differ significantly in the two matrices. The only congener with significantly higher concentrations

in plasma compared to feathers was CB 180 (V = 208, p = 2.861e-06). Preen oil concentrations of PCB congeners were all significantly higher than both plasma and feather concentrations (all $p < 0.001$). ΣPCBs were positively correlated between matrices ($0.56 \leq r_s \leq 0.81$, $p < 0.05$), with the strongest correlation between feathers and preen oil, as shown in Table 3. The mean ΣPCBs was higher in feathers compared to plasma ($V = 0$, $p = 0.011$), and preen oil ΣPCBs levels were higher than in feathers ($V = 0.45$, $p = 9.537e-07$).

p,p'-DDT was only detected in more than 50 % of the samples in plasma (DF=0.85), with a mean concentration of 0.063 ± 0.01 ng mL^{-1}. The metabolite *p,p'*-DDE was detected in all samples that were analyzed. It had the highest mean concentration of all compounds in all three matrices [plasma: 5.02 ± 0.82 ng mL^{-1}, feather: 31.8 ± 3.5 ng g^{-1}, preen oil: 1021 ± 165 ng g^{-1} lipids]. The mean concentration of *p.p'*-DDE in preen oil was more than 200 times higher than in plasma and approximately 30 times higher than the mean concentration in feathers. *p,p'*-DDE was positively and significantly correlated between matrices ($0.66 \leq r_s \leq 0.89$, $p < 0.05$), with the strongest correlation between plasma and preen oil (Table 3).

Of the chlordanes, OxC, TN and CN were detected in more than 50 % of the samples in plasma [OxC: 0.02-0.49 ng mL^{-1}, TN: 0.01-0.55 ng mL^{-1}, CN: 0.008-0.16 ng mL^{-1}]. In preen oil, both OxC and TN were detected in all samples [OxC: 1.38-20.7 ng g^{-1} lipids, TN: 1.0-35.9 ng g^{-1} lipids], while CN was detected in less than 50 % of the preen oil samples. In feathers, OxC was the only chlordane that was detected in more than 50 % of the samples [0.06-0.34 ng g^{-1}]. OxC concentrations were highest in preen oil ($V = 0$, $p = 9.537e-07$), while concentrations in plasma and feathers were not significantly different ($V = 108$, $p = 0.92$). OxC showed a significant correlation between plasma and preen oil only ($r_s = 0.62$, $p < 0.05$). α-HCH was not detected in any sample, only the β-isomer was detected in more than 50 % of the samples and in all three matrices [plasma: 0.01-0.06 ng mL^{-1}, feather: 0.075-1.1 ng g^{-1}, preen oil: 1.54-13.0 ng g^{-1} lipids], with significantly higher concentrations in preen oil compared to feathers ($V = 0$, $p = 9.537e-07$), and significantly higher concentrations in feathers compared to plasma ($V = 0$, $p = 9.537e-07$). β–HCH showed significant correlations between matrices for plasma and preen oil only ($r_s = 0.69$, $p < 0.01$). HCB was sufficiently detected for quantification in more than 50 % of the samples for all matrices, and was detected in all preen oil samples [3.14-37.5 ng g^{-1}]. The highest HCB concentrations were detected in preen oil ($V = 0$, $p = 9.537e-07$), while HCB levels in plasma [0.02-1.14 ng mL^{-1}]

and feathers [0.06-0.79 ng g^{-1}] were not significantly different (V = 78, p = 0.33). Like the OPFRs, a-DP was only detected in feathers, with a mean concentration of 1.58 ± 0.31 ng g^{-1}.

Table 3. Correlation analysis by non-parametric Spearman rank correlation. Correlation coefficient rho (r_s) and estimated significance level (p) of main groups of persistent organic pollutants (DF ≥ 0.50) between plasma, feathers and preen oil from northern goshawk nestlings (n=20). Main groups are mean summed concentrations of congeners detected in all three matrices. Significant correlations are marked in bold.

	Plasma - Feather		**Feather - Preen Oil**		**Plasma - Preen Oil**	
POP	r_s	p	r_s	p	r_s	p
ΣPCBs	**0.55**	**0.012**	**0.81**	**<0.001**	**0.71**	**<0.001**
***p,p'*-DDE**	**0.66**	**0.002**	**0.76**	**<0.001**	**0.89**	**<0.001**
OxC	0.13	0.54	0.20	0.37	**0.62**	**0.004**
HCB	**-0.64**	**0.005**	-0.08	0.71	0.22	0.32
β-HCH	0.16	0.46	0.38	0.09	**0.69**	**0.001**
ΣPBDEs	**0.79**	**<0.001**	**0.93**	**<0.001**	**0.77**	**<0.001**

3. 1. 2. 2. Brominated contaminants and PBDE profiles

The lowest investigated brominated PBDE-congener, BDE 28, was not detected above LOQ in any sample of the 20 individuals. BDE 183 was only quantifiable in plasma, and BDE 154 was sufficiently detected for quantification in preen oil only. This left four of the seven targeted PBDE-congeners for comparison between matrices: BDE 47, BDE 99, BDE 100 and BDE 153. All four congeners were detected in all plasma and preen oil samples, whilst the DF ranged from 0.65 to 0.90 in feathers. BDE 99 was the congener with the highest mean concentration in preen oil [12.9 ± 2.0 ng g^{-1} lipid] and in plasma [0.092 ± 0.017 ng mL^{-1}]. All commonly detected congeners in the three matrices had significantly higher concentrations in preen oil than in feathers (All V = 0, all $p < 0.05$), whilst feather levels were significantly higher than plasma levels (V = 0-3, all $p < 0.05$). Feathers had a higher percentage of the lower brominated BDE 47, and less of higher chlorinated BDE 100 and BDE 153 compared to plasma and preen oil, as shown in Figure 4. Plasma and preen oil had more similar congener compositions of PBDEs. ΣPBDEs showed significant and positive correlations between matrices, with the strongest correlation between feathers and preen oil (r_s = 0.91, $p <$ 0.05; Table 3).

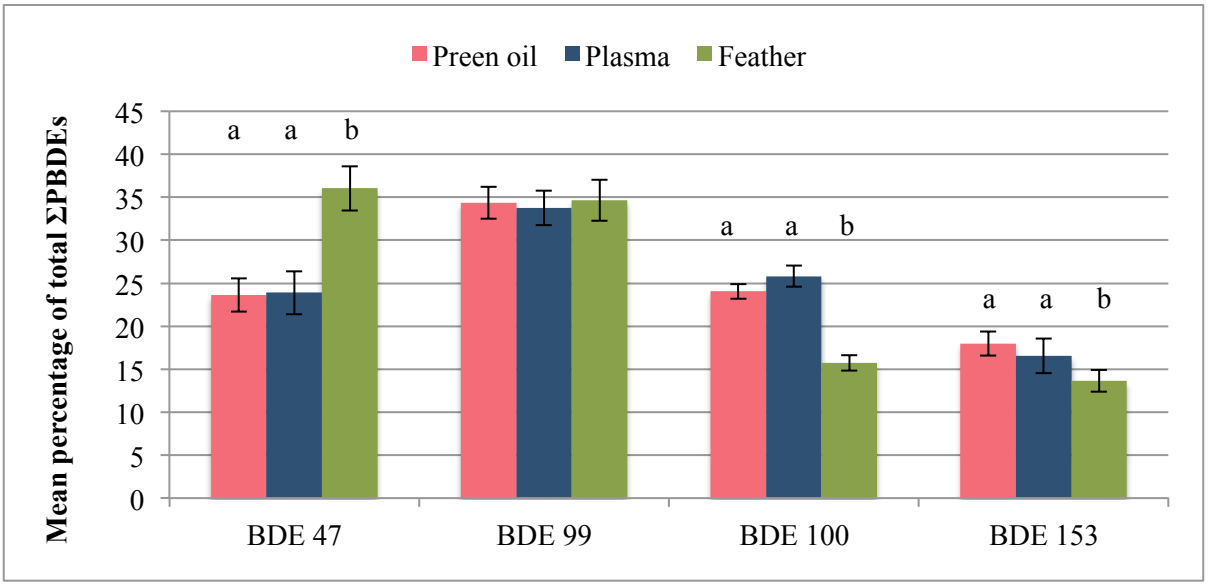

Figure 4. Mean percentage contribution ± SE of PBDE congeners to Σ PBDEs in preen oil, plasma and feathers from northern goshawk nestlings (n = 20) from Trøndelag. Significant difference ($p < 0.05$) between matrices denoted by different letters a, b and c.

3. 1. 2. 3. Total POP load and profiles

The mean percentage contributions of the different POP groups to the total POP load are shown in Figure 5. *p,p′*-DDE had the highest percentage contribution to the total load of POP groups in both feathers (71.2 %) and preen oil (68.5 %). In plasma, *p,p′*-DDE contributed 41.4% to the total POP load, with mean ΣPCBs being the only group with a higher contribution (52.4%). The summed percentage contribution of ΣPCBs and *p,p′*-DDE exceeded 90 % of the total POP load in all three matrices. The mean percentage contribution of OxC was highest in plasma (1.6 %), while the share of OxC was lower and more similar in preen oil and feathers (0.5 and 0.4 %, resp.). The load of HCB was higher in preen oil (1.1 %) and plasma (1.5 %), compared to feathers (0.6 %), while feathers had a higher percentage of *β*-HCH (0.8 %) than plasma (0.3 %) and preen oil (0.4 %). ΣPBDEs contributed most to the total POP load in feathers (4.0%), followed by preen oil (3.1%) and then plasma (2.4%).

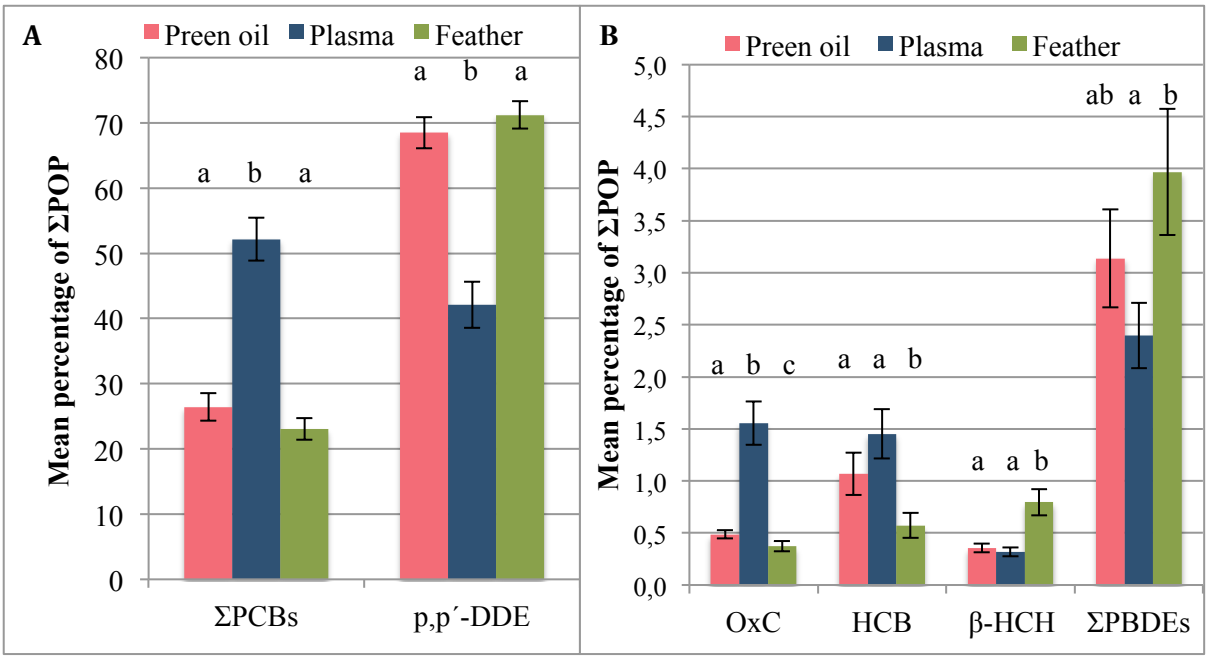

Figure 5. Mean percentage contribution ± SE of main POP groups (A: Σ PCBs and *p,p´*-DDE, B: OxC, HCB, *β*-HCH and ΣPBDEs) to total POP load in preen oil, plasma and feathers from northern goshawk nestlings (n = 20) from Trøndelag. Significant difference ($p < 0.05$) denoted by different letters a, b and c.

3. 1 .2. 4. Factors affecting contaminant levels in plasma

CI was not associated with age or sex (all $p > 0.05$), and CI was not correlated with contaminant level in plasma of the nestlings (see Table 4). CB 153, OxC and HCB were all significantly and negatively correlated with age (all $p < 0.05$; Table 4).

Table 4. Correlation analysis by non-parametric Spearman rank correlation. Correlation coefficient rho (r_s) and estimated significance level (p) between plasma levels of five persistent organic pollutants with age (days) and condition index (CI) in northern goshawk nestlings (n=20). Significant correlations are marked in bold.

	Age		**Condition Index**	
POP	r_s	p	r_s	p
CB 153	**-0.58**	**0.007**	0.34	0.13
p,p´-DDE	-0.01	0.94	0.11	0.63
OxC	**-60.0**	**0.005**	0.15	0.52
HCB	**-0.49**	**0.02**	0.17	0.46
BDE 99	0.07	0.76	0.41	0.07

The biplot (Figure SI-1 in Appendix A), showing the combined score and loadings, indicated that the contaminants were positively correlated, as most compounds were clustered in the positive section of the axis of PC1, which also was the PC explaining most of the variation

(63.7 %). The PCA also indicated that the contaminant load seemed to be negatively correlated with age. No clear pattern indicating differences in contaminant load between sexes was observed.

Of the sampled nestlings, 13 were females and 7 were males (Figure SI-2, Appendix A). Of the five contaminants investigated, only *p,p´*-DDE in plasma differed significantly between sexes, with males having a mean *p,p´*-DDE level that was 2.27 ng mL^{-1} higher than the mean level in females ($F_{18,1} = 6.56$, $p = 0.02$), as shown in Figure 6. Feather and preen oil levels of *p,p´*-DDE did however not differ significantly between sexes (feathers: $p = 0.15$, preen oil: $p = 0.09$).

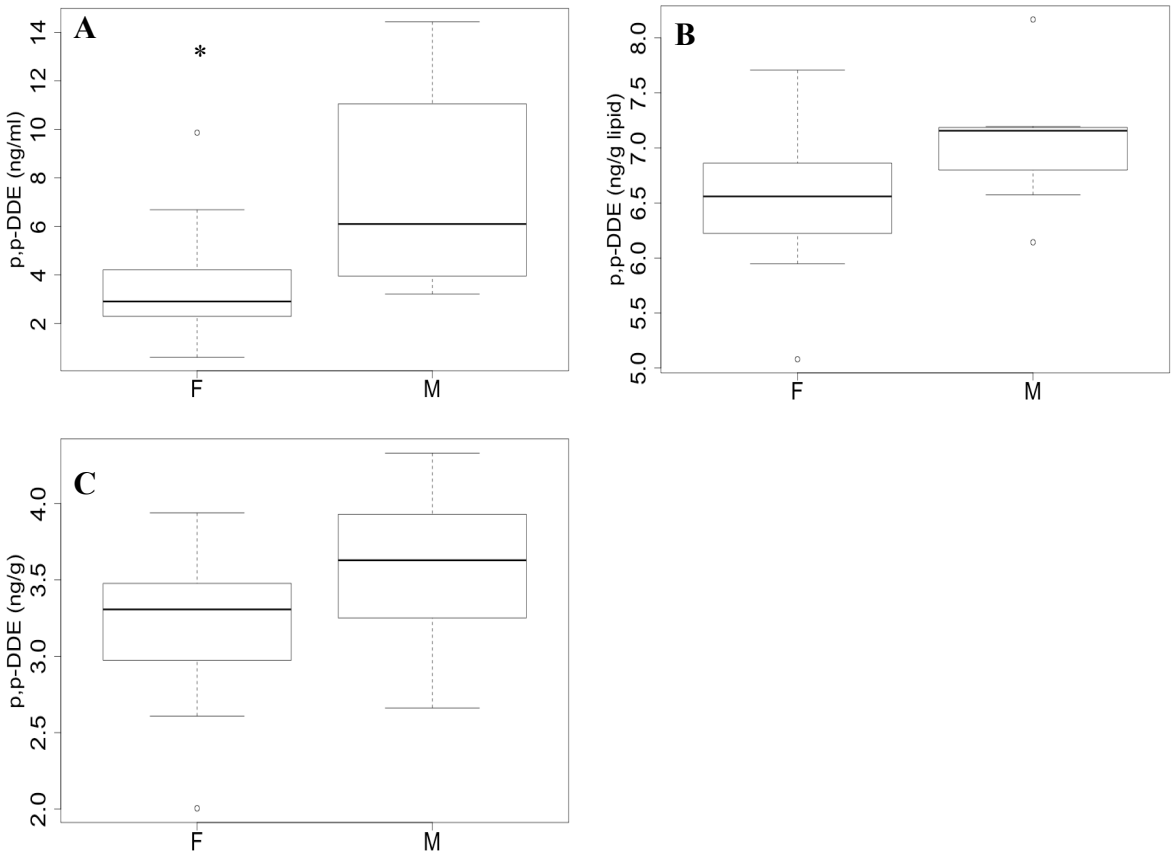

Figure 6. Levels of *p,p´*-DDE in (A) plasma (ng mL^{-1}), (B) preen oil (ng g^{-1} lipid) and (C) feather (ng g^{-1}) from female (**F**, n = 13) and male (**M**, n = 7) northern goshawks nestlings (n = 20) from Trøndelag. Boxplots represent median value (bold line) with upper 75th and lower 25th percentile, with whiskers showing the highest and lowest values within 1.5 times the interquartile range (upper and lower whiskers, resp.). Potential outliers (circles) are also shown. Significant difference between sexes denoted by * = $p < 0.05$.

Age significantly affected the variation in both CB 153 ($F_{18,1}$ = 11.2, p=0.003) and OxC ($F_{18,1}$ = 8.18, p=0.01) levels, with decreasing levels of the contaminants as the bird aged (see Figure 7). The best models of both CB 153 and OxC had AICc values that differed from the second best models by more than 2.0 (see Table SI-3 in Appendix B). Model parameters and test statistics for the best models explaining respectively CB 153 and OxC levels are shown in Table 5. The two best models explaining variation in HCB differed by just 0.8 in ΔAICc, and the simplest model had the lowest AICc (Table SI-3 Appendix B) where variation in HCB concentration was explained by age only (see Figure 7). The negative effect of increasing age on HCB was however not significant ($F_{18,1}$ = 3.9, p = 0.06). In the alternative model both age ($F_{18,1}$ = 4.75) and the interaction of CI and age ($F_{18,1}$ = 4.66) showed significant negative effects on HCB levels (both $p < 0.05$). Model parameters and test statistics for the two best models explaining HCB levels in plasma are shown in Table 6.

Table 5. Model statistics and back transformed parameter estimates for the significant effect of age (days) on respectively CB 153 (ng mL^{-1}) and OxC (ng mL^{-1}) plasma levels, estimated by linear regression. Significant effects are marked in bold. The sample size was n = 20 nestlings of northern goshawk. Contaminant parameters were transformed by natural logarithm (ln) to ensure normality, and age was mean centered.

Model:	lm(ln CB 153 ~ Age)	**R^2 = 0.35**	***p* = 0.003**	
Parameter	**Parameter estimate**	**Estimate SE**	***F-value***	***p***
Intercept	1.57	1.1		**< 0.01**
Age	0.84	1.0	**11.24**	**0.003**
Model:	lm(ln OxC ~ Age)	**R^2 = 0.27**	***p* = 0.01**	
Parameter	**Parameter estimate**	**Estimate SE**	***F-value***	***p***
Intercept	0.13	1.16		**< 0.01**
Age	0.86	1.17	**8.18**	**0.01**

Table 6. Model statistics and back transformed parameter estimates for the two best models showing the effect of age (days) and CI on HCB plasma levels, estimated by linear regression. The sample size was n = 20 nestlings of northern goshawk. Significant effects are marked in bold. Contaminant parameters were transformed by natural logarithm (ln) to ensure normality, age and CI were mean centered

Model:	lm(ln HCB ~ Age)	R^2 = 0.13	*p* = 0.06	
Parameter	**Parameter estimate**	**Estimate SE**	***F-value***	***p***
Intercept	-2.32	0.23		**< 0.01**
Age	-0.15	0.19	3.9	0.06
Model:	lm(ln HCB ~ Age+CI+Age:CI)	**R^2 = 0.27**	***p* = 0.04**	
Parameter	**Parameter estimate**	**Estimate SE**	***F-value***	***p***
Intercept	-2.19	0.21		< 0.01
Age	**-0.45**	**0.007**	**4.75**	**0.04**
CI	-0.0002	0.007	0.32	0.55
Age:CI	**0.004**	**0.002**	**4.66**	**0.03**

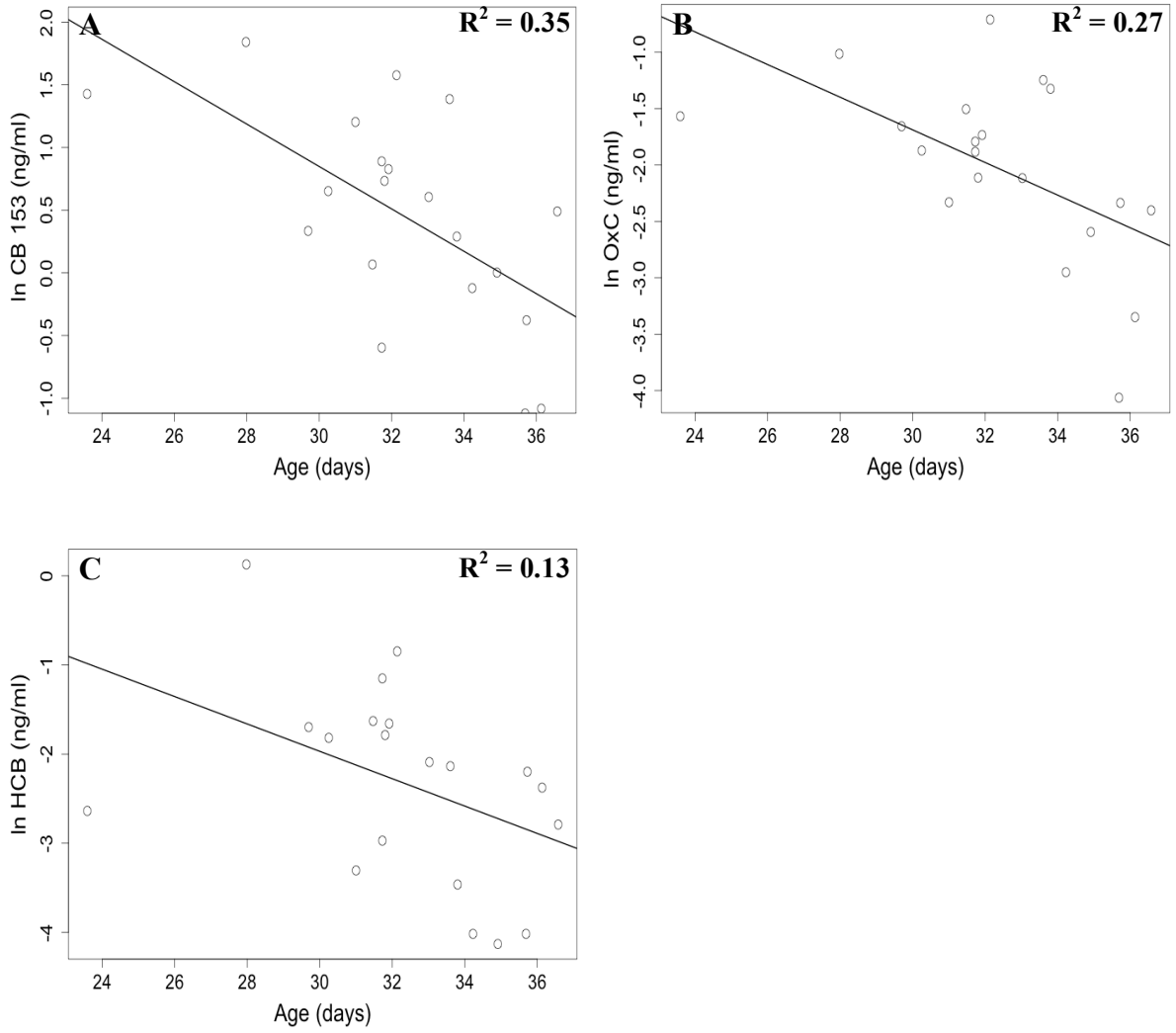

Figure 7. Linear regression with (A) CB 153 (R^2 = 0.35, p = 0.003), (B) OxC (R^2 = 0.27, p = 0.01) and (C) HCB (R^2 = 0.13, p = 0.06) in ng mL^{-1} plasma against age (in days) in northern goshawk nestlings (n = 20) from Trøndelag. Compounds were transformed by natural logarithm (ln) prior to analysis. R^2 denotes the adjusted R squared for the regression model.

3. 2. Enzyme activity

3. 2. 1. Glutathione reductase

No glutathione reductase activity was detected in the GR assay, as there was no difference in absorbance between the sample wells and the non-enzymatic wells, and no changes in absorbance during the six time intervals was observed for any sample (Figure SI-3 in Appendix A).

3. 2. 2. Glutathione peroxidase

The RBC levels of GPx ranged from 184 to 681 nmol min^{-1} mg^{-1} protein, with a mean concentration of 402 ± 28.6 nmol min^{-1} mg^{-1} protein. See Table SI-7 in Appendix B for listed enzyme activity per individual. The scores of PC1 showed a positive but non-significant effect on the activity of GPx (p = 0.15). The best regression models explaining GPx activity included sex as the only explanatory variable, except for the models with HCB, where the best model included only HCB (Table SI-4 in Appendix B). However, the variation in GPx levels was not significantly explained by any of the models that were tested, and the GPx activity did not differ significantly between sexes, as shown in Figure 8.A.

3. 2. 3. Catalase

Catalase levels ranged from 28.3 to 57.5 nmol min^{-1} mg^{-1} protein, with a mean concentration of 41.9 ± 1.4 nmol min^{-1} mg^{-1} protein. See Table SI-7 in Appendix B for listed enzyme activity for each individual. The scores of PC1 showed a positive but non-significant effect on the activity of catalase (p = 0.11). Variation in catalase levels was not significantly explained by age, CI or breath counts. Catalase activity in the northern goshawk nestlings differed significantly between sexes, with females having higher levels than males ($F_{1, 17}$ = 7.24; p = 0.014), as illustrated in Figure 8.B. The mean female level of catalase was 44.4 ± 1.27 nmol min^{-1} mg^{-1} protein, while mean male catalase level was 37.3 ± 1.2 nmol min^{-1} mg^{-1} protein. Both CB 153 ($F_{1,17}$ = 5.45; p = 0.03) and OxC ($F_{1,17}$ = 5.18, p = 0.03) were found to significantly affect the catalase activity in the nestlings, with an increase in catalase activity as the contaminant level increased (all $p < 0.05$), as illustrated in Figure 9. The effect of the contaminants did not differ between sexes, as the increases in catalase activity with increasing CB 153 and OxC were the same for both females and males, which also is shown by the parameter estimates for CB153 and OxC in Table 7.

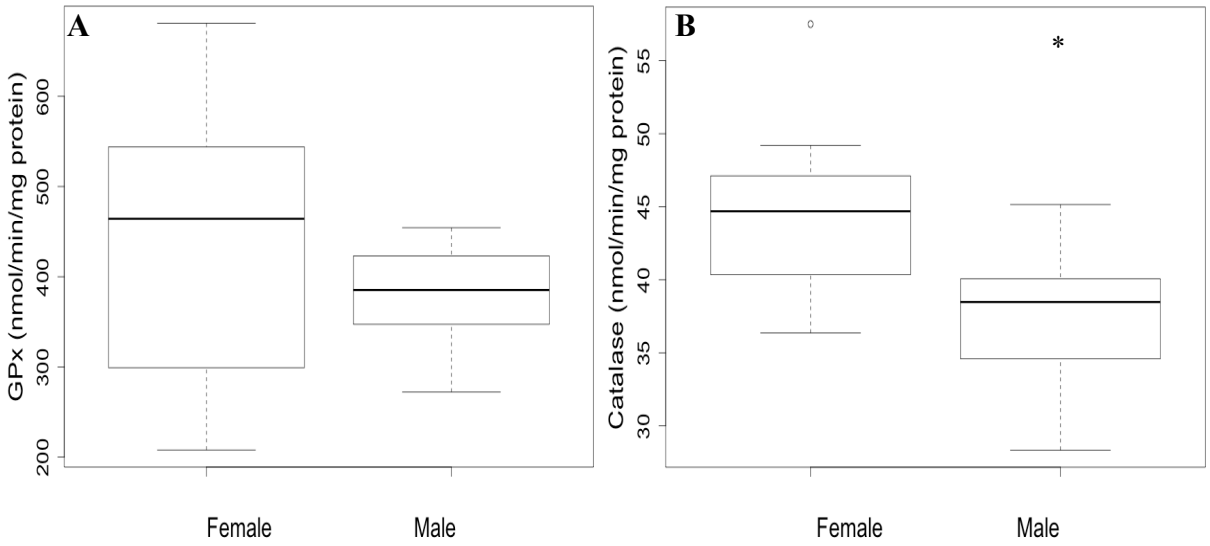

Figure 8. Levels of (A) GPx and (B) catalase (nanomol per min^{-1} per mg^{-1} protein) in red blood cells from female (n = 13) and male (n = 7) northern goshawk nestlings from Trøndelag. Box plots represent median value (bold line) with upper 75^{th} and lower 25^{th} percentile, with whiskers showing the highest and lowest values within 1.5 times the interquartile range (upper and lower whiskers, resp.). Potential outliers (circle) are also shown. Significant difference between sexes denoted by * = $p < 0.05$.

The tested models for variation in catalase activity explained by respectively CB 153 and OxC are listed in Table SI-5 in Appendix B. Model parameters and test statistic for the best regression models including the significant effect of respectively CB 153 and OxC on catalase activity in male and female goshawks are shown in Table 7.

Table 7. Model statistics and back transformed parameter estimates for the significant effect of respectively CB 153 and OxC on catalase level (nanomol per min^{-1} per mg^{-1} protein), estimated by linear regression. The sample size was n = 20 nestlings of northern goshawk. Compounds were transformed by natural logarithm (ln) to ensure normality.

Model:	lm(Catalase ~ sex + ln CB 153)	**R^2 = 0.39**	***p* = 0.005**	
Parameter	**Parameter estimate**	**Estimate SE**	***F-value***	***p***
Sex			**9.04**	**0.007**
Female	43.2	1.48		
Male	35.4	2.07		
CB 153	26.9	3.99	**5.45**	**0.03**
Model:	lm(Catalase ~ sex + ln OxC)	**R^2 = 0.38**	***p* = 0.005**	
Parameter	**Parameter estimate**	**Estimate SE**	***F-value***	***p***
Sex			**8.93**	**0.008**
Female	51.3	3.34		
Male	43.7	3.42		
OxC	27.8	4.31	**5.18**	**0.03**

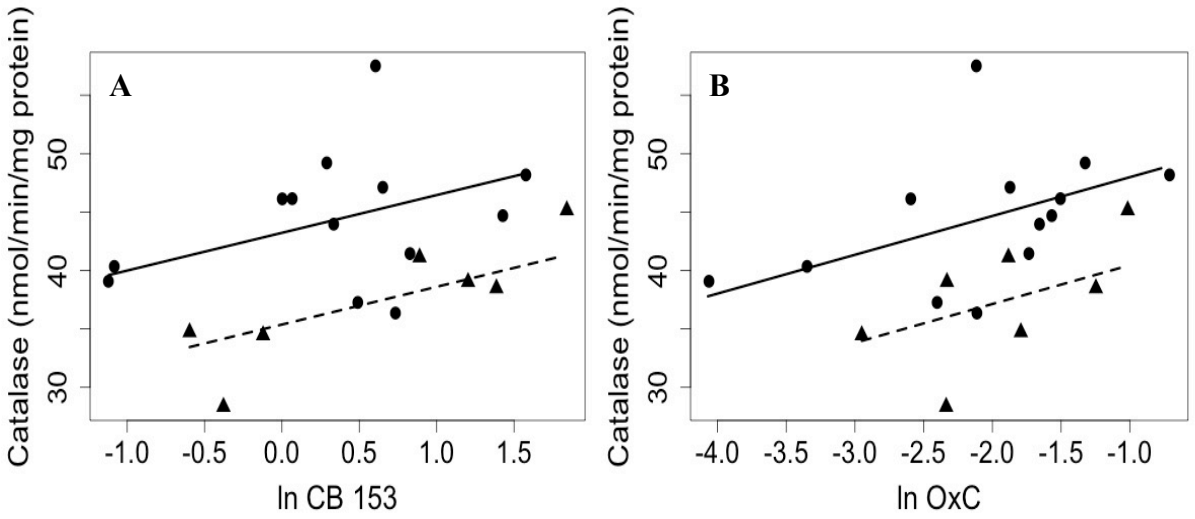

Figure 9. Significant increase in catalase activity (nanomol min^{-1} mg^{-1} protein) with increasing concentrations of (A) ln CB 153 (R^2 = 0.39, p = 0.005) and (B) ln OxC (R^2 = 0.38, p = 0.005) in female (circles, solid line) and male (triangle, dash line) goshawk nestlings (n = 20) from Trøndelag. The contaminants were transformed by natural logarithm (ln) prior to analysis.

4. DISCUSSION

In the present study, most of the targeted POPs were detected in the northern goshawk nestlings, confirming the exposure of the birds to these contaminants in Trøndelag. Plasma and preen oil contained the widest ranges of detected compounds, where preen oil content reflected accumulated contaminants and plasma content the present state of contamination in the birds (Eulaers et al., 2011b, Espín et al., 2016, Henriksen et al., 1998).

4. 1. Organic pollutants

4. 1. 1. Levels of emerging contaminants

4. 1. 1. 1. Novel brominated flame retardants

The low detection of the targeted nBFRs in all three matrices indicates that the presence of these compounds is low in nestlings of the northern goshawk from Trøndelag, probably due to their lower production and usage than PBDEs. This finding is in agreement with the previous, and relatively few, studies on these compounds in birds of prey. Eulaers et. al. (2014a) did not detect any of the targeted nBFRs in more than 50 % of plasma samples from white-tailed eagle nestlings, and feather levels were low. Verreault et al. (2007) detected BTBPE in plasma and eggs of glaucous gulls, but the compound was only found in the plasma of one individual, and contributed very little to the total load of brominated contaminants in the egg yolk. It was concluded that even though the compounds undergo LRT, their occurrence in the marine birds seems to be low. BTBPE was also detected in eggs and plasma of northern fulmars (*Fulmarus glacialis*) from the Faroe Islands, but the levels were 150 times lower than the levels of the traditional PBDEs reflecting the lower lipophilicity of the compound, and the lower usage compared to the traditional BFRs (Karlsson et al., 2006). The detection frequencies in the northern goshawks in the present study were even lower than in the previous studies on marine birds, as no detections of nBRFs were made in plasma. It therefore seems like the results in the present study are in line with previous findings, showing that the exposure levels of nBFRs in raptors are low due to the lower production levels of these contaminants. In addition, it might not be that nBFRs will increase in the future, as many other substitutes including OPFRs are available.

4. 1. 1. 2. Organophosphate flame retardants

The detected OPFR levels in feathers did not reflect levels in plasma, nor preen oil in the present study, as detection frequencies in both plasma and preen oil were below 0.50. Compared to the other detected compounds in the feathers, ΣOPFRs exceeded the levels of legacy BFRs, as well as both ΣPCBs and *p,p´*-DDE. As OPFRs have higher production and usage volumes compared to BFRs this might be expected (van der Veen and de Boer, 2012). Previous studies on herring gulls showed that targeted OPFRs were not detectable in plasma or liver, and that OPFRs seemed to be quickly metabolized in the birds, with fat and egg yolk levels reflecting deposition of metabolic residues (Greaves and Letcher, 2014). The theory of a rapid OPFR metabolism was supported by Su et al. (2014), who detected a higher level of OPFR metabolites compared to the levels of their precursor flame retardant in plasma of herring gulls. It could be that the OPFRs were quickly metabolized in the goshawks in the present study, but it could also be that the detected OPFRs in feathers were originating from another source than the nestlings, such as atmospheric deposition. High OPFR concentrations in feathers were also found in body feathers of Norwegian white-tailed eagle nestlings (Eulaers et al., 2014a). Eulaers et al. (2014a) detected only two OPFRs in plasma, but no significant associations between feather and plasma levels were found. It was therefore stated that feather levels of OPFRs most likely originated from atmospheric deposition. The median levels in the goshawk feathers in the present study [TCEP: 12.9 (4.6 – 30.7), TPhP: 4.4 (0.95 – 22.3), EHDPHP: 2.5 (0.75 - 23.4), ΣTCIPP: 26.8 (1.75 – 60.4)] were however considerably lower than the median levels detected in the white-tailed eagles [TCEP: 110 (14 - 3000), TPhP: 15 (5.9 - 250), EHDPHP: 12 (5.4 - 25), TCIPP: 91 (14 - 220)], which might be due to geographical and temporal differences in atmospheric contaminant levels between Tromsø and Trøndelag. In the present study TCIPP was the most dominant compound, followed by TCEP. These two compounds are the OPFRs that have been reported as most abundant in air, supporting the theory that feathers might act as passive samplers of atmospheric OPFRs (van der Veen and de Boer, 2012, Reemtsma et al., 2008). The results thereby suggested that OPFRs in feathers reflected external deposition from the atmosphere, and not internal levels in the goshawk.

4. 1. 2. Levels of legacy POPs

Contrary to the emerging contaminants, most targeted legacy POPs were detected in all of the three matrices investigated in the current study, where the most prominent POPs were *p,p´*-DDE and the PCBs. These compounds have commonly been reported as the dominant POPs in terrestrial predatory birds (Jaspers et al., 2006a, Yu et al., 2013).

4. 1. 2. 1. Chlorinated contaminants and PCB profiles

The persistence and clearance rate of PCBs in terrestrial birds of prey has been shown to be dependent on both the number and structure of chlorine substitutions on the molecule, as well as on how lipophilic the congener is (Drouillard et al., 2001). The pattern of detected congeners in the current study is in line with previous literature on the bioaccumulation potential of PCBs in juvenile terrestrial birds of prey (Drouillard et al., 2007). CB 153 was the dominating PCB congener in all three matrices, followed by CB 138 and CB 180 in plasma and preen oil, whilst CB 138 was more abundant than CB 180 in feathers. This pattern of the most abundant congeners in plasma has also been found in previous studies on Norwegian northern goshawk nestlings (Eulaers et al., 2011a, Sonne et al., 2010), as well as in liver and muscle of adult terrestrial raptors from Belgium (Jaspers et al., 2006a). These findings indicate that the goshawk nestlings metabolize and accumulate PCBs in the same pattern as adult raptors. This has previously been shown for nestlings of American kestrels (*Falco sparverius*) exposed to PCBs (Drouillard et al., 2007). The congener levels in the present study are however considerably lower than the levels in the adult raptors, most likely partly due to the different types of sample tissues, as Jaspers et al. (2006a) investigated muscle and liver samples, not plasma as in the present study. Other factors that can explain this difference are the different locations in the studies, as well as the nestlings having had a shorter time to accumulate the contaminants compared to adult birds. The mean ΣPCBs in plasma [7.23 (1.0 - 23.4) ng mL^{-1}] and feathers [9.90 (0.05 - 8.01) ng g^{-1}] in the present study were lower than the mean plasma and feather levels in northern goshawk nestlings from northern Norway, reported by Eulaers et al. (2011a) [plasma: 11.6 (2.76 - 42.2) ng mL^{-1}, feather: 39.7 (6.7 - 140) ng g^{-1}]. It should be noted that ΣPCBs reported by Eulaers et al. (2011a) contained common but fewer congeners than the ΣPCBs in the present study, and the levels were still higher in the previous study compared to the present.

p,p´-DDT was only detected in plasma, most likely reflecting recent uptake of the contaminant (Henriksen et al., 1998). As *p,p´*-DDE is more persistent than *p,p´*-DDT, it was expected that *p,p´*-DDE would be detected in all matrices (Vorkamp et al., 2009, Kelce et al., 1995). The mean plasma levels of *p,p´*-DDE in the present study [5.02 (0.61 - 14.4) ng mL^{-1}] appeared to be similar to the mean plasma levels from 2010 in northern goshawk chicks from northern Norway reported by Sonne et al. (2012) [4.8 (1.8 - 10) ng mL^{-1}]. As for ΣPCBs, the mean plasma and body feather [31.8 (7.4 - 75.9) ng g^{-1}] levels of *p,p´*-DDE in the present study were both remarkably lower than the mean detected levels in northern goshawk

nestlings [plasma: 9.17 (1.77 - 24.7) ng mL^{-1}, feather: 43.9 (7.31 - 145) ng g^{-1}] reported by Eulaers et al. (2011a).

OxC was the only chlordane detected in all matrices, while plasma contained all three targeted chlordanes: OxC, TN and CN. HCB [0.18 ± 0.05 ng mL^{-1}] was detected in similar mean concentrations as OxC [0.17 ± 0.03 ng mL^{-1}] in plasma in the present study, whilst feathers and preen oil both seemed to accumulate higher concentrations of HCB [feather: 0.23 ± 0.05 ng g^{-1}, preen oil: 11.7 ± 1.81 ng g^{-1} lipid] compared to OxC [feather: 0.16 ± 0.02 ng g^{-1}, preen oil: 6.69 ± 0.98 ng g^{-1} lipid]. The mean HCB plasma level in the present study [0.18 (0.01 - 1.13) ng mL^{-1}] was lower than the mean level detected in plasma from northern goshawk nestlings reported by Eulaers et al. (2011a) [0.61 (0.15 - 2.25) ng mL^{-1}] and Sonne et al. (2012) [0.9 (0.36 - 1.3) ng mL^{-1}], which were sampled in respectively 2008 and 2010. This is in line with the trend that was also seen for ΣPCBs and *p,p´*-DDE. These differences could be due to differences in geographical location and diet, or it could be possible that this difference reflects a decrease in environmental levels of *p,p´*-DDE and PCBs since 2008 and 2010, when the nestlings in the studies by respectively Eulaers et al. (2011a) and Sonne et al. (2012) were sampled. The only HCH isomer that was detected in all tissues was *β*-HCH, which has previously been reported as the dominant isomer of HCH in tissues of marine predatory bird nestlings (Olsson et al., 2000). In the study by Eulaers et al. (2011a), *β*-HCH was the only isomer that was detected in northern goshawk nestlings, and it was only detected in feathers. The mean feather concentration of *β*-HCH reported by Eulaers et al. (2011a) [0.30 (0.11 - 1.37) ng g^{-1}] was similar to the detected mean level in the present study [0.34 (0.08 - 1.12) ng g^{-1}].

4. 1. 2. 2. Brominated contaminants and PBDE profiles

The mean ΣPBDEs levels in plasma and feathers in the present study [plasma: 0.27 (0.02 - 0.67) ng mL^{-1}, feather: 1.68 (0.31 - 6.58) ng g^{-1}] were slightly lower than the levels detected in northern goshawk nestlings by Eulaers et al. (2011a) [plasma: 1.04 (0.02 - 3.97) ng mL^{-1}, feather: 3.54 (1.01 - 7.56) ng g^{-1}]. The ΣPBDEs reported by Eulaers et al. (2011a) did not contain BDE 153 as in the present study, but included BDE 49 in feathers, and the levels in the present study were still lower. The contributions of BDE 47 were lower in the metabolically active plasma and preen oil, compared to feathers. Higher contribution of BDE 47 in feathers compared to plasma has previously been found for both nestlings of northern goshawk and adult terrestrial raptors from Belgium (Jaspers et al., 2006a, Eulaers et al.,

2011a). This is most likely due to the higher halogenated PBDEs being metabolized to BDE 47 in the blood, before deposition and accumulation in feathers. In exposure studies on juvenile American kestrels, BDE 47 was metabolized and excreted in similar ways as the less persistent PCBs (Drouillard et al., 2007). In the present study, the higher brominated BDE 183 was detected in preen oil and in very low levels in plasma. This was as expected since higher brominated congeners are more lipophilic and persistent, as shown in the study by Drouillard et al. (2007). BDE 183 has previously been found in higher levels in liver and muscle tissue of terrestrial compared to marine raptors (Jaspers et al., 2006a), which might be further confirmed by the detection of BDE 183 in plasma and preen oil in the current study.

4. 1. 2. 3. Factors affecting POP levels in northern goshawk nestlings

HCB was the only contaminant of the five investigated POPs that was found to be significantly associated with body condition. However, the interaction effect of CI and age was very small with a relatively high standard error (0.004 ± 0.002), indicating that this interaction was not of great importance. Also, the effects of CI or age alone were not significantly associated with HCB, suggesting that variation in HCB was not explained well by any of the two best models. It should be kept in mind that a body condition index, based on the relationship between mass and body length measurements, not necessarily reflects the overall fitness of the individual. Additionally, mass might not be correlated with the lipid content, which also might not be the best predictor for fitness, as assumed by many indices (Wilder et al., 2016). It has also been shown that condition indices fail to reflect rapid changes that potentially affect overall fitness, such as increasing levels of POPs by lipid mobilization due to short-term starvation (Marteinson et al., 2016). The CI used in the present study has however been shown to be a good predictor of energy reserves in a wide range of animals, including birds (Peig and Green, 2009). The scaled mass index was also shown to better account for sexual dimorphism and age compared to conventional condition indices, enabling comparison between sexes and growing individuals (Peig and Green, 2010).

Plasma *p,p´*-DDE levels differed significantly between the sexes, with males having higher levels than females. As preen oil and feather levels of *p,p´*-DDE did not differ between sexes, the observed difference in plasma *p,p´*-DDE levels might be due to the relatively small sample size. This theory is supported by the relatively high variation in plasma levels of *p,p´*-DDE in the female goshawks. It has previously been shown that lower levels in females are attributed to transfer of chlorinated contaminants to eggs (Bustnes et al., 2008). However, as

the sampled individuals in the present study are juveniles, this cannot be the mechanism causing the observed differences in *p,p´*-DDE levels.

Plasma levels of CB 153 and OxC decreased with increasing nestling age in the present study; this indicated that the detected level of PCBs might reflect residues transferred from the mother to the egg. This decrease in contaminant level with increasing growth is referred to as growth dilution (Bustnes et al., 2013b). This observation was also found for northern goshawk nestlings by Bustnes et al. (2013b), where CB 153 in plasma was declining as the nestlings approached the age of fledging, reflecting the effect of maternal POP transfer, resulting in the observed growth dilution. Dauwe et al. (2006) also found that levels of persistent organohalogen contaminants (OHCs) in 15-day old nestlings of great tits most likely reflected levels from the eggs and not the diet, highlighting that the maternal source of contamination should be taken into consideration when using nestlings to assess local contamination. Unlike CB 153 and OxC, plasma levels of the highly detected *p,p´*-DDE did not seem to be negatively affected by age in the present study, and the level of this contaminant might therefore reflect dietary input. It has previously been shown that *p,p´*-DDE concentrations are closely related to trophic level and dietary proxies in northern goshawk nestlings, illustrated by an increase in plasma levels during growth until fledging (Bustnes et al., 2013b). The plasma levels of *p,p´*-DDE in the present study were however not found to be associated with age, rendering it difficult to say anything about the accumulation of the contaminant in the nestlings over time.

4. 1. 3. Nestling feathers – a useful tool in biomonitoring of OHCs

The POP profiles in feathers showed higher accumulations of lower halogenated PCBs and PBDEs compared to plasma and preen oil in the present study. This pattern was also found in feathers from northern goshawk nestlings by Eulaers et al. (2011a). Differences in congener pattern can be due to the different metabolic capacities of the matrices; as feathers are metabolically inactive, compounds will not be further metabolized in this matrix (Eulaers et al., 2011a). The contaminant load in feathers is affected by several pathways of exposure, as stated by Jaspers et al. (2008). Internal deposition via the blood stream and external deposition via preening and the environment all ultimately result in the pattern of contaminants observed in the feathers. As OPFRs were only detected in feathers, it is likely that some contaminant detections are associated with aerial depositions (Eulaers et al., 2014a). In profiles of total POP load, *p,p´*-DDE was the dominant POP in both preen oil and

feathers, while the ΣPCBs contributed more to the total POP load in plasma. This pattern was also found in northern goshawk nestlings from northern Norway (Eulaers et al., 2011a). In the present study, feather levels of the three main POP groups (ΣPCBs, *p,p´*-DDE and ΣPBDEs) showed strong and positive correlations with preen oil. This indicated that the detection of these POPs reflects an exogenous source due to preening, not aerial deposition, which was also found by Jaspers et al. (2008) The congener composition in feathers was also more similar to preen oil than plasma, as both exhibited a larger contribution of lower halogenated PCB congeners compared to the blood.

ΣPCBs, *p,p´*-DDE, and ΣPBDEs levels in feathers were all significantly and positively correlated with plasma levels. This result reflects the existing connection of the nestling feathers with the blood, which also enables the accumulation of POPs in feathers from the onset of feather growth until sampling (Eulaers et al., 2011a, Van den Steen et al., 2007). The accumulation of contaminants over time is reflected in most POPs being detected in higher levels in feathers than in plasma in the present study. This finding clearly shows the usefulness of nestling body feathers as a biomonitoring tool of POPs. This result also confirms the suitability of using nestlings as bio-indicators of POP exposure and accumulation in terrestrial food chains.

4. 1. 4. Non-destructive sampling – blood and preen oil

In the present study, both plasma and preen oil contained a higher number of detected legacy POPs (PCBs, PBDEs, organochlorine pesticides) than feathers, indicating their suitability in biomonitoring of these organic contaminants. The higher concentrations of POPs in preen oil compared to plasma and feathers, are due to the higher lipid percentage of preen oil. This makes it a suitable matrix for accumulation of lipophilic contaminants (Kocagoz et al., 2014). The higher percentage of lower halogenated PCBs and PBDEs in preen oil compared to plasma has been observed in other studies on raptors (Jaspers et al., 2013). This pattern could be due to the lower metabolic capacity in preen oil (Yamashita et al., 2007, Eulaers et al., 2011b). This would result in preen oil accumulating PCBs with less opportunity to be metabolized than in plasma, leading to the observed differences in POP profiles between the two matrices. Differences in metabolic capacity between matrices can also explain why levels of OxC and β-HCH in feathers were not found to correlate with plasma nor preen oil levels. It can also explain why HCB levels in preen oil did not correlate with plasma nor feather levels. As these contaminants occurred in much lower levels than ΣPCBs, *p,p´*-DDE and

ΣPBDEs, it would also be expected that differences in matrix metabolism would be more prominent for these contaminants compared to the dominating POP groups (Eulaers et al., 2011b, Yamashita et al., 2007).

How the preen oil levels in the northern goshawk nestlings are associated with internal organ levels in the present study is not known, as only non-destructive sampling was performed on the goshawks. However, the three most prominent groups of POPs (ΣPCBs, *p,p′*-DDE and ΣPBDEs) in preen oil were significantly and positively correlated with plasma levels. This finding indicated that preen oil reflected the recent exposure to these contaminants in the northern goshawk nestlings, as predicted. This also showed that preen oil could be a good non-invasive biomonitoring tool, as previously stated by Espín et al. (2016). In the present study, it was however experienced that sampling preen oil from nestlings can be challenging, as it was difficult to obtain preen oil from some of the birds. This was likely due to the goshawks not being fully developed, thereby producing less preen oil than adult birds (Eulaers et al., 2011b). This also underlines one of the practical differences by using nestlings compared to adult birds, as well as using preen oil compared to feathers, as feathers were easier to obtain.

4. 2. Oxidative stress in northern goshawk nestlings

4. 2. 1. Enzyme activity and effects of legacy POPs

Of enzymatic antioxidants, both CAT and GPx were detected in red blood cells of northern goshawk nestlings in the present study. Red blood cells contained higher levels of GPx activity compared to CAT, this was also found in hepatic tissues from black-legged kittiwake chicks by Hegseth et al. (2011b). This difference in activity could be due the fact that GPx has a wider range of substrates compared to catalase, which may lead to differences in their levels (Mates, 2000). As hypothesized in the current study, blood enzyme activity was found to be associated with plasma levels of POPs, as CAT activity increased significantly with increasing levels of CB 153 and OxC. This finding suggests that the detected levels of these organic contaminants might in fact induce oxidative stress in nestlings of northern goshawk. PCBs have previously been found to cause oxidative stress in nestlings of white-tailed eagles (Sletten et al., 2016) as well as chicks of seabirds (Hegseth et al., 2011b). In the study by Hegseth et al. (2011b), both GPx and CAT levels were found to increase with increasing PCB levels in seabird chicks. The GPx activity was however not affected by any of the POPs or factors investigated in the present study. It should be noted that Hegseth et al. (2011b)

investigated hepatic levels of enzymes and contaminants, which will likely contain higher levels than blood.

The results of the present study show that also in the terrestrial food chain, young birds accumulate sufficient levels of POPs to affect the homeostatic balance of some enzymatic antioxidants, most likely due to the production of ROS. As nestlings are growing, an elevated consumption of food and oxygen can increase the potential for ROS production as well as enzyme levels (Hegseth et al., 2011b). As ΣPCBs was the major contributor to the total POP load in plasma in the present study, and ΣPCBs was dominated by CB 153, the effect on enzyme levels by CB 153 in nestling blood might be expected. The mean plasma level of OxC in the present study was lower than the mean CB 153 level, this might suggest that OxC is relatively potent in affecting catalase activity compared to CB 153. Although most previous studies seem to have focused on the effect of PCBs on avian oxidative stress, OxC has been shown to be associated with a decrease of hepatic levels of superoxide dismutase (SOD), another enzymatic antioxidant, in chicks of white-tailed eagle (Sletten et al., 2016). Sletten et al. (2016) also found that SOD was negatively correlated with increased PCB, HCB and *p,p´*-DDE concentrations, demonstrating the potential effect of HCB and *p,p´*-DDE on other antioxidants not investigated in the present study. OxC was also found to negatively affect telomere length in blood samples from adult female kittiwakes, possibly by the mechanism of oxidative stress (Blévin, 2016). OxC was however not found to affect telomere length in white-tailed eagle nestlings (Sletten et al., 2016), which might be due to the lower contaminant levels in the nestlings compared to the adult kittiwakes (Blévin, 2016). In another study, Hegseth et al. (2011a) showed that the effect of PCBs on GPx and CAT in exposed chicks was only present in fasting birds, where the enzyme level decreased as the PCB levels increased. This indicates the presence of a toxic limit of contaminant exposure, where the compensating increase in enzyme activity can no longer balance out the ROS (Sletten et al., 2016). It also highlights the importance of how different stress factors in the environment might act in synergism, further complicating the oxidative stress response in free ranging birds. As the enzyme activity in red blood cells in the present study was found to increase with contaminant concentrations in plasma from the northern goshawks, the potential toxic limit in these individuals was most likely not yet reached. It is however important to keep in mind that plasma only offers a snapshot of the current state of the nestling, although it has been found that contaminant levels in plasma correlate well with internal tissues in adult birds of prey (Jaspers et al., 2013).

It should be kept in mind that the levels of both OxC and CB 153 were found to decrease with increasing nestling age in the current study, indicating that maternal transfer might be the main source of these contaminants in the nestlings. It might therefore not be dietary input of POPs that cause oxidative stress, but rather contaminants originating from the eggs. This finding might also suggest that oxidative stress could be higher at a younger age and decreases with age, if the contaminant levels decrease as the bird grows. For the nestlings in the present study, enzyme activity was not significantly associated with age, but for even younger birds than in the current study it might be the case based on the present findings. It has previously been noted that maternal transfer is one aspect in using nestlings that can reduce their usefulness as sentinel bio-indicators of local pollution (Dauwe et al., 2006).

Although CB 153 and OxC were the only two of the five contaminants that were found to significantly affect the enzyme levels, the detection of a broad range of POPs in the nestlings should be taken into consideration when evaluating the potential effects on oxidative stress. Considering that these individuals were growing, and possibly accumulating the compounds that were not currently declining with age in the present study (*p,p´*-DDE, BDE 99 and HCB), the total POP load can potentially exert a joint negative effect in the individual over time. Although BDE 99 was not found to significantly affect the enzyme levels at the time of sampling, the total accumulation of PBDEs and other POPs could affect the ROS levels over time, especially if the northern goshawk accumulates higher levels of the higher brominated and persistent PBDEs than marine raptors (Jaspers et al., 2006a).

The fact that GR was not detected was unexpected, as both GPx and GR are needed to complete the repeated cycle of glutathione reduction of ROS (Mannervik, 1987). GR has previously been detected in considerably lower levels than both GPx and CAT in liver tissues from chicks of different seabird species from Norway (Hegseth et al., 2011b) and in lower levels than CAT in lung tissue of great tits (*Parus major*) (Isaksson et al., 2009). This might suggest that the GR levels in the northern goshawk also could be low, if present. It could also be possible that the reaction of GR with hydrogen peroxide occurred too rapid for the assay to detect the activity, a potential source of error that would be strengthened by low levels of GR. Since the baseline levels of the different enzymes are not well known for goshawks or their nestlings, it is difficult to make a statement regarding the differences in enzyme levels in the present study.

4. 2. 2. Confounding factors

There is a wide range of factors that can potentially affect the enzymatic responses of a bird to exposure of contaminants. As found in the present study, enzyme levels can differ between sexes, as female nestlings exhibited higher levels of catalase compared to males. This is most likely due to the sexual dimorphism in northern goshawks, where females become larger than males and thereby are in need of more food. The higher food intake can lead to a higher metabolic turnover than in males, resulting in higher levels of ROS and thereby higher enzyme levels (Martinez-Padilla et al., 2004). Other factors that have been shown to affect the level of oxidative stress in birds are age (Costantini et al., 2006), parasite load and sibling competition (Martinez-Padilla et al., 2004, Costantini et al., 2006) as well as differences in dietary habits (Cohen et al., 2009). Differences in avian biochemistry can also lead to species differences in sensitivity and enzymatic responses to contaminant exposure (Jin et al., 2001). The comparisons between the present study and findings in marine birds should therefore be made with these factors in mind, as both biochemistry and diet will differ greatly between the species. Relatively high intra- and interplate coefficients of variation (CV) (CV % intraplate: 13.5 ± 8.7, CV% interplate: 18.1 ± 6.8) were observed for the catalase levels within the same individual, this should also be kept in mind when interpreting the results, as this could be a source of error in the current study.

5. CONCLUSIONS

In the present study, most of the targeted legacy POPs were detected in nestlings of the northern goshawk, proving that nestlings of this terrestrial raptor species are exposed to these contaminants in Trøndelag. The targeted nBFRs were generally not detected, indicating that exposure of the goshawk to these emerging contaminants is of less concern than to legacy POPs at the present time. However, as the nestlings seemed to reflect exposure to legacy BFRs well, their use in further biomonitoring of the nBFRs could be of importance. OPFRs were detected in feathers, but showed a low detection frequency in plasma and preen oil. This suggested that the feather levels of OPFRs originate mainly from the atmosphere, as found in previous studies on Norwegian raptor nestlings. Body feathers reflected plasma and preen oil levels of the most prominent POPs (ΣPCBs, *p,p′*-DDE and ΣPBDEs), proving their suitability as biomonitoring tools of these contaminants, as hypothesized. Regarding the oxidative stress parameters, GR was not detected in red blood cells, whilst GPx and catalase were detected. Catalase levels differed significantly between sexes, with higher enzyme activity in female goshawks. Catalase was the only enzyme that was associated with contaminant levels, with a significant increase in enzyme activity as CB 153 and OxC levels increased in plasma. These findings indicate that these compounds are associated with oxidative stress in the nestlings, as predicted in the current study. Plasma levels of CB 153 and OxC were however found to decrease by growth dilution, and as no increase in plasma levels of these contaminants was observed, it is likely that CB 153 and OxC originated from maternal transfer rather than dietary input.

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APPENDIX A

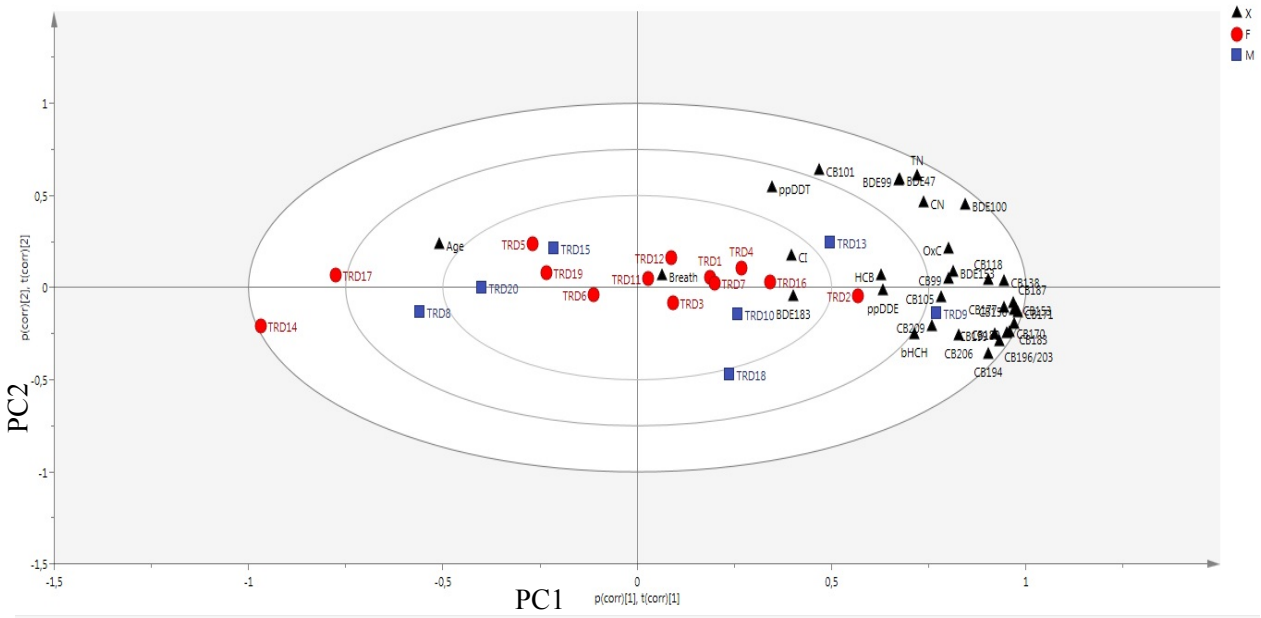

Figure SI-1. Principal component analysis illustrating the combined score plot of females (red circles) and males (blue squares) with loading plots (black triangles) of the detected POPs in plasma with age, breath count and condition index (CI) from northern goshawk nestlings (n = 20) from Trøndelag. Principal component 1 and 2 explained respectively 63.7 % and 9.0 % of the variation in the data set

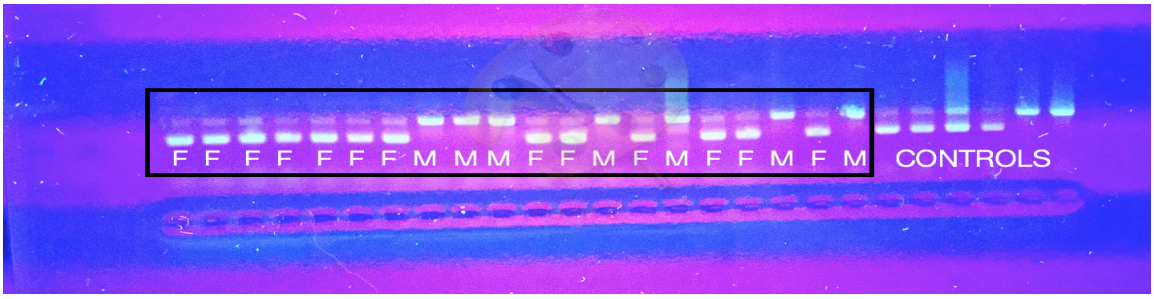

Figure SI-2. SYBR® Safe stained agarose gel, showing cular sex determination of northern goshawk nestlings (n = 20) from Trøndelag, by gelelectrophoresis of amplified CHDZ and CHDW genes extracted from red blood cells. Sex was determined by two visible lines for females (**F**, ZW heterozygote) and one visible line for males (**M**, ZZ homozygote). Samples are in well 1 – 20 (black box), with four female and two male controls at the end (marked controls).

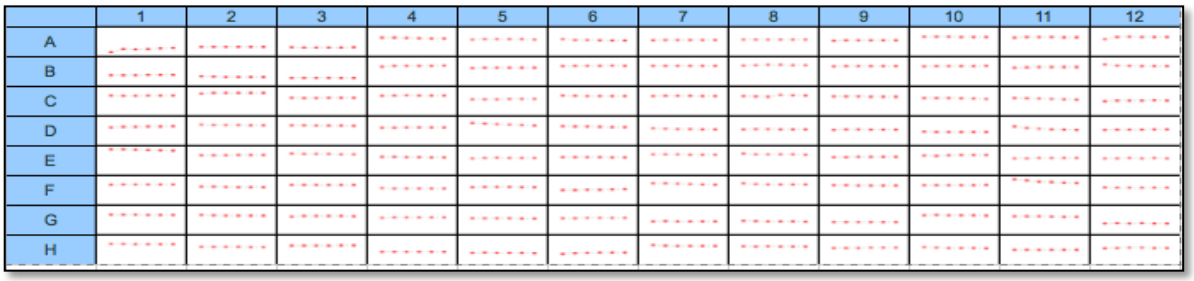

Figure SI-3. Absorbance measured at 340 nanometers at six time points (one minute intervals) in a 96 well plate with diluted red blood cells samples from northern goshawk nestlings (n = 20) investigated for glutathione reductase activity. Well A1-A3 and B1-B2 represent non-enzymatic wells with no sample added. No change in absorbance was detected.

APPENDIX B

Table SI-1. Non detected compounds (DF = 0) in plasma, feathers and preen oil from northern goshawk nestlings (n = 20) from Trøndelag.

Plasma	Preen oil	Feather
CB 28	CB 28	CB 28
CB 49	CB 49	CB 49
CB 52	CB 52	CB 52
CB 74	CB 74	CB 74
CB 95	CB 95	CB 95
		CN
α-HCH	*α*-HCH	*α*-HCH
BDE 28	BDE 28	BDE 28
		BDE 183
TBB	TBB	TBB
BTBPE		BTBPE
TBPH	TBPH	
TBEP	TBEP	TBEP
TCPP2	TCPP2	s-DP
	EHDPHP	a-DP

Table SI-2. Compounds with detection frequency (DF) less than 0.50 in plasma, feather and/or preen oil samples from northern goshawk nestlings (n = 20) from Trøndelag.

Plasma		Preen oil		Feather	
Compound	**DF**	**Compund**	**DF**	**Compound**	**DF**
γ-HCH	0.05	CN	0.35	CB 171	0.30
BDE 154	0.30	BDE 154	0.45	CB 156	0.35
s-DP	0.05	*p,p´*-DDT	0.30	CB 199	0.10
a-DP	0.10	BTBPE	0.05	CB 196/203	0.25
TCEP	0.05	s-DP	0.45	CB 194	0.15
TPhP	0.10	TCEP	0.05	CB 206	0.05
TDCPP	0.05	TPhP	0.10	CB 209	0.05
TCPP1	0.10	TDCPP	0.20	BDE 154	0.05
		TCPP1	0.20	*p,p´*-DDT	0.15
				TN	0.25
				TBPH	0.40

Table SI-3. Model selection by small sample size adjusted Akaikes information Criteria (AICc) of regression models explaining variation in level of five different POPs (CB 153, *p,p´*-DDE, OxC, BDE 99 and HCB) in plasma of northern goshawk nestlings (n = 20). Models included the effect of sex, age and CI (body condition index) on compound levels. Compounds were transformed by natural logarithm (ln). The best models explaining a significant effect on compound level are marked in bold.

POP	Model ID	Model	AICc	ΔAICc	AICcWt
CB 153	**1**	**CB 153 ~age**	**46.5**	**0**	**0.7**
	2	CB 153 ~sex+age	48.9	2.4	0.21
	3	CB 153 ~sex+age+CI	51.0	4.5	0.07
	4	CB 153 ~sex+age+CI+CI:age	54.7	8.2	0.01
	5	CB 153 ~sex+age+CI+sex:age+CI:age	58.7	12.2	0
	6	CB 153 ~sex+age+CI+sex:CI+sex:age+CI:age	64.0	17.5	0
	7	CB 153 ~sex*age*CI	66.2	19.7	0
p,p´-DDE	**1**	***p,p´*-DDE ~sex**	**47.2**	**0**	**0.7**
	2	*p,p´*-DDE ~sex + CI	49.3	2.1	0.25
	3	*p,p´*-DDE ~sex + age + CI	52.6	5.4	0.05
	4	*p,p´*-DDE ~sex+age+CI+age:sex	55.9	8.7	0.01
	5	*p,p´*-DDE ~sex+age+CI+sex:age+CI:age	60.5	13.3	0
	6	*p,p´*-DDE ~sex+age+CI+sex:CI+sex:age+CI:age	66.2	18.9	0
	7	*p,p´*-DDE ~sex*age*CI	70.8	23.7	0
OxC	**1**	**OxC ~age**	**46.6**	**0**	**0.71**
	2	OxC ~age + CI	49.7	3.1	0.15
	3	OxC ~sex+age+CI	50.6	3.9	0.1
	4	OxC ~sex+age+CI+age:CI	53.1	6.5	0.03
	5	OxC ~sex+age+CI+age:CI+sex:age	55.4	8.8	0.01
	6	OxC ~sex+age+CI+age:CI+sex:age+sex:age	60.9	14.3	0
	7	OxC ~sex*age*CI	66.4	19.8	0
BDE 99	1	BDE 99~CI	57.1	0	0.8
	2	BDE 99~age+CI	60.2	3.1	0.16
	3	BDE 99~sex+age+CI	63.7	6.6	0.03
	4	BDE 99~sex+age+CI+age:CI	66.4	9.3	0.01
	5	BDE 99~sex+age+CI+age:CI+sex:CI	70.9	13.8	0
	6	BDE 99~sex+age+CI+age:CI+sex+CI+sex:age	76.6	19.5	0
	7	BDE 99~sex*age*CI	78.2	21.1	0
HCB	1	HCB~age	63.5	0	0.49
	2	**HCB~age+CI+age:CI**	**64.3**	**0.8**	**0.33**
	3	HCB~age+CI	66.3	2.8	0.12
	4	HCB~sex+age+CI+age:CI	68.5	5.0	0.04
	5	HCB~sex+age+CI+age:CI+sex:CI	71.2	7.7	0.01
	6	HCB~sex+age+CI+age:CI+sex:CI+sex:age	75.4	11.9	0
	7	HCB~sex*age*CI	80.7	17.2	0

Table SI-4. Model selection by small sample size adjusted Akaikes Information Criteria (AICc) of regression models explaining glutathione peroxidase (GPx) activity in red blood cells of northern goshawk nestlings (n = 20). The effect of five different POPs (CB 153, OxC, *p,p´*-DDE, BDE 99 and HCB) was tested separately in models including potential effect of sex, age, CI (body condition index) and breath (number of breaths in 60 seconds). Compounds were transformed by natural logarithm (ln) to ensure normality. The best models explaining a significant effect on GPx activity are marked in bold.

POP	Model ID	Model	AICc	ΔAICc	AICc Wt
CB 153	1	GPx~sex	254.9	0.00	0.51
	2	GPx~sex+CB 153	255.5	0.53	0.39
	3	GPx~sex+breath+CB 153	258.6	3.71	0.08
	4	GPx~sex+age+breath+CB 153	261.6	6.70	0.02
	5	GPx~sex+CI+age+breath+CB 153	265.9	11.00	0
	6	GPx~sex+CI+age+breath+CB 153+age:CB 153	268.7	13.80	0
	7	GPx~sex+CI+age+breath+CB 153+age:CB 153+CB 153:CI	274.7	19.80	0
	8	GPx~sex+CI+age+breath+CB 153+sex:CI+age:CB 153+CB 153:CI	281.6	26.60	0
	9	GPx~sex+CI+age+breath+CB 153+sex:CI+CB 153:breath+age:CB 153+CB 153:CI	291.1	37.20	0
	10	GPx~sex+CI+age+breath+CB 153+sex:CI+CB 153:breath+sex:CB 153+age:CB 153+CB 153:CI	305.6	50.7	0
OxC	1	GPx~sex	254.9	0	0.51
	2	GPx~sex+OxC	255.5	0.53	0.39
	3	GPx~sex+breath+OxC	258.6	3.71	0.08
	4	GPx~sex+breath+OxC+sex:CI	261.6	6.70	0.02
	5	GPx~sex+age+breath+OxC+sex:CI	265.9	11.0	0
	6	GPx~sex+age+breath+OxC+sex:CI+age:OxC	268.7	13.8	0
	7	GPx~sex+CI+age+breath+OxC+sex:CI+age:OxC	274.7	19.8	0
	8	GPx~sex+CI+age+breath+OxC+sex:CI+age:OxC+OxC:CI	281.6	26.6	0
	9	GPx~sex+CI+age+breath+OxC+sex:CI+sex:OxC+age:OxC+OxC:CI	292.1	37.2	0
	10	GPx~sex+CI+age+breath+OxC+sex:CI+OxC:breath+sex:OxC+age:OxC+OxC:CI	305.7	50.7	0
BDE 199	1	GPx~sex	252.6	0	0.73
	2	GPx~sex+breath+BE 99	255.0	2.42	0.22
	3	GPx~sex+breath+BE 99+BDE 99:breath	258.4	5.81	0.04
	4	GPx~sex+breath+BE 99+BDE 99:breath+sex:BDE 99	261.7	9.12	0.01
	5	GPx~sex+age+breath+BE 99+BDE 99:breath+sex:BDE 99	266.6	14.0	0
	6	GPx~sex+age+breath+BE 99+BDE 99:breath+sex:BDE 99+age:BDE 99	272.3	19.7	0
	7	GPx~sex+age+breath+BDE 99+BDE 99:breath+sex:BDE 99+age:BDE 99	277.8	25.2	0

	8	GPx~sex+CI+age+breath+BDE 99+BDE 999:breath+sex:BDE 99+age:BDE 99	284.2	31.5	0
	9	GPx~sex+CI+age+breath+BDE 99+sex:CI+BDE 99:breath+sex:BDE 99+age:BDE 99	294.1	41.5	0
	10	GPx~sex+CI+age+breath+BDE 99+sex:CI+BDE 99:breath+sex:BDE 99+age:BDE 99+BDE 99:CI	307.6	55.0	0
p,p´-DDE	1	GPx~sex	254.9	0	0.76
	2	GPx~sex+breath	257.6	2.70	0.2
	3	GPx~sex+breath+*p,p´*-DDE	261.0	6.15	0.04
	4	GPx~sex+age+breath+*p,p´*-DDE	265.1	10.2	0
	5	GPx~sex+age+breath+*p,p´*-DDE+age:*p,p*-´DDE	269.7	14.8	0
	6	GPx~sex+age+breath+*p,p´*-DDE+*p,p´*-DDE:breath+age:*p,p*-´DDE	272.6	17.7	0
	7	GPx~sex+age+breath+*p,p´*-DDE+*p,p´*-DDE:breath+sex:*p,p´*-DDE+age:*p,p*-´DDE	277.1	22.2	0
	8	GPx~sex+CI+age+breath+*p,p´*-DDE+*p,p´*-DDE:breath+sex:*p,p´*-DDE+age:*p,p*-´DDE	285.4	30.5	0
	9	GPx~sex+CI+age+breath+*p,p´*-DDE+*p,p´*-DDE:breath+sex:*p,p´*-DDE+age:*p,p*-´DDE+*p,p´*-DDE:CI	295.7	40.7	0
	10	GPx~sex+CI+age+breath+*p,p´*-DDE+sex:CI+*p,p´*-DDE:breath+sex:*p,p´*-DDE+age:*p,p*-´DDE+*p,p´*-DDE:CI	309.2	54.20	0
HCB	1	GPx~HCB	252	0.00	0.57
	2	GPx~sex+HCB	253.5	1.45	0.27
	3	GPx~sex+breath+HCB	255.7	3.65	0.09
	4	GPx~sex+breath+HCB+HCB:breath	256.7	4.65	0.06
	5	GPx~sex+CI+breath+HCB+HCB:breath	259.8	7.80	0.01
	6	GPx~sex+CI+breath+HCB+sex:CI+HCB:breath	265.2	13.1	0
	7	GPx~sex+CI+age+breath+HCB+sex:CI+HCB:breath	270.4	18.3	0
	8	GPx~sex+CI+age+breath+HCB+sex:CI+HCB:breath+age:HCB	277.7	25.6	0
	9	GPx~sex+CI+age+breath+HCB+sex:CI+HCB:breath+sex:HCB+age:HCB	283.6	31.6	0
	10	GPx~sex+CI+age+breath+HCB+sex:CI+HCB:breath+sex:HCB+age:HCB+HCB:CI	296.0	43.9	0

Table SI-5. Model selection by small sample size adjusted Akaikes Information Criteria (AICc) of regression models explaining catalase activity in red blood cells of northern goshawk nestlings (n = 20). The effect of five different POPs (CB 153, OxC, *p,p´*-DDE, BDE 99 and HCB) was tested separately in models including potential effect of sex, age, CI (body condition index) and breath (number of breaths in 60 seconds). Compounds were transformed by natural logarithm (ln) to ensure normality. The best models explaining a significant effect on catalase activity are marked in bold.

POP	Model ID	Model	AICc	ΔAICc	AICcWt
CB 153	**1**	**Catalase ~ sex + CB 153**	**128.8**	**0.00**	**0.65**
	2	Catalase ~sex	131.2	2.40	0.20
	3	Catalase ~sex+CB 153 + age	132.2	4.35	0.12
	4	Catalase ~sex CB 153 + age + sex: CB 153	134.9	6.22	0.03
	5	Catalase ~sex + age + CB 153 + sex: CB153 + age: CB153	138.5	9.74	0.01
	6	Catalase ~sex + CI + age + CB 153 + sex:CB 153 + age: CB 153	142.7	13.9	0
	7	Catalase ~sex + CI + age + CB 153 + sex:CB 153 + age: CB 153 + sex:CI	184.4	19.6	0
	8	Catalase ~sex + CI + age + CB 153 + sex: CB 153 + age:CB 153 + sex:CI + breath	156.8	28.0	0
	9	Catalase ~sex + CI + age + CB 153 + sex:CB 513 + age:CB 153 + sex:CI + breath + breath:CB 513	167.3	38.6	0
	10	Catalse ~sex + CI + age + CB 153 + sex:CB 153 + age:CB 153 + sex:CI + breath + breath:CB 153 + CB 153:CI	180.6	51.8	0
OxC	**1**	**Catalase ~sex + OxC**	**129.0**	**0.00**	**0.64**
	2	Catalase ~sex	131.2	2.16	0.22
	3	Catalase ~sex + OxC + age	132.2	3.24	0.13
	4	Catalase ~sex + OxC + age + CI	136.1	7.08	0.02
	5	Catalase ~sex + OxC + age + CI + CI:OxC	139.9	10.8	0
	6	Catalase ~sex + OxC + age + CI + CI:OxC + sex:OxC	143.8	14.7	0
	7	Catalase ~sex + OxC + age + CI + CI:OxC + sex:OxC + sex:CI	150.3	21.3	0
	8	Catalase ~sex + OxC + age + CI + CI:OxC + sex:OxC + sex:CI + breath	158.8	29.7	0
	9	Catalase ~sex + OxC + age + CI + CI:OxC + sex:OxC + sex:CI + breath + OxC:breath	169.2	40.2	0
	10	Catalase ~sex + OxC + age + CI + CI:OxC + sex:OxC + sex:CI + breath + OxC:breath + OxC:age	181.2	52.2	0
BDE 99	**1**	**Catalase ~ sex**	**131.2**	**0.00**	**0.47**
	2	Catalase ~ sex +age	131.4	0.22	0.42
	3	Catalase ~ sex +CI+age	134.6	3.40	0.09
	4	Catalase ~ sex +CI+age+sex:CI	137.7	6.52	0.02
	5	Catalase ~ sex +CI+age+BDE 99+sex:CI	142.4	11.2	0
	6	Catalase ~ sex +CI+age+BDE	147.4	16.3	0

		99+sex:CI+sex:BDE 99			
	7	Catalase ~ sex +CI+age+BDE 99+sex:CI+sex:BDE 99+CI:BDE 99	153.8	22.6	0
	8	Catalase ~ sex +CI+age+breath+BDE 99+sex:CI+sex:BDE 99+CI:BDE 99	162.2	31.0	0
	9	Catalase ~ sex +CI+age+breath+BDE 99+sex:CI+ BDE 99:breath+sex:BDE 99+CI:BDE 99	172.7	41.6	0
	10	Catalase ~ sex +CI+age+breath+BDE 99+sex:CI+ BDE 99:breath+sex:BDE 99+age:BDE 99+CI:BDE 99	181.8	50.6	0
p,p´-DDE	**1**	**Catalase ~ sex**	**131.1**	**0.00**	**0.42**
	2	Catalase ~ sex + age	131.4	0.22	0.38
	3	Catalase ~ sex + age + *p,p´*-DDE	132.9	1.71	0.18
	4	Catalase ~ sex + CI + age + *p,p´*-DDE	136.9	5.72	0.02
	5	Catalase ~ sex + CI + age + *p,p´*-DDE + sex:CI	140.8	9.60	0
	6	Catalase ~ sex + CI + age + *p,p´*-DDE + sex:CI + sex:*p,p´*-DDE	145.9	14.8	0
	7	Catalase ~ sex + CI + age + *p,p´*-DDE + breath + sex:CI + sex:*p,p´*-DDE	152.6	21.4	0
	8	Catalase ~ sex + CI + age + *p,p´*-DDE + breath + sex:CI + sex:*p,p´*-DDE + CI:*p,p´*-DDE	161.0	29.9	0
	9	Catalase ~ sex + CI + age + *p,p´*-DDE + breath + sex:CI + sex:*p,p´*-DDE + age:*p,p´*-DDE+CI:*p,p´*-DDE	171.6	40.4	0
	10	Catalase ~ sex + CI + age + *p,p´*-DDE + breath + sex:CI + *p,p´*-DDE:breath + sex:*p,p´*-DDE + age:*p,p´*-DDE+CI:*p,p´*-DDE	183.8	52.6	0
HCB	**1**	**Catalase ~ sex**	**131.2**	**0.00**	**0.47**
	2	Catalase ~ sex + age	131.4	0.22	0.42
	3	Catalase ~ sex + age + HCB	134.4	3.14	0.10
	4	Catalase ~ sex + CI + age + HCB	138.2	7.00	0.01
	5	Catalase ~ sex + CI + age + breath + HCB	142.9	11.8	0
	6	Catalase ~ sex + CI + age + breath + HCB + HCB:breath	146.7	15.6	0
	7	Catalase ~ sex + CI + age + breath + HCB + sex:CI + HCB:breath	148.2	17.0	0
	8	Catalase ~ sex + CI + age + breath + HCB + sex:CI + HCB:breath + age:HCB	155.8	24.6	0
	9	Catalase ~ sex + CI + age + breath + HCB + sex:CI + HCB:breath + sex:HCB + age:HCB	166.3	35.2	0
	10	Catalase ~ sex + CI + age + breath + HCB + sex:CI + HCB:breath + sex:HCB + age:HCB + CI:HCB	179.7	48.6	0

Table SI-6. Median with maximum (max) and minimum (min) concentrations of organic pollutants detected in more than 50 % of samples (plasma, feather and preen oil) from northern goshawk nestlings from Trøndelag (n = 20). Nd denotes not detected in more than 50 % of the samples. For OPFRs n = 19.

	PLASMA ng ml^{-1}			FEATHER ng g^{-1}			PREEN OIL ng g^{-1} lipids		
Compound	**Median**	**Min**	**Max**	**Median**	**Min**	**Max**	**Median**	**Min**	**Max**
CB 101	0.04	0.02	0.31	0.47	0.17	1.27	6.31	0.85	28.8
CB 99	0.10	0.03	0.31	1.01	0.17	4.83	15.4	0.90	68.1
CB 105	0.04	0.01	0.13	0.23	0.08	1.04	3.34	0.67	23.3
CB 118	0.18	0.03	0.66	0.75	0.17	2.86	15.5	4.44	100.1
CB 153	1.73	0.33	6.30	3.15	0.78	8.02	120.9	32.3	525.7
CB 138	0.84	0.09	3.47	1.41	0.57	5.41	40.1	10.4	112.6
CB 187	0.74	0.12	2.36	0.68	0.29	1.45	28.7	9.87	79.1
CB 183	0.16	0.02	0.62	0.17	0.08	0.45	7.51	1.79	29.7
CB 177	0.06	0.01	0.24	0.09	0.05	0.24	3.06	0.95	9.72
CB 171	0.05	0.01	0.19	Nd			2.61	0.85	9.75
CB 156	0.09	0.01	0.37	Nd			3.15	0.90	12.5
CB 180	1.30	0.17	5.39	0.43	0.17	1.45	45.2	10.3	178.8
CB 170	0.43	0.06	1.79	0.32	0.13	1.18	16.5	3.47	72.1
CB 199	0.16	0.03	0.59	Nd			5.54	1.67	16.9
CB 196/203	0.15	0.02	0.49	Nd			5.42	1.25	19.3
CB 194	0.14	0.03	0.63	Nd			6.45	1.41	25.1
CB 206	0.03	0.01	0.16	Nd			1.44	0.85	12.8
CB 209	0.02	0.01	0.06	Nd			1.27	0.80	7,60
ΣPCBs	6.92	1.00	23.40	8.79	0.05	8.01	332.0	92.9	1204
OxC	0.15	0.02	0.49	0.14	0.06	0.34	6.03	1.38	20.7
TN	0.12	0.01	0.56	Nd			5.12	1.02	35.9
CN	0.03	0.01	0.17	Nd			Nd		
HCB	0.11	0.02	1.14	0.15	0.06	0.79	9.74	3.14	37.5
***p,p´*-DDE**	3.87	0.61	14.43	27.32	7.42	75.96	799.4	160.8	3523
***p,p´*-DDT**	0.04	0.02	0.18	Nd			Nd		
***β*-HCH**	0.03	0.02	0.06	0.27	0.08	1.12	4.43	1.54	13.0
***γ*-HCH**	Nd			0.08	0.05	1.05	0.83	0.50	47.1
BDE 47	0.04	0.01	0.20	0.45	0.09	2.46	7.15	1.19	45.4
BDE 100	0.05	0.01	0.14	0.24	0.07	0.85	7.95	1.26	24.5
BDE 99	0.07	0.01	0.31	0.40	0.09	2.99	11.86	1.74	38.2
BDE 154				Nd			1.20	0.40	5.68
BDE 153	0.04	0.003	0.09	0.17	0.07	0.45	5.75	1.81	11.7
BDE 183	0.01	0.002	0.03	Nd			Nd		
ΣPBDEs	0.22	0.02	0.67	1.26	0.31	6.58	34.70	6.00	94.6
a-DP	Nd			Nd			1.68	0.26	6.11
TCEP	Nd			12.9	4.64	30.7	Nd		
TPhP	Nd			4.40	0.95	22.3	Nd		
EHDPHP	Nd			2.46	0.75	23.4	Nd		
ΣTCIPP	Nd			26.8	1.75	60.4	Nd		

Table SI-7. Enzyme activity (nanomol per minute per milligram protein) of catalase and glutathione peroxidase (GPx) in red blood cells of northern goshawk nestlings (n = 20).

ID	Catalase activity (nmol min^{-1} mg^{-1} protein)	GPx activity (nmol min^{-1} mg^{-1} protein)
TRD 1	57.50	543.9
TRD 2	48.17	465.4
TRD 3	47.11	494.4
TRD4	41.44	460.4
TRD5	46.14	558.2
TRD 6	43.96	464.2
TRD 7	36.35	681.0
TRD 8	34.71	351.2
TRD 9	45.15	454.3
TRD 10	41.12	385.2
TRD 11	37.26	604.9
TRD 12	49.20	207.9
TRD 13	38.47	448.7
TRD 14	39.07	375.4
TRD 15	28.33	272.0
TRD 16	44.69	280.4
TRD 17	40.34	255.4
TRD 18	39.01	343.3
TRD 19	46.12	299.0
TRD20	34.46	397.2