



A retrospective investigation of feather corticosterone in a highly contaminated white-tailed eagle (*Haliaeetus albicilla*) population

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ABSTRACT

Exposure to persistent organic pollutants (POPs), such as organochlorines (OCs) and polybrominated diphenyl ethers (PBDEs), is associated with adverse health effects in wildlife. Many POPs have been banned and consequently their environmental concentrations have declined. To assess both temporal trends of POPs and their detrimental impacts, raptors are extensively used as biomonitors due to their high food web position and high contaminant levels. White-tailed eagles (WTEs; *Haliaeetus albicilla*) in the Baltic ecosystem represent a sentinel species of environmental pollution, as they have suffered population declines due to reproductive failure caused by severe exposure to dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCB) during the 1960s through 1980s. However, there is a lack of long-term studies that cover a wide range of environmental contaminants and their effects at the individual level. In this study, we used 135 pooled samples of shed body feathers collected in 1968–2012 from breeding WTE pairs in Sweden. Feathers constitute a temporal archive for substances incorporated into the feather during growth, including corticosterone, which is the primary avian glucocorticoid and a stress-associated hormone. Here, we analysed the WTE feather pools to investigate annual variations in feather corticosterone (fCORT), POPs (OCs and PBDEs), and stable carbon and nitrogen isotopes (SIs; dietary proxies). We examined whether the expected fluctuations in POPs affected fCORT (8–94 pg. mm⁻¹) in the WTE pairs. Despite clear temporal declining trends in POP concentrations ($p < 0.01$), we found no significant associations between fCORT and POPs or SIs ($p > 0.05$ in all cases). Our results do not support fCORT as a relevant biomarker of contaminant-mediated effects in WTEs despite studying a highly contaminated population. However, although not detecting a relationship between fCORT, POP contamination and diet, fCORT represents a non-destructive and retrospective assessment of long-term stress physiology in wild raptors otherwise not readily available.

1. Introduction

Persistent Organic Pollutants (POPs), which comprise halogenated organic compounds such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and polybrominated diphenyl ethers (PBDEs), are anthropogenic contaminants that persist in the environment, are ubiquitously distributed, and have the capacity to

bioaccumulate and biomagnify in living organisms (Harrad, 2009). Apex predators, susceptible of accumulating high levels of bio-magnifying contaminants, have therefore been extensively used as biomonitors of environmental pollution (Rattner, 2009). The white-tailed eagle (WTE; *Haliaeetus albicilla*), the largest raptor in northern Europe, is a well-documented environmental sentinel species (Helander et al., 2008; Herrmann et al., 2011). On the Swedish coast of the Baltic Sea,

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WTE territories have been closely monitored since the 1960s following observed reproductive failures and population declines, mainly due to high POP contamination (Helander et al., 2002).

In avian ecotoxicology, the stress of being contaminated has been a subject of growing interest where corticosterone (CORT), the main glucocorticoid and stress-associated hormone in birds (Romero and Romero, 2002), has been proposed as a predictive tool for pollutant-mediated health effects in wild birds (e.g., Bourgeon et al. (2012); Nordstad et al. (2012); Strong et al. (2015); Tartu et al. (2014); Verboven et al. (2010)). Current findings on the relationship between CORT and anthropogenic contaminants are however ambiguous, as several studies on free-living birds report weak or no relationships (e.g., Bourgeon et al. (2012), Goutte et al. (2018), Monclus et al. (2019), Tartu et al. (2015c), and Randulff et al. (2022)). Discrepancies between previous studies can be due to CORT being influenced by additional environmental factors such as low or suboptimal food availability (Catitti et al., 2022; Patterson et al., 2015; Will et al., 2015) and inclement weather (de Bruijn and Romero, 2018; Kouwenberg et al., 2016). In addition, most effect studies investigating the influence of contaminants on CORT concentrations have been conducted within short time periods (maximum 1–2 sampling years except Goutte et al. (2018)), limiting the concentration range of the contaminants as well as the environmental stressors that individuals are exposed to. Many anthropogenic contaminants display clear declining temporal trends due to global regulations of compounds, and wild bird populations have thus experienced large variations in the magnitude of contaminant exposure over the last century (Sun et al., 2020).

Long-term studies on CORT-contaminant relationships in bird species are largely lacking, mainly pertaining to methodological limitations. Indeed, such retrospective studies can only be achieved by using a suitable matrix, which is not sensitive to time of conservation. As such, feathers allow the assessment of both POPs (Jaspers et al., 2019) and CORT concentrations (Bortolotti et al., 2008). While blood plasma represents immediate concentrations of circulating CORT (Romero and Romero, 2002), feather concentrations (hereafter fCORT) reflect the deposition of CORT during the time of feather growth (days to weeks; Bortolotti et al. (2008)) although studies report that fCORT can be used as a proxy of plasma CORT concentrations (Fairhurst et al., 2013a). Analysing CORT in feathers has therefore become increasingly popular since feathers can be collected from either live or dead birds (including museum specimens; Fairhurst et al. (2015)). In addition, sampling of shed feathers does not require capturing individuals (Sun et al., 2020) and allows investigation of fCORT also in non-breeding individuals (Fairhurst et al., 2015). Therefore, feather corticosterone represents a non-destructive and retrospective measure of stress physiology in wild avian species.

As part of a Swedish national WTE rescue project (Helander, 2003) and WTE monitoring program (Helander et al., 2008), shed feathers of territorial adults have been opportunistically collected during nest visits resulting in a collection of archived feathers that spans multiple decades. These archives create a unique opportunity to assess fCORT concentrations and POP contamination in adult pairs present at their nest (successful or unsuccessful breeders) of a sentinel raptor species exposed to a broad range of POP levels over many years. In total, 135 unique feather pools collected in the period 1968–2012 from pairs of WTEs were selected for this study. One pool consisted of 10 body feathers collected at the same nest, of which one was used to analyse fCORT (expressed as pg. per length unit) and the remaining 9 were homogenized and analysed for 47 POP compounds (39 organochlorines (OCs) and 6 polybrominated diphenyl ethers (PBDEs)) and stable isotope signatures (i. e., dietary proxies; $\delta^{15}\text{N}$ indicating trophic level and $\delta^{13}\text{C}$ indicating habitat of prey (Kelly, 2000)). Since WTEs are resident, monogamous birds that feed on similar prey and exhibit shared parental care during chick rearing, the use of pooled feathers can be considered a reliable proxy for pollution and dietary status within their territory. Previous studies have found no significant differences in POP concentrations

between male and female WTEs (Jaspers, 2013; Krone, 2006), further supporting the use of pooled feathers as a representative sample. Further, as WTEs do not differ in feather plumage pigmentation and as sex was not shown to influence CORT levels in wild birds (Apfelbeck et al., 2017; Tartu et al., 2015a, 2015b; Verboven et al., 2010) including raptor species (Strong et al., 2015), fCORT was measured in a single feather (i.e., either male or female). This was done to minimize the high variations within and between individuals, as previously reported in poultry studies (Häffelin et al., 2021).

Our objectives were (1) to investigate the temporal variations in fCORT and POP contamination in the highly contaminated Baltic Sea WTE population over the study period 1968–2012, and (2) to examine whether the predicted strong fluctuations in contaminant levels affected fCORT. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopes were also tested as potential drivers since feeding ecology might influence fCORT concentrations (Fairhurst et al., 2015). The exploitation of this unique archive of WTE feathers allowed a retrospective long-term effect study in an avian apex predator.

2. Material and methods

2.1. Feather sampling

The present study used a collection of WTE body feathers comprising 135 samples from 25 breeding territories sampled at least once or multiple times in the period 1968–2012 (Table S1) and stored at the Swedish Museum of Natural History. The WTE population at the central Swedish Baltic coast (Fig. 1) has been monitored annually since 1968 and moulted body feathers of breeding eagle pairs were collected at individual nests in their specific territory (Helander et al., 2008). Feathers were stored in separate polypropylene bags upon collection and subsequently stored in dark polypropylene boxes at ambient temperature and humidity conditions. The feather collection has not been subjected to any preservative treatment before or during storage at the museum. Approximately ten body feathers per territory at a given year were extracted from the collection and stored in a similar fashion until chemical analysis.

2.2. Contaminant analysis

After reserving one body feather per territory per year for CORT measurement (see section 2.4), the remaining nine feathers were thoroughly cleaned using distilled water and dried overnight before they were cut into <1 mm pieces and homogenized to one pooled sample as described in Sun et al. (2020). The pooled sample was analysed for a wide range of POPs and stable carbon and nitrogen isotopes (section 2.3). By washing feathers in distilled water only, we were able to retain preen oil on the feather surface, as preen oil originates from internal sources of the bird and has been found to be significantly correlated with internal muscle concentrations (Jaspers et al., 2011, 2019). The analysis of contaminants was performed at the Toxicological Centre (University of Antwerp, Belgium). All feather pools were analysed for the following organochlorine (OC) compounds: 20 polychlorinated biphenyl (PCB) congeners (CB 49, 52, 74, 95, 99, 101, 105, 110, 118, 138, 149, 153, 156, 170, 171, 177, 180, 183, 187, and 194), *p,p'*-dichlorodiphenyltrichloroethane (*p,p'*-DDT) and its metabolites, *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) and *p,p'*-dichlorodiphenyl dichloroethane (*p,p'*-DDD), five chlordane-related compounds (CHLs: *cis*- and *trans*-nonachlor (CN and TN), *cis*- and *trans*-chlordane (CC and TC), and oxychlordane (OxCh)), hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs; α -, β - and γ -HCH), seven polybrominated diphenyl ether (PBDE) congeners (28, 47, 99, 100, 153, 154, 183) and two PBDE metabolites (6-MeO-BDE-47 and 2-MeO-BDE-68).

The analytical methods for OCs have already been described in Sun et al. (2020). The analysis for PBDEs was conducted as following: each individual subsample (0.12–0.41 g) was spiked with internal standards (BDE 77 and BDE 128) and incubated overnight at 45 °C with hexane:

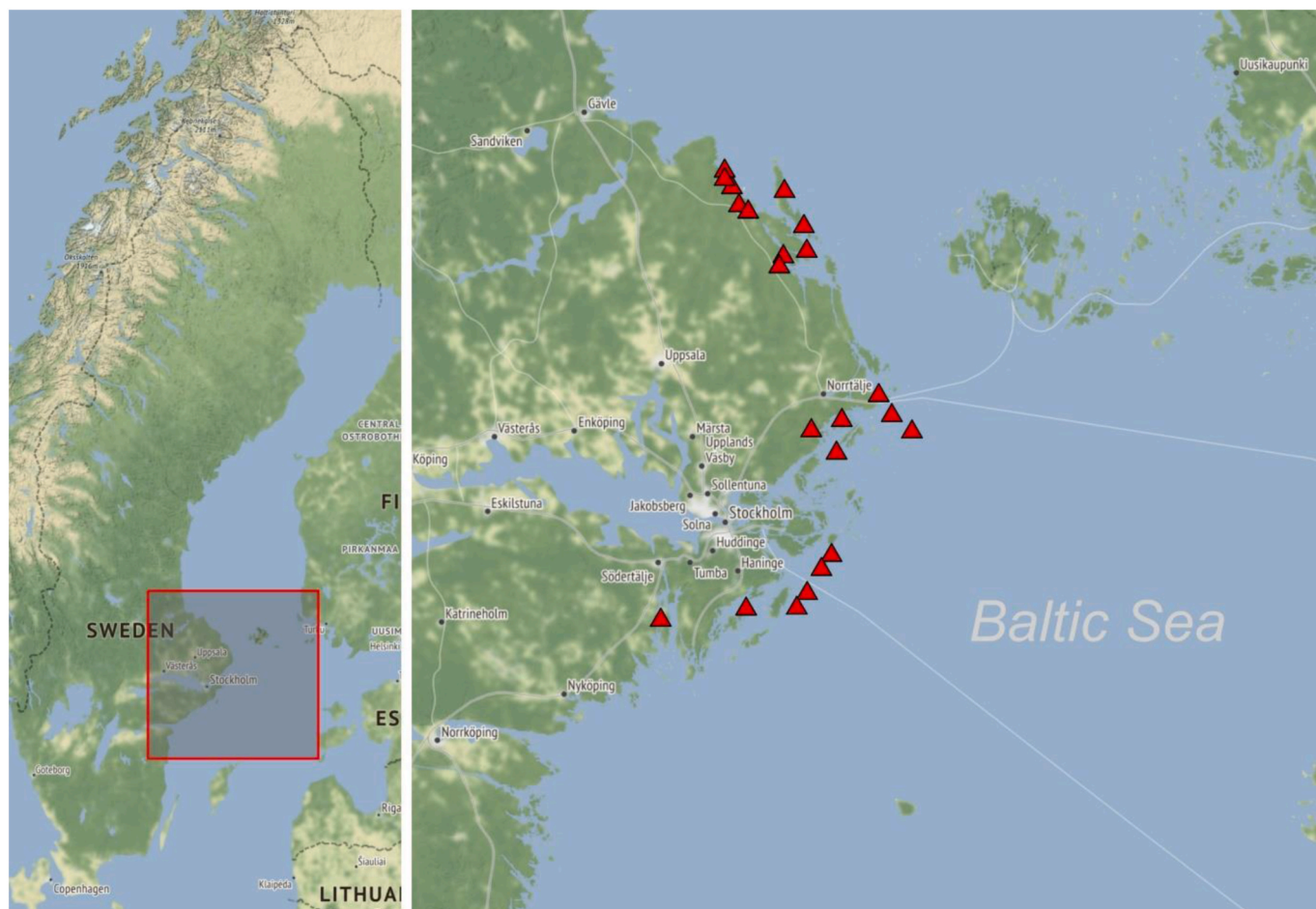


Fig. 1. Baltic coastline of Sweden indicating locations (red triangles) where shed body feathers in nests of breeding pairs of white-tailed eagles were collected in the period 1968–2012.

dichloromethane (4:1; v:v) and HCl (1 M). After liquid-liquid extraction, the extracts were fractionated on prepacked Supelclean™ ENVITM-Florisil® SPE cartridges (3 mL; 500 mg; Supelco, Bellefonte, PA, USA) topped with anhydrous Na₂SO₄. A first fraction, containing PBDEs, was eluted with 10 mL of hexane and cleaned-up on acidified silica (10% H₂SO₄), while a second fraction, containing PFRs, was obtained in light of another study. The PBDE fraction was concentrated until dryness under a gentle nitrogen flow and reconstituted in iso-octane. All PBDEs were quantified using gas chromatography coupled to electron capture negative ionisation mass spectrometry.

Pesticide-grade solvents (Merck KGaA Chemicals, Darmstadt, Germany and Acros Organics, Geel, Belgium) were used throughout and every 23rd sample a procedural blank was analysed. Internal standard recoveries ranged between 86 and 106% and the obtained certified reference material concentrations were within 3.7 SD from the indicative values. All compounds were blank subtracted using average procedural blank values. The limit of quantification (LOQ) was set at 3*SD of the procedural blank values, or, for analytes not detectable in blanks, was calculated from a 10:1 signal to noise ratio.

2.3. Stable isotope analyses

The analysis was performed at the Laboratory of Oceanology of the University of Liège, Belgium. The stable isotope ratios and the analytical protocol and quality assurance were previously reported in Sun et al. (2019). The stable isotope ratios for carbon and nitrogen are expressed as δ values (‰) relative to their respective international measurement standards Vienna Pee Dee Belemnite and atmospheric N₂.

2.4. Corticosterone analysis

The analysis for feather corticosterone (fCORT) concentrations was performed on one whole body feather per sample at the Department of Arctic and Marine Biology at UiT - the Arctic University of Norway, Tromsø. Prior to CORT extraction, the length of each feather (from base to tip, without the calamus) was recorded to the nearest millimetre. Extraction of CORT followed the methanol-based extraction method reported earlier by Bortolotti et al. (2008). Final extracts were assessed for CORT with an enzyme immunoassay kit (901–097, Assay Designs Inc., USA). The quality of the assay was validated using serial dilutions of feather extracts (displacement curves), which confirmed the absence of interfering substances in the extract. Moreover, the cross-reactivity of the assay is reported to be high with CORT (100%) but low with related steroids (e.g., progesterone: 1.7%; cortisol: 0.05%; β -estradiol: 0.03%). Each feather extract was measured in duplicate, and for all four separate plates used in the present study we reported intra- and inter-assay variability of 2.6% ($n = 16$) and 0.8% ($n = 4$), respectively. The final concentration of CORT was calculated using a standard curve run on each plate and the unit is given as pg. mm⁻¹.

2.5. Statistical analyses

The statistical analyses were conducted using R version 4.2.1 (R Development Core Team, 2022). All tests performed were two-tailed and the null hypothesis was rejected at an alpha level of 0.05. To attain the assumptions of normality and homogeneity of variance, fCORT and POP concentrations were natural log-transformed (ln) prior to statistical

analyses. Since some WTE territories were sampled multiple times across the study period, we included territory as a random variable in a linear mixed effect model using function *lme* the *nlme*-package (Pinheiro et al., 2017). However, the *lme* revealed low among-territory variability (i.e., low random intercept variance; results not shown) and thus the random effect of territory in the models was negligible and therefore not included in the models. Model assumptions were visually assessed and validated through residual plots, quantile-quantile plots, and histograms of residuals following the protocol by Zuur et al. (2010).

We only included POP compounds with a detection frequency of more than 75% in the statistical analyses (Hansen et al., 2022; Sletten et al., 2016). The resulting compounds were 29 PCB congeners (28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 151, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206 and 209), four chlordane-related compounds (CN, TN, OxC and CC), *p,p'*-DDT and metabolites (*p,p'*-DDE and *p,p'*-DDD), HCB, β - and γ -HCH, and six PBDE congeners (28, 47, 99, 100, 153 and 154). Concentrations below the level of quantification were replaced with zero for the calculation of sums. Due to the high number of compounds and high correlations among the OCs and PBDEs, respectively, we conducted principal component analysis (PCA; function *prcomp* (Zuur et al., 2007)) on the 39 OC compounds and six PBDEs separately (PCA biplots, scree and correlational plots for OCs and PBDEs are presented in Figs. S1–S3). We then extracted values of the first and second components, which together explained >75% of the variation for OCs and PBDEs, respectively, and included them in the models as predictors.

Two sets of analyses were performed using linear regression models (function *lm*): one to investigate the temporal variation in fCORT, POPs (OCs and PBDEs separately), and SIs ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), and one to investigate the relationship between fCORT, POP contamination and feeding ecology ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of the breeding pairs. In the temporal analyses, the main effect of year (a numeric variable) or its second-order polynomial (Year²; to allow non-linearity) were used as predictors. $\delta^{13}\text{C}$ values were corrected for the oceanic Suess effect (as explained in Sun et al. (2019)), which is the temporal decline of atmospheric $\delta^{13}\text{C}$ and consequently oceanic organic baseline $\delta^{13}\text{C}$ values due to the large quantity of CO₂ released from fossil fuel burning in recent industrial times (Gruber et al., 1999). Next, in the relationship analyses (i.e., between fCORT, POPs and SIs), two models were performed in which principal components (PC) of OCs and PBDEs, respectively, were placed in separate models due to their expected differing temporal trends in concentrations. $\delta^{15}\text{N}$ (trophic position proxy) and $\delta^{13}\text{C}$ (habitat proxy) were also included in these models to assess whether fCORT concentrations were related to feeding ecology of the WTEs. To avoid problems with multicollinearity (Zuur et al., 2010), we assessed collinearity between predictor variables based on the variance inflation factor (VIF, *VIF* function in the package *regclass*; Pietre (2020)). All variables had a VIF <2, which indicated low collinearity among variables (Zuur et al., 2010).

3. Results and discussion

This study examined fCORT in feather samples ($N = 135$) from pairs of Baltic WTE adults sampled in 1968–2012 encompassing historical periods of peak exposures to OCs and PBDEs followed by decreases driven by mitigation measures (Bjurliid et al., 2018; Jinhui et al., 2017; Sun et al., 2020). Our original methodological approach exploiting archived feathers allowed a retrospective and long-term assessment of contamination stress in breeding pairs of birds of prey exposed to a 38- and 114-fold difference in ranges of OC and PBDE levels, respectively (Table S2: min-max concentrations and Sun et al., 2020 for OC data).

3.1. Temporal variation in POP and fCORT concentrations

As expected, feather concentrations of OCs and PBDEs changed over the study period but had distinct peak periods (Figs. 2 and 3). OC

concentrations were highest in the 1960s followed by a steady linear decline (Fig. 2, Table S3; $F_{(1, 133)} = 210.5$, $p < 0.01$), while PBDE concentrations followed a bell-shaped curve with peak concentrations in the 1990s (Fig. 3, Table S3; $F_{(2, 132)} = 278.6$, $p < 0.01$). Our findings are in line with studies on trends in levels of POPs reported in aquatic wildlife in the Baltic ecosystem (Bignert and Helander, 2015; Bjurliid et al., 2018; Kierkegaard et al., 2004), where clear declines in concentrations matched the global regulations of OCs in the mid-seventies (Jones and de Voogt, 1999) and pentaBDEs (commercial mixture of PBDE congeners analysed in this study) in the 1990s (Jinhui et al., 2017). Importantly, our results provide evidence that the selected pools of feathers were a suitable and reliable matrix to measure temporal variation in environmental levels of lipophilic POPs such as OCs and PBDEs (Jaspers et al., 2019). Using shed feathers at the nest as passive monitors of environmental POP contamination is also particularly valuable since adult WTEs cannot be easily live captured and collecting eggs requires an invasive procedure that in addition does not provide a good proxy for measuring maternal CORT concentrations (Rettenbacher et al., 2005). Therefore, shed feathers at the nests were the only representative matrix available for analysis in the 1960s until 1980s when the reproductive output in the Swedish WTE population was at its lowest (Helander et al., 2002).

In contrast to the strong temporal trends in POP concentrations highlighted in the WTEs, we did not detect any clear temporal patterns in fCORT concentrations over the 44-year study period (Fig. 4, Table S3; $F_{(1, 133)} = 0.04$, $p = 0.84$). The lack of a temporal trend in fCORT is however in line with findings in a previous study that measured fCORT in a pelagic seabird over a 153-year period (Fairhurst et al., 2015), even though the WTEs displayed a relatively wide concentration range of fCORT. Median concentrations of fCORT in the present study were 18 pg. mm⁻¹ and we reported a tenfold difference in range between the lowest (8 pg. mm⁻¹) and highest (94 pg. mm⁻¹) concentrations (Fig. 3, Table S2). There are no previous records of fCORT in adult WTEs, but fCORT measured in 70 WTE nestlings sampled in two locations in Norway showed median concentrations of 3.26 and 2.62 pg. mm⁻¹, respectively (Losest et al., 2019), which indicates that breeding WTE

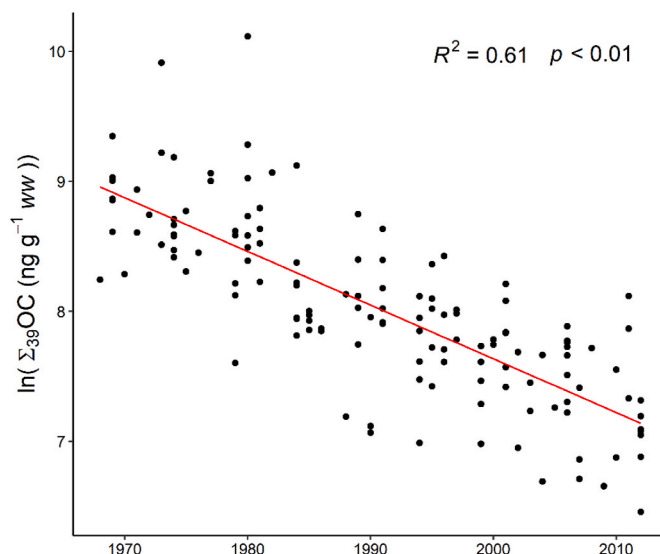


Fig. 2. Annual concentrations of the sum of 39 organochlorine (OC; list of compounds in section 2.5) in feather pools ($N = 135$) from breeding pairs of adult white-tailed eagles (*Haliaeetus albicilla*) in Sweden in 1968–2012. Concentrations are natural log (\ln) transformed, and concentrations (filled dots) are calculated from a pool of feathers in which one pool represented a breeding pair. Number of feather pools analysed for pollutants were 1–9 per year, except in 1978, 1983, 1987, 1992–1993, and 1998, when feathers were not sampled. Estimate (red line) from the linear model investigating the temporal variations in OC concentrations is given in the supplementary information, Table S3.

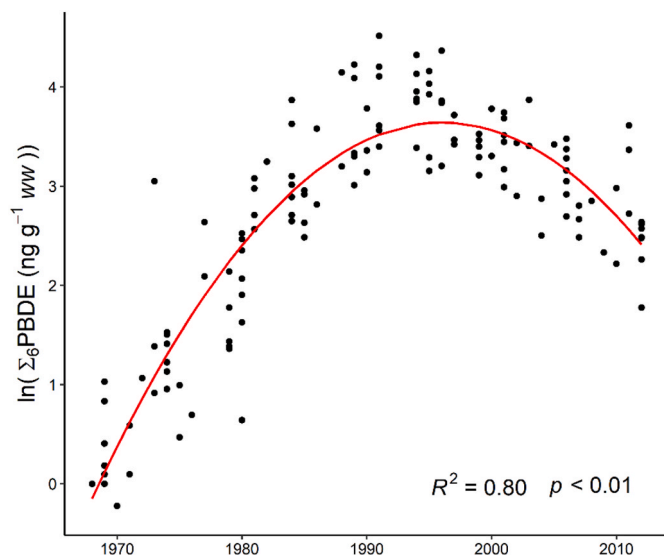


Fig. 3. Annual concentrations of the sum of 6 polybrominated diphenyl ether (PBDE; list of compounds in section 2.5) in feather pools ($N = 135$) from breeding pairs of adult white-tailed eagles (*Haliaeetus albicilla*) in Sweden, 1968–2012. Concentrations are natural log (\ln) transformed, and concentrations (filled dots) are calculated from a pool of feathers in which one pool represented a breeding pair. Number of feather pools analysed for pollutants were 1–9 per year, except in 1978, 1983, 1987, 1992–1993, and 1998, when feathers were not sampled. Estimate (red line) from the linear model investigating the temporal variations in PBDE concentrations is given in the supplementary information, Table S3.

adults in the Baltics displayed 6 times higher fCORT concentrations than nestlings in Norway. In contradiction, a previous study investigating differences in fCORT among age classes of red kites (*Milvus*) within the same population showed that fCORT concentrations were 1.5 times higher at age 1 year compared to 7–11 year-old birds (López-Jiménez et al., 2017). Other studies have reported spatial differences in fCORT among populations of the same species (Bourgeon et al., 2012; Treen et al., 2015; Voit et al., 2021). Therefore, the difference in fCORT between Norwegian WTE nestlings (Loseth et al., 2019) and adult WTEs in the current study is likely a result of studying different age classes and populations in different environments.

3.2. fCORT in relation to POP contamination and diet variability of breeding WTE pairs

In general, stressful stimuli activate the hypothalamic-pituitary-adrenal (HPA) axis, which leads to the release of glucocorticoids (i.e., CORT) from adrenal tissue that mobilizes a suite of behavioural, physiological and endocrinological responses to maintain homeostasis (McEwen and Wingfield, 2003). While an acute activation of the HPA axis is crucial to survive a stressor, chronic stress is associated with deleterious health effects such as immunosuppression and reproductive impairment (McEwen and Wingfield, 2003). Direct negative population effects from POP exposure have been well documented in the Swedish WTEs (Helander et al., 2002, 2008; Sonne et al., 2020), but much less is known about potential health effects at the individual level. To fill this gap, we investigated whether the clear patterns in contaminants correlated with fCORT concentrations over the study period. We also included stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in the models due to the potential influence of feeding ecology on fCORT (Fairhurst et al., 2013b, 2015; Will et al., 2015). We did however not detect any significant relationships between fCORT and pollutants, i.e., OC-PCs (Table 1; $F_{(4, 110)} = 0.57$, $p > 0.005$ in all cases) nor PBDE-PCs

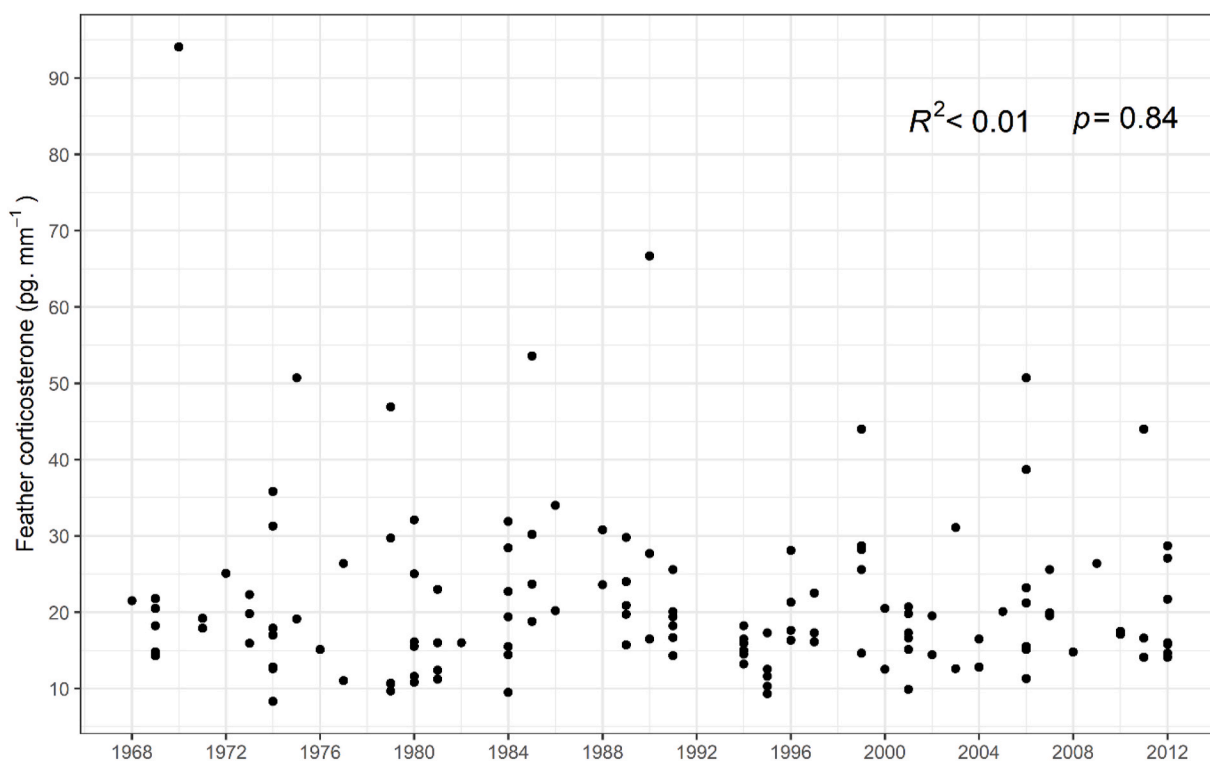


Fig. 4. Corticosterone concentrations (pg. mm^{-1}) in individual body feathers ($N = 135$) of adult white-tailed eagles (*Haliaeetus albicilla*) in Sweden, 1968–2012. The number of feathers analysed for corticosterone were 1–9 per year, except in 1978, 1983, 1987, 1992–1993, and 1998, when feathers were not sampled. One feather per nest was analysed. Estimate from the linear model investigating the temporal variations in feather corticosterone is given in the supplementary information, Table S3.

(Table 1; $F_{(4, 100)} = 1.08$, $p > 0.005$ in all cases). fCORT was also not significantly related to diet (proxied by $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$; Table 1, $p > 0.05$ in all cases).

The plethora of sampling procedures (mainly determined by the accessibility and biology of the investigated species) and methodological approaches available to researchers may contribute to discrepancies among studies. Similar to our study, Randulff et al. (2022) found no associations between fCORT and POP levels in Norwegian goshawk (*Accipiter gentilis*) nestlings, while two studies on red kites and cinereous vultures (*Aegypius monachus*) both reported positive relationships between fCORT and POP concentrations (Monclus et al. (2018) and Monclus et al. (2019), respectively). In contrast, Loseth et al. (2019) reported that fCORT decreased with increasing levels of perfluoralkyl substances (PFAS) in the Norwegian WTE nestlings although ranges in PFAS and fCORT levels encompassed different study locations. Thus, disentangling whether it was the direct effect of greater PFAS exposure that negatively affected fCORT in the Norwegian nestlings, or an effect of heterogeneous habitats, is difficult. Spatial differences in the fCORT-contaminant relationship have been previously reported; for example, Bourgeon et al. (2012) compared Great Skuas (*Stercorarius skua*) from populations breeding at different locations (Iceland, Shetland, and Bjørnøya). They reported a negative relationship between fCORT and PBDE levels in the Iceland population only (i.e., the ecological context; Bourgeon et al. (2012)). As such, spatial variations in fCORT are more likely to be driven by heterogeneous environmental conditions among locations/populations rather than pollutant exposure *per se*. Although anthropogenic pollutants have been proposed as a chronic stressor in wildlife (Ganz et al., 2018), findings in the current and above-mentioned studies by Loseth et al. (2019), Monclus et al. (2018, 2019), and Randulff et al. (2022) do not support that POPs influence CORT physiology. In addition, studies investigating other pollutants such as toxic metals (Strong et al., 2015) and other matrices (blood: e.g., Tartu et al. (2015a)) also reported contradicting findings on the CORT-contaminant relationship, further emphasizing the complexity behind establishing CORT as a proximal mechanism underlying the effects of contaminant exposure in avian wildlife.

To further investigate the impact of habitat on stress physiology, feathers do also allow for analysis of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopes that describe broad patterns in diet by indicating the trophic level and habitat at which an individual forage, respectively (Inger and Bearhop, 2008). Nutritional effects on CORT have previously been reported in studies on migrating seabirds (Fairhurst et al., 2015, 2017; Will et al., 2015), and Fairhurst et al. (2015) suggested a nutritional effect on fCORT in Leach Storm petrels (*Oceanodroma leucorhoa*), where higher $\delta^{15}\text{N}$ (i.e. trophic position of prey, of which also had higher food quality) were correlated with lower fCORT concentrations. We did however not report an effect

Table 1

Parameter estimates for the linear models of the relationship between feather corticosterone concentrations (pg. mm^{-1}) and the principal components (PCs) of organochlorines (OC-PC1 and OC-PC2) and polybrominated diphenyl ethers (PBDE-PC1 and PBDE-PC2), $\delta^{15}\text{N}$ (‰) and $\delta^{13}\text{C}$ (‰) in feather pools ($N = 135$) from breeding pairs of white-tailed eagles in Sweden between 1968 and 2012. Feather corticosterone and pollutant concentrations were ln-transformed prior to analysis, and calculations of the PCs of the OCs and the PBDEs are described in section 2.5 and PCA plots are provided in Figs. S1–S3.

Response variable	Coefficients	Estimate	Std. error	t-value	P-value
fCORT	Intercept	2.832	0.878	3.223	0.001
	OC-PC1	-0.008	0.007	-1.074	0.285
	OC-PC2	-0.010	0.019	-0.573	0.568
	$\delta^{15}\text{N}$	0.022	0.036	0.619	0.537
	$\delta^{13}\text{C}$	0.011	0.037	0.311	0.756
	fCORT	Intercept	2.163	0.842	2.569
fCORT	PBDE-PC1	-0.022	0.021	-1.069	0.287
	PBDE-PC2	0.031	0.312	1.014	0.313
	$\delta^{15}\text{N}$	0.005	0.376	0.158	0.874
	$\delta^{13}\text{C}$	-0.041	0.037	-1.114	0.268

of $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ on fCORT (Table 1), which corroborates previous findings on raptor nestlings (Loseth et al., 2019; Randulff et al., 2022). In addition, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were relatively stable across our study period (Figs. S3 and S4), which suggest that there were no substantial changes in diet over time. Year had however a weak positive effect on $\delta^{15}\text{N}$ (Table S3; $F_{(1,133)} = 5.48$, $p = 0.02$) but excluding potential extreme $\delta^{15}\text{N}$ -values (Fig. S5, values above >16) also removed the significant year-effect (results not shown). The outlier values did however not influence the estimates of the relationship models (Table 1) and therefore no $\delta^{15}\text{N}$ -values were excluded.

Overall, including ancillary information on biological and ecological variables appears essential to increase our understanding on how CORT responds to a defined stressor such as contaminant exposure in wildlife (Romero and Beattie, 2021). Namely, biological and environmental factors such as species differences (Cockrem, 2007; Strong et al., 2015), life-history stage (Boves et al., 2016; Goutte et al., 2010; López-Jiménez et al., 2017; Wilcoxon et al., 2011), nutrition (Fairhurst et al., 2015; Will et al., 2015, 2019) and climatic conditions (Hau et al., 2010; Treen et al., 2015) are likely more important variables than pollutants explaining variations in fCORT. In the Baltic WTE population, the most notable effect of pollution during the 1960s through 1980s was abnormal egg formation (eggshell thinning and egg desiccation), of which the latter was correlated with lowered productivity and subsequent population declines (Helander et al., 2002).

4. Conclusion

Despite the reported strong effects from pollutants on WTE reproduction during the study period, our results suggest that exposure to high POP levels did not impact the physiological stress of adults as measured by fCORT concentrations over the study period and the lack of association between POPs and fCORT. This study nonetheless provides novel information about fCORT concentrations in adult WTEs over a multi-decadal period covering periods during which this population suffered massive reproductive failure from being exposed to high POP contamination. Although we did not report significant relationships between fCORT, POP contamination or feeding ecology of the breeding pairs, analysis of fCORT represents a non-destructive and retrospective assessment of long-term stress in wild adult raptors otherwise not readily available. Since multiple biological and environmental factors likely affect CORT (e.g. Romero and Beattie (2021)), future studies using CORT as a biomarker of pollution exposure should consider the multiple stressor perspective, i.e., acknowledge that pollutants work in orchestra with other stressors when investigating the underlying mechanisms driving physiological stress in wild animals. In essence, feathers constitute a temporal archive for substances and remain the one matrix enabling a non-invasive and retrospective analysis of CORT physiology and POP exposure simultaneously in wild birds that have been exposed to harmful levels of environmental pollutants.

Credit author statement

Elisabeth Hansen: Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Jiachen Sun:** Formal analysis, Investigation, Writing – review & editing. **Björn Helander:** Investigation, Writing – review & editing. **Jan Ove Bustnes:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing. **Igor Eulaers:** Conceptualization, Resources, Writing – review & editing. **Veerle L. Jaspers:** Funding acquisition, Writing – review & editing. **Adrian Covaci:** Funding acquisition, Resources, Writing – review & editing. **Marcel Eens:** Funding acquisition, Resources, Writing – review & editing. **Sophie Bourgeon:** Conceptualization, Resources, Supervision, Writing – review & editing.

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Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2023.115923>.

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