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Feathers as integrated archives of environmental stress: Direct and indirect effects of metal exposure and dietary ecology on physiological stress in a terrestrial raptor

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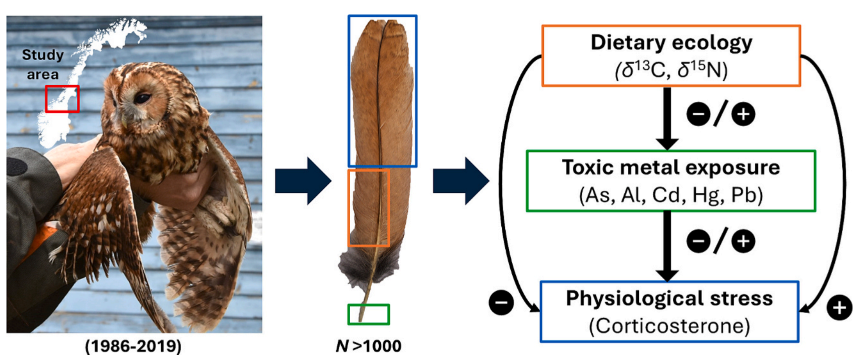
HIGHLIGHTS

- Over 1000 feathers sampled from female tawny owls in central Norway over 34 years.
- Feathers analyzed for toxic metals, corticosterone, and stable isotopes.
- Contrasting links found between corticosterone, metals, and stable isotopes.
- Diet and metal exposure can modulate physiological stress in tawny owls.
- Feathers can serve as integrated and non-destructive tools in ecotoxicology.

GRAPHICAL ABSTRACT



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ABSTRACT

Metal pollution is a global environmental issue with adverse biological effects on wildlife. Long-term studies that span declines in metal emissions due to regulation, resulting in varying levels of environmental contamination, are therefore well-suited to investigate effects of toxic metals, while also facilitating robust analysis by incorporating fluctuating environmental conditions and food availability. Here, we examined a resident population of tawny owls in Norway between 1986 and 2019. Tail feathers from females were collected annually, resulting in over 1000 feathers. Each feather served as an archive of local environmental conditions during molt, including the presence of metals, and their dietary ecology, proxied by stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$), as well as corticosterone levels (CORTf), the primary avian glucocorticoid and a measure of physiological stress.

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We analyzed feathers to examine how exposure to toxic metal(loid)s (Al, As, Cd, Hg, and Pb) and variability in dietary proxies modulate CORTf. Using structural equation modelling, we found that increased Al concentrations and δ^{15}N values, linked directly to increased CORTf. In opposite, we found that increased Hg concentrations and δ^{13}C related to decreased CORTf concentrations. δ^{15}N was indirectly linked to CORTf through Al and Hg, while δ^{13}C was indirectly linked to CORTf through Hg. This supports our hypothesis that metal exposure and dietary ecology may individually or jointly influence physiological stress. Notably, our results suggest that dietary ecology has the potential to mediate the impact of metals on CORTf, highlighting the importance of considering multiple variables, direct and indirect effects, when assessing stress in wildlife. In conclusion, feathers represent an excellent non-destructive biomonitoring strategy in avian wildlife, providing valuable insights not easily accessible using other methods. Further research is warranted to fully comprehend implications of alterations in CORTf on the tawny owl's health and fitness.

1. Introduction

Pollution by various metals, mostly due to anthropogenic activities such as mining and smelting, is a global environmental issue (Ali et al., 2019). While some metals (e.g., copper, iron, and zinc) are essential at trace levels and highly biologically regulated, non-essential metals can be toxic even at trace levels (Ali and Khan, 2019). Examples of non-essential toxic metals and metalloids (hereof metals for simplicity) include Aluminum (Al), Arsenic (As; metalloid), Cadmium (Cd), Mercury (Hg), and Lead (Pb) (Ali and Khan, 2019). Exposure of wildlife to high levels of toxic metals is related to adverse biological effects (Scheuhammer, 1987; Ackerman et al., 2024), which has led to the implementation of regulatory efforts to reduce their emissions worldwide (Harmens et al., 2015). Consequently, wildlife populations have been exposed to changing concentrations of metals in their environment over the last century (Burger, 2013; Berglund et al., 2015; Devalloir et al., 2023). Long-term studies are particularly well suited to monitoring exposure to and potential detrimental effects of metals by spanning temporal differences in environmental concentrations. However, long-term studies investigating the detrimental health effects of metal exposure in free-living birds are difficult and therefore scarce.

Corticosterone (CORT) is the primary glucocorticoid and stress-associated hormone in birds (Romero and Romero, 2002) and is often used as a biomarker of health in avian ecology and conservation research (Romero and Beattie, 2021). In general, CORT helps birds regulate energy in response to both normal life processes, such as during breeding, and unpredictable environmental perturbations (Lattin et al., 2016). When birds are exposed to stressors, CORT concentrations rise within two minutes from baseline to stress-induced levels (i.e., the stress-induced response), resulting in immediate physiological changes that maximize survival during stressful events including the activation of the fight or flight response (Romero and Romero, 2002). However, when birds are chronically exposed to stressors (e.g., contaminants), this can disrupt the stress response by maintaining elevated baseline CORT levels and hindering a proper stress-induced response (Rich and Romero, 2005). Chronic stress and elevated CORT can have adverse effects, including immunodeficiency and reproductive impairment (Chrousos, 2009) and were shown to affect individual fitness (Koren et al., 2012).

Obtaining both baseline and stress-induced plasma CORT concentrations in wild birds, particularly in long-term studies, is challenging. Feathers represent an alternative matrix to study CORT physiology, as they integrate both baseline and stress-induced CORT levels (Bortolotti et al., 2008) in addition to being easier to collect and store. When a feather grows and is connected to the bloodstream, CORT is deposited into the feather over a relatively long period of days to weeks (Bortolotti et al., 2009). Previous studies have shown that feather and blood CORT concentrations are closely related (Fairhurst et al., 2013a; Lattin et al., 2016). Importantly, CORT remains stable in the grown feather, which allows for retrospective studies (Beattie and Romero, 2023). As such, feather CORT (hereof CORTf) provides a long-term integrated measurement of individual stress physiology (Bortolotti et al., 2008). Variations in CORTf have been successfully used as a proxy of stress in relation to reproductive and fitness-related traits (Crossin et al., 2013; Crossin et al., 2017; Fairhurst et al., 2017; Monclús et al., 2020), climate variability (Romero et al., 2000; Treen et al., 2015; Crino et al., 2020), and food availability (Patterson et al., 2015; Will et al., 2015; Catitti et al., 2022). In addition, contaminant-induced stress has been of recent growing interest in avian ecotoxicology, and several studies have reported links between metal exposure and CORT in wild birds (Eeva et al., 2005; Baos et al., 2006; Strong et al., 2015; Powolny et al., 2020; White et al., 2022).

To assess the impact of environmental contaminants on avian wildlife, birds of prey are often used as biomonitors due to their high trophic position resulting in elevated contaminant exposure through biomagnification processes (Helander et al., 2008; Shore and Taggart, 2019; Badry et al., 2020). The tawny owl (*Strix aluco*), a non-migratory and territorial raptor common in Europe (Millon et al., 2010), has recently been identified as one of the most suitable candidates for pan-European biomonitoring programs (Ratajc et al., 2022). Tawny owls typically inhabit areas at the interface between urban and natural habitats near settlements, where they primarily feed on small mammals (such as voles) and passerine birds (Sunde et al., 2001). As a result, they are prone to accumulating contaminants associated with industrialized and urban environments, such as metals (Kekkonen, 2017; Devalloir et al., 2023). The tawny owl has been used in several long-term studies on environmental contaminants considering different matrices such as eggs (Bustnes et al., 2022) and feathers (Garcia-Seoane et al., 2017; Devalloir et al., 2023). Feathers, in particular, serve as an excellent non-destructive strategy for assessing local metal exposure (Lodenius and Solonen, 2013).

In the present study, we sampled tail feathers of tawny owls from a resident nest box population in Central Norway annually during breeding (i.e., April) in the period 1986–2019. Since tawny owls molt their tail feathers either annually or biannually after breeding (Petty, 1993), the collected feathers are assumed to encapsulate metabolites from the autumn with a lag of one or two years. Different sections of a single tail feather were used to analyze metals (lower shaft) and CORT (distal part). In addition, stable isotopes (middle shaft) of carbon (δ^{13}C) and nitrogen (δ^{15}N) were assessed to evaluate diet sources and trophic level (Inger and Bearhop, 2008), due to their influence on individual contaminant burden (Ratajc et al., 2022) and stress levels (Fairhurst et al., 2013b; Fairhurst et al., 2015; Will et al., 2015). Analyzing all metabolites in the same feather ensured that feather concentrations reflected the same overarching period (i.e., last molt) regardless of the timing of molting. In addition, only females were sampled as only females incubate and occupy the nest box (Sunde et al., 2003), eliminating possible sex-biased feather concentrations of metals and CORTf in our study.

We hypothesized that metals (Al, As, Cd, Hg, and Pb) and the dietary proxies (δ^{15}N and δ^{13}C) modulate CORTf in the tawny owl, and we investigated these relationships using structural equation modelling (Lefcheck et al., 2018). This allowed us to examine direct and indirect effects of stable isotopes and metals on CORTf. For example, we *a priori* expected that both diet and metals could negatively modulate CORTf concentrations (see e.g., Fairhurst et al. (2015), Fairhurst et al. (2013b), Will et al. (2015), Powolny et al. (2020), Strong et al. (2015) and White et al. (2022)). Diet can also influence metal exposure (Tasneem et al., 2020). Thus, we aimed to examine whether stable isotopes could indirectly influence CORTf through their association with metal exposure.

2. Materials and methods

2.1. Field sampling and data collection

The study was carried out in the period 1986–2019 in the area surrounding Trondheim (63.42° N, 10.23° E), Norway, where >150 tawny owl nest boxes have been deployed. Each nest box was visited annually in late April or early May around hatching when the females were caught to collect one of the two mid-positioned tail feathers. The collected tail feathers were stored individually in paper envelopes (or taped to paper sheets) at room temperature until chemical analysis. We used different sections of one feather per female to analyze corticosterone, stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), and metals (Supplementary information: Fig. S1). In total, 486 unique individuals (identified by ring number) were sampled in this study, with some females being sampled repeatedly in subsequent breeding seasons (Table S1). The number of owls caught annually varied between 8 and 71, and the total number of sampled tail feathers across the 34-year study period was 1202.

2.2. Analysis of metal and metalloid elements

Analyses of metal and metalloid elements were conducted at the heavy metal laboratory at NINA, Trondheim. In the current study, feathers from 2017 to 2019 were analyzed following the same protocol as for metal measurements in tawny owl feathers from the same population from 1986 to 2016 already described in detail by Devalloir et al. (2023). Only the lower shaft of the feather was used in the analyses, as it better represent the internal body burden of metals (Seoane et al., 2018). In brief, the shaft was acid digested in concentrated HNO_3 (ultra-pure grade) and maintained in a microwave oven (Milestone Mls Mega) at 180 °C for 45 min (going from 20 °C–180–20 °C). MilliQ-water diluted the HNO_3 acid solution (q.s. 0.6 M). Chemical analyses were carried out using HR-ICP-MS Element 2 (Thermo Electronics) with certified multi-element calibration solutions, matrix-matched for ion strength and acidity, and verified against certified reference material in solution (SPS-SW2). Measurement accuracy and uncertainty were confirmed using certified reference material (CRM). Contamination was monitored with at least three blanks, and repeatability was assessed by calculating the relative standard deviation (RSD) from repeated measurement values as already described in Devalloir et al. (2023). Among the 39 metallic elements that were analyzed (Bustnes et al., 2013), the present study focuses on five non-essential metals based on their potential toxicity (Devalloir et al., 2023), namely Al, As (metalloid), Cd, Hg, and Pb. Not all elements could be analyzed in all feather samples. We successfully acquired data for 1133 samples for Al, 1155 for As, 973 for Cd, 1169 for Hg and 1129 for Pb.

2.3. Feather corticosterone analysis

The analysis of feather CORT (CORTf) was performed at UiT, Tromsø, and the protocol is based on the description given by Bortolotti et al. (2008). A methanol-based extraction technique was used to extract CORT from feathers ($n = 1199$). First, the total weight and length of the feather (including the rachis) were measured before the distal 70 mm was cut straight across perpendicular to the rachis. Final extracts were assessed for CORT with an enzyme immunoassay kit (901–097, Assay Designs Inc., USA). The assay quality was validated using serial dilutions of feather extracts (displacement curves), which confirmed the absence of interfering substances in the extract. Each feather extract was measured in duplicate in 36 separate plates, and for all separate plates used in the present study, we reported intra- and inter-assay variability of 14.97 % ($n = 148$) and 14.71 % ($n = 164$), respectively. In addition, a pooled calibrator feather extract (prepared from 70 random feather extracts) was assayed on each plate and showed an inter-assay variability of 13.05 % ($n = 36$). The final value of CORTf was calculated using a standard curve run on each plate with a unit of pg mm^{-1}.

2.4. Stable isotope analysis

The analysis of stable carbon (^{13}C and ^{12}C) and nitrogen isotopes (^{15}N and ^{14}N) was performed at the Stable Isotope Lab of the RPTU Kaiserslautern-Landau, Germany. A total of 500 feathers covering the entire study period were randomly selected for stable isotope analysis. Feathers were washed in distilled water and dried overnight before the narrowest side of the vane (along the rachis) was cut off. The pieces cut from each feather were transferred to a Precellys MK28 hard tissue grinding tube containing stainless steel beads (Bertin, France) and ground to a fine powder in the Precellys 24 (Bertin, France) for 2 × 50 s at 6500 rpm with 15 s breaks. Finally, 1.00 ± 0.20 mg of ground feather sample were packed into tin capsules (8 × 5 mm; Sercon SC0009). Stable isotope ratios of carbon (^{13}C:^{12}C) and nitrogen (^{15}N:^{14}N) were determined in bulk homogenised feather material using a Flash 2000 HT elemental analyser coupled via a ConFlo IV interface to a Delta V Advantage isotope ratio mass spectrometer (all Thermo Fisher Scientific, Bremen, Germany). The stable isotope ratios are expressed as δ values (‰) relative to their respective international measurement standards Vienna Pee Dee Belemnite and atmospheric N_2. For quality control, an internal reference material (i.e., casein) was measured in duplicate every ten samples revealing a precision (±*SD*) ≤0.06 ‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Because atmospheric CO_2 has become progressively depleted in $\delta^{13}C$ during the last 150 years due to the burning of fossil fuels (known as the "Suess effect"; Keeling, 1979), $\delta^{13}C$ values were corrected using the SuessR package by applying the *SuessR* function in *R* with the sub-arctic North Atlantic as the specified study region (Clark et al., 2021).

2.5. Statistical analysis

The statistical analyses were conducted using R version 4.2.1 (R Core, 2023). All tests performed were two-tailed and the null-hypothesis was rejected at an alpha level of 0.05. 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). To attain the assumptions of normality and homogeneity of variance, CORTf and metal concentrations were ln-transformed. We considered one CORTf value, which exceeded 10,000 pg mm^{-1}, an outlier and subsequently removed it from the analyses.

Since 240 out of 486 owls had been repeatedly sampled in our study, we explored the repeatability of CORTf by calculating the intra-class correlation coefficient (ICC) (Wolak et al., 2012). Previous studies on hormone repeatability have suggested the presence of a glucocorticoid phenotype, characterized by stable between-individual differences in glucocorticoid concentrations (Taff et al., 2018). The ICC for CORTf was low (0.07; Table S2) and below 0.5, indicating poor repeatability (Wolak et al., 2012). This finding suggests the absence of an apparent "CORT titer phenotype" in feathers, which could have influenced our subsequent analyses if left unaccounted for.

We used structural equation modelling (SEM), to investigate both direct and indirect effects of stable isotopes and metals on CORTf (Lefcheck et al., 2018). SEM allows including more than one response variable in models, which is beneficial when working with variables that can act as both predictor and response, as in the case with stable isotopes and metals (Lefcheck et al., 2018). Since we need paired data for all variables, the SEM was conducted on a subsample because we had stable isotope data for only 500 feathers. Model fit was assessed using 'Goodness of Fit'-testing (see supplementary materials for details).

Five SEMs were built, each representing one metal, using the function *psem* (Lefcheck et al., 2018), with sub-models specified as linear models (*lm* function). The decision to build separate SEMs for each metal was made due to the relatively strong correlations among metals causing problems with collinearity, which made it difficult to determine the individual effect of the metals (Fig. S2). Each sub-model included one metal (response or predictor variable), $\delta^{13}C$ and $\delta^{15}N$ (predictor variables) and CORTf (response variable; Table S3a–e). In the SEM including Hg, $\delta^{13}C$ and $\delta^{15}N$ were excluded in the CORTf sub-model due to their relatively strong correlation with Hg, which caused collinearity problems (Fig. S2, Table S3e). Consequently, the direct effects of stable isotopes on CORTf could not be estimated in the SEM including Hg. Therefore, the estimates of the direct effects of stable isotopes on CORTf were retrieved from the SEMs including Al, As, Cd, and Pb (Table S3a–d).

We provide standardized path coefficients (i.e., the default parameters reported in the picewiceSE-package) retrieved from the SEMs to describe relationships between variables. In addition, we calculated 'Total effects' of stable isotopes (i.e., the sum of their direct effect and indirect effects) and 'Mediator' effects (i.e. the sum of all indirect paths which operate through each individual mediator - here, metals) with a 95 % confidence interval (CI) using the semEff package (Murphy, 2022).

3. Results

3.1. Descriptive statistics of metals, stable isotopes and CORTf

Among metals, Al contributed the most with 59.3 %, followed by Hg (36.1 %), Pb (2.4 %), and As (2.1 %), while Cd contributed the least (0.1 %) to the mean of the total targeted metal load (Table 1). The stable isotopes showed $\delta^{13}C$ ranging from −24.57 ‰ to −20.83 ‰ and $\delta^{15}N$ ranging from 4.33 ‰ to 11.63 ‰ (Table 1). For CORTf, the median value was 17.68 pg mm^{-1} but displayed a wide variability spanning over a 100-fold range from 2.91 to 327.0 pg mm^{-1} (Table 1).

3.2. Direct and indirect effects on CORTf

The SEMs applied in the present study revealed both direct and indirect effects of metal exposure and stable isotopes on CORTf in the tawny owl (Fig. 1; see Appendix Table S3a–e for full model summaries). Parameter estimates showed that Al had a direct positive effect on CORTf (path coefficient: 0.17), while Hg had a direct negative effect on CORTf (path coefficient: −0.17; Figs. 1 and 2). Further, $\delta^{15}N$ showed a direct positive effect on CORTf (path coefficient: 0.12–0.15 depending on SEM; Table S3a–d, Figs. 1 and 2), Al and Pb (path coefficients: 0.11 for both, Figs. 1 and 3), and a direct negative effect on Hg (path coefficient: −0.16; Figs. 1 and 3). $\delta^{13}C$, on the other hand, showed a direct negative effect on CORTf (path coefficient: −0.12–(−0.13) depending on SEM; Table S3a–d, Figs. 1 and 2) and a direct positive effect on Cd and Pb (path coefficients: 0.11 and 0.15, respectively; Figs. 1 and 3). The strongest relationship within the path analysis was the direct positive effect of $\delta^{13}C$ on Hg (path coefficient: 0.59; Figs. 1 and 3). The effect of Hg on CORTf is mediated if the indirect effects of $\delta^{15}N$ and $\delta^{13}C$ are considered (−0.07, 95 % CI: −0.12–[−0.03]). The total effects of stable isotopes on CORTf were 0.14 (95 % CI: 0.03–0.24) and −0.13 (95 % CI: −0.23–[−0.02]) for $\delta^{15}N$ and $\delta^{13}C$, respectively.

Table 1
Descriptive statistics of raw data for feather concentrations (dry weight) of metal and metalloid elements (Aluminum [Al], Arsenic [As], Cadmium [Cd], Mercury [Hg], and Lead [Pb]), stable isotope values ($\delta^{13}C$ and $\delta^{15}N$), and corticosterone (CORTf) in tawny owls from 1986 to 2019. Some metals exhibited concentrations below the level of detection (LOD), hence different sample sizes of metals. In addition, a subset of the total sample size was analyzed for stable isotopes.

	n	Median	Mean (±SD)	Min	Max
Al (μg g^{-1})	1133	0.87	1.74 (±5.21)	<0.17 (LOD)	146.1
As (μg g^{-1})	1155	0.04	0.06 (±0.14)	<0.008 (LOD)	2.38
Cd (μg g^{-1})	973	0.001	0.004 (±0.01)	<0.001 (LOD)	0.19
Hg (μg g^{-1})	1169	0.75	1.06 (±1.25)	0.08	17.80
Pb (μg g^{-1})	1129	0.02	0.07 (±0.20)	<0.001 (LOD)	5.03
$\delta^{13}C$ (‰)	500	−22.54	−22.57 (±0.70)	−24.57	−20.83
$\delta^{15}N$ (‰)	500	7.13	7.29 (±1.083)	4.330	11.63
CORT (pg mm^{-1})	1199	17.68	23.37 (±21.05)	2.91	327.0

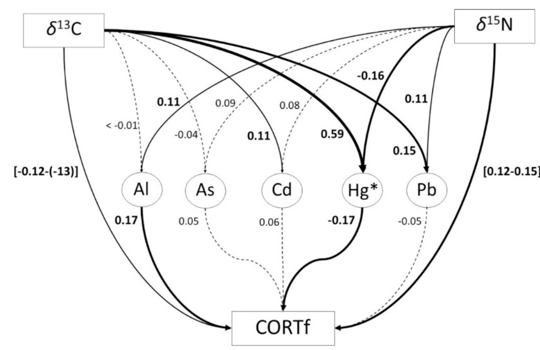

Fig. 1. Path diagram compiled by the five structural equation models (SEMs; Table S3a–e) to illustrate the relations among dietary ecology (proxied by $\delta^{13}C$ and $\delta^{15}N$), metal exposure (Al, As, Cd, Hg, or Pb), and physiological stress (CORTf). Like linear models, a positive path coefficient (i.e., positive effect) implies an increase in the response variables (here, CORTf or metals), and a negative path coefficient (i.e., negative effect) means a decrease. Filled lines and path coefficients in bold represent significant relationships, and dashed lines represent non-significant relationships. The width of the path lines is proportional to the strengths of the significant relationship (indicated by the standardized path coefficients). *In the SEM including Hg, $\delta^{13}C$ and $\delta^{15}N$ were not included in the CORTf sub-model due to problems with collinearity (Table S2e; see Section 2.5 for details). Therefore, the path coefficients for direct effects of stable isotopes on CORTf are derived from the SEMs including Al, As, Cd, and Pb, resulting in a small range for path coefficients.

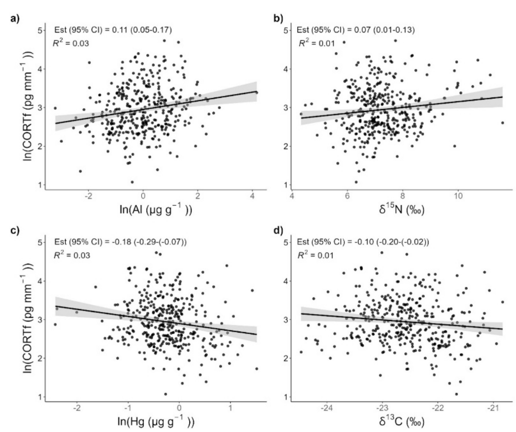

Fig. 2. Feather corticosterone (CORTf) levels in adult female tawny owls are positively related to aluminum (Al) (a) and $\delta^{15}N$ (b), but negatively related to mercury (Hg) (c) and $\delta^{13}C$ (d). The fitted lines, coefficient estimates (Est) with 95 % confidence intervals (CI) and adjusted R^2-values are derived from simple linear regression models (function lm). Both CORTf values and metal concentrations are natural log (ln) transformed.

4. Discussion

In this study, we illustrate how feathers can be used to unravel

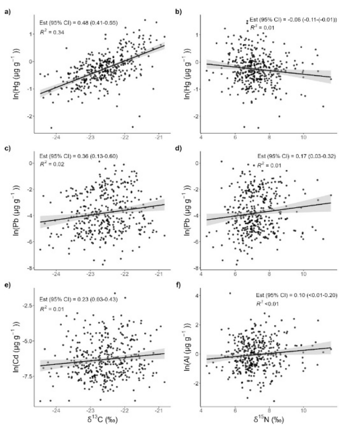

Fig. 3. Feather concentrations of 4 out of 5 investigated metals (ln-transformed) were significantly related to stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in adult female tawny owls. Mercury (Hg) is positively related to $\delta^{13}C$ (a) and negatively related to $\delta^{15}N$ (b). Lead (Pb) is positively related to both $\delta^{13}C$ (c) and $\delta^{15}N$ (d). Cadmium (Cd) is positively related to $\delta^{13}C$ only (e), while aluminum (Al) is positively related to $\delta^{15}N$ only (f). The fitted lines, coefficient estimates (Est) with 95 % confidence intervals (CI) and adjusted R^2-values are derived from simple linear regression models (function lm).

potential sublethal effects of environmental stress by investigating the interrelationships between metal exposure, dietary proxies, and physiological stress (CORTf) in a terrestrial raptor. Our findings reveal indirect and direct effects of $\delta^{13}C$, $\delta^{15}N$, and direct effects of toxic metals (Al and Hg) on CORTf supporting our hypothesis that these factors may influence CORTf levels in the tawny owl. We provide valuable insights into the complex dynamics of environmental stressors and their possible impact on avian physiology, highlighting the importance of considering multiple interacting factors in ecotoxicological and/or wildlife conservation studies.

The direct and positive relationship between Al and CORTf suggests that higher concentrations of Al elevate stress hormone levels in tawny owls. This finding corroborates previous studies that reported increases in CORTf with increasing concentrations of non-essential metals in wild birds, including raptors (Strong et al., 2015; Meillere et al., 2016; Powolny et al., 2020). In contrast, we found that CORTf was negatively related to Hg, suggesting that owls with higher Hg concentrations have lower CORTf levels. Metals can influence CORT physiology in wild birds by interacting with the adrenal glands and/or the hypothalamic-pituitary-adrenal axis, potentially activating or suppressing synthesis or metabolism which affects the secretion and/or release of CORT (Hidalgo and Armario, 1987; Wayland et al., 2002; Pollock and Machin, 2009). Determining whether elevated or lowered CORTf levels are indicative of physiological stress is, however, challenging. Higher CORTf could indicate chronic stress or the accumulation of multiple stressful events during molt (Romero and Beattie, 2021). Conversely, lower CORT levels are not always indicative of individuals in good health; they may reflect difficulties in coping with stressors (Romero and Beattie, 2021) or suppressed baseline CORT levels, which can detrimentally affect various physiological functions (Whitney and Cristol, 2018).

In ecotoxicological research, it is also crucial to consider that wildlife is exposed to a mixture of contaminants in their environment (Powolny et al., 2020). Therefore, the impact of metal exposure on physiological indicators such as CORT may result from additive, synergistic, or antagonistic interactions among various contaminants (Lin et al., 2016; Powolny et al., 2020). As such, metals that contributed most to the total targeted metal load in the tawny owl, namely Al and Hg, could have masked or mitigated potential effects on CORTf by the other metals (As, Cd and Pb), which contributed to <5 % of the total targeted metal load.

Wildlife is primarily exposed to contaminants through their diet. Therefore, dietary proxies such as $\delta^{13}C$ and $\delta^{15}N$ often explain variations in metal concentrations (Badry et al., 2019; Tasneem et al., 2020; Lidman and Berglund, 2022). For $\delta^{15}N$, the wide range in the observed values suggest that the prey items consumed by the tawny owls originated from various trophic levels (since each trophic level is successively enriched by ∼3–4 ‰; Kelly, 2000). Notably, common prey of the tawny owl such as omnivorous and insectivorous animals including passerine birds and amphibians typically exhibit higher $\delta^{15}N$ values than herbivorous animals such as voles (Swan et al., 2020). Tawny owls are opportunistic feeders, primarily preying on rodents like voles, especially when abundant (Sunde et al., 2001; Solonen, 2009). Long-term studies have shown a dampening of these cycles since the 1980s (Hörnfeldt, 2004; Cornulier et al., 2013), altering prey availability for predators such as tawny owls (Millon et al., 2014; Avotins et al., 2023).

$\delta^{13}C$ values in the present study aligned with the typical range observed in predators that feed in the terrestrial ecosystem, that is, below −20 ‰ (Inger and Bearhop, 2008). In contrast to $\delta^{15}N$, $\delta^{13}C$ is usually only marginally enriched between trophic levels (0–1 ‰) and therefore not deemed suitable to distinguish among trophic levels of prey (Inger and Bearhop, 2008). However, a study by Villegas et al. (2021) found that $\delta^{13}C$ in herbivorous finches was higher than in insectivorous finches, indicating that $\delta^{13}C$ might be useful for distinguishing dietary guilds.

In the present study, we found that Al and Pb were positively related to $\delta^{15}N$, suggesting higher concentrations of these metals in higher trophic prey. In contrast, we found a negative relationship between $\delta^{15}N$ and Hg, contrary to the generally documented positive correlation (Carravieri et al., 2018; Badry et al., 2019; Chetelat et al., 2020). Compared to other metals, Hg exhibits higher bioavailability (especially in its organic methylated form) causing it to accumulate readily in biota (Chetelat et al., 2020). Hg biomagnification is well-documented in marine ecosystems, with higher trophic species showing elevated Hg concentrations (Chetelat et al., 2020). In the terrestrial ecosystems where tawny owls reside, Hg biomagnification is typically less pronounced (Dietz et al., 2022). Yet, it still occurs, influenced by variations in food chain structure, species composition, and environmental conditions (Douglas et al., 2012). We did, however, observe a relatively strong positive relationship between $\delta^{13}C$ and Hg, indicating that variability in the carbon source (e.g., prey types) is an important driver of Hg exposure in the tawny owl. Al and Pb were also linked to more positive $\delta^{13}C$ values, but to a lesser extent than Hg.

Since both Al and Hg were directly related to CORTf, dietary patterns likely influence exposure to these two metals and consequently their effect on CORTf in the tawny owl. We found that more positive $\delta^{13}C$ is linked to lower CORTf in the tawny owl, while owls with higher $\delta^{15}N$ (i.e., higher trophic prey) were associated with higher CORTf. This suggests that changes in dietary patterns (e.g., changes in prey composition or availability of specific prey) may modulate CORTf, consistent with previous research on wild birds (Fairhurst et al., 2013b; Fairhurst et al., 2015; Will et al., 2015). The influence of variation in dietary patterns on CORT physiology may stem from the fluctuating availability of preferred prey, leading individuals to feed on less favorable prey with, for example, lower nutritional value (Fairhurst et al., 2015) or potentially higher energetic costs of capture (Solonen et al., 2017). Vole populations exhibit cyclical fluctuations (Oli and Dobson, 2001), significantly influencing tawny owl breeding performance (Solonen, 2009; Solonen, 2022; Orlando et al., 2023). The variability in vole populations likely contributes to the observed patterns in dietary proxies and CORTf in this study, potentially altering the owl's preferred prey selection and subsequently affecting their stress physiology.

Overall, the SEM pathways in our study reveal diverse scenarios of how CORTf can be influenced, and potentially mediated, by changes in dietary patterns and the magnitude of metal exposure in tawny owls. It is essential to consider the strength of these path coefficients (ranging from −1 to 1), as some factors exert stronger effects than others. For instance, the direct effect of $\delta^{13}C$ on Hg was 0.59, while the direct impact of metals on CORTf appeared weaker (0.17 for Al and −0.17 for Hg). This suggests that while some metals modulate stress hormone levels, their individual contributions may be less influential when accounting for other variables, such as dietary proxies. For example, our results showed that δ^{13}C and δ^{15}N mediated the direct effect of Hg on CORTf, reducing the strength of the effect from −0.17 (direct effect) to −0.7 (mediated effect). Consequently, the total effect of the stable isotopes was greater than the mediated effect of Hg on CORTf, with path coefficients of −0.13 for δ^{13}C and 0.14 for δ^{15}N. Additionally, δ^{15}N were directly linked to Al, indicating that the effect of Al on CORTf is likely influenced by dietary ecology, although to a lesser degree than Hg. Thus, focusing solely on metal exposure may overlook dietary ecology's broader influence on tawny owls' stress physiology.

In conclusion, when assessing stress in wild animals, it is essential to consider multiple variables given the complexity of factors influencing the stress response (Romero and Beattie, 2021). Our study highlights the use of feathers as an excellent non-destructive integrated biomonitoring strategy for long-term retrospective analysis of stress physiology, metal exposure, and dietary ecology in avian wildlife. Indeed, feathers offer unique advantages compared to other sampling methods, providing valuable insights not easily accessible in wild birds (Ganz et al., 2018). Further research is warranted to explore the underlying mechanisms behind the divergent relationships between metals and CORTf and to fully understand the implications of alterations in CORTf on the health and fitness of tawny owls. In addition, future studies should analyze the prey items and stable isotopes in the primary prey of tawny owls to better understand how toxic metal exposure affects these owls in the context of a dynamic ecosystem.

CRediT authorship contribution statement

Elisabeth Hansen: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Jan Ove Bustnes:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Dorte Herzke:** Writing – review & editing, Supervision, Conceptualization. **Georg Bangjord:** Writing – review & editing, Resources, Investigation, Data curation. **Manuel Ballesteros:** Writing – review & editing, Formal analysis. **Bård-Jørgen Bårdsen:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Eric Bollinger:** Writing – review & editing, Formal analysis. **Ralf Schulz:** Writing – review & editing, Resources. **Igor Eulaers:** Writing – review & editing, Resources, Formal analysis, Conceptualization. **Sophie Bourgeon:** Writing – review & editing, Supervision, Project administration, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.176324.

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