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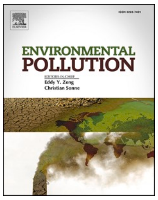

Fecal calcium levels of bird nestlings as a potential indicator of species-specific metal sensitivity☆

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ABSTRACT

Sensitivity of bird species to environmental metal pollution varies but there is currently no general framework to predict species-specific sensitivity. Such information would be valuable from a conservation point-of-view. Calcium (Ca) has antagonistic effects on metal toxicity and studies with some common model species show that low dietary and circulating calcium (Ca) levels indicate higher sensitivity to harmful effects of toxic metals. Here we measured fecal Ca and five other macroelement (potassium K, magnesium Mg, sodium Na, phosphorus P, sulphur S) concentrations as proxies for dietary levels in 66 bird species to better understand their interspecific variation and potential use as an indicator of metal sensitivity in a wider range of species (the main analyses include 39 species). We found marked interspecific differences in fecal Ca concentration, which correlated positively with Mg and negatively with Na, P and S levels. Lowest Ca concentrations were found in insectivorous species and especially aerial foragers, such as swifts (Apodidae) and swallows (Hirundinidae). Instead, ground foraging species like starlings (Sturnidae), sparrows (Passeridae), cranes (Gruidae) and larks (Alaudidae) showed relatively high fecal Ca levels. Independent of phylogeny, insectivorous diet and aerial foraging seem to indicate low Ca levels and potential sensitivity to toxic metals. Our results, together with information published on fecal Ca levels and toxic metal impacts, suggest that fecal Ca levels are a promising new tool to evaluate potential metal-sensitivity of birds, and we encourage gathering such information in other bird species. Information on the effects of metals on breeding parameters in a wider range of bird species would also help in ranking species by their sensitivity to metal pollution.

1. Introduction

Sensitivity of birds to environmental pollution varies among species (Blus and Henny, 1997; Fair and Myers, 2002; Peakall and Burger, 2003; Head et al., 2008; Berglund et al., 2011). This may result from differences in exposure (e.g. through the diet), physiological sensitivity (e.g. differences in detoxification ability) or different sensitivity to indirect pollution effects (e.g. via pollution-related changes in food availability or habitat quality). However, information on species-specific sensitivity is very limited in wildlife (Spurgeon et al., 2020). Diet is in many cases the largest source of environmental pollutants for birds and hence an important source of variation in exposure levels (Scheuhammer, 1987; Orłowski et al., 2015, e.g. Ali and Khan, 2019; Lidman and Berglund, 2022). Information on dietary pollutant levels of species are therefore important in estimating pollution-related effects. Yet they do not reveal the response of a species, and relatively similar exposure levels may produce variable responses among species e.g. in reproductive success (Eeva and Lehikoinen, 1995). Reasons to species-specific responses often

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remain unclear without more detailed studies, although from the conservation point-of-view such information would be valuable.

Potentially toxic metals (often called heavy metals) are a classical group of environmental contaminants and their accumulation in birds depends on diet quality (e.g. Belskii and Belskaya, 2013; Orłowski et al., 2015). An important essential dietary nutrient metal is calcium (Ca) and, besides other vital biochemical roles (e.g. in blood coagulation, muscle function, energy metabolism and cell signaling), the importance of Ca for bird eggshell formation and reproduction is well documented (Graveland and Drent, 1997). Rich Ca level in the diet diminishes absorption of toxic metals from food such as lead (Pb) and cadmium (Cd), and mitigate harmful effects of other metals (Scheuhammer, 1996; Eeva and Lehikoinen, 2004; Dauwe et al., 2006). This is because many metals as divalent cations resemble Ca ions and show similar mobility but antagonistic relationship with Ca in the body (Barton et al., 1988; Marchetti, 2013). Essential elements like Ca and P compete with toxic metals for absorption in the gastrointestinal tract reducing their uptake (Blake and Mann, 1983; Goyer, 1997). Calcium can also form complexes with other metals, and these metal ligand complexes are less readily absorbed by the body and more likely excreted through feces (Flora and Pachauri, 2010). Finally, when absorbed, toxic metals interact metabolically with nutritionally essential metals, e.g. interfering with Ca receptors and impairing normal Ca homeostasis in cells (Goyer, 1997).

A good example of Ca-related species-specific differences in metal sensitivity are two common cavity-nesting insectivorous passerines, the great tit (*Parus major*) and pied flycatcher (*Ficedula hypoleuca*). Developing *P. major* nestlings have proved more resistant to metal exposure in a polluted environment adjacent to a Finnish copper-nickel smelter, whereas *F. hypoleuca* suffered from thin egg shells, deficit bone development and decreased nestling survival at relatively similar exposure levels in the same study area (Eeva and Lehikoinen, 1995, 1996). The key factor for better pollution resistance of *P. major* has been shown to be their up to four times higher dietary Ca level, which shows up in their nestlings' fecal and blood plasma Ca concentrations (see also Mänd and Tilgar, 2003; Eeva and Lehikoinen, 2004; Espín et al., 2016a). The question remains whether this observation applies to a wider range of bird species and could fecal Ca levels be a useful and more general indicator of metal-sensitivity?

The idea of species-specific Ca-related metal sensitivity has not been tested among a wider range of bird species. This is partly because there is currently not much information on species-specific dietary Ca levels aside from a few common model species in avian ecology. In order to meet this challenge, we gathered fecal samples as a proxy measure from nestlings of 66 Finnish bird species to estimate levels of their dietary Ca and five other macroelements (potassium K, magnesium Mg, sodium Na, phosphorus P, sulphur S). As compared to Ca, nutritive aspects of the latter five elements are much less studied in wild birds but for example Mg and P have important roles in Ca homeostasis (Shastak and Rodehutscord, 2015; Loughrill et al., 2016) and could hence be potential indicators of metal sensitivity. Feces are considered good indicators of dietary metal levels and, for example, reflect closely the dietary level of Ca since the majority of ingested Ca is normally excreted via urine and feces (Reynolds, 1997; Bendsen et al., 2008; Ross et al., 2011; Eeva et al., 2020). Dietary Ca is known to be especially critical for successful breeding (e.g. relative to egg shell thinning) of small-sized passerines because they have limited capacity to store Ca in their skeleton for the needs of reproduction, and more specifically, for production of Ca carbonate containing egg shells (Graveland and van Gijzen, 1994). This is why also our sampling and analyses mainly focus on Ca and passerines, although a few species from other orders were included too.

In this study we compared levels of fecal Ca among species, bird families, diets (insectivores, omnivores and granivores) and foraging niches (e.g. aerial screening, arboreal cleaning, ground foraging etc.) in order to rank and classify bird species according to their potential metal sensitivity. Since insects and seeds have shown to be relatively poor sources of Ca (Graveland and van Gijzen, 1994), we hypothesized that insectivorous and granivorous species should show generally lower fecal Ca levels than omnivorous species. Our analysis focused on nestling stage because fast growing young are generally considered most prone for detrimental effects of metals, partly due to their high food intake and need of Ca (Scheuhammer, 1987; Ross et al., 2011). In addition to the former, we reviewed information in the literature on fecal Ca levels in birds.

2. Materials and methods

2.1. Fecal sampling and measurements

We collected fresh fecal sacks from nestlings of 66 bird species (Supplement S1) during their ringing and measuring in 2015–2021. Fresh fecal sacks were collected in plastic 1.5 ml Eppendorf-tubes, avoiding contamination. We used brood as a sampling unit by combining fecal sacks from chicks within a brood in the same tube (n = 412). Average sample number per species was 12.4 (range 1–35 broods). Nests were intentionally searched for fecal sampling, or samples were collected in some previously established nest box populations. In addition, some nests were found randomly when doing other field work. Primarily we focused on passerines (Passeriformes) but occasional species from 7 other orders were also sampled (Table S1). We collected samples in a relatively wide geographic area, but most samples (65%) were taken in southwestern Finland (Fig. 1). Within Finland, soils are generally not rich in Ca, soil pH values are relatively low (mean ± SD: 4.8 ± 1.27) and there are no strong geographical gradients in topsoil acidity (Ballabio et al., 2019). We did not restrict the sampling

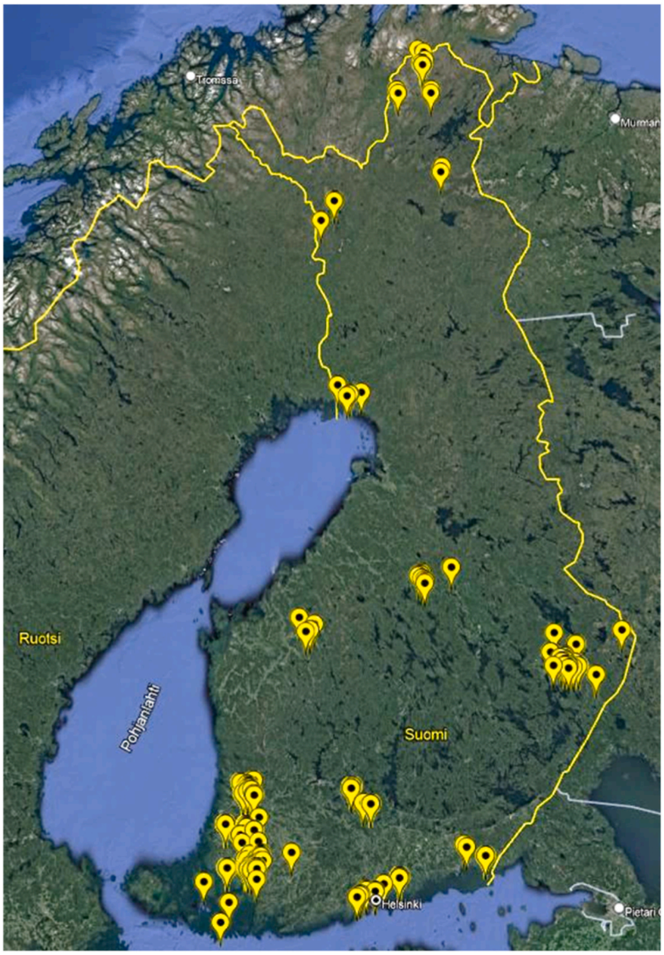

Fig. 1. Geographical distribution of fecal samples (n = 412) in Finland. Map source: Google Earth Pro.

environment, except that sampling in the vicinity of known heavily polluted industrial locations (e.g. a copper smelter) was avoided. Ringing and handling of birds was based on the Finnish policy of ringing licenses issued by Finnish Museum of Natural History and Centers for Economic Development, Transport and the Environment (VARELY/959/January 07, 2012, VARELY/3622/2017, VARELY/1344/2018, VARELY/2341/2019, VARELY/913/2020, VARELY/1134/2020, VARELY/1914/2021).

The following information was recorded at sampling: species, date, location (coordinates) and wing length (mm; maximum method) of nestlings. Age of nestlings varied at sampling and wing length was used for age determination. However, because the sampled species varied a lot in their nestling development period duration and adult size we used relative wing length (RWL) instead of age as a measure of developmental stage of nestlings. RWL was calculated as a percentage of nestling wing length (brood average) of the wing length of adult birds. Wing lengths of adult birds were taken from Piha and Lehikoinen (2016) and Piha et al. (2019). For some broods (ca. 8%), wing length was not available and we used the ringer's own estimate of the nestling age. To calculate RWL value also for these broods, ages were converted to wing length estimates by using wing growth data from our own databases from Kevo and Harjavalta (Culina et al., 2021), database of Finnish Ringing Centre, or data from literature (Tiainen, 1978; Ricklefs and Peters, 1979; Soler and Soler, 1990; Johnston, 1993; Helm, 2003). For conversion we fitted to those data species-specific logistic growth curves with MS Excel Solver function.

2.2. Macronutrient analyses

In the laboratory, samples were dried for 72 h at 50 °C and ground to a powder. Dried samples were analyzed for their Ca, K, Mg, Na, P and S content with OES-MS analyzer in CEBAS-CSIC laboratory in University of Murcia (Spain). A set of some potentially toxic elements (e.g. arsenic, As) were also measured but since we avoided collecting samples from polluted environments, their levels are generally low (e.g. less than 10% of As values were above the detection limit) and not analyzed here further. Samples (dry mass 0.0072–0.50 g) were placed in digestion tubes together with a mixture of 4 ml HNO_3 (70 %) and 1 ml H_2O_2 (33 %). Samples were then submitted to a thermal microwave treatment and, after which they were diluted in ultrapure water before analysis. Commercial reference material BCR–063 R Skim milk powder by European Virtual Institute for Speciation Analysis (EVISA) were used as certified reference samples (n = 5). Recoveries (% of certified value ± SD) were as follows: Ca 101 ± 3.4%, K 100 ± 3.5%, Mg 95 ± 3.7%, Na 95 ± 3.4%, P 108 ± 5.2% (no certified value for S). One sample was discarded from all analyses as an outlier due to very high values for all the elements. The final sample size is therefore 412 (Table S1). All concentrations were expressed per dry weight (d.w.).

2.3. Ecological classification

Sampled species were classified by their taxonomy (Family), main dietary preference (insectivores, omnivores and granivores) and primary foraging niche during the breeding period (Table S1). Diet classes are based on Bandelj et al. (2015), Pearman et al. (2014) and Tobias and Pigot (2019) with a few modifications (e.g. *Turdus* ssp. are all considered omnivorous). Note, however, that most of the birds classified as non-insectivores feed varying proportion of insects or other animal food to nestlings. Foraging niche classification (AE = Aerial screening, AG = Arboreal gleaning, AS = Aerial sallying, AQ = Aquatic surface, BG = Bark gleaning, FG = Foraging generalist, GF = Ground foraging) follows Tobias and Pigot (2019) with one modification (*Sturnus vulgaris* considered as a ground forager instead of arboreal gleaner).

2.4. Literature review

Web of Science database and the internet were searched for to find published fecal calcium values for wild free-living birds. We did not aim to make a systematic review but rather to collect examples of fecal Ca levels in nestling birds of different species for a comparison to our own values. We used keywords such as *feces*/faeces, *fecal/faecal, bird*/avian, nestling** and *calcium* in different combinations. Since mean values are published as arithmetic and geometric means, we report separate values for them. The gathered information is presented in Supplement S2.

2.5. Statistics

Because the samples were collected from a relatively large geographical area, we first checked with linear mixed models (LMM with species as a random effect) if there were strong spatial gradients in our element concentration. All concentrations were $\log_{10}$-transformed before the analyses to better conform the normal distribution. None of the elements showed significant latitudinal or longitudinal gradients (e.g. for Ca; latitude: $F_{1,344} = 1.03$, $p = 0.31$; longitude: $F_{1,344} = 0.01$, $p = 0.91$) and these spatial variables were not included in the further models. Next, we explored if Ca values in bird feces reflected local soil acidity. For this we downloaded LUCAS 2018 TOPSOIL data from the European Soil Data Centre (ESDAC; Panagos et al., 2022), including topsoil pH (in H_2O) values around the Europe (Ballabio et al., 2019). We combined the soil data to our bird data in QGIS (QGIS Development Team, 2023), by always using the average values of three nearest soil measurements for each fecal sampling point (a mean distance to our sampling points was 15.8 km). The analysis (LMM with species as a random factor) indicated no significant relationship between soil acidity and fecal Ca level of birds ($F_{1,345} = 0.11$, $p = 0.74$) and this soil variable was not included in the further models.

First, we tested with linear models (LM) interspecific differences in $\log_{10}$-transformed concentrations of six elements for those 39 species for which we had at least three samples (n = 380 broods). RWL (a measure of developmental stage) was first included as a covariate, but retained in the model only for Na since the effect was not statistically significant for the other elements. Normality was checked visually and with Kolmogorov-Smirnov test from model residuals. Estimates were transformed back to the original scale for figures and tables. Second, we tested family level differences in element concentrations with LMMs by including those 17 families for which we had at least five samples (compared to species level analysis, a slightly larger cut point value could be used here because family level samples sizes are generally higher). This analysis included 56 species (n = 398 broods) and we used species as a random factor in the model. Multidimensional preference analysis (SAS Institute Inc, 2013) was further used for visual presentation (principal component biplot; based on the correlation matrix) of relationships between the six elements among bird families. For all these analyses we used SAS 9.4 software (SAS Institute Inc, 2013).

Taxonomically closely related species are not statistically independent because of more recently shared common ancestry and therefore we wanted to explore how much the phylogeny would explain of the observed Ca level pattern. All the phylogenetic statistical analyses were run in R version 4.2.2. with RStudio (R Core Team, 2022). To incorporate phylogenetic relatedness between bird species in the models, we first constructed 100 phylogenetic trees (Nexus format) with branch lengths of 39 bird species by using the webpage application at Birdtree.org (https://birdtree.org/). Of those we created a consensus tree with mean edge length method by using the ape package (Paradis et al., 2004) in R.

Pagel's λ (Pagel, 1999; Freckleton et al., 2002) was used to measure the phylogenetic signal in fecal Ca levels. Pagel's λ varies between 0 and 1: value 0 indicates a random trait value with respect to phylogeny (i.e. no phylogenetic signal), whereas value 1 indicates phylogenetic trait conservatism (i.e. phylogenetic dependence; Freckleton et al., 2002).

Pagel's λ was calculated with the R function phylosig in the phytools package (Revell, 2012). Finally, we tested whether Ca levels differed between diet classes and foraging niche of study species ($n = 39$) with (phylogenetic linear mixed model, PGLM; package caper in R) and without (LMM in Glimmix procedure of SAS) phylogenetic structure. PGLM was ran with $\log_{10}$-transformed species-specific means and LMM with $\log_{10}$-transformed individual values by using species as a random factor.

3. Results

All the fecal levels of elements showed significant differences among species (Table 1). Four lowest Ca levels included purely insectivorous species and aerial foragers such as *Apus apus*, *Delichon urbicum*, *Muscicapa striata* and *Hirundo rustica* (Fig. 2). Species with highest Ca levels were ecologically variable but contained notably many ground foraging species such as *Plectrophenax nivalis*, *Sturnus vulgaris*, *Passer montanus*, *Turdus merula* and *Grus grus* (Fig. 2). Similar pattern was shown by Mg, which correlated positively with Ca, while Na, P and S correlated negatively with Ca, and K showed no significant correlation (Fig. 3). Sodium was the only element significantly positively associated with the developmental stage (RWL) (Table 1). Interspecific differences in Ca levels were large, species-specific means ranging from 365 to 20,186 mg/kg, d. w. (Fig. 2). Of the six minerals, Ca showed highest interspecific variation, coefficients of variation being: Ca = 1.02, K = 0.30, Mg = 0.27, Na = 0.55, P = 0.29, S = 0.25 ($n = 39$ species; Table S1).

All the elements, except Mg, showed significant differences among Families (Table 1). Lowest Family-level Ca values included Apodidae, Hirundinidae, Picidae and Certhiidae (Fig. 4). Highest Family-level Ca values included Sturnidae, Passeridae, Gruidae and Alaudidae (Fig. 4). The other elements, except Mg, showed generally opposite patterns to Ca, e.g. Gruidae, Sturnidae, Turdidae, Passeridae and Alaudidae showing relatively low total levels (Fig. 5). In the multidimensional preference analysis, the first two principal components (PC1 and PC2) showed eigenvalues >1 and explained 76% of the variation in the element concentrations. PC1 got highest negative factor loadings by Ca and Mg, and highest positive loadings by S and P (Fig. 6). PC2 got highest positive loadings by K and P and negative loadings by especially Na (Fig. 6). Families Sturnidae, Passeridae, Gruidae, Alaudidae and Turdidae locate at Ca rich, while Apodidae, Certhiidae, Hirundinidae and Muscicapidae locate at Ca poor end of the continuum (Fig. 6). Families Certhiidae, Apodidae, Acrocephalidae and Hirundinidae were further associated to relatively high S and Na levels (Fig. 6).

We found no clear (i.e. deviating significantly from zero) phylogenetic signal for species-specific geometric means of Ca concentration ($\lambda = 0.00006$, $p = 1.0$, $n = 39$ species). This is apparent, for example, among closely related species of Paridae (*Parus*, *Periparus*, *Poecile*, *Cyanistes*) and Turdidae (*Turdus* spp.), both families showing relatively large interspecific variation in Ca values (Fig. 7). Due to this, the results in LMM and the PGLM were rather similar. We found significant variation in Ca levels among dietary groups (PGLM: $F_{2,36} = 5.83$, $p = 0.0064$; LMM: $F_{2,40.1} = 5.10$, $p = 0.011$). Insectivorous species showed lower fecal Ca levels (3010 [CL: 2160−4190] mg/kg) than omnivorous species (6740 [CL: 3830−11,900] mg/kg; Tukey's test: $t = 2.50$, $p = 0.044$) while granivorous species showed still higher (10,500 [CL: 3810−28,900] mg/kg) but not significantly different Ca level as compared to the omnivorous species (Tukey's test: $t = -0.77$, $p = 0.72$). Note, however, that relatively low number of samples especially for granivorous species ($n = 21$ broods) makes the test power low (0.23) for this comparison. Among the foraging habitats, the Ca levels differed significantly in the PGLM ($F_{5,33.0} = 6.54$, $p = 0.00025$) and in the LMM ($F_{5,27.9} = 8.42$, $p < 0.0001$). Aerial foragers (Apodidae and Hirundinidae) showed the lowest and ground foragers the highest fecal Ca levels (Fig. 8).

Information on nestling's fecal Ca levels for wild bird species was scanty in literature. All in all, we found 14 studies (26 datasets) reporting mean values for only 9 species (Table 2). These, mostly common avian model species, follow the same order in terms of Ca levels than in our data, except that *Passer montanus* showed higher levels than *P. major*. *Apus apus* and two swallow species showed lowest Ca levels, followed by aerially sallying species, *Sialia sialis* and *F. hypoleuca*. By far the highest mean value was found for *Gypaetus barbatus* − a species specialized in eating bones. Pearson correlation between $\log_{10}$-transformed means of the current study (geometric) and literature (arithmetic or geometric) was 0.75 for the seven overlapping species.

4. Discussion

Our multi-species comparison of fecal Ca levels indicates considerable inter-specific variation in fecal Ca levels and this variation reflects the food and foraging ecology of species, independent of their phylogeny. Based on these results we can hypothesize that strictly insectivorous species, and especially those foraging on aerial arthropod fauna (e.g. swifts and swallows), could be sensitive to toxic metals due to their low dietary Ca levels and potentially harmful effect of toxic metals on their Ca metabolism (Goyer, 1997). An analogous case are human populations with low dietary Ca, being at the risk of metal toxicity (Blake and Mann, 1983). This risk is further enhanced in insectivorous species, both birds and mammals, due to their tendency to easily accumulate toxic metals (e.g. Wijnhoven et al., 2007; Leonzio et al., 2009; Ackerman et al., 2019). An extreme case are swifts, facultative aerial foragers, and our study confirms the earlier findings that these pure insectivores show especially low dietary Ca levels (Orłowski et al., 2015). On the other hand, species often foraging on the ground and/or using more variable diet (omnivores) could be less sensitive due to mitigative effect of higher dietary Ca levels against absorption and impact of toxic metals (Dauwe et al., 2006; Sánchez-Virosta et al., 2019). Contrary to our expectation, the species classified as granivores also showed relatively high Ca levels in nestling feces but their small number in our sample call for more studies on this dietary group, and still other groups (e.g. nectarivores, frugivores and carnivores) and more taxa should be studied in the future. More detailed and direct quantification of diet quality (e.g. by metabarcoding of fecal samples) would also help in interpreting the connection between diet and Ca levels (see Orłowski et al., 2015).

Table 1

F-test statistics for differences in fecal concentrations of six macroelements among 39 bird species (Model 1)[a] and 17 families (Model 2)[b]. N = number of broods. RWL = relative wing length (a measure of developmental stage).

Models		N	F					
			Ca	K	Mg	Na	P	S
Model 1	Species	380	12.2***	5.55***	5.10***	5.82***	6.94***	6.29***
	RWL		ns	ns	ns	9.43**	ns	ns
Model 2	Family	398	3.41**	7.78***	1.38	3.88**	3.23**	3.59**
	RWL		ns	ns	ns	12.0***	ns	ns

[a] Linear model with relative wing length (RWL) as a covariate. Model estimates for Ca shown in Fig. 2.

[b] Linear mixed model with species as a random factor. Model estimates are shown in Figs. 4 and 5. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns: variable dropped from the model when non-significant.

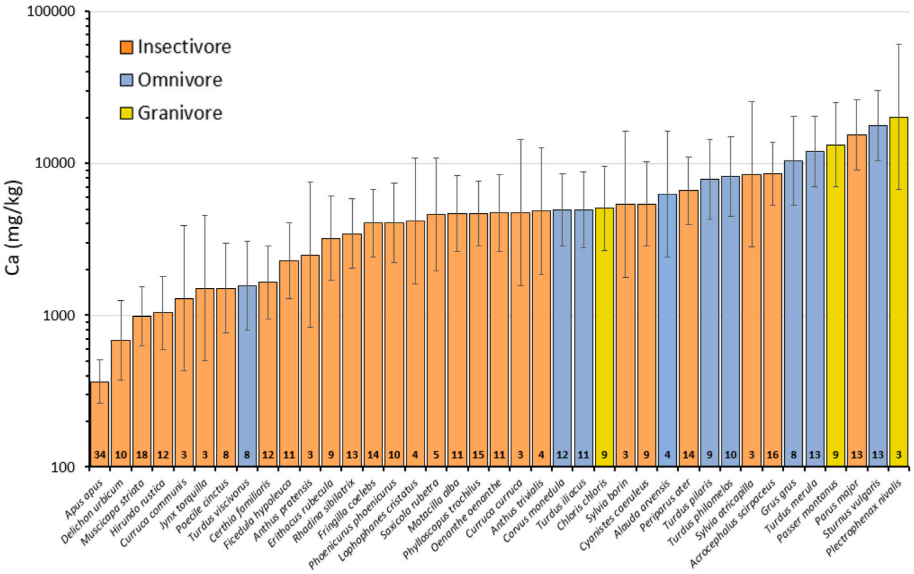

Fig. 2. Geometric means (±95% confidence limits) of Ca concentrations in feces of those 39 bird species (n = 380 broods) for which we had at least three samples. Species with predominantly insectivorous diet are shown in orange but also most of the other species feed varying amount of animal food to nestlings. Sample sizes are shown at the base of the bars. Estimates come from the model 1 in Table 1. Note the logarithmic scale of the y-axis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

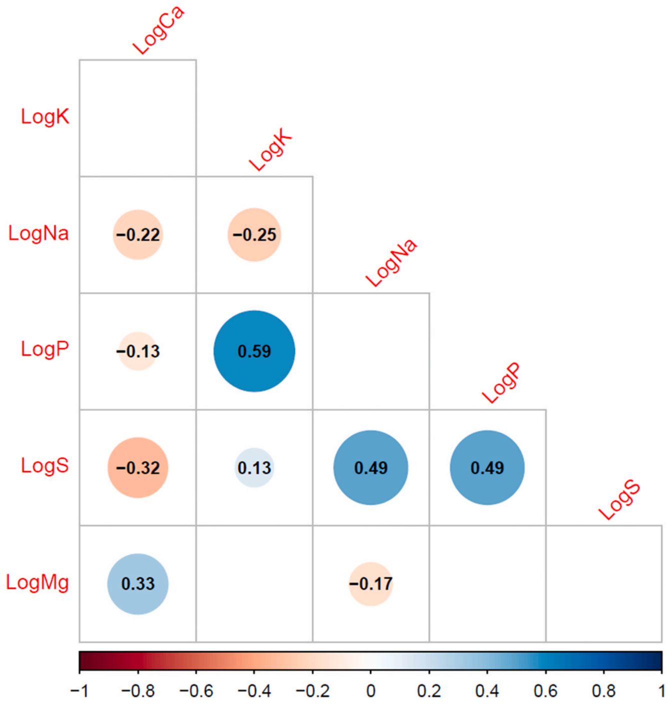

Fig. 3. Pearson correlation coefficients among the six element concentrations measured in feces of 39 bird species (n = 380 broods). Symbol size and color reflect the value and direction of the correlation coefficient, respectively. Concentrations were $\log_{10}$-transformed before the analysis. Only statistically significant correlations are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Why is the diet of aerial foragers and other strictly insectivorous species relatively low in Ca, especially compared to ground foraging omnivores? Flying insects are primary food sources for aerial insectivores (Cramp et al., 1985; Chung et al., 2021; Forsman et al., 2022) but they contain relatively little Ca (Graveland and van Gijzen, 1994; Johnson and Barclay, 1996; Finke, 2002). Especially small passerines need extra Ca besides their normal insect food for successful breeding, to cover their Ca requirements for good quality egg shells and proper bone formation of nestlings (Tilgar et al., 1999, 2004). Typical Ca-rich food items are e.g. small snails (Gastropoda), millipedes (Diplopoda) and woodlice (Isopoda) (Graveland and Drent, 1997; Bureš and Weidinger, 2003; Eeva and Lehikoinen, 2004). These ground or vegetation dwelling invertebrates are less used by aerial foragers, although even aerial insectivores (Hirundinidae), feed pieces of snail shells, egg shells and grit to their nestlings, and also use clay for their nests (Cramp, 1988; Poulin and Brigham, 2001). Instead, such Ca-rich invertebrates are more frequently available to ground foraging omnivores, together with abiotic Ca sources like soil mineral particles (Cramp, 1988; Dhondt and Hochachka, 2001; Downs et al., 2019). For example, earthworms are common food for some ground foraging species (e.g. Turdidae and Sturnidae) and a good source of Ca through their gut soil contents (Bilby and Widdowson, 1971). Most typical earthworm eating bird species in our sample were all included in the omnivore group (containing no non-earthworm eaters), which may partly explain relatively high Ca levels in this dietary group. Lack of Ca-rich food has resulted in intensified accumulation of toxic metals and Ca-related impairment of breeding success in metal-polluted environments in some insectivorous passerines (e.g. Eeva and Lehikoinen, 1995; Dauwe et al., 2006; Belskii and Grebennikov, 2014), but we are not aware of any studies focusing on the interaction between Ca and dietary exposure to toxic metals in purely aerial foragers.

Limited Ca availability could also apply to pure granivores, since seeds also show relatively low Ca content (Graveland and van Gijzen, 1994). However, in our study the species classified as granivores showed relatively high Ca levels, which could be because many granivores include varying number of invertebrates in the food given to growing nestlings, including Ca-rich items like snails (Cramp and Perrins, 1994; Cramp et al., 1994). To help break down their food, granivorous birds consume more grit to be used as gizzard stones than insectivores and omnivores (Best and Bulletin, 1996). The types of gizzard stones vary from hard siliceous to softer and Ca-rich calcareous grit (Martinez-Haro et al., 2009). Ca-rich gizzard stones, if available, could be one explanation to relatively high fecal Ca levels of granivores. Although soils at our sampling locations are mostly Ca-poor, as suggested by generally low pH values, birds might also use some anthropogenic Ca-rich sources

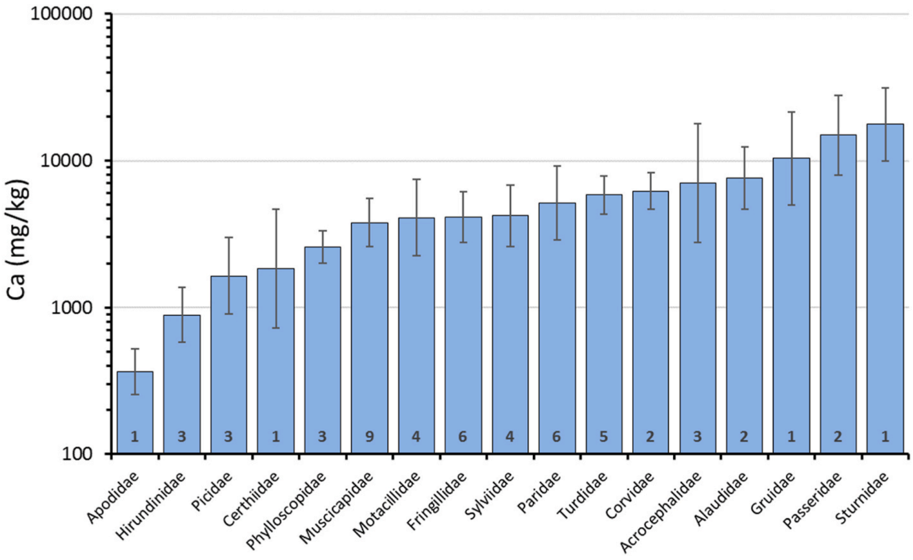

Fig. 4. Geometric means (±95% confidence limits) of Ca concentrations in feces of those 17 bird families for which we had at least five samples. Number of sampled species (n = 56) in each Family are shown at the base of the bars. Note the logarithmic scale of the y-axis. Estimates come from the model 2 in Table 1. N = 398 broods.

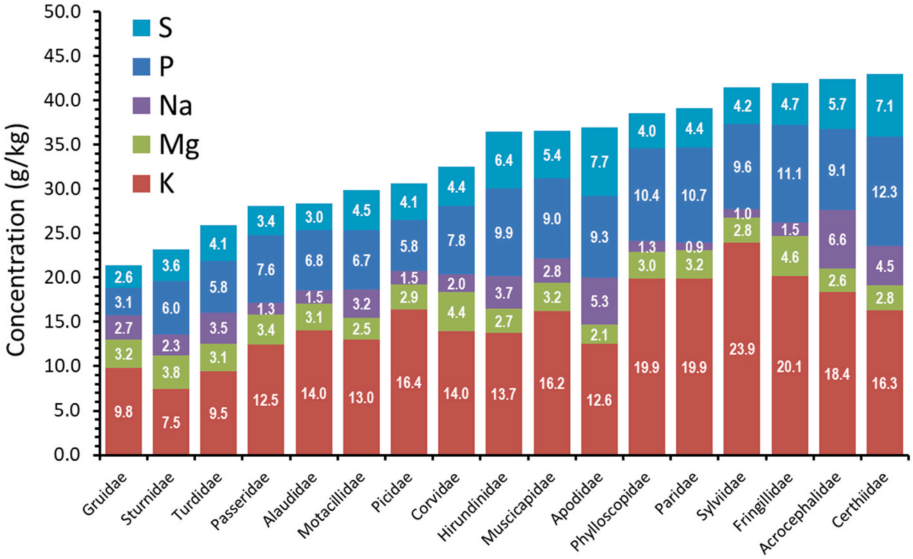

Fig. 5. Geometric means of K, Mg, Na, P and S concentrations in feces of 17 bird families (including 56 species). Families are arranged by their total element concentration. Estimates come from the model 2 in Table 1. N = 398 broods.

to acquire their gizzard stones.

At the end, the question is not just of Ca availability but also adaptation: some species, like pure insectivores, have adapted to breed at low Ca level and their dietary Ca input would likely stay at relatively low level even if there would be unlimited access to Ca-rich food (Espín et al., 2016b). An experimental study, where a pure insectivore, *F. hypoleuca,* was offered extra Ca-rich food during breeding, indicated that ample Ca availability did not markedly increase nestlings' fecal or plasma Ca levels (Espín et al., 2016a). Likewise, Reynolds et al. (2004) concluded that Ca supplementation will normally be ineffective if natural food provides sufficient dietary Ca for the birds' breeding requirements. Therefore, insectivores like *F. hypoleuca*, may retain their low-Ca diet despite an abundant Ca availability but also suffer more from toxic metals when exposed to those (Eeva and Lehikoinen, 1995).

To become a useful indicator of metal sensitivity, fecal Ca levels should be studied together with toxic metal exposure and their impact on health and breeding success. Such information is currently very limited. Obviously, there is no clear Ca level threshold where a species can be considered as metal-sensitive, since the impacts will at least depend on the toxic metal, its speciation and the level of exposure. Calcium absorption efficiency could also vary among species but we are not aware of any comparative studies on this. Furthermore, spatial variation in Ca availability produces intraspecific variation in dietary Ca levels (Graveland, 1996). We could not make strong inferences on spatial effects with our data because individual species were often sampled in a relatively restricted geographical area and habitat quality was not included in our data. A species range and habitat can, however, be seen as inherent parts of their ecology, making them more or less sensitive to Ca-related toxic effects. However, species studied in a common metal polluted environment provide a good opportunity to estimate Ca-related sensitivity. Three insectivorous bird species, *F. hypoleuca* (Fh), *Cyanistes caeruleus* (Cc) and *P. major* (Pm) showed Ca

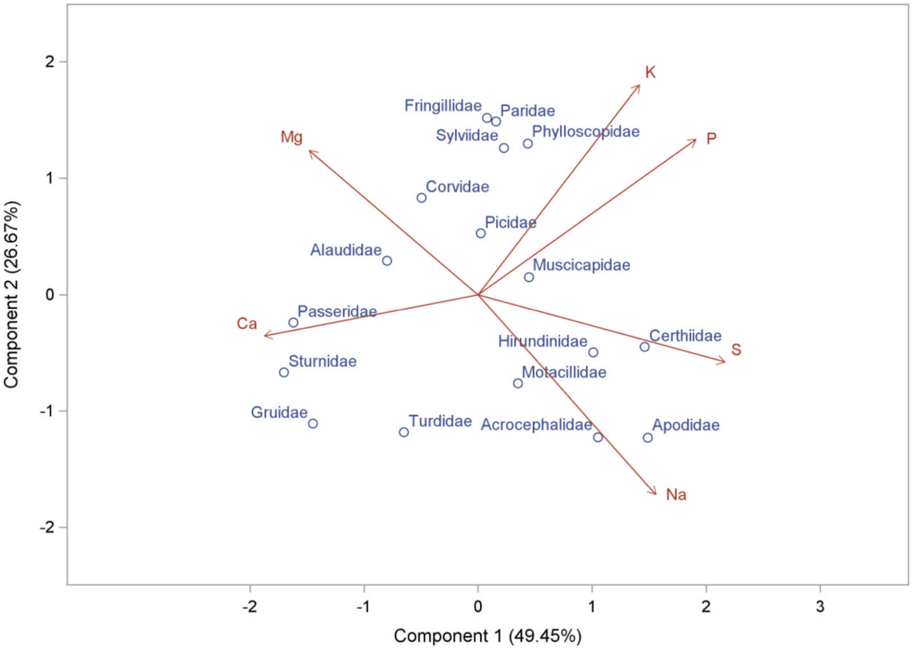

Fig. 6. Principal component analysis biplot for fecal concentrations of six macroelements. N = 398 broods of 56 species. The dimensions of the biplot are the first two principal components.

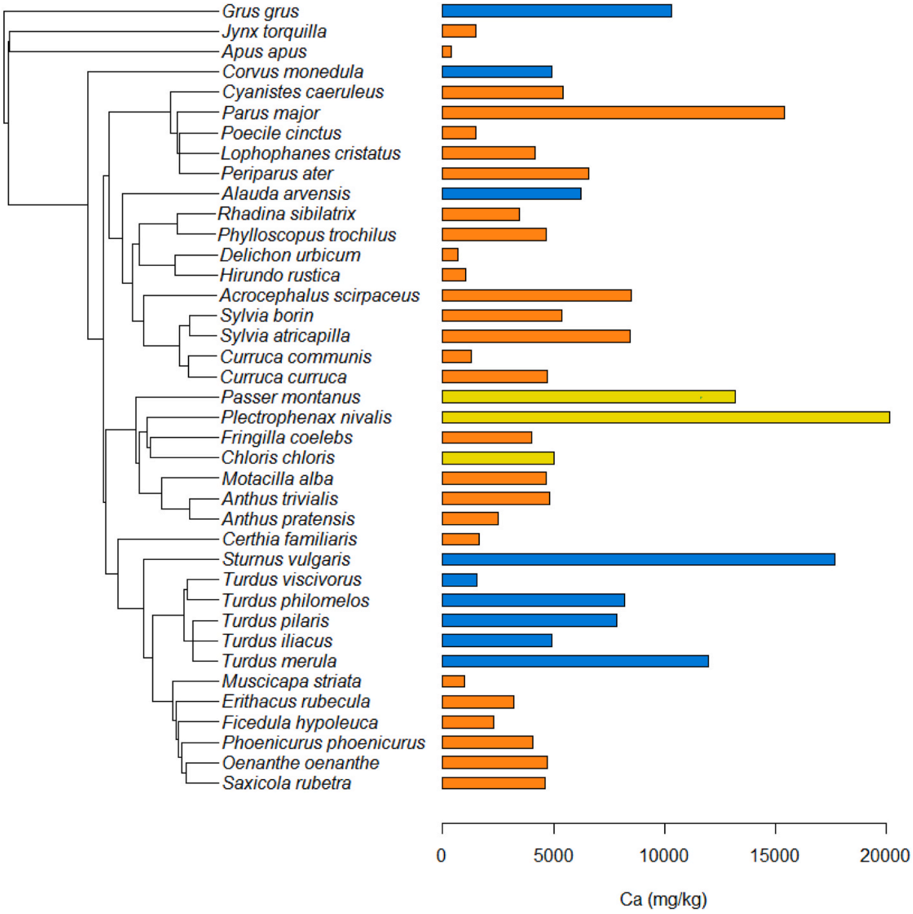

Fig. 7. Phylogeny of 39 bird species used to compare mean fecal Ca levels. Main diet of bird species is indicated with colors: orange - insectivore, blue – omnivore, yellow - granivore. Phylogeny is based on 100 trees downloaded from Birdtree.org database (see Material and Methods section for further details). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

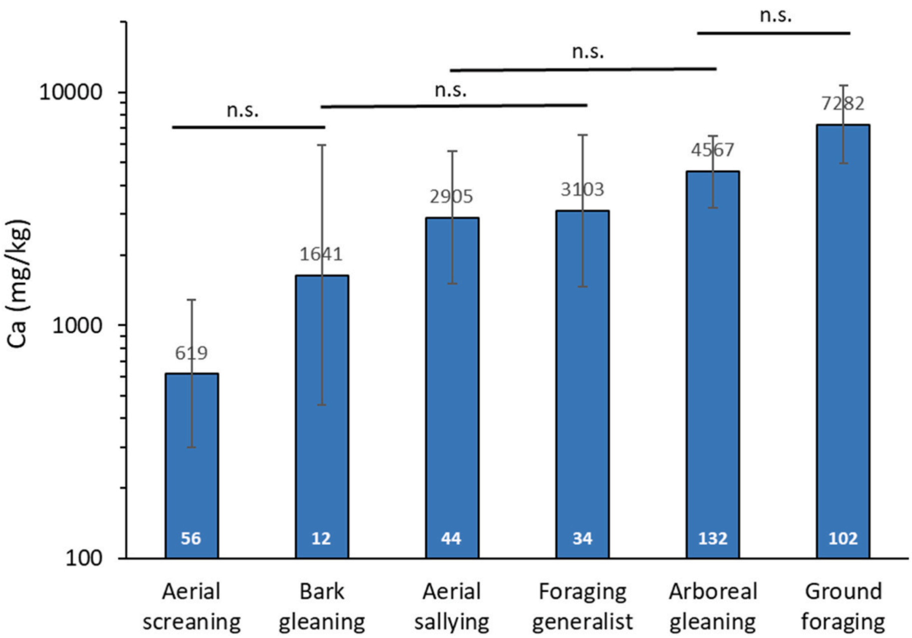

Fig. 8. Mean (CL) fecal Ca levels (mg/kg) for different foraging niches (n = 380 broods of 39 species). Numbers at the bottom of the bars indicate the number of broods and values above the bars indicate geometric means. Mean values come from the LMM model where species was included as a random factor. Horizontal lines show non-significantly different groups in pairwise comparison while all the other differences are significant (Tukey's test).

Table 2
Mean avian nestling fecal calcium level (mg/kg, d.w.) values from literature. Arithmetic and geometric means (±SD) are presented separately. Study-specific values are presented in the Supplement S2. N = number of datasets. NA = not applicable.

Species	Mean type	n	Mean	SD	References
Apus apus	Arithmetic	1	728	NA	Orłowski et al. (2015)
Delichon urbicum	Arithmetic	1	894	NA	Orłowski et al. (2015)
Hirundo rustica	Arithmetic	1	1250	NA	Orłowski et al. (2015)
Sialia sialis	Arithmetic	2	1946	597	Hungerford et al. (1993), Burns (2016)
Ficedula hypoleuca	Arithmetic	4	2510	230	Eeva and Lehikoinen (2004), Eeva et al. (2005), Berglund et al. (2011), Espín et al. (2016a)
Ficedula hypoleuca	Geometric	2	2916	1392	Rainio et al. (2013), Eeva et al. (2020)
Cyanistes caeruleus	Geometric	2	3450	1202	Eeva et al. (2009), Rainio et al. (2013)
Parus major	Geometric	6	9350	4700	Eeva et al. (2009), Rainio et al. (2013), Ruuskanen et al. (2019)
Parus major	Arithmetic	5	10,627	1092	Eeva and Lehikoinen (2004), Eeva et al. (2005), Berglund et al. (2011), Costa et al. (2012), Espín et al. (2016a)
Passer montanus	Arithmetic	1	26,800	NA	Ding et al. (2020)
Gypaetus barbatus	Arithmetic	1	287,700	NA	Margalida et al. (2020)

levels of 3900, 5700 and 14,200 mg/kg, respectively, in the same metal-polluted study area near a copper-nickel smelter, emitting a mixture of toxic elements like As, Cd, Cu, Ni and Pb (Rainio et al., 2013). While Fh has suffered from eggshell thinning, smaller clutch and egg sizes, decreased hatching success and impaired development of nestlings' bones in this area (see also Nyholm, 1981), such effects were weak or non-existing in Pm (Eeva and Lehikoinen, 1995, 1996). In this respect, Cc resembles more Pm than Fh, laying relatively normal eggs and clutches with normal hatching success (Eeva et al., 2009). We suggest that in this area the threshold for sensitivity is below the fecal Ca level of Cc, and likely closer to the level of Fh. Pm again is relatively tolerant to metal exposure due to its high dietary Ca level. In accordance with this, no marked effects on egg shells of Pm and Cc were either found near a non-ferrous smelter in Belgium, where these species were exposed to relatively high levels of Pb (Janssens et al., 2003; Dauwe et al., 2004).

Macroelements like Na, P and S showed to some extent opposite patterns to Ca. From the viewpoint of metal sensitivity indication, there is no similar empirical and theoretical support for those than for Ca, but among them especially interesting could be P because low Ca:P ratio is well known to affect bone health (Bonjour, 2011). Being important minerals for bone tissue structure, an adequate dietary intake of both elements is crucial for bone development and health (Loughrill et al., 2016). Besides low circulating Ca level, high dietary P intake may activate parathyroid hormone (PTH) secretion, which again increases bone resorption (Kemi et al., 2010). Too much of P relative to Ca could then be harmful, especially when other metals disturb the Ca metabolism (Takeda et al., 2014). Some bird families representing pure insectivores, such as Certhiidae, Apodidae, Acrocephalidae and Hirundinidae showed relatively high S and Na levels. At least in Certhiidae and Acrocephalidae this might reflect relatively high dietary proportion of spiders (Araneae) (Suhonen and Kuitunen, 1991; Chernetsov and Manukyan, 1999). As compared to insects, spiders contain higher levels of some S-rich amino acids, like taurine, cysteine, and methionine (Ramsay and Houston, 2003; Wiesenborn, 2012). On the other hand, in swifts (Apodidae) the dietary proportion of spiders may be relatively small (Lack and Owen, 1955). Spiders and aquatic insects also seem to contain higher levels of Na than terrestrial insects (Schowalter and Crossley, 1983). These two dietary item groups might explain the relatively high Na levels in Acrocephalidae, which feed on spiders and insects in aquatic reed beds (Grim and Honza, 1996).

Besides Ca, Mg is another important mineral for bone formation and for Ca homeostasis (Shastak and Rodehutscord, 2015). In our sample, levels of Mg correlated overall positively with those of Ca, and Mg has shown similar antagonistic relationship with some toxic metals like cadmium (Matović et al., 2010; Espín et al., 2020), making Mg another potential metal sensitivity indicator. Impaired reproduction by both Mg deficiency and excess have been reported in poultry, but we are not aware of any such reports in wild birds, which may indicate that Mg deficiency is relatively rare in the wild. Furthermore, and possibly from the abovementioned reason, in our study Mg showed much less interspecific variation than Ca and might in this sense be less suitable indicator of metal sensitivity.

5. Conclusions

Our results suggest that fecal Ca levels could become a simple, useful and general indicator of avian metal sensitivity but more needs to be done to combine information on Ca levels, metal exposure and impacts on breeding. As mentioned, sensitivity has several different meanings and our study does not document the susceptibility of a species to become exposed to metals, which is another aspect often associated to pollution sensitivity: species prone to accumulate pollutants may be sensitive to harmful effects due to their high exposure. Next, we would need information on the effects of metals on breeding parameters in a wider range of bird species. For example aerial insectivores, due to their low dietary Ca level and potential exposure to toxic metals, would be an interesting but currently understudied group (Barton et al., 2023). We encourage collecting similar data on wider range of birds species and also some other potentially Ca limited animals, like bats (Studier et al., 1994). Collecting fecal samples from nestlings is relatively fast, easy and non- or minimally invasive technique, enabling measuring element composition and dietary composition (with DNA metabarcoding) from the same sample. However, one should pay attention to adequate sample size due to relatively high variation in fecal Ca levels (Eeva et al., 2020).

CRediT authorship contribution statement

S. Espín: Conceptualization, Investigation, Methodology, Writing – review & editing. **T. Andersson:** Investigation, Writing – review & editing. **M. Haapoja:** Investigation, Writing – review & editing. **R. Hyvönen:** Investigation, Writing – review & editing. **E. Kluen:** Investigation, Writing – review & editing. **H. Kolunen:** Investigation, Writing – review & editing. **T. Laaksonen:** Investigation, Writing – review & editing. **J. Lakka:** Investigation, Writing – review & editing. **L. Leino:** Investigation, Writing – review & editing. **K. Merimaa:** Investigation, Writing – review & editing. **J. Nurmi:** Investigation, Writing – review & editing. **M. Rainio:** Investigation, Writing – review & editing. **S. Ruuskanen:** Investigation, Writing – review & editing. **K. Rönkä:** Investigation, Writing – review & editing. **P. Sánchez-Virosta:** Investigation, Writing – review & editing. **J. Suhonen:** Formal analysis, Investigation, Visualization, Writing – review & editing. **P. Suorsa:** Investigation, Writing – review & editing. **T. Eeva:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.123181.

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