White-tailed eagle (*Haliaeetus albicilla*) and great cormorant (*Phalacrocorax carbo*) nestlings as spatial sentinels of Baltic acidic sulphate soil associated metal contamination

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ABSTRACT

Sulphate soils, characterized by low pH conditions, are found worldwide, and are potentially large sources of metal contamination, often exceeding industrial emissions. Metal leaching from sulphate soils has been shown to be harmful to aquatic organisms, but the cascading effect on exposure in apex avian predators has not been studied earlier. With the present study we aimed at evaluating the potential of white-tailed eagle (*Haliaeetus albicilla*) and great cormorant (*Phalacrocorax carbo*) nestlings, collected from nests located either in sulphate soil or control areas, for monitoring spatial contaminant trends of metals typically associated with sulphate soils.

In blood of white-tailed eagles, the concentrations of aluminium and cobalt were significantly higher in sulphate soil areas. In blood of great cormorants, the concentrations of copper and manganese were so, while the concentration of zinc was found to be lower. Also, we observed an interaction between the latitude and soil type in cobalt and lithium concentrations of great cormorants, showing that concentrations in the sulphate soil associated nestlings rose more steeply towards the north than in the control group. Latitudinal trends of higher concentrations in the south were found in cadmium, manganese, and copper of white-tailed eagle nestlings, while thallium of white-tailed eagle nestlings, and thallium and zinc of great cormorant nestlings showed a latitudinal trend of higher concentrations in the north. Concentrations of several metals correlated positively within a species indicating covariation in metal exposure. Generally, the metal concentrations in both species were similar to levels reported to be below toxicity thresholds in other species. These results indicate, that white-tailed eagle and great cormorant nestling metal burdens may indicate environmental contamination from acidic sulphate soil runoff, and that they may act as indicators of latitudinal gradient identifying different contamination sources.

Keywords: acidic sulphate soil, Baltic Sea, elements, metals, biomonitoring, bird

1. INTRODUCTION

Acidic sulphate soils, cited as “the nastiest soils in the world” by Dent and Pons (1995), are found globally on multiple continents, e.g. Australia, Asia and Africa. They are characterised by low pH conditions, which causes metals and metalloids present in the soils to leach into the environment (Dent and Pons 1995). Acidic sulphate soils do not contain higher concentrations of metals than other soil types, but low pH conditions increase mobilisation and, thus leaching (Sohlenius and Öborn 2004). Sulphur acid is formed in dry soil, and metals are washed away by rainfall, and along with the runoff, metals are carried to near-by water bodies (Sohlenius and Öborn 2004; Fältmarsch et al. 2008). Such leaching of many metals, amongst which manganese, aluminium, nickel and zinc, can be multiple times that of local industrial metal emissions (Sundström et al. 2002).

The largest areas of acidic sulphate soils in Europe are found along the Finnish western and southwestern Baltic Sea coast, their coverage being up to 3000 km^2 (Fältmarsch et al. 2008). There, sulphate soils were formed under anoxic benthic conditions of the Littorina Sea, the geological brackish water stage of the Baltic Sea 7,500 to 4,000 years ago. Such soils have high sulphur concentrations, and become acidic as they are exposed to atmospheric oxygen due to land uplift and agricultural land use (Dent and Pons 1995). The acidity and the composition of metals leaching from sulphate soils can vary spatially (Fältmarsch et al. 2008; Wallin et al. 2015), with the highest amounts of metals observed to leach into the environment in the Kvarken region of the Gulf of Bothnia (Roos and Åström 2006).

Metals mobilised from sulphate soils end up in the brackish estuaries at the Baltic coast, where they can spread widely, especially during seasons of high flow, such as autumn and spring (Nystrand and Österholm 2013; Nystrand et al. 2016). In estuaries, metals can end up in the sediment, or in the water column, from which they may become available to aquatic estuarine organisms through bioconcentration (Nystrand et al. 2016).

A risk assessment of 14 estuaries affected by acidic sulphate soils in the Western Finnish coast revealed elevated metal concentrations in both the water column and sediments (Wallin et al. 2015). Moreover, Wallin et al. (2015) reported deteriorated benthic invertebrate communities in many sulphate soil affected estuarine sites. The ecological risk caused by acidic sulphate soils was assessed to be high or moderate in several of the studied estuaries, demonstrating the relevance of the ecological impacts caused by the acidic sulphate soils (Wallin et al. 2015).

In Australia, acidic sulphate soil effluents have been found to affect oyster feeding behaviour and histology (Dove and Sammut 2007a), to decrease their survival (Dove and Sammut 2007b), and to decrease the normal development of oyster embryos (Wilson and Hyne 1997). In fresh water invertebrates and fish, metal effluents have been observed to cause morphological abnormalities to aquatic insect larvae (Vuori and Kukkonen 1996). Also, sulphate soil effluents can affect fish reproduction negatively, and even mass fish kills have been reported (Fältmarsch et al. 2008). Although sulphate soil effluents seem to be harmful to aquatic organisms, there is no knowledge on the extent to which vertebrates higher up or at the top of the food web are potentially affected. While some metals are essential for proper metabolic functioning, excessive metal contamination is known to be able to cause both acute and chronic toxic effects on organisms, depending on the dose and mode of toxicity. Moreover, some metals accumulate into various tissues and organs over time (e.g. Lebedeva 1997; Nam et al. 2005; Berglund 2018), bioaccumulate (Barwick and Maher 2003; Nfon et al. 2009; Cui et al. 2011; Guo et al. 2016), and biomagnify to apex species.

The present study’s objective is to determine, whether the proximity to and spatial variation in acidic sulphate soils impacts metal concentrations in nestlings of apex avian species of the Baltic Sea coastal food web, and whether these nestlings may consequently act as valuable sentinels of sulphate soil leaching.

We investigated the blood burdens of nestlings of two avian apex predators, the white-tailed eagle (*Haliaeetus albicilla*) and great cormorant (*Phalacrocorax carbo*), collected from nests in both sulphate soil and control areas along the western Finnish coast. Our hypotheses are that 1) nestlings in the proximity of sulphate soils exhibit higher blood concentrations of metals than those further away from sulphate soils; that 2) due to coinciding leaching of several sulphate soil-associated metals we expect to find correlations between concentrations within a species; and that 3) due to spatial covariation in the metal contamination and their potential biomagnification in the aquatic food web, there are spatial correlations in the metal concentrations found in the two apex predator species. Because our sampling areas extended over a latitudinal range of 600 km, with varying environmental conditions, e.g. lowering salinity towards the north, differences in climate, and consequent differences in species composition (HELCOM 2018), we also explored possible latitudinal trends in metal contamination.

2. MATERIALS AND METHODS

2.1. Study species and sample collection

The white-tailed eagle is the largest bird of prey in the Baltic Sea region feeding at the top of the marine and terrestrial food chains. In the past, the Baltic white-tailed eagle population declined due to persecution and environmental contaminants, such as mercury (Hg) and persistent organic pollutants (POPs), but the population is on the rise to recover after legal restrictions on the production and use of these compounds (Helander et al. 2008, Saurola et al. 2013). In Finland, the white-tailed eagle is a year-round resident mainly in the coastal areas, where the nesting population is non-migratory, though immature individuals may migrate in a larger area around the Baltic, Central Europe, and Russia before settling in territories (Saurola et al. 2013). Based on food remnants collected around the white-tailed eagle nests during the breeding season, the diet of

Finnish white-tailed eagles consists mostly of waterfowl and fish, mammals occupying a small proportion of the diet (Sulkava et al. 1997; Ekblad et al. 2016).

The great cormorant has a global distribution and reappeared on Finland's list of breeding bird species in 1996 after disappearing from the Finnish coastal areas for few a hundred years (Lehikoinen 2006). In 2018, 26,700 cormorant nests were counted along the Finnish coast (Finnish Environmental Institute 2018). Great cormorants are migratory, the main wintering areas being in Central Europe and the Mediterranean (Saurola et al. 2013). In the Baltic Sea, they nest in colonies and are piscivorous, and mainly feed on smaller fish, such as common roach (*Rutilus rutilus*), European perch (*Perca fluviatilis*) and viviparous eelpout (*Zoarces viviparus*) (Lehikoinen 2005; Lehikoinen et al. 2011).

The nestlings of both species are immobile for several weeks after hatching and are being fed by the parents with prey from around the nesting site and near-by-areas (Krone et al. 2013, Thaxter et al. 2012, Hentati-Sundberg 2018), acting thus as sentinels for local metal contamination. We chose blood as the target tissue, as it is does not require the termination of the individual what the collection of internal tissues would require, and blood can be collected even from young individuals with less developed feathers. Blood metal concentrations represent recent dietary exposure, but blood can also be used as an indicator of long-term accumulation for some metals (Berglund 2018).

We collected blood samples from 31 great cormorant and 16 white-tailed eagle nestlings during May and June 2017 along the Finnish west coast at sites that were either on sulphate-rich soils or control areas. We also had access to archived erythrocyte samples from eight white-tailed eagle nestlings sampled during May and June 2016 (Figure 1). From each white-tailed eagle territory, we sampled one nestling, and from each cormorant colony, three nestlings, except for one colony, where we sampled only one cormorant nestling. The nestlings were captured on the nest, ringed, and sampled for blood from the ulnar vein using a 21 G hypodermic needle and syringe. From each individual, a 5 ml blood sample was drawn. The samples were stored in a cooler for transportation. Blood samples were centrifuged on the day of collection in 3000 rpm for 10 min. Plasma and erythrocytes were transferred and stored at -18 °C until chemical analysis.

2.2. Spatial study design for sulphate soil effects

We assigned each sampling point (white-tailed eagle territory or great cormorant colony) to one of two soil types: control or sulphate soil. We measured the distance from each sampling point to the closest rivers. The nests and colonies were located 2-30 km distance outward to the sea from the nearest river mouth. For each sampling point, we collected data on the magnitude of metal contamination from the sulphate soil in the closest river or estuary using reported metal contamination levels from Beucher et al. (2014), Roos and Åström (2005, 2006), Saarinen et al. (2010), Wallin et al. (2015), and Nyberg et al. (2012). To further assess the presence of the sulphate soils along the rivers close to the sampling points, we also used sulphate soil measurement data from a map produced by the Finnish Institute of Geology (https://gtkdata.gtk.fi/Hasu/index.html, accessed 25.6.2019). Sampling points in the proximity of estuaries or rivers notably contaminated by sulphate soils were assigned to the sulphate soil group, and vice versa. As metal concentrations in the water column decrease with increasing distance from the estuary (Åström et al. 2012; Nystrand et al. 2016), sampling points with a long distance (> 15 km) to the closest river estuaries were always assigned to the control group. While assigning sampling points to the treatment groups, other near-by rivers were also taken into consideration. However, due to the spatial distribution of the sampling points, the points in the sulphate soil group were in proximity of clearly contaminated rivers, or there were no other notable rivers near-by.

2.3. Metal analysis

We investigated erythrocyte concentrations of aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), lithium (Li), manganese (Mn), nickel (Ni), thallium (Tl) and zinc (Zn) for the metal analyses, as they have been reported to be associated with acidic sulphate soil effluents (Sohlenius and Öborn 2004; Fältmarsch et al. 2008; Nordmyr et al. 2008a, b; Nyberg et al. 2012; Nystrand and Österholm 2013; Wallin et al. 2015; Nystrand et al. 2016). In addition, we analysed chromium (Cr). Chromium is mobilised in lower pH conditions than other metals associated with sulphate soils, e.g. Zn and Al (Palko and Yli-Halla 1990; Åström 2001; Sohlenius and Öborn 2004), but the solubility of chromium increases in highly acidic conditions (pH<3.5) (Åström 2001).

All chemical analyses were carried out at ALS Scandinavian, Luleå, Sweden, using an accredited Inductively Coupled Plasma Mass Spectrometry method. Full details on the methods have been earlier reported by Rodushkin et al. (2000, 2001).

2.4. Statistical analyses

To detect the overall response of metals we initially conducted a PCA for the metal concentration data, separately for both species, and then used the PC axis values in an ANOVA with soil type and latitude as the explanatory variables. Because the PCA resolutions needed five components to cumulatively explain > 80 % of the total variation, we conducted separate ANOVAs for the principal components one to five (Electronic Supplement 1). As we found significant effects, we continued analysing each metal separately: We used general linear mixed models (GLMM) to test the difference in nestling erythrocyte metal concentrations between control and sulphate soil areas. To test simultaneously the effect of latitudinal location of the sampling point on the metal concentration, we added standardized latitude and the soil type-by-latitude interaction in the models as fixed factors. When the interaction of the treatment and latitudinal location was non-significant, we removed the interaction from the model. In the models for the great cormorants, we added the sampling point (i.e. the colony) as a random factor to control for the non-independence of nestlings within the same colonies.

We visually checked the normality and heteroscedasticity assumptions of the GLMM from residual plots, Shapiro-Wilk's test, and by Levene's test. We used log-normal transformation for those metals not fulfilling the assumptions (for white-tailed eagles: Al, Cd, Co, Cr, and Ni; for great cormorants: Al, Cd, Co, Cr, Mn, Ni, and Tl). For white-tailed eagles, Ni concentrations did not show normal distribution nor heteroscedasticity due to one likely outlier. The maximum concentration of Ni without outlier was 5.57 µg L^{-1} (outlier 59.5 µg L^{-1} in the control group). Therefore, GLMM were run for Ni with this outlier removed, and did show that normality and heteroscedasticity were met. Also, though fitting the model assumptions, there was a putative outlier of 16.3 µg L^{-1} in the white-tailed eagle Cr data, while the maximum without the outlier was 2.52 µg L^{-1}. For Cr, we present results with and without the outlier.

We derived the estimated marginal means of the metal concentrations for each soil type with their 95% confidence limits (LS means statement in SAS). For the models using log-normal transformed data we back-transformed the means and confidence limits to original scale. $P < 0.05$ was considered statistically significant, while p-values between 0.05 – 0.1 were considered to be marginally non-significant, and indicative of possible true difference.

Given the non-normally distributed data we calculated Spearman's correlations to test for spatial correlations in metal concentrations in great cormorant and white-tailed eagle nestlings, as well as intraspecific correlations among the different metals. For calculating the correlations, we associated each sampling point with the nearest sulphate soil or control area and calculated for each area the mean concentrations of each metal for both species separately. Thus, each area formed one data point in the correlation.

3. RESULTS

For white-tailed eagles, Al ($F_{1, 21}$ = 5.85. p = 0.03) and Co ($F_{1, 21}$ = 12.00, p = 0.002) concentrations were higher in the sulphate soil group (Figure 2A). The concentration of Cr differed significantly between control and sulphate soil areas when tested using all data ($F_{1, 21} = 5.84$, $p = 0.03$), but when we removed the putative outlier, the difference was marginally non-significant ($F_{1, 20} = 3.91$, $p = 0.06$) (Figure 2 A). Also, there was a similar marginally non-significant difference between the soil-types, concentrations of Mn ($F_{1, 21} = 3.70$, $p = 0.07$) and Li ($F_{1, 21} = 3.32$, $p = 0.08$) being higher in sulphate soil, and concentrations of Cu ($F_{1, 21} = 2.98$, $p = 0.099$)being higher in the nestlings from the control soils (Figure 2 A). In white-tailed eagle nestlings, there were no significant interactions between soil type and latitude.

In great cormorant nestlings, the concentrations of Cu ($F_{1, 8.23} = 17.2$, $p = 0.003$) and Mn ($F_{1, 8.54} = 5.63$, $p = 0.04$) were higher in the sulphate soils than in control soils (Figure 2 B). Unexpectedly, concentrations of Zn ($F_{1, 8.67} = 5.20$, $p = 0.050$) were higher in control than in sulphate soils, with similar marginally non-significant difference in concentrations of Tl ($F_{1, 8.1} = 3.82$, $p = 0.09$) (Figure 2 B). In Co and Li of great cormorants', there was a statistically significant interaction between soil type and latitude (Co $F_{1, 7.05} = 9.1$, $p = 0.019$; Li $F_{1, 27} = 4.26$, $p = 0.049$), and the concentrations of both increased with the latitude. For both Co and Li, the concentrations in birds rose more steeply towards the northern latitudes in sulphate soil areas than in the control areas (Figure 3 A and 3 B).

There were mainly positive correlations between metal concentrations in the intraspecific correlation analyses. In white-tailed eagle nestlings, all significant ($p < 0.05$) and near-significant ($p < 0.10$) correlations between metals were positive, except for the correlations between Li and Cd and between Li and Zn (Table 1). A strong correlation ($r_s > 0.80$) occurred between Cu and Zn (Table 1). All other correlations were between $r_s = 0.40$-0.80. In great cormorant nestlings, all significant and near-significant correlations between metals were positive, except for the correlation between Cu and Tl (Table 2). The strongest correlation ($r_s > 0.80$) occurred between Al and Cr (p =

0.002). All other correlations were also strong (r_s = 0.5-0.8). In both species, there were positive correlations between Al and Cr, and between Co and Li.

When examining spatial correlations in metal concentrations between great cormorant and white-tailed eagle nestlings from the same area, we found a significant and moderately strong positive correlation in the concentrations of Tl (Table 3). There were no correlations observed for the other elements (Table 3, all p-values ≥ 0.3).

There were latitudinal gradients in metal concentrations of both species (Fig 3). In white-tailed eagles, the Cd (Fig 3 E; $F_{1,21}$ = 9.5, p = 0.006) and Mn (Fig 3 G; $F_{1, 21}$ = 6.70, p = 0.02) concentrations were higher in the southern than northern latitudes, and Cu showed a similar tendency (Fig 3 F; $F_{1, 21}$ = 4.15. p = 0.05). Tl concentrations, on the other hand, showed to be higher concentrations in the northern latitudes (Fig 3 H; $F_{1, 21}$ = 3.89, p = 0.06). In great cormorants, Tl (Fig. 3 C; $F_{1, 9.28}$ = 7.41, p = 0.02) and Zn (Fig 3 D; $F_{1, 10.4}$ = 5.67, p = 0.04) concentrations were higher in the northern latitudes.

4. DISCUSSION

4.1. Occurrence of acidic sulphate soil metals in white-tailed eagle and great cormorant nestlings

Our results suggest that acidic sulphate soils are a source of contamination of certain metals for white-tailed eagle and great cormorant nestlings in the Finnish coast. In white-tailed eagle nestlings, the concentrations of Al and Co, and in great cormorant nestlings the concentrations of Cu and Mn were higher in nestlings reared in the neighbourhood of sulphate soils. Also, Cr and Mn concentrations in white-tailed eagles tended to be higher in the sulphate soil areas. This is the first evidence suggesting uptake of sulphate soil metals by apex avian species through the food chain. However, in case of many metals, the differences between the two groups were small, and possibly not of toxicological relevance. Also, for many metals, there were no differences between the two groups, or, the control group estimate was higher than that of the sulphate soil group.

Al, Co, Mn, and Cu are all prominent metals in the sulphate soil effluents due to their increased mobility in low pH conditions (Åström 2001; Fältmarsch et al. 2008; Nordmyr et al. 2008b; Wallin et al. 2015; Nystrand et al. 2016). Concentrations of Al, Co and Cu in the water column appear to decrease closer to the river mouth, while Mn is more persistent and deposited further from the estuary (Åström et al. 2012; Nystrand et al. 2016), which could explain why there were higher concentrations of Mn in the sulphate soil areas in both species. Elevated concentrations of all the above-mentioned metals have been observed in the sediments of acidic sulphate soil affected rivers (Nordmyr et al. 2008a; Wallin et al. 2015), where they could end up in fish and other benthic species, to become further transferred along the food web into white-tailed eagle and great cormorant nestlings. The relationship between Cr and acidic sulphate soils is more complex, as although Cr has been associated with sulphate soils, it is less soluble in low pH conditions than other sulphate soil associated metals, e.g. Al, Co, and Zn (Palko and Yli-Halla 1990; Åström 2001). However, the solubility of Cr increases in highly acidic conditions ($pH < 3.5$) (Åström 2001), thus Cr being possibly leached from very acidic sulphate soils.

Contrary to our hypothesis, in great cormorants, we found higher Zn concentrations in the control areas compared to the sulphate soil areas. These results indicate that the acidic sulphate soils are not the primary source of Zn contamination, at least not for great cormorants. The difference in Zn levels between the soil type groups was, although significant, only 8%. Overall, there was only little variation in the Zn concentrations of both groups, with no single colonies standing out and explaining higher concentrations in the control areas. Zn is an essential metal, and birds can regulate Zn levels efficiently (Beyer et al. 2004), possibly explaining the small variance in Zn levels. Also, as normal values of zinc can show variation within bird species (Puschner et al. 1999, Osofsky et al. 2001), it is possible that the observed difference could be due to natural variation, and not due to the treatment.

In case of many metals, we found no differences between sulphate soil and control areas in either species. This could be due to various reasons. Due to changes in water chemistry, the concentrations of most metals in the water column reduce quickly when the acidic fresh river water reaches saline and more basic estuarine waters (Nordmyr et al. 2008b; Åström et al. 2012; Nystrand et al. 2016). The bioavailability of the metals decreases as they are precipitated and sedimented (Nordmyr et al. 2008b). Lowered bioavailability results in lower bioconcentration and bioaccumulation in low trophic species and hence lower biomagnification in apex birds.

Also, it is possible that the parent birds carry food for the nestlings over long distances, outside the immediate range of sulphate soil leaching, thus reducing their metal contamination in the proximity of sulphate soil areas. Foraging distances of great cormorants and white-tailed eagles in the Baltic region are poorly known. White-tailed eagles in lakes of northern Germany have been found to mainly have small home ranges (Krone et al. 2013), although some long distance flights were observed. Haworth et al. (2010) found another large apex avian species, the golden eagle (*Aquila chrysaetos*), to have smaller foraging distances during the breeding season than during rest of the year in Scotland. Great cormorants have been found to feed close to the colony in a Baltic island environment (Hentati-Sundberg 2018). Thaxter et al. (2012) estimated 5 km mean breeding season foraging distances for great cormorants, with maximum foraging ranges up to 35 km from the colony. From the energetic point of view, it would be tempting to assume that parenting birds carry food to the nest from the vicinity, in which case the metal burden in the nestlings would be acquired recently from near-by areas, thus reflecting the local metal contamination. However, foraging over long distances (> 10 km) may happen in both species, possibly contributing to the lack of differences or to the pattern in some metals opposing our hypothesis of higher contamination in sulphate soil than control areas.

4.2. Spatial variation and covariation in nestling metal concentrations

Except for Mn in white-tailed eagle nestlings, we did not find higher concentrations in sulphate soil areas compared to control areas in metals for which we found a latitudinal gradient (for white-tailed eagle nestlings' Cd, Cu, and Tl, and for great cormorant nestlings' Tl and Zn). These results indicate, that the sulphate soils are not a primary source of contamination of the metals with latitudinal trends for apex birds, even though they are released to the environment from the soils.

The latitudinal trends show that the magnitude of metal contamination varies along the Finnish coast, and that metals differ in their contamination patterns, indicating different sources of environmental contamination for different metals along the latitudinal gradient. Different parts of the Finnish Baltic coast differ in their physical and chemical properties. One of the most characteristic aspects of the Baltic Sea is the salinity gradient, salinity being higher in the south than in the north (HELCOM 2018). As concentrations of most sulphate soil associated metals get lower when the proportion of saline sea water in the solution increases (Nystrand et al. 2016), the gradients of higher concentrations in the north than in the south found in the Tl of both species, and in Li, Co, and Zn of great cormorants could be explained by the lower salinity in the northern Baltic.

For both Li and Co in great cormorant nestlings, we also found an interaction of latitude and soil type, where the concentrations rose towards the north though more steeply so in the sulphate soil than in the control areas. The steeper rising trend of both Li and Co in the sulphate soil areas can be explained by the location of the northernmost sampling points that are in the hot-spot region for acidic sulphate soil emission in the Finnish coast having the most contaminated rivers (e.g. Roos and Åström 2005, 2006). Though sulphate soils affect also the more southern parts of the Finnish coast, the emissions from the more northern sulphate soil affected rivers are much higher, explaining the steeper rise in Co and Li concentrations in the sulphate soil areas compared to control areas.

Trends of higher concentrations in the southern latitudes were found in Cd, Mn and Cu concentrations in white-tailed eagle nestlings. One explanation for this pattern could be riverine runoff of metals from Gulf of Finland and southern Baltic, and the transference of air-borne industrial emissions from the middle and south Europe. For Cd, riverine run-off from southern Baltic and air-borne emissions are known to be a relevant source of contamination (HELCOM 2010). The higher concentrations of Mn and Cu in the southern latitudes may indicate their higher European air-borne fallout or diffuse riverine pollution from southern catchment area of the Baltic.

For Tl we found a similar latitudinal trend for both species, concentrations being higher in the northern latitudes. Also, Tl concentrations in white-tailed eagle and great cormorant nestlings from the same areas correlated positively, being the only metal with concentrations correlating between species. Together these geographical patterns indicate, that nestlings from both species are subjected to similar Tl contamination pathways, originating from spatially coinciding sources. Although Tl has been associated with sulphate soils (e.g. Roos and Åström 2005), we did not find differences in Tl concentrations between sulphate soil and control areas. Tl sources are very heterogeneous as this metal is released into the environment from both natural and anthropogenic sources, such as industrial smelters, and it can be transferred as atmospheric emissions (Karbowska 2016; Belzile and Chen 2017).

We expected to find between-species correlations in metal concentrations for more metals. A lack of these correlations may be due to differences in the diet, possibly leading to differential exposure to metals depending on the prey. This also seems to be confirmed by a varying range of metals for which we in fact detected species-specific concentration differences among sulphate soil and control regions. Great cormorants are fully piscivorous, while the diet of white-tailed eagles consists also of birds and mammals in addition to fish. Also, composition of fish species in the diet differs between the species (Sulkava et al. 1997; Lehikoinen 2005; Lehikoinen et al. 2011; Ekblad et al. 2016).

4.3. The toxicological implications in white-tailed eagles and great cormorants

We found intraspecific correlations between several metals. With a few exceptions, almost all intraspecific correlations were positive, indicating exposure to multiple metals in the same areas simultaneously. Simultaneous exposure to multiple metals at the same time can pose a risk to toxic additive effects and possibly also to interactive effects, that differ from the effects caused by each metal individually, and these possible combination effects of multiple metals are hard to predict, as metals can function in both synergistic and antagonistic ways (Pan et al. 2015).

Although concentrations of some metals in white-tailed eagle and great cormorant nestlings were higher in the sulphate soil than in the control areas, the concentrations were generally low. Concentrations of Al, Cd, Co, Cr, Cu, Mn, and Ni were at a level of those reported previously in nestlings (Al: Dolan et al. 2017; Cd: Dolan et al. 2017, Maia et al. 2017; Co: Maia et al. 2017; Cr: Maia et al. 2017; Cu: Maia et al. 2017; Mn: Maia et al. 2017; Ni: Dolan et al. 2017, Maia et al. 2017) and adult individuals (Cd: Fenstad et al. 2017, Maia et al. 2017; Cr: Fenstad et al. 2017, Maia et al. 2017; Cu: Fenstad et al. 2017, Maia et al. 2017) of other European bird species, and not found to be above toxic thresholds. The Zn levels in white-tailed eagles and great cormorant nestlings were the highest of all elements included in this study, and slightly higher than those reported in Baltic common eiders (*Somateria mollissima*) (Fenstad et al. 2017), white-storks (*Ciconia ciconia*) (Maia et al. 2017) and northern goshawks (*Accipiter gentilis*) (Dolan et al. 2017). Blood toxicity levels for birds have not been established for Zn, but a high zinc concentration can partly be explained by its metabolic necessity. For Tl, Stout et al. (2010) reported blood concentrations below the detection limit of 50 µg L^{-1} not likely posing harm, and our concentrations were only 0.1 – 2% of that. For Li, reference values for bird blood are not available. Some caution should be taken with making comparison as we used erythrocytes rather than full blood, though levels are roughly in the same order of magnitude.

5. CONCLUSIONS

Our results indicate, that acidic sulphate soils may be a contamination source for multiple metals in white-tailed eagle and great cormorant nestlings in the Finnish coast. However, the elevated exposure in nestlings reared on or close to sulphate soils was quite small, and likely not of toxicological relevance. Moreover, some metals showed an opposite trend of concentrations being higher in the control areas, and therefore indicate the importance of contamination sources other than sulphate soils. This is also indicated by the latitudinal trends in metal exposure found in many metals. Overall, the metal concentrations were at low levels, but it should be noted that we sampled nestlings, which haven’t had time to accumulate metals for long periods. Sulphate soil emissions might be more of a concern for adult birds due to possible bioaccumulation of metals into tissues over long time periods, potentially causing long-term cumulative impacts such as fitness effects, especially in species higher up in the food web. Also, as different metals seem to have different contamination patterns, quantitative identification of the sources and pathways of metals through a food web should be further studied.

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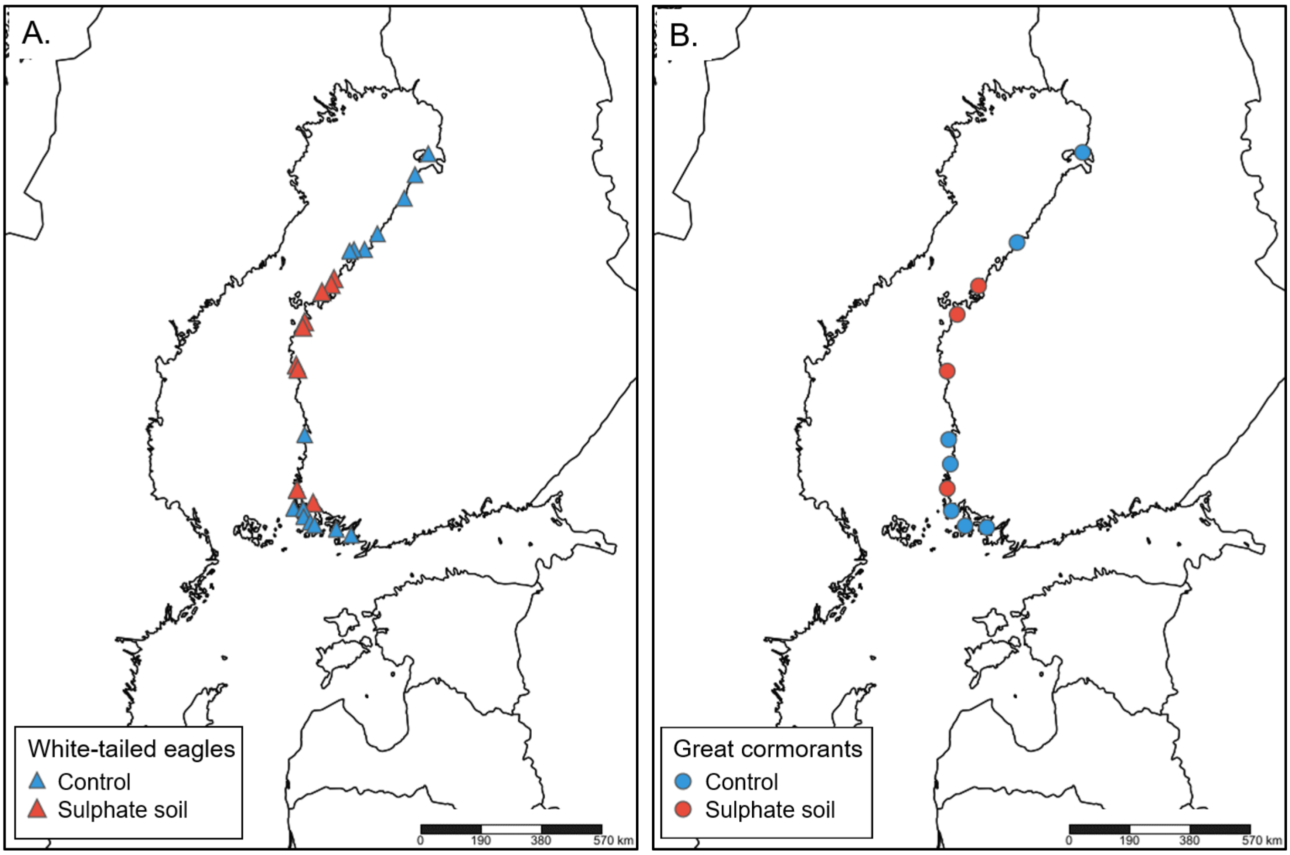

Figure 1. Sampling locations of the A. white-tailed eagle territories and B. great cormorant colonies.

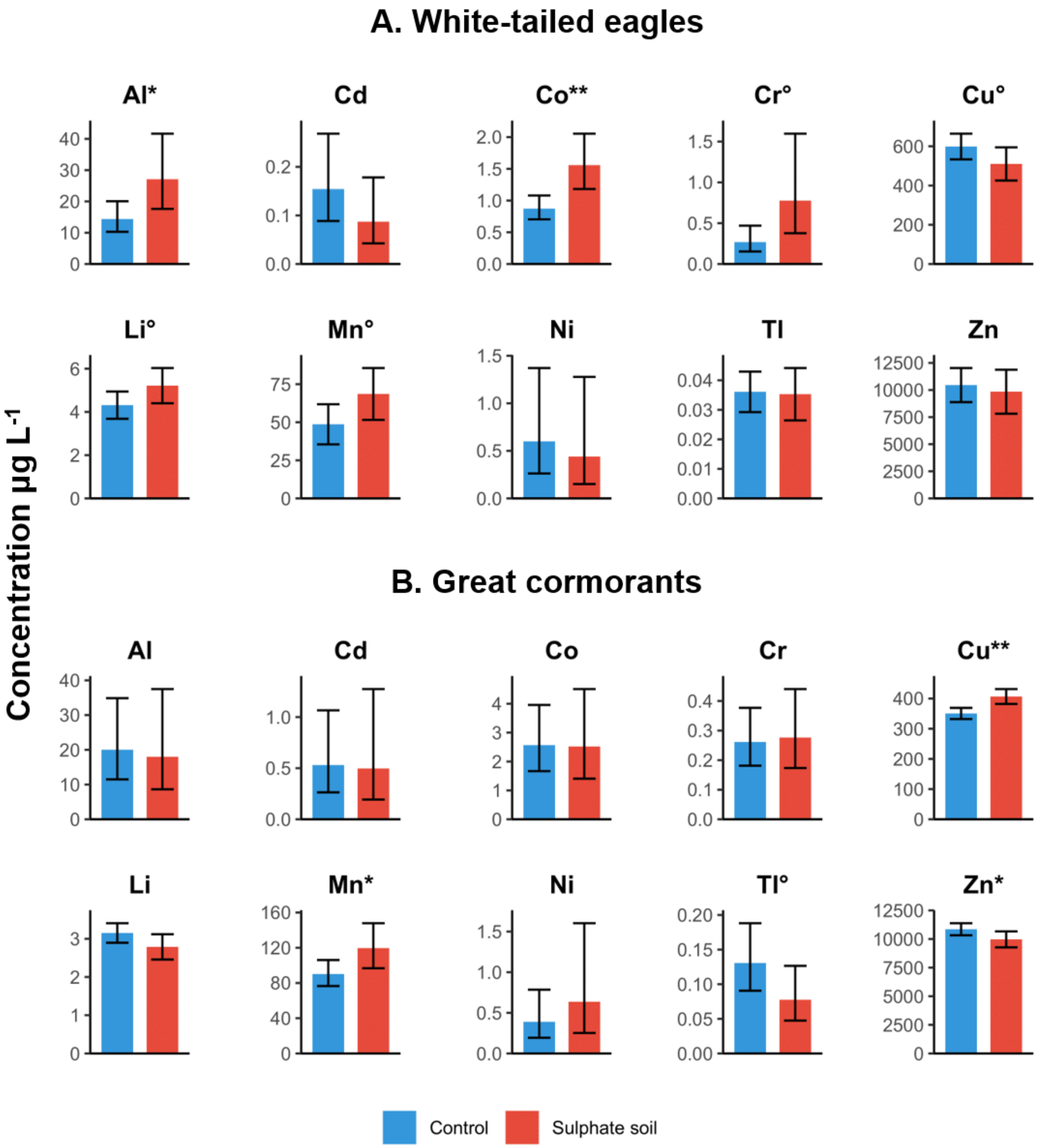

Figure 2. The estimated marginal means and their 95 % confidence intervals of blood metal concentrations in control and sulphate soil areas for A. white-tailed eagle nestlings (n = 24, except for Cr: n = 23) and for B. great cormorant nestlings (N = 31). °: $P \leq 0.10$, *: $P \leq 0.05$, **: $P \leq 0.01$. Results Co and Li of great cormorant nestlings without the interaction between latitude and treatment group are included for comparison. For Co and Li in great cormorant nestlings with the interactions, see Fig 3.

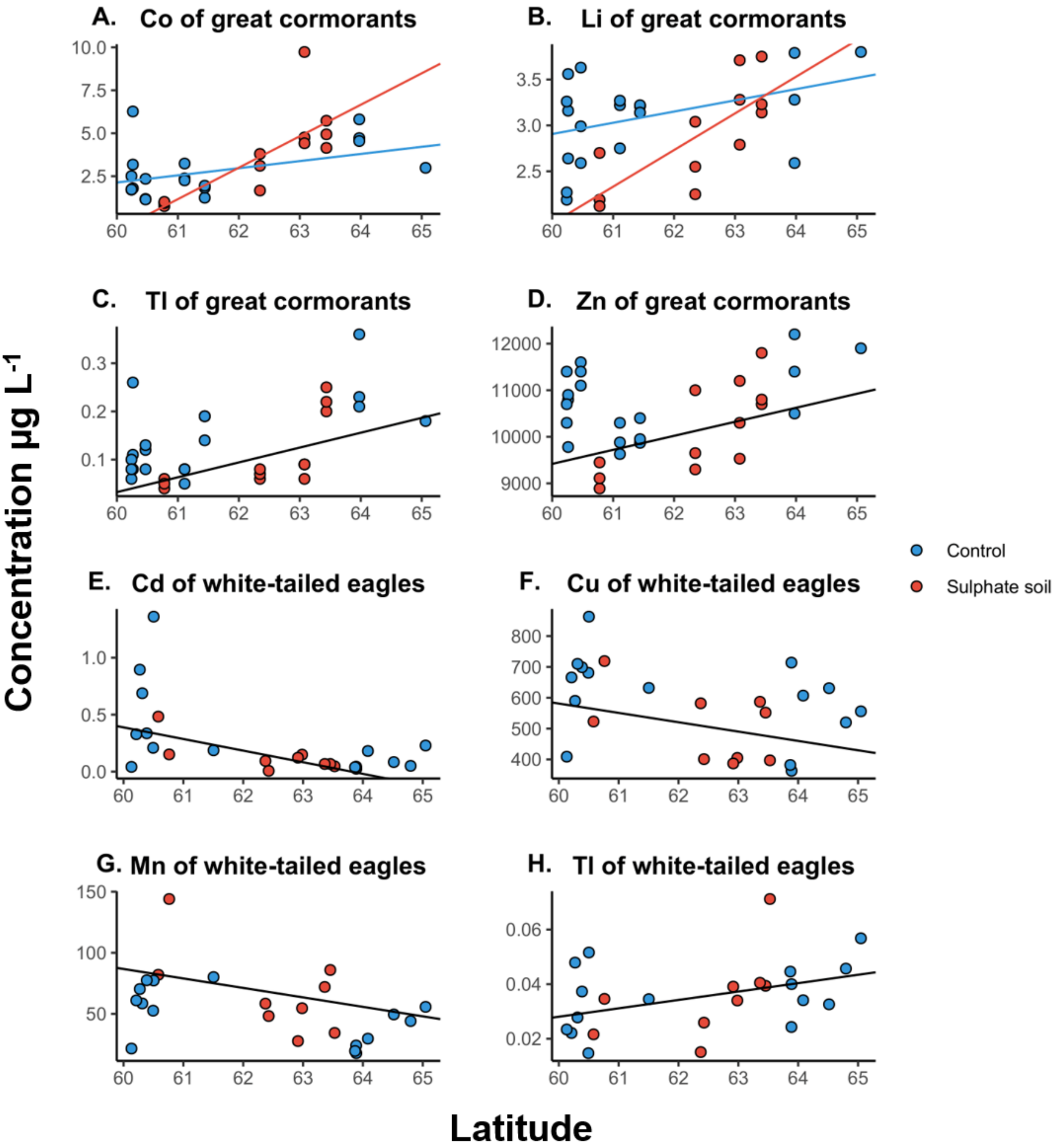

Figure 3. Interactions between latitude and treatment group (control versus sulphate soil) for A. Co and B. Li and latitudinal trends in E. Tl, and F. Zn concentrations in blood of nestlings of great cormorants. Latitudinal trends in E. Cd, F. Cu, G. Mn, and H. Tl concentrations in blood of nestlings of white-tailed eagles.

Table 1. Intraspecific Spearman's correlation coefficients (r_s) for metals in white-tailed eagle nestlings. For each correlation, r_s is above the adjacent p-value. Correlations with $p < 0.10$ are highlighted with green colour, and significant ones are bolded.

	Al	**Cd**	**Co**	**Cr**	**Cu**	**Li**	**Mn**	**Ni**	**Tl**	**Zn**
Al										
p-value										
Cd	-0.36									
p-value	0.19									
Co	**0.65**	-0.29								
p-value	**0.01**	0.29								
Cr	**0.70**	-0.27	**0.71**							
p-value	**<0.01**	0.33	**<0.01**							
Cu	-0.13	**0.55**	-0.34	-0.10						
p-value	0.66	**0.03**	0.22	0.73						
Li	0.41	**-0.54**	**0.56**	0.38	-0.29					
p-value	0.12	**0.04**	**0.03**	0.16	0.29					
Mn	0.10	**0.76**	-0.05	0.09	**0.59**	-0.34				
p-value	0.71	**<0.01**	0.85	0.74	**0.02**	0.22				
Ni	0.05	-0.21	0.10	0.36	-0.17	0.11	-0.22			
p-value	0.85	0.44	0.71	0.19	0.55	0.69	0.44			
Tl	0.01	0.09	0.22	0.19	0.01	0.04	0.01	-0.02		
p-value	0.96	0.74	0.44	0.51	0.97	0.88	0.98	0.94		
Zn	-0.05	**0.62**	-0.23	-0.18	**0.81**	-0.50	**0.61**	-0.41	0.05	
p-value	0.85	**0.01**	0.42	0.53	**<0.01**	0.06	**0.02**	0.13	0.86	

Table 2. Intraspecific Spearman's correlation coefficients (r_s) for metals in great cormorant nestlings. For each correlation, r_s is above the adjacent p-value. Correlations with p < 0.10 are highlighted with green colour, and significant ones are bolded

	Al	**Cd**	**Co**	**Cr**	**Cu**	**Li**	**Mn**	**Ni**	**Tl**	**Zn**
Al										
p-value										
Cd	-0.24									
p-value	0.48									
Co	0.25	-0.19								
p-value	0.45	0.57								
Cr	**0.83**	-0.18	-0.26							
p-value	**<0.01**	0.59	0.43							
Cu	0.01	-0.45	-0.15	0.20						
p-value	0.98	0.16	0.65	0.56						
Li	0.46	0.22	**0.72**	0.07	-0.45					
p-value	0.15	0.52	**0.01**	0.83	0.17					
Mn	-0.22	0.23	0.10	-0.25	0.42	-0.06				
p-value	0.52	0.50	0.77	0.47	0.20	0.85				
Ni	-0.05	-0.09	-0.12	-0.10	0.22	-0.43	-0.25			
p-value	0.87	0.79	0.73	0.77	0.52	0.19	0.45			
Tl	0.44	0.32	0.47	0.16	-0.55	**0.72**	0.06	-0.42		
p-value	0.18	0.34	0.14	0.63	0.08	**0.01**	0.85	0.20		
Zn	**0.62**	0.02	0.36	0.35	-0.36	0.56	-0.18	-0.10	**0.80**	
p-value	**0.04**	0.95	0.28	0.29	0.28	0.07	0.59	0.76	**<0.01**	

Table 3. Between-species Spearman correlation coefficients (r_s) and their p-values for each metal. P-values < 0.05 are marked with *. For each correlation n = 10 areas.

	Al	**Cd**	**Co**	**Cr**	**Cu**	**Li**	**Mn**	**Ni**	**Tl**	**Zn**
r_s	-0.33	-0.35	0.24	-0.03	0.04	-0.21	0.18	0.02	0.66	-0.02
p-value	0.35	0.33	0.51	0.93	0.91	0.56	0.63	0.96	*0.038	0.97

Electronic supplement 1.

We conducted a PCA for the metal concentration data, separately for each species, and then used the PC axis values in an ANOVA with soil type, latitude and their interaction as the explanatory variables. Because the PCA resolutions needed 5 components to cumulatively explain > 80 % of the total variation (Table 1 for white-tailed eagle and Table 2 for great cormorant nestlings), we conducted separate ANOVAs for the PCA1 to PCA5. For cormorants the sampling point (colony) was added as a random factor to control for the non-independence of nestlings within the same colonies. The interaction of soil type and latitude was non-significant in all models ($p > 0.05$), and thus removed.

We found significant effects of either sulphate soil treatment or latitude, or both, in several analyses (Table 3 for white-tailed eagle and Table 4 for great cormorant nestlings).

Table 1. Standard deviations, proportion of variance explained, and cumulative proportion of variance explained for PC-axes of white-tailed eagle nestlings

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard deviation	1.80220	1.35560	1.18560	1.10520	0.96950	0.76593	0.58310	0.45269	0.39537	0.24871
Proportion of variance explained	0.32480	0.18380	0.14030	0.12210	0.09400	0.05866	0.03400	0.02049	0.01563	0.00619
Cumulative proportion	0.32480	0.50850	0.64890	0.77100	0.86500	0.92369	0.95770	0.97818	0.99381	1.00000

Table 2. Standard deviations, proportion of variance explained, and cumulative proportion of variance explained for PC-axes of great cormorant nestlings

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard deviation	1.56030	1.43230	1.14600	1.11060	0.98694	0.81865	0.75240	0.65508	0.46250	0.33735
Proportion of variance explained	0.24350	0.20520	0.13130	0.12330	0.09741	0.06702	0.05661	0.04291	0.02139	0.01138
Cumulative proportion	0.24350	0.44860	0.57990	0.70330	0.80069	0.86770	0.92432	0.96723	0.98862	1.00000

Table 3. Results of ANOVAs for PCA1-PCA5 of white-tailed eagle nestlings.

PC-axis	Explanatory variable	F	df	p
PC1	treatment	2.21	1, 21	0.1517
PC1	latitude	4.87	1, 21	0.0387
PC2	treatment	8.46	1, 21	0.0084
PC2	latitude	0.99	1, 21	0.332
PC3	treatment	5.91	1, 21	0.0241
PC3	latitude	0.13	1, 21	0.7192
PC4	treatment	0.67	1, 21	0.4216
PC4	latitude	0.11	1, 21	0.7414
PC5	treatment	0.1	1, 21	0.7583
PC5	latitude	0.29	1, 21	0.5928

Table 4. Results of ANOVAs for PCA1-PCA5 of great cormorant nestlings.

PC-axis	Explanatory variable	F	df	p
PC1	treatment	7.75	1, 7.78	0.0244
PC1	latitude	13.8	1, 8.89	0.0049
PC2	treatment	2.06	1, 7.48	0.1921
PC2	latitude	0.69	1, 9.29	0.4255
PC3	treatment	6.4	1, 8.72	0.0331
PC3	latitude	1.85	1, 10.6	0.2015
PC4	treatment	0.01	1, 8.7	0.9243
PC4	latitude	0	1, 10.5	0.9818
PC5	treatment	0.03	1, 8.17	0.8768
PC5	latitude	0.02	1, 10.1	0.8986