SHORT COMMUNICATION



Exposure of White-throated Dippers to heavy metals in acidified and non-acidified streams in Norway

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Abstract

We examined the exposure of White-throated Dippers (*Cinclus cinclus*) to aluminum and mercury in acidified and non-acidified streams in two regions of Norway. Both metals showed significantly higher concentrations in the body tissues of dippers in acidified streams at southern sites compared to non-acidified streams in central Norway. Elevated concentrations of aluminum in acidified areas could not be explained by a corresponding elevated level of aluminum in the invertebrate foods of dippers. During our study period in 1993–1995, deposition rates of mercury by long-range air pollution were higher in southern than central Norway. High levels of mercury in dippers could have been due to higher levels of atmospheric deposition or higher water acidity in southern Norway. We found a high correlation between mercury levels in body tissues of females and their eggs, but not for aluminum. Thus, eggs are suitable for monitoring mercury levels but not for aluminum in female dippers. Our data provide baseline estimates of exposure to heavy metals in freshwater ecosystems in Norway that will be useful for monitoring future trends.

Keywords Acidified streams · Bioindicator · Heavy metals · Long-range pollution

Zusammenfassung

Belastung von Wasseramseln durch Schwermetalle in versauerten und nicht versauerten Fließgewässern in Norwegen Wir untersuchten die Belastung von Wasseramseln (*Cinclus cinclus*) durch Aluminium und Quecksilber in versauerten und nicht versauerten Fließgewässern in zwei Regionen Norwegens. Beide Metalle zeigten signifikant höhere Konzentrationen im Körpergewebe von Wasseramseln an den versauerten Fließgewässern im Süden, verglichen mit den nicht versauerten Fließgewässern in Mittelnorwegen. Erhöhte Aluminiumkonzentrationen in versauerten Gegenden ließen sich nicht durch korrespondierende erhöhte Aluminiumwerte in der Wirbellosennahrung der Wasseramseln erklären. Während des Untersuchungszeitraums von 1993–1995 lagen die Ablagerungsraten von Quecksilber durch weiträumige Luftverschmutzung im Süden höher als in Mittelnorwegen. Die hohen Quecksilberwerte bei den Wasseramseln könnten durch stärkere atmosphärische Ablagerungen oder einen höheren Säuregehalt des Wassers in Südnorwegen verursacht worden sein. Es zeigte sich eine starke Korrelation zwischen dem Quecksilbergehalt im Körpergewebe der Weibchen und dem der Eier, beim Aluminiumgehalt allerdings nicht. Eier eignen sich somit zur Dokumentation der Quecksilberbelastung, nicht jedoch der Aluminiumbelastung von Wasseramselweibchen. Unsere Daten liefern eine Basisschätzung der Schwermetallbelastung norwegischer Süßwasserökosysteme, welche bei der Beobachtung zukünftiger Entwicklungen von Nutzen sein werden.

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Introduction

The effects of industrial pollutants and acid rain on the ecology of freshwater streams have a long history of research in northern Europe. As early as the first decades of the previous century the first observations of fish death in southern Norway were recorded (Dahl 1921; Huitfelt-Kaas 1922), and a connection to acidic water was suggested in 1926 (Dahl 1926; Sunde 1926). In the late 1950s, a theory linking fish



mortality to acid rain was introduced by Dannevig (1959). The causal relationship between long-range air transported acidic components, water acidification, and fish mortality in Norway was established in the 1970s by the national project 'Acid Precipitation—Effects on Forest and Fish' (SNSF, Overrein et al. 1980). The SNSF project was comprehensive and also dealt with effects of acid rain on soils, forests and invertebrates in terrestrial ecosystems. However, the potential indirect effects of acidification on birds were not studied in Norway or elsewhere until Nyholm and Myhrberg (1977) reported reproductive problems of passerine songbirds breeding close to an acidic lake in northern Sweden. Their discovery led to the beginning of scientific work on indirect effects of acidification on birds feeding on aquatic organisms in Sweden (Eriksson et al. 1980; Nyholm 1981; Eriksson 1984), Britain (Ormerod et al. 1985, 1988) and North America (DesGranges 1982; DesGranges and Darveau 1985; Longcore et al. 1988). Previous to our project, only two field studies on potential effects of acidification on birds had been conducted in Norway, including a breeding study of Pied Flycatchers (Ficedula hypoleuca) and Great Tits (Parus major, Jåbekk 1985) and a population study on Whitethroated Dippers (Cinclus cinclus) in the acidified areas of southern Norway (Jerstad 1991).

Concerns about environmental exposure to heavy metals followed from early work on organismal responses to acid rain. Heavy metals were found to have a similar deposition pattern to acid rain in 1978-79 in a monitoring program for trace elements in air and precipitation (Hanssen et al. 1980) and in surveys based on analyses of mosses (Steinnes 1980). In the mid-1980s, the potential influence on longrange deposition of trace metals on metal accumulation in birds was established by Fimreite and Barth (1986), Steinnes and Brevik (1987) and Herredsvela and Munkejord (1988). Later, a literature review by Pedersen and Nybø (1990) concluded that levels of lead (Pb), mercury (Hg), cadmium (Cd) and possibly aluminum (Al) were elevated in some species of birds and mammals and that these levels possibly could be increased by long-range air pollution. Spatial deposition patterns of trace metals were similar to acidifying compounds, with the highest deposition rates in southern Norway and decreasing northwards (Overrein et al. 1980; Berg et al. 1995). Long-range air pollution includes a range of trace metals: arsenic (As), bismuth (Bi), cadmium (Cd), mercury (Hg), molybdenum (Mo), lead (Pb), antimony (Sb), thallium (Tl), vanadium (V) and zinc (Zn), but not aluminum (Al) (Berg et al. 1995).

The solubility and mobility of certain toxic metals, especially aluminum and cadmium, increases with increasing acidity in freshwater systems (Tyler et al. 1987; Nelson and Campbell 1991; Wällstedt and Borg 2003). Acidification of freshwater systems is known to increase the concentrations of aluminum in invertebrate organisms (Lien et al. 1996;

Walton et al. 2010; Harmon and Wiley 2011). Water acidification may also increase mercury bioavailability (Stein et al. 1996; Winfrey and Rudd 1990). Acidification does not increase the amount of dissolved inorganic mercury in freshwater lakes (Nelson and Campbell 1991), but the net methylation rate of inorganic mercury increases with decreasing water pH (Xun et al. 1987; Scheuhammer and Graham 1999; Kelly et al. 2003). As methyl mercury is more easily absorbed than inorganic mercury, negative correlations between water pH and mercury levels are common among invertebrates, fish, birds, and mammals living in acidified waters (Scheider et al. 1979; Wren and MacCrimmon 1983; Scheuhammer 1991; Wren and Stephenson 1991; Scheuhammer and Blancher 1994; Mattieu et al. 2013). Few studies have investigated metal exposure in bitrophic systems with the potential for accumulation of aluminum concentrations in bird species feeding on aquatic invertebrates (Magali et al. 2010).

We selected White-throated Dippers as a study species because they are an iconic species in Norway that are associated with natural freshwater ecosystems. Our studies were motivated by the early work of Jerstad (1991), who suggested that the productivity of dippers in southern Norway was reduced due to acidification because food quality was altered in waters with a lower pH (Scheuhammer 1991). Food quality might also be reduced due to increased bioavailability of trace metals, particularly aluminum, cadmium and mercury. Aquatic systems are more affected by acid precipitation than terrestrial systems, and dippers are likely to be a good sentinel species for the effects of acidification because the species is a year-round resident in freshwater habitats, diving underwater to feed on aquatic invertebrates and fish, with more than 90% of the population overwintering in Norway (Ormerod et al. 1987; Gjershaug et al. 1994; Tyler and Ormerod, 1994; O'Halloran et al. 2003). Based on their unique natural history, close association with riverine habitats, and a wide geographic distribution, White-throated Dippers were designated as the national bird of Norway in 1963. Other species of aquatic birds may be affected by acidification of streams, but exposure might be reduced if they move among habitats at different stages of their annual cycle (Eriksson et al. 1980; Nyholm 1981; Eriksson 1984).

In previous work, we showed that White-throated Dippers in southern Norway had higher rates of exposure to lead in southern Norway, but not to copper, cadmium or zinc (Nybø et al. 1996). In the present paper, our goal was to examine whether aluminum and mercury levels of dippers also differed between acidified and non-acidified habitats. In ecotoxicology, different assays have been used to test for exposure to heavy metals. A common practice is to analyze concentrations of trace elements, organic compounds, and other chemicals in important body tissues such as the brain, liver, kidney, bones, or adipose tissue. However, tissue analyses



require destructive sampling of animals collected from the field or analyses of individuals found dead. In recent years, alternative non-invasive methods have been developed to reduce impacts on natural populations, such as feather samples or abandoned eggs of wild birds. Eggs are considered good indicators of spatial and temporal patterns of organochlorine pesticides due to maternal transfer of contaminants during egg formation (Ormerod and Tyler 1994; Kallenborn et al. 1998; Scharenberg and Ebeling 1998; Braune et al. 2001). However, egg deposition rates for mercury and other heavy metals are less well known (O'Halloran et al. 2003). Similarly, feathers are difficult to assay for aluminum because they are challenging to prepare without contamination and it is difficult to obtain repeatable analytical results when the materials are collected and analyzed (S. Lierhagen, pers. com.). Here, we report on chemical analyses on a small sample of female White-throated Dippers and eggs collected from wild populations in two different regions of Norway. We evaluated aluminum and mercury levels in four different body tissues (liver, kidney, eggshell gland, and bone), and collected eggs to evaluate whether eggs are suitable for monitoring metal levels among breeding birds.

Methods

Our two main study areas included a non-acidified river Høylandsvassdraget (pH in river water 6.91) and its tributaries in central Norway (64° 44′ 00" N, 12° 20′ 00" E) and three acidified rivers Kvina, Litlåni and Mandalselva (pH in river water 4.94) and their tributaries in southern Norway (58° 34′ 00" N, 7° 18′ 00" E). A detailed description of the two study areas, deposition rates of long-range air pollution and water acidity are given by Nybø (1997) and Nybø et al. (1996). Our field studies of the possible effects of acidification on White-throated Dippers were conducted in 1993-1995 as part of a large national research program on the effects of acid rain in Norway during the 1980–1990s. Our results for exposure to lead, cadmium and other contaminants have been published elsewhere (Kallenborn et al. 1996, 1998; Nybø 1997; Nybø et al. 1996, 1997). Female dippers were captured during egg-laying with mist nets along watercourses and humanely euthanized by decapitation. A sample of 1–2 eggs was collected from dipper nests and analyzed for aluminum and mercury. To avoid pseudoreplication, we used the mean value of 1–2 eggs per female in our statistical analyses. All collecting and sampling of wild birds was conducted under permits for scientific research from the Directorate of Nature Management, Norway (ref. no. 94/5281 AS 446.7 and ref. no. 94/3494 AS 446.7).

Chemical analyses were conducted on liver, kidneys, eggshell gland, eggs and tibia bone at laboratory facilities of the Norwegian Institute for Nature Research (NINA). Metal concentrations were determined by atomic adsorption spectroscopy (AAS) (Perkin Elmer Model 1100B). A graphite furnace (HGA 700) with an automatic sampler (AS 70) was used for aluminum and a hydride system (FIAS 200) with an automatic sampler (AS 90) for mercury. The accuracy of the analytical procedures was checked against the National Bureau of Standards (NBS) for Bovine liver 1577A (Al, Hg), dogfish liver DOLT-1 (Hg) and dogfish muscle DOLM-1 (Hg). Laboratory procedures and methods for analyses are described in detail by Kålås et al. (1995).

We estimated the effects of contaminant exposure in female dippers with the tools of meta-analysis (Gurevitch et al. 2018). All analyses were conducted with functions of the meta package in an R environment (ver. 3.6.0, R Core Team 2019; Schwarzer 2007) (see online Appendix 1 for details). We calculated levels of two metals (aluminum and mercury) for five different types of tissues that were likely to have different turnover rates (eggs, eggshell gland, liver, kidney, tibia bone, Vander Zanden et al. 2015). In addition, we calculated levels of aluminum in metatarsus bone for validation of our lab procedures. We used metatarsus bone for cross-validation among different labs because our samples of tibia bone were not adequate to complete analyses of all elements along with additional screenings for validation. We calculated metal concentrations for each combination of metal and tissue (mean, standard error and sample size). We set acidified streams in southern Norway as the experimental treatment and considered non-acidified streams in central Norway to be a baseline control. We then used the metacont function of the meta package to calculate the standardized mean difference (SMD) as an estimate of effect size and to test for heterogeneity among comparisons of the different tissues. An effect size of zero would indicate no difference, whereas effect sizes ranging from 0.2, 0.5 and 0.8 would indicate small, medium, and large effects (Nakagawa and Cuthill 2007). We treated metal as a fixed effect and tissue as a random effect to calculate overall estimates of effect size.

Results

We sampled a total of 12 birds and eggs from 9 clutches from acidified areas of southern Norway and 10 birds and eggs from 9 clutches from non-acidified habitats in central Norway. In four of our analyses, metal concentrations were below the detectable levels of 0.6 mg kg⁻¹ for aluminum (eggs, eggshell gland, and kidney) and 0.1 mg kg⁻¹ for mercury (tibia bone), and no comparisons could be made between the two sites. Aluminum concentrations were 5.9–6.2 mg kg⁻¹ in liver and 10–16 mg kg⁻¹ in bone, whereas mercury concentrations were 1.4–2.5 mg kg⁻¹ in liver and kidney, and 0.15–0.91 mg kg⁻¹ in the eggshell gland and eggs (Table 1). We were able to calculate effect sizes for seven analyses, and all effect sizes were



Table 1 Concentrations of aluminum and mercury (mg kg⁻¹ dry weight) in the body tissues and eggs of female White-throated Dippers in non-acidified streams in central Norway versus acidified streams in southern Norway, 1993–1995

Metal	Body tissue	Non-acidified			Acidified		
		Mean	SE	N	Mean	SE	N
Aluminum	Liver	5.9	3	10	6.2	3.6	12
	Tibia bone	10.1	5.8	10	16.0	5.1	11
	Metatarsus bone	10.0	6.9	10	15.0	7.6	11
Mercury	Liver	1.42	0.89	10	2.00	2.42	12
	Kidney	1.51	0.44	10	2.50	2.03	12
	Eggshell gland	0.52	0.14	9	0.91	0.61	12
	Eggs	0.15	0.04	9	0.25	0.19	9

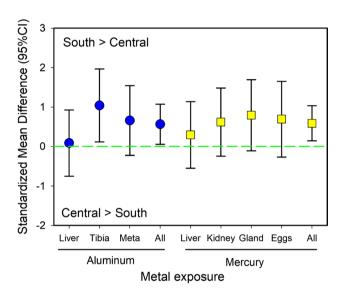


Fig. 1 Estimates of the effect size for concentrations of aluminum and mercury in the body tissues and eggs of female White-throated Dippers in central and southern Norway, 1993–1995

positive, indicating higher metal concentrations among dippers from southern than central Norway (Fig. 1). Estimates of the standardized mean difference ranged from +0.09 for aluminum in the liver and up to +1.04 for aluminum in tibia bones. The effect size for aluminum concentrations in tibia bones was significantly different from zero, but the 95% confidence intervals included zero for the other 6 of 7 effect sizes. We found no evidence for heterogeneity in the effect sizes among tissues $(Q_6=3.0,\,P=0.81)$ or between the two metals $(Q_1<0.01,\,P=0.95)$. The overall effect sizes for the two different metals indicated that female dippers had moderate but significant levels of exposure to both aluminum (SMD=0.568, 95% CI0.022 to 1.115, k=3) and mercury (SMD=0.588, 95% CI0.143 to 1.032, k=4).



Discussion

Our chemical analyses revealed that female dippers in acidified areas of southern Norway have body tissues and eggs with higher levels of aluminum and mercury (this study) and lead (Nybø et al. 1996). Higher concentrations of aluminum in female dippers in southern Norway are consistent with regional variation in other terrestrial organisms that show similar patterns of elevated exposure near industrial sources (Pedersen and Nybø 1990, K. Jerstad unpubl. data). Higher tissue concentrations of aluminum in dippers from southern Norway cannot be explained by higher aluminum concentrations in their invertebrate food items in the same study area (Nybø 1996). In related work in Britain, Ormerod et al. (1988) also concluded that aluminum levels were not related to water acidity in three genera of freshwater invertebrates (Ephemeroptera, Plecoptera, and Trichoptera). Additional work is needed to identify the sources and pathways leading to higher aluminum levels in dippers inhabiting acidified areas. Despite high aluminum levels in females, exposure may not affect embryo development because we found limited evidence for the transfer of aluminum from laying females to their eggs. Due to the low deposition rates, eggs are unlikely to be suitable for monitoring aluminum levels of female dippers. Birds excrete elements into growing feathers, and element concentrations may be higher and easier to monitor in feathers than other body tissues. Feathers can be used as a non-invasive method to monitor contaminants in birds (Ansara-Ross et al. 2013), but maybe less suitable for aluminum because of the challenges of avoiding contamination in the chemical analyses.

Mercury concentrations were also significantly higher among dippers in the acidified southern area than in non-acidified central Norway. Our findings are consistent with previous work with other birds in Sweden and northern Canada. Eriksson et al. (1989) also found higher mercury levels among ducklings of Common Goldeneyes (*Bucephala clangula*) feeding on invertebrates in acidic lakes compared to limed lakes. Similarly, Eastern Kingbirds (*Tyrannus tyrannus*) are an insectivorous species and had higher mercury levels in acidic wetlands compared to circum-neutral

wetlands (Glooschenko et al. 1986). Ospreys (*Pandion haliaetus*) and other species of birds associated with freshwater habitats in Norway do not show regional differences in mercury accumulation, but migratory birds might have less exposure than resident species like dippers (Holt et al. 1979; Frøslie et al. 1986).

The deposition of mercury by air pollution during our study period in the late 1990s was consistently higher in southern than central Norway (Steinnes and Anderson 1991). Higher levels of mercury in dippers could have been due to higher rates of atmospheric deposition, lower water acidity in southern Norway, or some combination of these two factors. O'Halloran et al. (2003) reported low levels of mercury in eggs of dippers in Ireland, and the potential for maternal transfer of mercury and other heavy metals to eggs was unclear. Here, we found that concentrations of mercury in body tissues and eggs were highly correlated, and eggs may be suitable for monitoring exposure. In summary, our analyses provide new baseline estimates for exposure of White-throated Dippers to aluminum and mercury in freshwater ecosystems in the late 1990s. In future work, similar analyses could be conducted to examine current levels of exposure after two decades of concerted effort to reduce levels of environmental pollution.

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