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Barn owl feathers as biomonitors of mercury: sources of variation in sampling procedures

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Abstract Given their central role in mercury (Hg) excretion and suitability as reservoirs, bird feathers are useful Hg biomonitors. Nevertheless, the interpretation of Hg concentrations is still questioned as a result of a poor knowledge of feather physiology and mechanisms affecting Hg deposition. Given the constraints of feather availability to ecotoxicological studies, we tested the effect of intra-individual differences in Hg concentrations according to feather type (body vs. flight feathers), position in the wing and size (mass and length) in order to understand how these factors could affect Hg estimates. We measured Hg concentration of 154 feathers from 28 un-moulted barn owls (*Tyto alba*), collected dead on roadsides. Median Hg concentration was 0.45 (0.076–4.5) mg kg^{-1} in body feathers, 0.44 (0.040–4.9) mg kg^{-1} in primary and 0.60 (0.042–4.7) mg kg^{-1} in secondary feathers, and we found a poor effect of feather type on intra-individual Hg levels. We also found a negative effect of wing feather mass on Hg concentration but not of feather length and of its position in the wing. We hypothesize that differences in feather growth rate may be the main driver of between-feather differences in Hg concentrations, which can have implications in the interpretation of Hg concentrations in feathers. Finally, we recommend that, whenever possible, several feathers from the same individual should be analysed. The five innermost primaries have lowest mean deviations to both between-feather and intra-individual mean Hg concentration and thus should be selected under restrictive sampling scenarios.

Keywords Biomonitor · Barn Owl · Mercury · Feathers · Intra-individual variations

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Introduction

Mercury (Hg) is a metal naturally present in the environment (prolific in coal and metal-rich geologic deposits) and also an introduced contaminant—its main anthropogenic sources are mining and fossil fuel combustion (Krabbenhoft and Sunderland 2013). Hg is mostly available to living organisms after conversion in its toxic organic form of methylmercury (MeHg), which is reported to be harmful both to humans and wildlife, mainly due to neurological and immunological effects, and reproductive impairment (Evans et al. 1982; Burger and Gochfeld 1997; Scheuhammer et al. 2007). Methylation of the element can occur in aquatic environments, and so Hg ecotoxicological studies have been focused mainly in aquatic organisms (Seewagen 2010). Nevertheless, toxicity thresholds have also been reported in terrestrial organisms in agricultural wetlands (Ackerman and Eagles-Smith 2010; Ackerman et al. 2010). Despite Hg compounds were banned as plant protection products in Europe since 1991 (Commission Directive 91/188/EEC), Hg availability appears to be increasing globally through atmospheric deposition

(Windham-Myers et al. 2014), highlighting the urgent need for Hg contamination biomonitors in farmlands for ecological and food safety concerns (Jiang et al. 2014; Chen et al. 2015).

Given the pronounced bioaccumulation and biomagnification of Hg in food webs, the highest concentrations are often attained in top predator species (Lindberg and Odsjö 1983; Dietz et al. 2000; Lourenço et al. 2002). Both owls (Strigiformes) and diurnal raptors (Accipitriformes, Falconiformes) have been used as sentinels of environmental contamination in Europe since the late 1950s (Gómez-Ramírez et al. 2014), and most monitoring schemes used feathers as a non-invasive sampling method for several contaminants, including metals (García-Férnandez et al. 2008; Castro et al. 2011; Bustnes et al. 2013). Since feathers can be collected from both live and dead individuals, they are extremely versatile as reservoirs of contaminants, allowing for monitoring direct effects in contemporary populations, as well as for studying long time trends, using for instance specimens stored in museum collections (Bustnes et al. 2013; Gómez-Ramírez et al. 2014).

Feathers are the key excretory pathway for Hg in birds because they hold from 50 % to more than 90 % of the body Hg burden (Honda et al. 1986; Braune 1987; Lewis and Furness 1991; Agusa et al. 2005). Mercury concentrations in feathers result mostly from endogenous deposition of blood-circulating Hg and are not or slightly affected by external deposition (Burger and Gochfeld 1997; Dauwe et al. 2003). Since the transfer of blood-circulating substances in feathers is interrupted after total feather growth, Hg is trapped and remains stable, bonded to keratin fibbers, mainly in the form of MeHg (Furness et al. 1986).

However, the interpretation of Hg concentrations in feathers for biomonitoring purposes is not straightforward. There is no general agreement on which factors influence Hg deposition, and the biological meaning of the observed between-feathers variation is still unclear. While some authors recommend the use of smaller body feathers for Hg quantification (Furness et al. 1986; Solonen and Lodenius 1990), others state that feathers cannot be indiscriminately selected and therefore flight feathers (remiges) should be used, given they can be consistently located (Bortolotti 2010). The correlations found in many bird species between Hg concentration in primary feathers and species-specific moulting sequence (i.e. feathers replaced earlier have higher Hg concentrations) are generally interpreted as a cause-effect pattern linked to Hg deposition: (1) circulating Hg levels drop as this metal is retained in growing feathers (Lindberg and Odsjö 1983; Furness et al. 1986; Dauwe et al. 2003); or (2) individuals select less contaminated prey during the moult than before (Lindberg and Odsjö 1983). However, it is also hypothesized that this pattern is an artefact of variation in feather mass for elements whose incorporation is time dependent, such as Hg. Thus heavier (and often longer) feathers show a more diluted concentration since they have a wider growth period (Bortolotti 2010). Moreover, there is evidence that the decrease in Hg concentrations along with the moult sequence is not generalized to all species. For instance, a study with barn owl (*Tyto alba*) primaries did not show any relationship between the two (Dauwe et al. 2003).

Owing to its ecological requirements and its closeness to humans, the barn owl is potentially a good sentinel of environmental Hg contamination, particularly in farmland habitats. This owl is a generalist and opportunistic predator that hunts in open farmland, feeding mostly on small mammals, and in many regions using man-made structures (e.g. barns, sheds, old houses) as nesting sites (Bunn et al. 1982; Roulin 2002). The same nests may be continuously monitored for long time periods: at nest sites, feathers can be collected from nestlings and sometimes shed flight feathers (from adults' moult) are also available (adults can also be captured to take feather samples). Another straightforward source of barn owl feathers for ecotoxicological analysis is collecting carcasses on roadsides. Owls are frequent victims of collision with vehicles, as for example the road-killing estimates of 0.35–0.49 owls/km/year for Southern Portugal (Silva et al. 2008; Gomes et al. 2009; Grilo et al. 2014). Literature reporting Hg levels measured in owl feathers is still modest (see review in Espín et al. 2014), and to the best of our knowledge only a few studies have analysed Hg in barn owl feathers (Westermark et al. 1975; Denneman and Douben 1993; Dauwe et al. 2003; Lourenço et al. 2002). None of these studies examined the implications of feather sampling methods.

For ethical and legal reasons sampling live birds requires the use of non-invasive methods. Body feathers from the breast are therefore frequently used, since they are easy to pluck and it is possible to collect a few without causing harmful effects to the bird. Also, since body feathers can be collected from both live and dead individuals (while not possible for blood samples) these tissues are good candidates for a standard assessment of environmental contamination levels. Therefore, considering that many ecotoxicological studies rely mostly on opportunistic sampling, i.e. with access to a limited number and/or type of tissue samples, it is important to understand how the characteristics of the available samples affect the accuracy of the results and thus the quality of the conclusions.

Our main goal in this study is to verify if feathers of different types and also flight feathers (remiges) varying in size and position in the wing show considerable variation in Hg levels, independently of feather age, with

implications in the use of barn owl feathers as biomonitors and in sampling procedures. We focused on feathers collected from road killed un-moulted barn owls (moult starts in the 2nd calendar year; Martínez et al. 2002), thus restricting the analysis to feathers from the same generation, which were simultaneously developed while the birds were nestlings (i.e. in each individual the available Hg in blood during growth is identical for all feathers). We tackled the following issues: (1) is the variation in Hg concentration between body and flight feathers small, so that these feather types can be interchangeably used to compare contamination levels in different sites? and (2) is the Hg concentration in flight feathers similar despite feather length, mass and position in the wing, so that remiges (primary and secondary feathers) can be indiscriminately used to assess environmental Hg contamination?

Methods

Study area

Samples were collected along roads in central Portugal, between Vila Franca de Xira and Évora (7°53′–8°59′W; 38°32–38°59′N). The climate in the study area is Mediterranean, with mild winters and hot dry summers, and the rain period mainly concentrated in winter. Landscape is mostly plain or undulating and is dominated by cork oak *Quercus suber* and holm oak *Quercus rotundifolia* traditional woodland systems named 'montados', with varying tree density. 'Montados' are managed for different uses (e.g. cork extraction, grazing, cereals), resulting in a multifunctional landscape. Agricultural areas occupy 10–30 % of the study area and consist mainly of irrigated annual cultures, rice fields, rainfed cereal crops, vineyards and olive groves.

Sampling procedures

A total of 154 feathers were plucked from 28 barn owl carcasses collected on roadsides from 2009 to 2012: 29 samples of body feathers, 62 primary feathers and 64 secondary feathers. Whenever possible, five feather samples were collected from each individual: (1) at least three body feathers from the breast, and (2) one primary feather from the outermost group (P10–P6), (3) one primary feather from the innermost group (P5–P1), (4) one secondary feather from the outermost group (S1–S6) and (5) another secondary feather from the innermost group (S7–S12), in order to represent all the wing length. Feathers were stored in transparent plastic bags until analysis. We followed the feather numbering system of Martínez et al. (2002). Regarding position in the wing, flight feathers were numbered from 1 to 24 from the outermost primary (P10) to the innermost secondary (S14). Feather mean mass (dry weight) and length were obtained by weighing and measuring all flight feathers from the right wing of two barn owls in the range of extreme wing lengths for the species (277 and 296 mm; range in our data: 269–295 mm ($n = 12$ individuals); range in Martínez et al. 2002: 270–300 mm). Prior to weighing, feathers were dried in an oven for 2 h at 35 °C in order to remove excess moisture resulting from freeze storing. Feathers were weighed on a precision scale (0.1 mg) and measured with a wing ruler (1 mm).

Mercury analysis

Total Hg concentration in feather samples was determined by thermal atomization followed by atomic absorption spectroscopy, using an AMA-254 spectrophotometer (LECO, Czech Republic). This methodology is simple, and requires minimum sample handling prior to analysis, since no digestion procedure or sample pre-treatment is necessary. Homogenized, dried samples are placed into a pre-cleaned combustion boat and inserted in a quartz combustion catalytic tube. The sample is firstly dried at 120 °C prior to combustion at 680–700 °C in an oxygen atmosphere. The mercury vapour is collected in a gold amalgamator and after a delay period heated at 900 °C. The released mercury is transported to a heated (120 °C) cuvette and then quantified by atomic absorption spectroscopy using a silicon UV diode detector (for more details please see Costley et al. 2000). Given the reduced mass of a single body feather, for analytical reasons mean Hg concentration was calculated analysing a pool of body feathers per individual. Concerning single flight feathers, Hg concentration was determined using the mean of the measurements in successive cuts starting from the distal part of the feather. All Hg concentrations are presented in mg kg^{-1} on a dry weight basis.

Quality assurance

Precision, accuracy and analytical detection limits were continuously monitored as means of assessing analytical performance, and hence the validity of results. Sample treatment and analyses were performed using ultra-clean protocols. All glassware used was previously soaked for at least 24 h in a bath containing 5 % Decon, then 24 h in 25 % HNO_3 and finally thoroughly rinsed with ultra-pure water obtained from a Millipore Milli-Q Integral System.

Precision was assessed through the analysis of dispersion between replicate analyses. Acceptance criteria were established (three replicate results with relative standard deviation below 10 %) above which samples were re-analysed.