Predatory Bird Monitoring Scheme

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Mercury (Hg) concentrations and stable isotope signatures in golden eagle eggs 2009-2013: a Predatory Bird Monitoring Scheme (PBMS) report

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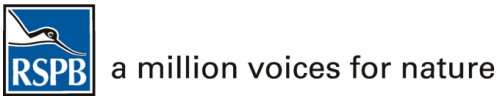

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Contents

1. Executive Summary

The Predatory Bird Monitoring Scheme (PBMS; http://pbms.ceh.ac.uk/) is the umbrella project that encompasses the Centre for Ecology & Hydrology's National Capability activities for contaminant monitoring and surveillance work on avian predators. The PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife.

Mercury (Hg) is a neurotoxin and there has been global concern over its impact on humans and wildlife. It has been predicted that global Hg emissions may rise in the future because of increased coal-fired power generation, but, in 2013, the United Nations Environment Programme (UNEP) agreed The Minamata Convention on Mercury, a global treaty to protect human health and the environment from the adverse effects of mercury. An overarching aim of the convention is to control the anthropogenic releases of Hg to the environment. Therefore, long-term trends in environmental Hg concentrations are uncertain. One cost-effective means of assessing such trends is to monitor exposure in sentinel wildlife species.

Golden eagles *Aquila chrysaetos* breed and forage in the Scottish uplands and could prove a sentinel for changing Hg deposition in upland terrestrial areas and associated wildlife exposure. We measured Hg residues in failed golden eagle eggs with the aim of providing baseline data on current levels of exposure. Specifically, we measured Hg concentrations in failed eggs laid between 2009 and 2013 in inland (> 3km from the coast) and coastal (<3 km from the coast) nests. We distinguished nests in this way because coastal nesting birds can feed on seabirds that can accumulate high levels of Hg themselves. Marine dietary Hg inputs could potentially obscure any changes in Hg accumulation associated with altered upland terrestrial Hg deposition, and so we hypothesized that only eggs from inland nests may be useful sentinels. In conjunction with Hg measurements, we examined stable isotope (SI) signatures (carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulphur ($\delta^{34}S$)) to determine if they differed between eggs from inland and coastal nests in a manner consistent with feeding primarily on terrestrial and marine prey, respectively. We also examined Hg concentrations and SI signatures of failed white-tailed sea eagle (*Haliaeetus albicilla*) eggs from nests on the west coast of Scotland. We used these measurements as a comparator against which to assess the extent to which SI and Hg measurements in eggs from coastal golden eagle nests might be indicative of feeding on marine prey and scavenge.

We found that SI signatures (particularly $\delta^{34}S$ isotopic ratios) and Hg concentrations were similar in golden eagle eggs from coastal nests and white tailed sea eagle eggs. SIs and Hg concentrations in eggs from inland nests were much more variable, and a third had SI signatures that were the same as those of eggs from coastal nests, suggesting that they too were laid by females feeding on a coastal diet. A cluster of seven eggs from inland nests had distinctive $\delta^{34}S$ and $\delta^{15}N$ values (below 11.0 ‰ and 5.7 ‰ respectively) and it was inferred that these were most likely laid by females feeding terrestrially. Hg concentrations were non-detectable in these seven eggs whereas the median concentration in golden eagle eggs associated with coastal feeding was 0.412 µg/g dry weight, similar to that (0.569 µg/g dry weight) in white tailed sea eagle eggs. Hg concentrations in all eggs were below those thought to be associated with embryotoxic effects.

The lack of detectable Hg concentrations in GE eggs associated with upland terrestrial feeding is problematic if these eggs are to be used as sentinels of change in upland Hg concentrations. Re-analysis of a set of eggs using a more sensitive analytical technique may resolve this issue and should be explored, otherwise other sentinels may need to be investigated.

2. Introduction

1.1 Background to the PBMS

The Predatory Bird Monitoring Scheme (PBMS; http://pbms.ceh.ac.uk/) is the umbrella project that encompasses the Centre for Ecology & Hydrology's long-term contaminant monitoring and surveillance work on avian predators. The PBMS is a component of CEH's National Capability activities.

By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and, in particular, to vertebrate wildlife. Our monitoring provides scientific evidence of how chemical risk varies over time and space. This may occur due to market-led or regulatory changes in chemical use and may also be associated with larger-scale phenomena, such as global environmental change. Our monitoring also allows us to assess whether detected contaminants are likely to be associated with adverse effects on individuals and their populations.

Overall, the PBMS provides a scientific evidence base to inform regulatory and policy decisions about sustainable use of chemicals; a key example in the context of the current report is the United Nations Environment Programme (UNEP) Minamata Convention on Mercury (see section 1.2). In addition, the outcomes from our monitoring are used to assess whether effects are likely to occur in wildlife, whether mitigation of exposure is needed and what measures might be effective. Monitoring also provides information by which the success of mitigation measures can be evaluated.

Currently the PBMS has two key general objectives:

(i) to detect temporal and spatial variation in exposure, assimilation and risk for selected pesticides and pollutants of current concern in sentinel UK predatory bird species and in species of high conservation value

(ii) in conjunction with allied studies, to elucidate the fundamental processes and factors that govern food-chain transfer and assimilation of contaminants by top predators.

Further details about the PBMS, copies of previous reports, and copies of (or links to) published scientific papers based on the work of the PBMS can be found on the PBMS website.

1.2 Mercury (Hg) in predatory birds

Mercury (Hg) is a highly toxic nonessential heavy metal emitted into the environment from a variety of natural and anthropogenic sources (Nriagu, 1989). It has been predicted that global Hg emissions are likely to increase, partly driven by the expansion of coal-fired electricity generation in the developing world, particularly Asia (Streets *et al.*, 2009). Mercury occurs in the environment both in inorganic and organic form and both can be ingested by wildlife. However, methyl-mercury (MeHg) is highly bioavailable and is biomagnified through the food web; apex predators, such as raptors, can therefore be exposed to relatively high dietary concentrations. Methyl-mercury is a neurotoxin and can affect reproduction indirectly by altering parental behavior and directly through toxicity to the embryo (Shore *et al.*, 2011).

The possible impacts of Hg on Man and the environment have aroused global concern. In January 2013, the United Nations Environment Programme (UNEP) agreed The Minamata Convention on Mercury, a global treaty to protect human health and the environment from the adverse effects of Hg. The major highlights of the convention include a ban on new Hg mines, the phase-out of existing ones, control measures on air emissions, and the international regulation of the informal sector for artisanal and small-scale gold mining http://www.mercuryconvention.org/Convention/tabid/3426/Default.aspx. An overarching aim of the convention is to control the anthropogenic releases of Hg to the environment.

It is unclear to what extent the implementation of the Minamata Convention may be successful in limiting the rise of, or even reducing, anthropogenic Hg emissions and whether this will lead to any change in exposure of wildlife and Man to Hg. One means of assessing this is to monitor accumulation in sentinel wildlife species. Long-term changes in tissue residues reflect changes in the bioavailable fraction of environmental Hg and can be used as a key indicator of risk of adverse effects.

1.3 Aims of the current study

The golden eagle, *Aquila chrysaetos*, population in Britain is confined to mountainous areas, predominantly in the Highlands and Islands of Scotland (Watson, 1997). This species has been used by the PBMS as a sentinel species for upland habitats and various contaminants, including Hg, have been measured previously in golden eagle eggs. When Hg monitoring has been conducted, Hg levels in eggs from nests classed as < 3 km and > 3km from the coast (hereafter called coastal and inland nest) have been considered separately where possible (Walker *et al.*, 2011). This is because golden eagles (GEs) nesting near the coast predate seabirds that can accumulate high levels of Hg themselves (Thompson, 1996). Thus, exposure pathways (marine vs terrestrial) may differ for GEs depending on the location of their breeding territories. Indeed, we have found previously that egg Hg concentrations differ between inland and coastal nests, likely reflecting differences in terrestrial and marine feeding and associated Hg transfer(Walker *et al.*, 2011).

Classification of nests as inland and coastal is somewhat arbitrary however. This is because the provenance information provided for nests is restricted (sometimes to a named glen, stated region of variable size, or even county), so as to prevent precise location information being inadvertently accessed by illegal egg collectors. Nests are classified as coastal if the nearest coastal border of the stated region of provenance is less than 3 km from the nearest coastline. Furthermore, GEs

have variably-sized territories with breeding densities ranging between 4 and 25 pairs per 1,000 km^2 (Haworth *et al.*, 2006, Watson *et al.*, 1992). It is possible that GEs occupying some of the nests classed as inland may still feed partly or extensively on seabirds. Altogether, the uncertainty about the extent to which GEs from inland nests may be accessing marine diet means that any changes in Hg accumulation due to changes in upland Hg deposition may be obscured by marine Hg inputs to the diet of some birds.

The aim of the current study was two-fold.

(i) We examined the stable isotope (SI) signature (carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulphur ($\delta^{34}S$)) of GE eggs and in particular examined whether signatures varied between eggs that were from coastal and inland nests. We aimed to determine if SI signatures could be used as a diagnostic indicator to distinguish between likely feeding on terrestrial and marine prey and scavenge by breeding female GEs. We also compared the SI signatures with those of failed white-tailed sea eagle *Haliaeetus albicilla* eggs from nests on the west coast of Scotland. White-tailed sea eagles (WTSE) take a large proportion of fish in their diet (Watson *et al.*, 1992). GE eggs with SI signatures (particularly $\delta^{34}S$) similar to those of WTSE eggs are likely to be laid by females feeding on substantial amounts of marine fauna.

(ii) We determined recent levels of total Hg in failed GE eggs (laid in the years 2009-2013) and compared concentrations in: (a) eggs from coastal and inland nests, (b) in the same eggs but separated into three groups (terrestrial feeding, coastal feeding, uncategorised) on the basis of the SI signatures of the eggs. We used the discrimination based on SI signature to determine current Hg concentrations in eggs from birds that were probably primarily feeding on terrestrial upland prey. It is those birds that are most likely to be useful as sentinels for change in Hg deposition and accumulation in upland areas.

3. Methods

Between 2009 and 2013 addled and deserted eggs were collected by licensed individuals visiting GE and WTSE nests as part of population status monitoring studies. These eggs were submitted to the Predatory Bird Monitoring Scheme. A total of 23 GE and 6 WTSE eggs from separate clutches were collected (Table 1). The contents of each egg was homogenized and stored at - 20ºC prior to analysis.

Stable isotope analysis was carried out in the Stable Isotope Facility at CEH Lancaster and the Lancaster Environment Centre. A 1g sample of homogenised egg contents was dried at 70ºC for 2 hrs prior to analysis. Samples were then weighed into tin capsules and combusted using a Eurovector elemental analyser. Resultant CO_2 and N_2, from combustion were analysed for $\delta^{13}C$ and $\delta^{15}N$ using a Micromass Isoprime isotope-ratio mass spectrometer (IRMS). The standard deviation for duplicate and QC samples was not more than 0.30‰ for $\delta^{15}N$ and 0.35 for %N. For carbon the standard deviation of QC samples was not more than 0.09‰ for $\delta^{13}C$ and 2.25 for %C. Samples analysed for sulphur ($\delta^{34}S$) stable isotopes were combusted at 1120ºC on Tungstic Oxide in a Vario Pyrocube EA and the $\delta^{34}S$ isotopes analysed on an Isoprime100 IRMS. Repeat standards were run to an internal and external precision of <0.2‰ standard deviations, while the difference for duplicate and QC samples was not more than 2.3% for $\delta^{34}S$.

Egg contents were analysed for Hg concentrations by microwave digesting an accurately weighed sub-sample (approximately 1 g wet weight) in 10ml of 70% ultrapure nitric acid (Baker, Ultrex II) at 200ºC for 15 minutes. The digests were made up to an initial volume of 25 ml with ultrapure water (Millipore, MilliQ), then further diluted 10-fold with ultrapure water immediately prior to analysis by inductively couple plasma mass spectrometry (Perkin Elmer DRCII ICPMS). The moisture content of the sample was determined by drying a 0.5g sub-sample at 70ºC for a minimum of 24 hours. Dry weight concentrations were calculated based upon the wet weight of the analysed sample and the moisture content of the sub-sample. The instrumental limit of detection (LoD) for Hg (0.01 µg/L) was calculated as 4.03 times the standard deviation of six replicate blank determinations. Taking into account the digest volume, dilution of the digest and the sample weight, the mean tissue LoD was 0.131 µg/g dry weight (dry wt.).

Unless otherwise stated, concentrations are presented as µg/g dry wt. of sample and have been statistically analysed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA). Eggs with non-detectable Hg concentrations were assigned a value of half the limit of detection (0.065 µg/g dry weight).

Table 1. Summary of number of golden eagle and white-tailed sea eagle eggs collected each year and the proximity of the nests to the coast

Species	Proximity to the coast	Year collected					
		2009	2010	2011	2012	2013	Total
Golden eagle (GE)	< 3 km	2	2	0	2	0	6
Golden eagle (GE)	> 3 km	6	1	4	2	2	15
Golden eagle (GE)	unknown	1	0	0	0	1	2
White tailed sea eagle (WTSE)	< 3 km	1	3	-	-	2	6

4. Results and Discussion

3.1 Stable isotope profile in golden eagle and white-tailed sea eagle eggs

Stable isotope (SI) profiles in the tissues of an animal can provide information on the trophic level at which an animal feeds and the dominant sources of the food consumed. The $\delta^{13}C$ values indicate whether this carbon has originated from fixation by C3 or C4 plants, while $\delta^{15}N$ values tend to increase with trophic level. Most importantly in the context of the present study, individuals with higher $\delta^{34}S$ values in their tissues tend to have fed more from marine systems rather than terrestrial systems (Michener and Lajtha, 2007).

The SI signatures of GE eggs from coastal and inland GE nests and for WTSE eggs showed some separation with $\delta^{34}S$ and $\delta^{15}N$ but little for $\delta^{13}C$ (Figure 1). The plot of $\delta^{34}S$ against $\delta^{15}N$ was the most useful for discriminating between eggs from inland and coastal GE nests. In particular, $\delta^{34}S$ values for GE eggs from coastal nests were tightly clustered and closely overlapped those for WTSE eggs (Figures 1 and 2). This suggested that laying GEs from all coastal nests had diets that were similar to WTSEs and likely contained a significant marine component.

The 15 eggs from inland GE nests had a relatively wide range of $\delta^{34}S$ and $\delta^{15}N$ values (Figures 1 and 2). Seven formed a cluster on the $\delta^{34}S$/ $\delta^{15}N$ plot (Figure 1 and 2) that could be defined as eggs with $\delta^{34}S$ and $\delta^{15}N$ values below 11.0 ‰ and 5.7 ‰ respectively (three standard deviations (3SDs) above the mean values). Their $\delta^{34}S$ and $\delta^{15}N$ values were clearly distinct from those of eggs from coastal nests. In contrast, five other eggs from inland nests had $\delta^{34}S$ and $\delta^{15}N$ values that were within 3SDs of the means for eggs from coastal nests, suggesting that laying females from these inland nests had diets that were similar to those of females occupying coastal nests. Finally, the three remaining eggs from inland nests had isotope signatures that were less distinctive in that $\delta^{15}N$ values were similar to, but $\delta^{34}S$ values (10.1-12.4 ‰) were below, values associated with eggs from coastal nests (Figure 2). The isotope signatures for these three GE eggs may have perhaps been indicative of a more mixed feeding strategy.

The reported isotope values cannot be taken as diagnostic of diet, particularly as we had no isotope values for prey that the GEs may have taken. However, given the apparent clustering of eggs, we inferred three categories from the $\delta^{34}S$ and $\delta^{15}N$ values so we could assess potential dietary influences on egg Hg concentrations. These categories were:

> *Coastal feeding*: $\delta^{34}S$ values > 13.6 ‰ (which is 3SDs below the mean value for GE eggs from coastal nests) were inferred to indicate likely significant feeding on marine prey and scavenge, as might also be found in WTSEs. All six eggs from coastal nests, five eggs from inland nests, and two eggs from nests for which we had no provenance information had such $\delta^{34}S$ values.
>
> *Upland terrestrial feeding:* This category contained the cluster of seven eggs from inland nests that had $\delta^{34}S$ and $\delta^{15}N$ values less than 11.0 ‰ and 5.7 ‰ respectively and were clearly distinct from eggs associated with coastal feeding. It was inferred that these eggs were laid by eagles that were likely to have been feeding largely on upland terrestrial scavenge and diet.
>
> *Uncategorised:* Eggs with $\delta^{34}S$ values between 11.0 ‰ and 13.6 ‰ could not be readily classed as coastal or terrestrial feeding. Three eggs, all from inland nests, fell into this category.

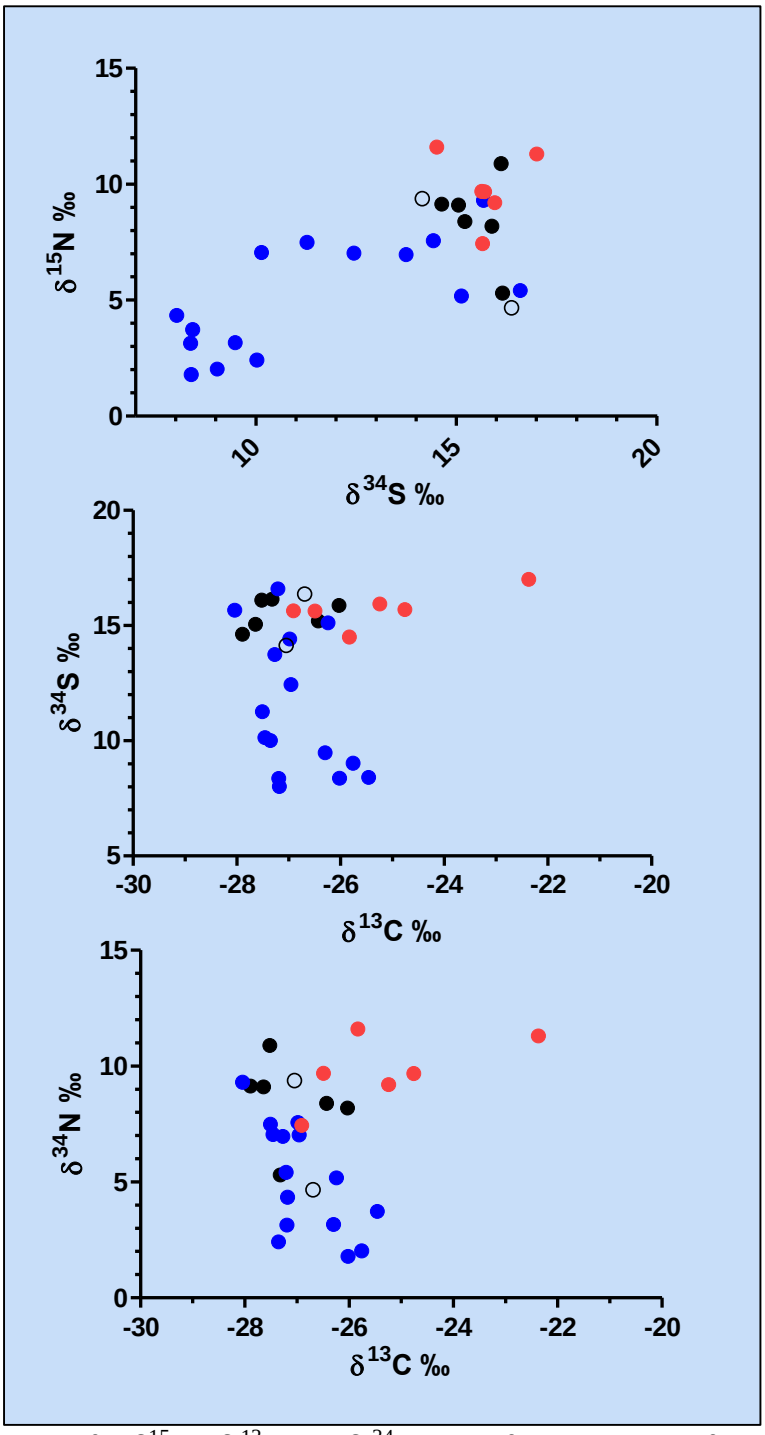

Figure 1. Isotope ratios for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ in: (i) failed GE eggs from coastal nests (●), inland nests (●) and nests of unknown location (O); (ii) failed WTSE eggs - coastal nests (●).

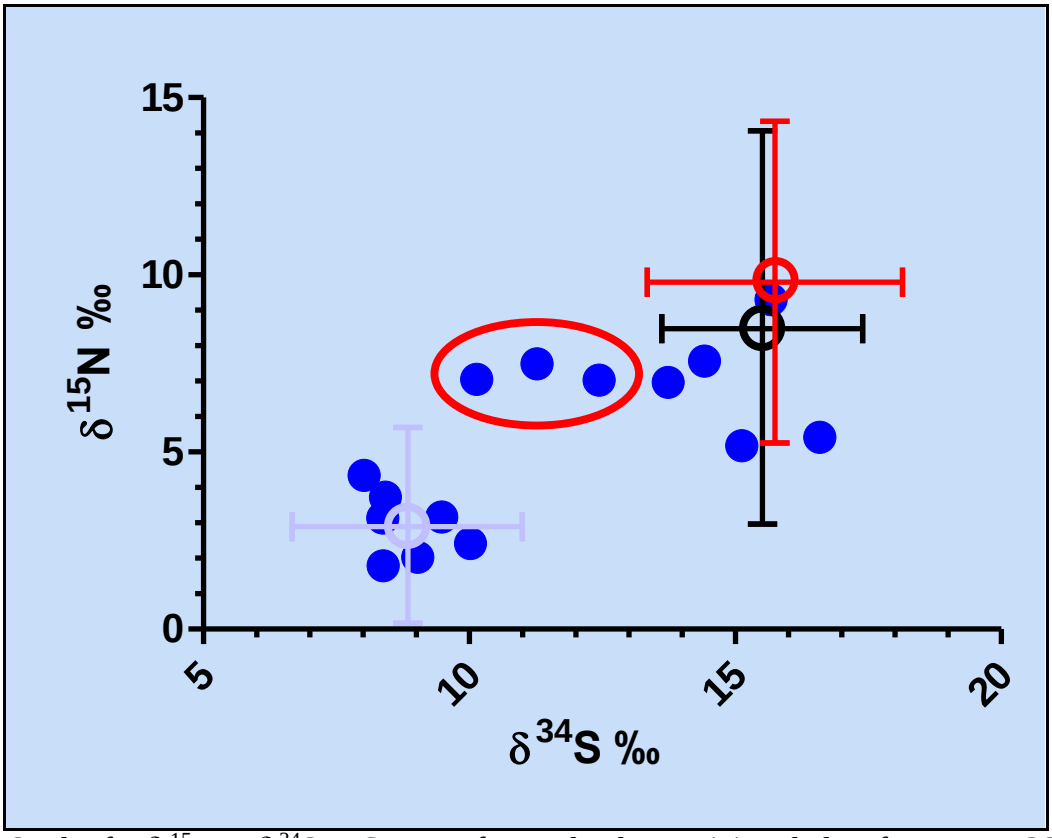

Figure 2. Plot for δ ^{15}N vs δ ^{34}S in GE eggs from inland nests (●) and plots for mean ± 3SDs for δ^{15}N and δ^{34}S in GE eggs from coastal nests (black circle and confidence bars) and WTSE eggs (red circle and confidence bars). The ranges (mean ± 3SD) for isotope values associated with the cluster of 7 GE eggs (from inland nests) with relatively low δ^{15}N and δ^{34}S values is also shown, while eggs with intermediate SI values are ringed (see text for details).

3.2 Hg concentrations in golden eagle and white-tailed sea eagle eggs

When eggs were categorized as coming from inland or coastal nests, 12 of the 15 eggs from inland nests had total Hg concentrations below the limit of detection and three had concentrations of between 0.324 and 0.436 µg/g dry wt. When eggs were re-categorised on the basis of their SI signatures, all seven eggs associated with upland terrestrial feeding had non-detected total Hg concentrations whereas 11 of 13 GE eggs associated with coastal feeding had detectable concentrations (Figure 3). The difference in detection rate between these two groups was significant (Fishers exact test, P<0.0005) and the median total Hg concentration in eggs associated with coastal feeding was significantly above the detection limit (Wilcoxon Signed ranks test, W=180, P=0.003; Figure 3). Total Hg concentrations in WTSE eggs were similar to those previously reported (Walker *et al.*, 2008) and did not differ from those in GE eggs associated with coastal feeding (Mann-Whitney U test, U = 32, P = 0.57; Figure 3) .

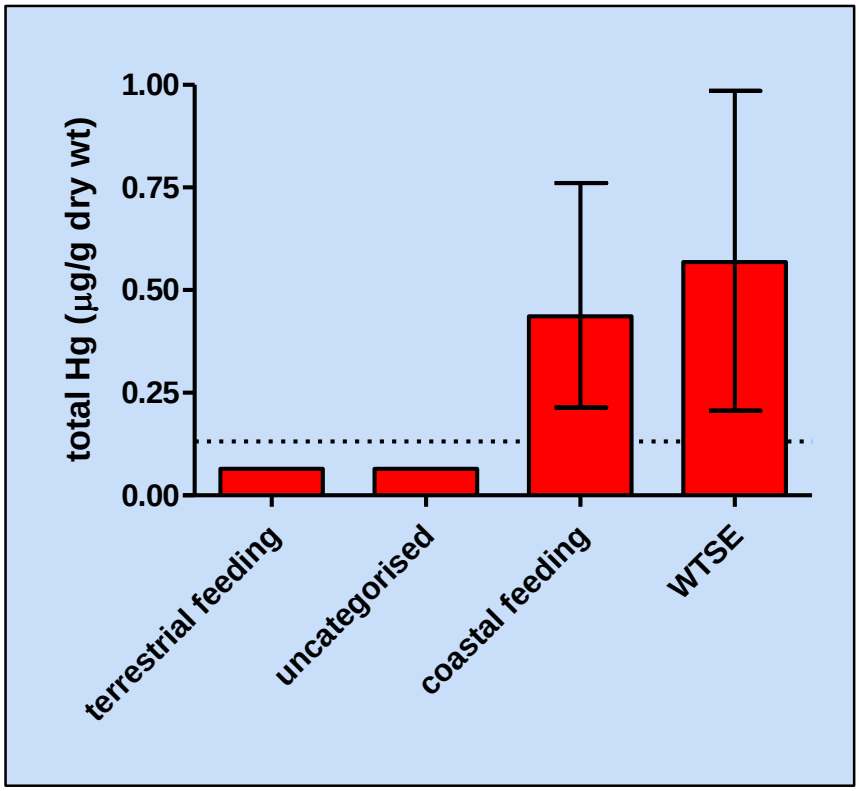

Figure 3. Median (and interquartile range) total Hg dry weight concentrations in: GE eggs associated with SI signal inferred as indicative of terrestrial [upland] feeding in laying females (n = 7 eggs); (ii) GE eggs associated with SI signal that was uncategorised (n = 3 eggs); GE eggs associated with SI signal inferred as indicative of coastal feeding (n = 13 eggs); WTSE eggs (n=6). The dotted line indicates the Limit of Detection (LoD) and all GE eggs associated with terrestrial feeding or uncategorised had total Hg concentrations below the LoD.

In terms of potential embryotoxic effects, concentrations greater than approximately 0.6 µg/g wet wt. have been proposed as a generic indicative value that may be associated with impaired avian reproduction (Shore et al., 2011), although values may be higher, or occasionally lower, for individual species. When the Hg concentrations in the GE and WTSE eggs were expressed on a wet weight rather than dry weight basis, the maximum Hg wet weight concentrations were 0.26 µg/g wet weight in both species, and so unlikely to have had adverse effects.

5. Conclusions

This study has demonstrated that SI signatures can be used to categorize golden eagle eggs in terms of likely feeding strategies of laying birds. Such categorisation is likely to better define the effect of diet on Hg accumulation than relying on provenance information for the nest.

Our results suggest that differences in diet, as inferred from the SI signal, is a major determinant of Hg concentrations in GE eggs. If we are correct in inferring that GE eggs with $\delta^{34}S$ and $\delta^{15}N$ values below 11.0 ‰ and 5.7 ‰ respectively are associated with terrestrial feeding by laying females, the lack of detectable Hg in such eggs is problematic. Current levels of Hg deposition in upland areas and subsequent food-chain transfer would appear to be too low to result in detectable Hg concentrations in GE eggs using the ICPMS analysis employed in the present study. This analytical technique has been used by the PBMS as it yields concentration data on a wide range of metals in addition to Hg, and has been of sufficient sensitivity to detect concentrations of toxicological concern. However, if the aim is to use eggs from terrestrial feeding birds as a sensitive monitor of potential changes in upland mercury concentrations, lower limits of detection for Hg are clearly needed. Concentrations in eggs need to be detectable so that future increases or decreases in Hg concentrations are detectable. Re-analysis using atomic fluorescence detection of those eggs identified from stable isotope analysis as likely being representative of terrestrial feeding is merited to ascertain whether levels of Hg contamination are detectable. Detection limits are likely to be < 10 ng/g dry weight, an order of magnitude more sensitive than ICPMS analysis.

If we are still unable to detect Hg in golden eagle eggs with more sensitive techniques, then it may be necessary to consider other sentinels. Other species , such as merlin (*Falco columbarius*), breed in upland areas and Hg was detectable in most merlin eggs collected from northern England and Scotland between 1979 and 2008. Annual total Hg concentrations ranged between 1.74 and 7.72 µg/g dw (Walker *et al.*, 2011), much higher than current concentrations in GE eggs. However, the number of failed merlin eggs received annually by the PBMS from 2004 and 2013 has varied between 2 and 24 (median = 8) and so sample sizes may be limiting in some years. Perhaps, a more significant issue is that merlins feed chiefly on small birds. Their dietary Hg intake may thus reflect recent [potentially non-upland] habitat use by relatively mobile prey species rather than reflect Hg contamination in upland areas.

Overall therefore, we conclude that use of SI analysis on GE eggs is valuable in terms of identifying which eggs are likely to be representative of terrestrial feeding birds. Use of GE eggs would provide a means of integrating the contamination signal over relatively large spatial upland areas, but this is dependent on ability to measure detectable Hg concentrations in eggs. This potential needs to be explored using more sensitive analytical techniques for Hg.

6. Acknowledgements

We thank all the members of the public who have submitted addled and deserted eggs to the Predatory Bird Monitoring Scheme. Their efforts are key to the success of the scheme. The Predatory Bird Monitoring Scheme was co-funded in 2014-15 by the NERC Centre for Ecology & Hydrology, the Department for the Environment, Food and Rural Affairs (Defra), Natural England (NE), the Royal Society for the Protection of Birds (RSPB), the Scottish Environment Protection Agency (SEPA) and Scottish Natural Heritage (SNH).

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