Predatory Bird Monitoring Scheme
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Mercury (Hg) concentrations in predatory bird livers and eggs as an indicator of changing environmental concentrations: a Predatory Bird Monitoring Scheme (PBMS) report

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1. Executive Summary

Concern over the potential health effects of mercury (Hg) has prompted an international agreement, the Minamata Convention on Mercury, that aims to control anthropogenic releases to the environment and reduce potential impacts on humans and wildlife. Monitoring is required to determine to what extent the convention is successful. The PBMS has monitored long-term trends in environmental Hg concentration using raptors and fish-eating birds as sentinels to track changes in exposure. Overall, PBMS monitoring of Hg in predatory birds provides an evidence base by which the impact of the Minamata Convention on environmental mercury concentrations in Britain can be assessed.

The current study consisted of four main aims that would help rationalize and inform our long-term Hg monitoring.

(i) *Updating long-term data for liver Hg concentrations in sparrowhawks, (*Accipiter nisus*), a sentinel for exposure in lowland terrestrial habitats.*

(ii) *Exploration of the use of alternative tissues for monitoring Hg in sparrowhawks.*

(iii) *Comparison of trends in liver Hg residues in sparrowhawks and kestrels (*Falco tinnunculus*) to examine if trends in sparrowhawks, which feed on relatively mobile avian prey, reflect those in kestrels which mainly feed on small mammals that are more likely to reflect local contamination.*

(iv) *Completion of work initiated last year to explore the potential for using Hg concentrations in the eggs of inland-feeding golden eagles (*Aquila chrysaetos*) as a sentinel to track changes in Hg bioavailability and uptake by biota in upland terrestrial systems.*

We measured liver Hg residues in sparrowhawks that had died in 2013 and 2014 to quantify current Hg exposure in lowland terrestrial habitats and to add to previously reported long-term data. Mercury residues in birds that died in 2013 and 2014 were largely consistent with those reported in recent previous years and were below those associated with mortalities. Three birds had residues higher than those associated with potential adverse effects on reproduction.

Analysis of long-term data (1990-2014) indicated liver Hg residues in sparrowhawks vary with age and sex; concentrations are highest in adult males. Starvation also elevates liver Hg concentrations. Taking age and sex into account and using only data for non-starved birds, we investigated temporal trends and found that, although there has been between-year variation in liver Hg concentrations, there has been no consistent upward or downward trend. We used the long-term dataset to define “current baseline” liver Hg concentrations against which levels in future years, and consistent time trends, can be quantitatively and rapidly assessed.

We found that total Hg concentrations in sparrowhawk liver, kidney and brain were closely related. We conclude it is possible to transfer our long-term monitoring of Hg in sparrowhawks (including retrospective calculation of “current baseline concentrations”) to analysis of kidney or brain. This would preserve [what are relatively small] sparrowhawk livers for other analyses.

Comparison of historic trends in liver Hg in sparrowhawks and kestrels indicated that rates of decline during 1980-1998 were similar in the two species. This is consistent with the premise that sparrrowhawks are as likely as kestrels to be representative of changes in environmental exposure to (and associated bioaccumulation of) Hg in lowland terrestrial systems.

The conclusion of our work on Hg concentrations in golden eagle eggs enabled us to quantify a “baseline concentration” for eggs laid by females feeding predominantly on terrestrial prey. We can use this to identify significant changes in future exposure and associated bioaccumulation and thereby use our measurements as sentinel of future change in Hg bioavailability in upland habitats in northern Britain.

2. Introduction

2.1. 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.

The PBMS is a citizen science based monitoring scheme in that it relies on members of the public to submit to the scheme dead birds that they find. Eggs that are analysed by the scheme are likewise collected by volunteers who are specifically licensed by the relevant countryside agencies to collect the failed eggs of raptors for scientific purposes.

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 **2.2**). 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.

2.2. 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, largely 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. It has been predicted that global Hg emissions are likely to increase, largely driven by the expansion of coal-fired electricity generation in the developing world, particularly Asia (Streets *et al.*, 2009). However, ,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. Major aspects of the convention include a ban on new mercury 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 a reduction 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.

The PBMS has monitored long-term trends in environmental Hg concentration using raptors and fish-eating birds as sentinels to track changes in exposure in different habitats (Table 1). In addition, we have started to explore the potential for using measurements of mercury concentrations in failed golden eagle (*Aquila chrysaetos*) eggs to monitor change in the availability of Hg in upland habitats (Walker *et al.*, 2015b), and completion of that work is one of the aims of the current study (see Section 2.3). Overall, PBMS monitoring of Hg in predatory birds provides an evidence base by which the impact of the Minamata Convention on environmental mercury concentrations in Britain can be assessed.

Table 1. Summary of the types of measurement and species used as sentinel measurements to indicate long-term changes in Hg availability in different habitats

Species		Mercury Measurement	Habitat
Eurasian sparrowhawk	*Accipiter nisus*	Liver concentrations	Lowland terrestrial
Northern gannet	*Morus bassanus*	Egg concentrations	Marine
Eurasian otter	*Lutra lutra*	Liver concentrations	Fresh- waters

2.3. Aims of the current study

The current study consisted of four main aims that overall would help rationalize and inform long-term mercury monitoring by the PBMS.

(i) *Updating long-term data for liver mercury concentrations in sparrowhawk (Section 4)* . In this report, we_report Hg residues in livers in birds that had died in 2013 and 2014, including an analysis of whether age, sex or nutritional status of birds on residue magnitude. We also demonstrate how such annual data can be used to generate Shewhart type control charts that can be used to readily detect changes in concentrations over time.

(ii) *Exploration of the use of alternative tissues for monitoring Hg in sparrowhawks (Section 5).* The PBMS has utilized sparrowhawk livers extensively to quantify spatial and temporal variation in a range of contaminants. While use of the same organ gives a measure of consistency in approach in our studies, it can result in the depletion of remaining sample. We investigated whether kidney tissue might be used as an alternative to the liver for monitoring Hg and compared both liver and kidney residues to those found in brain tissue, a potential site of toxicity for mercury.

(iii) *Comparison of trends in liver Hg residues in sparrowhawks and kestrels (Section 6).* Although we have used sparrowhawks as sentinels of exposure for lowland terrestrial systems, sparrowhawks feed predominantly on birds which, because of their mobility, may not necessarily reflect environmental contamination of the local area. It can be argued that the common kestrel (*Falco tinnunculus*) may be more representative of local habitat because they feed extensively on small mammals, which will have restricted home ranges. We compared liver Hg concentrations in sparrowhawks and kestrels for years in which measurements had been made on both species, so as to determine the extent to which trends in liver hg concentrations in kestrels are reflected in sparrowhawks.

(iv) *Hg concentrations in golden eagle eggs (Section 7).* Last year, we explored the potential for using Hg concentrations in the eggs of inland-feeding golden eagles (*Aquila chrysaetos*) as a sentinel measure for tracking potential changes in Hg exposure of biota in upland terrestrial systems. We assessed whether laying females had been feeding predominantly on terrestrial or marine foodwebs by examining the stable isotope signature of the egg contents (Walker *et al.*, 2015b). Some of the key samples that we analysed using inductively couple plasma mass spectrometry (ICP-MS) techniques had non-detected concentrations. An abundance of non-detected concentrations limits the value of such samples for monitoring as it is not possible to detect any decrease in concentrations. In this study, we re-ran those samples using fluorescence analysis, which has approximately a 10 fold greater sensitivity than ICPMS, and additionally analysed eggs collected in 2013 and 2014 but that had not been run previously. The aim of this analysis was to determine if it was possible to generate a more robust baseline for Hg concentrations in inland feeding eagle eggs against which future change in concentrations could be judged.

3. Methods

3.1. Sample selection and chemical analysis of sparrowhawk livers for mercury

Sparrowhawk that had died from a variety of causes between 2013 and 2014 were collected by the PBMS. The carcasses were necropsied and the nutritional status, sex and age class of each bird was determined; individuals hatched in the current or previous year to that in which they were found dead were classed as juveniles. Various tissue samples, including liver, kidney (left and right hand combined), and brain, were excised and stored at -20ºC prior to analysis. In all, 50 sparrowhawk livers were analyzed for total Hg concentrations (Table 2).

Table 2. Summary of sparrowhawks for which liver Hg concentrations were determined.

	Juvenile		Adult		
Year collected	Female	Male	Female	Male	Total
2013	7	5	7	6	25
2014	6	8	8	3	25
Total	13	13	15	9	50

A 1g wet weight sample of the liver was digested in 10ml of 70% ultrapure nitric acid (Baker, Ultrex II) in a microwave digestion system at 200ºC for 15 minutes. The digested samples were made up to an initial digest volume of 25ml using ultrapure water (Millipore, MilliQ). They were further diluted 10-fold using ultrapure water immediately prior to being analysed for Hg by ICPMS (Perkin Elmer DRCII operating under standard conditions. 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 concentration of the analysed sample and the gravimetrically determined moisture content of a separate sub-sample.

The ICPMS 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 0.09 µg/g dry wt.. Three replicate samples of two certified reference materials, Tort 2 and Dolt 4 (both from National Research Council Canda, Ottawa, Canada) were run concurrently with the sparrowhawk and red kite tissues. The mean recoveries for total Hg from samples of Tort 2 and Dolt 4 were 109.8% and 95.6%, respectively.

3.2. Sample selection and chemical analysis for mercury of sparrowhawk kidneys and brains livers

Samples of brain and kidney (both kidneys combined) from 24 birds for which liver Hg concentrations had been measured in previous PBMS monitoring activities were analysed for total Hg concentrations. Birds from which brain and kidney samples were taken for analysis were selected on the basis of their liver Hg concentrations, the aim being to analyse birds with a broad range of liver concentrations. Liver Hg concentrations in selected birds ranged between 0.10 and 19.3 µg/g dry wt. Desiccation had occurred during storage of the brain and kidney samples, which limited the amount of sample available for analysis and, on average, 0.6g and 0.4g of brain and kidney were analysed respectively; sub-samples were taken to gravimetrically determine dry weight conversion factors. Samples for Hg analysis were prepared by microwave assisted acid digestion and measured using ICPMS as described for the liver samples.

3.3. Analysis of golden eagle eggs for Hg and stable isotopes

Twelve golden eagle eggs (laid in years 2009 to 2013) previously analysed by ICP-MS techniques and six additional eggs (laid between 2012 and 2014) that had not been previously analysed were digested as described above for liver samples. Egg digests were further diluted 100 times with ultrapure water and analysed for Hg using atomic fluorescence spectrometry (PS Analytical Millennium Merlin AFS instrument). Two replicate samples of two certified reference materials, Tort 2 and Dolt 4 (both from National Research Council Canada, Ottawa, Canada) were run concurrently with the golden eagle eggs samples. The mean recoveries for total Hg from samples of Tort 2 and Dolt 4 were 119.7% and 101.4%, respectively. Duplicate samples of four eggs were run with between sample differences ranging between -5.9% and +3.8% with a mean difference of -0.7%.

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 70^{o}C 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 δ^{13}C and δ^{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 δ^{15}N and 0.35 for %N. For carbon the standard deviation of QC samples was not more than 0.09‰ for δ^{13}C and 2.25 for %C. Samples analysed for sulphur (δ^{34}S) stable isotopes were combusted at 1120^{o}C on Tungstic Oxide in a Vario Pyrocube EA and the δ^{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 δ^{34}S

3.4. Data and statistical analysis

Sparrowhawk liver Hg concentration from this study were combined with previously reported data (Walker *et al.*, 2011) for long-term trend analysis of mercury concentrations sparrowhawks that died between 1990 and 2012. Liver Hg concentrations for individual sparrowhawks were Johnson-transformed prior to general linear model analysis.

Unless otherwise stated, concentration are presented here as µg/g dry weight of sample and have been statistically analysed using either GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA) or Minitab version 16.1 (Minitab Inc., Coventry, U.K.).

4. Total mercury concentrations in livers of sparrowhawks

4.1. Current concentrations

Liver Hg residues in the sparrowhawks studied ranged from 0.123 to 10 µg/g dry wt.. The geometric mean Hg concentration was 1.61 µg/g dry wt. with a 95% confidence interval of 1.256-2.059 µg/g dry wt. (Fig. 1). However, analysis of Log_{10}-transformed data using a general linear model showed that liver Hg residues were significantly higher in adults than juveniles ($F_{1,46}$=4.30, P=0.044) and in males than females ($F_{1,46}$=5.28, P=0.026). Both observations are consistent with the analysis of the long term data (see below). The interaction term between age and sex was not significant ($F_{1,46}$=2.10, P=0.154). When data were broken down by sex and age class, it was evident that adult male sparrowhawks had the highest geometric mean liver Hg concentrations which was 2-3 folder higher than those for other age/sex classes but the difference was only statistically significant (Tukey's *post-hoc* test) between adult males and juvenile females, perhaps because of limited sample size (Fig. 1).

Proposed indicative liver Hg concentration associated with mortality and effects on reproduction in birds is 20 µg/g wet wt. and 2 µg/g wet wt., respectively (Shore et al., 2011). These concentrations are equivalent to 70 µg/g and 7 µg/g dry weight in bird livers, assuming a 3.5-fold correction factor between wet and dry weight concentrations, and are suggested values that are likely to be protective for most species; they are not specific to sparrowhawks. None of the birds that had died in 2013 and 2014 had liver Hg residues higher than those associated with mortality but three birds, all adult males, had residues higher than those associated with reproductive effects.

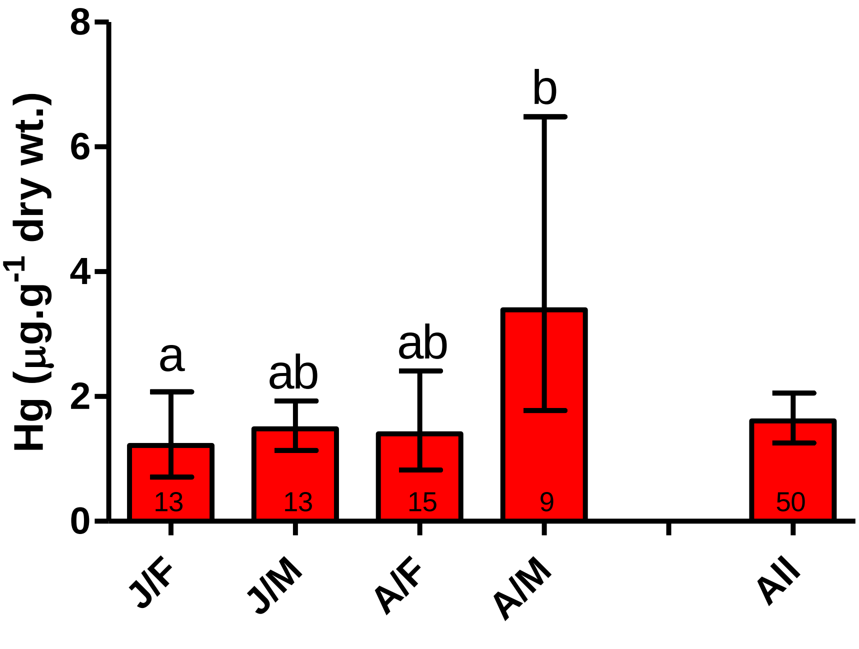

Figure 1. Geometric mean (±95% Confidence Interval) Hg liver concentrations in juvenile female (J/F), juvenile male (J/M), adult female (A/F) and adult male (A/M) sparrowhawks that died in 2013 and 2014 (combined). Sample numbers are shown in the column for each demographic group and significant (P<0.05) differences between groups are indicated by different letters.

4.2. Long-term trend analysis of total mercury concentrations in livers of sparrowhawks

The liver Hg concentrations in sparrowhawk livers described above were combined with those from a previous PBMS report (Walker et al 2014) that analysed residues in sparrowhawks between 1990 and 2012. Data were restricted to birds for which we had a recoded fat score and so evidence of the nutritional state at the time of death. Birds with a fat score of 0 (typically no evidence of fat deposits including those around the heart) or 1 (trace amounts of fat deposits, typically around heart but may be little elsewhere in the body) were scored as being in a starved state; birds with higher fat scores were classed as non-starved. Exploratory analysis of liver mercury residues using a GLM indicated that age, sex, nutritional state (as determined by fat score) and year, and multiple interactions between these factors, were significant in explaining variation in liver Hg concentrations. We therefore simplified the statistical analysis by examining data only for birds in a non-starved condition (fat scores ≥ 2) as starvation-induced liver wastage potentially could increase variability in liver Hg concentrations in an unpredictable manner.

Examination of data for non-starved birds only indicated that year of death was a significant factor explaining variation in liver Hg but only when included as a factor ($F_{23, 515} = 2.10$, P=0.002) rather than as a covariate in the model ($F_{1, 536} = 0.81$, P=0.37), indicating that while there was variation between years, there was no long-term trend over the period 1990- 2014. Age and sex were also significant factors in the model, with liver Hg residues higher in adults than first-year birds ($F_{1, 515} = 15.2$, P<0.001) and in males than females ($F_{1, 515} = 7.55$, P=0.006), although sex had not been found to be a significant factor when we analysed the data in our earlier report (Walker et al 2014).

Given there was no significant trend in liver concentrations over time, we constructed Shewhart control charts, based the sparrowhawk liver Hg data collected between 1990 and 2014, so that we could identify future years in which concentrations prove to be higher or lower than would be expected by chance. These charts (Fig. 2) were constructed for adult and first year males and females separately given the influence of age and sex on liver Hg concentrations and were based on annual median concentrations. Data were only included for years in which the number of sparrowhawks in the age and sex class was ≥ 5, although data for consecutive years were sometimes pooled when sample numbers within each year were low, and the liver mercury value was ascribed to the mid-point of the pooled years. The average number of birds (and range) upon which annual medians were based was 6 (5-9) for adults and 11 (5-24) for first-year birds. These charts can provide easy and rapid identification of years in which liver Hg values in sparrowhawks are unusually high or low and rapid visualization of developing trends in contamination.

Overall for the period 1990 to 2014, the annual median liver Hg concentrations in sparrowhawk livers were approximately 35 fold and 3.5 fold lower than the generic concentrations suggested as potentially indicative of mortality and of harmful effects on reproduction in non-marine birds, respectively (Shore *et al.*, 2011). None of the liver concentrations in individual birds exceeded the concentration associated with mortality but just over 2.5% of non-starved females had liver residues higher than those potentially indicative of harmful effects on reproduction.

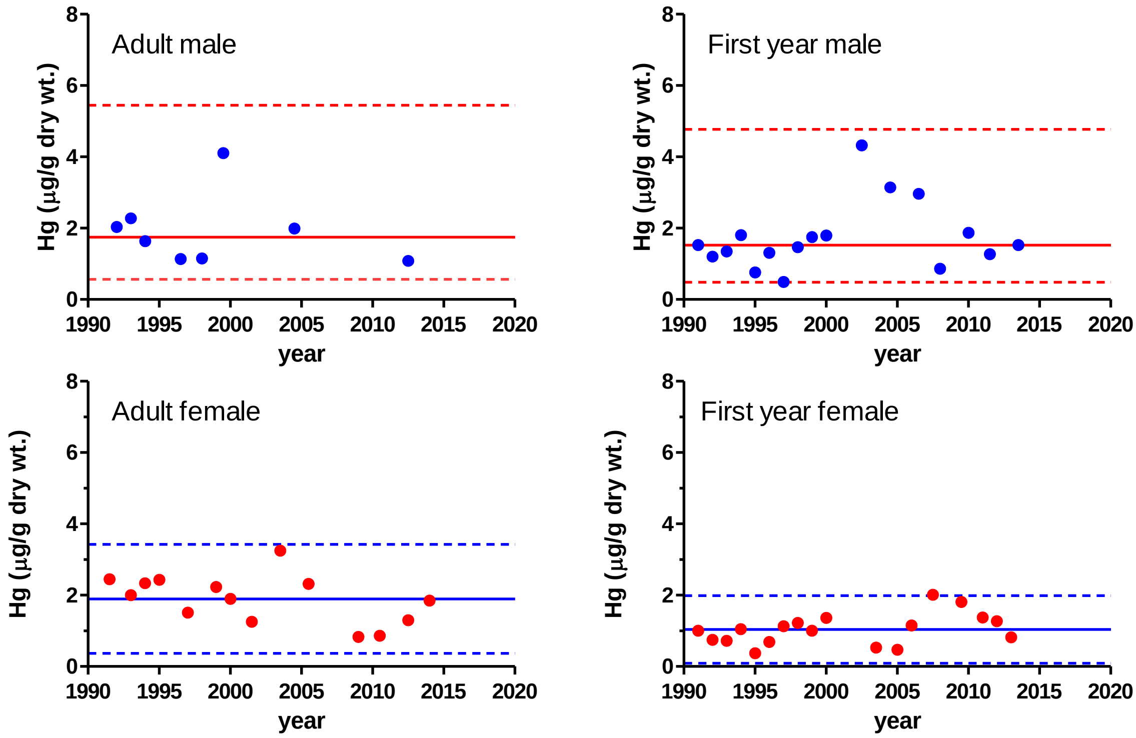

Figure 2. Annual median liver Hg concentrations in non-starved (fat scores ≥ 2) adult and first-year male and female sparrowhawks. Solid and dotted lines represent mean and 95% prediction intervals based on the annual medians. Values are geometric means and geometric prediction intervals for males (data for annual medians were log-normally distributed) and arithmetic means and precition intervals for females (data normally distributed). Each annual median value is derived from an annual sample size of ≥ 5 (range 5-24) birds.

5. Comparison of mercury concentration among tissue types in sparrowhawks.

Sparrowhawks have been used by the PBMS as a sentinel for pollution contamination in terrestrial habitats and data sets for a range of contaminants have been measured in this species. However, the sparrowhawk is one of the smaller resident raptors in the UK and the amount of liver available for multiple analyses is limited; the [arithmetic] mean (95% CI) liver weight is only 5.0 g (4.7-5.3 g) in females (n=138) and 3.3 g (3.1-3.5 g) in males (n=99). Mercury however accumulates in relatively high concentrations in the kidney (Shore *et al.*, 2011) and so it may be possible to monitor Hg residues in kidney instead of the liver which could be retained for other analyses. Furthermore, a key target organ for Hg is the brain but we have little information as to how Hg residues that we have monitored in the liver compare to concentrations in the brain.

Our aim in this study was to compare the Hg concentrations in liver, kidney and brain of a selection of sparrowhawks. We wanted to determine the extent to which concentrations were related to each other. We also wanted to examine if liver concentrations that we have already measured could be used to reconstruct Hg concentrations in other tissues such that those predicted concentrations might form a basis for long-term monitoring.

We measured total Hg concentrations in archived kidney and brain samples from 24 sparrowhawks; liver Hg concentrations had been previously determined in all the birds. Selection of sparrowhawks for inclusion in the study was on the basis of their known liver concentrations, the aim being to examine a set of birds that varied widely in their liver Hg concentrations and so were representative of the spectrum of likely environmental exposures. We also had ancillary information on the birds in terms of their age and sex class and year of death. Overall, the sample of 24 birds was comprised of two adult females, seven first year females, five adult males and 10 first-year males. Birds had died between 1975 and 2000. We had information on putative cause of death of all individuals and information on nutritional state at the time of death (assessed from fat score) for 17 of the birds. Eleven were in a starved state when they died (fat scores of 0 or 1) while 6 were not in a starved state (fat scores of 2-5). The remaining 7 birds died of causes other than starvation but may or may not have been in a starved state at the time of their death.

Arithmetic mean (and range) Hg concentrations in the liver, kidney and brain were 6.88 (0.10-19.3), 5.84 (0.53-16.2) and 2.63 (0.10- 6.37) µg/g dry weight respectively. All samples had Hg concentrations above the limit of detection.

There were significant linear relationships between Hg concentrations in all three organs. When data for all 24 birds were analysed, R^2 values in the regression models varied between 0.537 and 0.873 (Fig. 3, left hand graphs). These values were poorest when liver Hg concentration was the predictor. This is likely to have been due to inclusion in the analysis of liver Hg concentrations in starved birds. Starvation induces liver wastage and results in elevation in liver Hg concentration. Such wastage is less likely in kidney and particularly brain and so Hg concentrations in these two tissues is largely unaffected by starvation. Consequently, the overall effect of including data for starved birds in models where liver Hg concentration is the predictor (x variable) would be to depress the gradient of the regression line. Liver concentrations would therefore underestimate kidney and brain Hg concentrations. This effect was indeed evident when the regression models were re-analysed. This first involved excluding birds known to have been in a starved state (middle graphs in Fig. 3) and finally, the analysis was performed only using data from birds with a known fat score of ≥ 2 (right hand graphs, Fig. 3). The gradients of the regression models (where liver Hg was the predictor) progressively steepened and, despite the reduction in the number of replicates, R^2values increased such that they all exceeded 0.95 for analyses involving only birds with a fat score ≥ 2 (Fig. 3, right hand graphs).

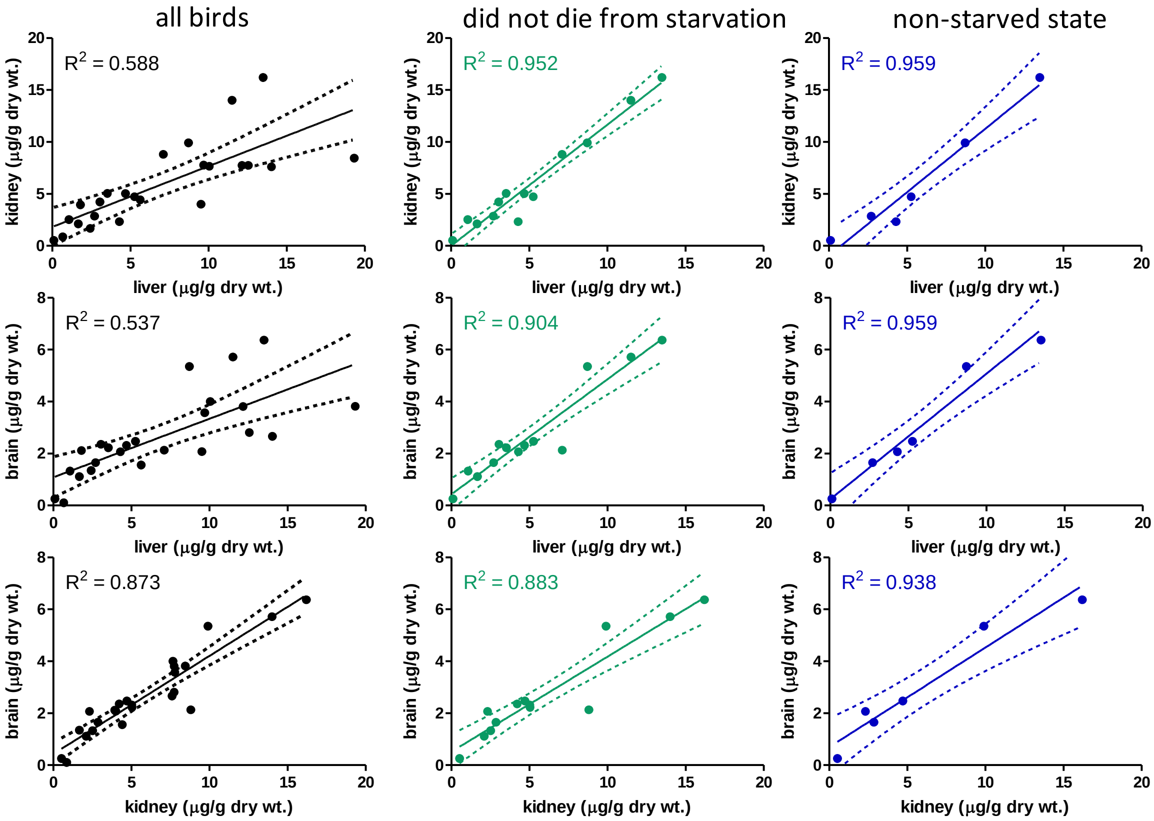

Figure 3. **Relationships between dry weight Hg concentrations in liver, brain and kidney of sparrowhawks.** Solid line indicate linear regression model with 95% confidence limits (dashed lines). Models are plotted for all 24 birds analysed (left hand graphs), birds with a fat score ≥ 2 or with an unknown fat score but known not to have died from starvation (middle graphs), or birds only with a fat score ≥ 2 (right hand graphs and Table 3).

These analyses suggest that, in sparrowhawks with fat scores ≥2, it is possible to use measurement of Hg concentrations in one organ (out of liver, kidney or brain) to predict the concentrations in others, but the accuracy of this prediction is likely to be poorer in starved birds. Given that Shewhart charts in Section 4 were constructed for non-starved (fat scores ≥ 2) birds only, it would be possible, on the basis of the relationships in Table 3, to reconstruct those charts such that they were relevant for kidney or brain Hg concentrations, although some limited further concurrent analysis of kidney and brain samples would be merited to increase the extent of replication and robustness of the regression model. Long-term monitoring of Hg trends in sparrowhawks could thus be conducted using analysis of brain or kidney, thereby enabling retention of liver tissue for other analyses.

Table 3. Results from linear regression analysis of Hg residues in liver, kidney and brain samples in non-starved (fat scores ≥ 2) sparrowhawks. Linear regression expressed as y = a + b*x.

Predictor (x axis)	Response (y axis)	a	b	R^2	P value
Liver	Kidney	-0.938	1.220	0.959	0.0006
Liver	Brain	0.245	0.483	0.959	0.0007
Kidney	Brain	0.691	0.388	0.938	0.0015

The indicative liver Hg residue values for effects on avian mortality and reproduction proposed by Shore et al. (2011) as protective for most species are approximately equivalent to 70 µg/g and 7 µg/g dry weight (se Section 4.1). Based on the equations in Table 3, these concentrations would correspond to 84 µg/g and 8 µg/g dry weight respectively for kidney Hg residues and 34 µg/g and 4 µg/g dry weight respectively for brain Hg residues. Monitoring of sparrowhawk kidneys or brains could therefore be related to potentially significant toxicological threshold values both for measurements in individuals and the annual median concentrations (See Section 4.2).

6. Comparison of liver mercury concentrations between kestrels and sparrowhawks.

The PBMS has widely used the sparrowhawk as a sentinel of exposure of biota in lowland terrestrial habitats and thereby examine spatial and temporal trends in exposure and bioaccumulation (Leslie *et al.*, 2011, Jagannath *et al.*, 2008, Walker *et al.*, 2014, Walker *et al.*, 2008, Crosse *et al.*, 2013, Crosse *et al.*, 2012, Walker *et al.*, 2015a). However, it can be argued that because sparrowhawks feed predominantly on birds which themselves are relatively mobile, exposure in sparrowhawks may not be as indicative of local exposure as other species, such as the kestrel, that feeds predominantly on small mammals which have relatively restricted home ranges.

The PBMS measured Hg concentrations in both sparrowhawks and kestrels in most years between 1980 and 1998 and these data provided a means to examine whether temporal rends in exposure in kestrels are indeed mirrored by sparrowhawks. We therefore compared liver Hg concentrations in sparrowhawks and kestrels for years in which measurements had been made on both species.

Our previous analyses (see sections 4 and 5) demonstrated that age, sex and nutritional state and can all affect liver Hg residues in sparrowhawks at least. We did not have information on the fat score for individuals in many of the years for which we had residue data for both sparrowhawks and kestrels. Therefore, we attempted to reduce spurious starvation-related variability by restricting our analyses to sparrowhawks and kestrels that had died from causes other than starvation; some individuals however would likely still have been in a starved state. We aimed to contrast temporal trends in liver Hg in the two species by comparing annual median Hg concentrations. The number of measurements in each year (upon which to calculate a median value) was relatively small for adult kestrels and sparrowhawks and so we further restricted our analysis to first-year birds, because within year sample sizes were larger. Overall, the number of sparrowhawks from which we could calculate annual median liver Hg concentrations ranged from 7 to 30 (mean of 16) per year for males and 11-36 (mean of 20) for females. These sample sizes allowed us to calculate annual median values for each sex separately and comparison of annual medians between males and females confirmed earlier analysis (section 4) that indicated liver Hg concentrations were higher in males than females (Wilcoxon matched pairs test, W = 130, P=0.009). Our data for kestrels was too sparse to calculate annual median concentrations for males and females separately. However, initial exploration of the raw data suggested that there was no significant difference in liver Hg residues between males and females ($F_{1,170} = 0.12$, P=0.73) and this was also true when annual median values for males and females were compared (Wilcoxon matched pairs test, W = 11, P=0.82). We therefore combined liver residue data for males and female kestrels and sample sizes on which annual median concentrations were based ranged from 6 to 19 (mean of 11).

There were statistically significant declines in annual median liver Hg concentrations in kestrels (sexes pooled; $F_{1,15}=8.45$, P=0.011) and in male and female sparrowhawks ($F_{1,16} \geq 6.58$; P<0.03 in both cases; Fig 4). The rate of decline in liver Hg did not differ between the three groups of birds ($F_{2,47} = 0.960$, P=0.39) but the elevations of the slopes were different ($F_{2,49} = 28.1$, P<0.0001), reflecting greater accumulation by male than female sparrowhawks and higher concentrations in sparrowhawks overall compared with kestrels. Annual median liver concentrations of male and female sparrowhawks and kestrels were all highly correlated with each other (Pearson r ≥ 0.555, P≤ 0.02).

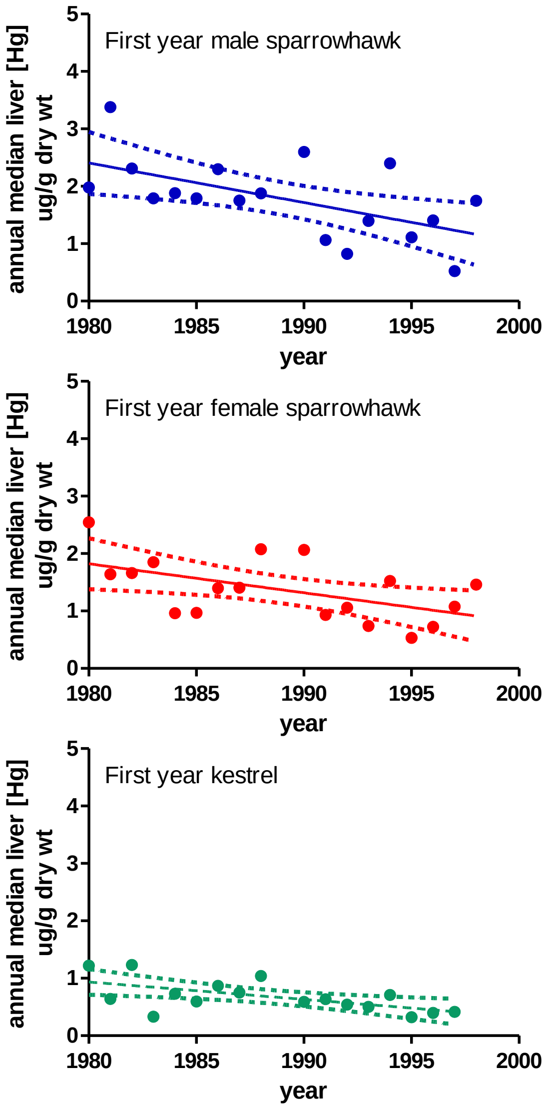

Figure 4. Annual median dry weight liver Hg concentrations in non-starved, first year male sparrowhawks (top graph), female sparrowhawks (middle graph) and kestrels (sexes pooled-bottom graph). Statistically significant linear regression models (with 95% Confidence Limits) are shown by solid and dashed lines respectively.

These results indicate that there was no difference between male or female sparrowhawks and kestrels in terms of indicating a significant decline over time in bioaccumulation of Hg in the liver. It is consistent with the concept that sparrowhawks are as likely to be as good an indicator as kestrels in terms of acting as broad sentinels of change in exposure to Hg in terrestrial habitats.

7. Mercury (Hg) concentrations and stable isotope signatures in golden eagle eggs.

In a previous study, we explored the potential of using golden eagle eggs as a sentinel for monitoring potential changes in Hg exposure in biota in the terrestrial upland habitats (Walker *et al.*, 2015b) This involved measuring total Hg concentrations in egg contents but a potential confounding factor was that golden eagles can feed on terrestrial and marine prey and so any changes in egg Hg over time could reflect changes in the extent to which birds feed on terrestrial or marine foodwebs rather than changes in Hg levels in terrestrial systems. A potential way to overcome this problem was to analyse the stable isotope (SI) signatures of the eggs to reveal predominant feeding pattern. Analysis of eggs laid between 2009 and 2013 indicated that the SI signatures could distinguish between what appeared to be predominantly terrestrial feeding and coastal feeding birds. However, we then found in our earlier study that the majority of the eggs with a terrestrial- feeding SI signature had Hg concentrations below the limit of detection. This limited their value as a sentinel for assessing change in Hg exposure in inland-feeding birds as any decrease in exposure [and associated concentrations in eggs] would not be measurable.

In the present study, we re-analysed the Hg concentrations in those eggs that previously had non-detectable concentrations but this time used a fluorescence technique that provided a 10-fold greater sensitivity compared with ICP-MS. We also measured the Hg concentrations and SI signatures in a further six eggs submitted to PBMS in 2014, thereby increasing the degree of replication in our study. The aim was to determine if it was possible to generate a baseline for Hg concentrations in inland feeding eagle eggs against which future change in environmental Hg exposures could be assessed.

Four of the six eggs submitted in 2014 had stable isotopic signatures that were consistent with those that we had previously identified (Walker *et al.*, 2015b) as likely being characteristic of terrestrial feeding by laying females, while the remaining two eggs had signatures consistent with coastal feeding females (Fig. 5).

The eggs that previously had concentrations below the Limit of Detection when analysed by ICP-MS and the newly submitted eggs all had detectable Hg concentrations when measured using fluorescence analysis. We used this larger and better characterised dataset to repeated the analysis reported by Walker et al. (2015) in which we compared Hg concentrations in golden eggs that had SI signatures indicative of either inland-feeding or coastal-feeding females. We additionally included a dataset on Hg concentrations in white-tailed sea eagle (*Haliaeetus albicilla*) eggs that we had measured previously (Fig 6), and hypothesised that Hg concentrations in sea eagle eggs would be similar to those in eggs from coastal nesting golden eagles and both would be higher than Hg concentrations in eggs laid by terrestrial-feeding birds.

Analysis by ANOVA of log-transformed Hg concentrations indicated significant between golden eagle eggs with differing SI signatures ($F_{(2,29)} = 22.3$, $P<0.001$). Mercury concentrations in eggs laid by coastal feeding GE eggs and white tailed sea eagles did not differ but were significantly higher in than levels in eggs laid by terrestrial feeding birds (Tukey's post-hoc test $P<0.05$; Fig. 6).

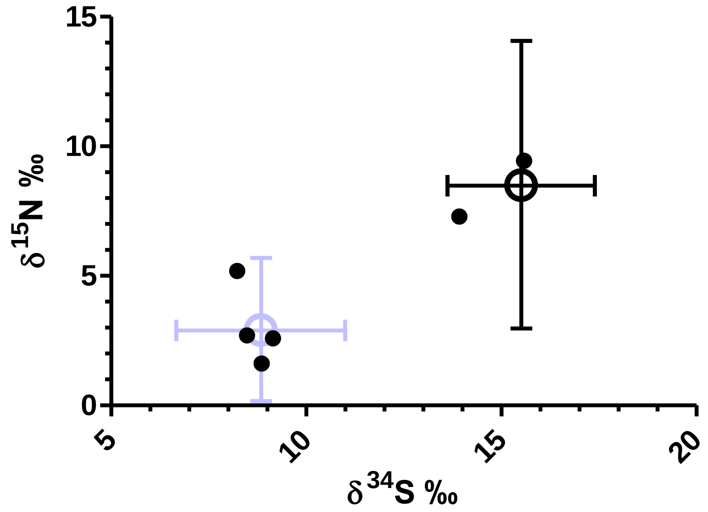

Figure 5. δ^{15}N vs δ^{34}S plots (filled circles) of GE eggs received in 2014. The mean ± 3SDs for δ^{15}N and δ^{34}S signatures in GE eggs from coastal feeding (black circle and confidence bars) and inland feeding birds (grey circle and confidence bars) as defined previously (Walker *et al.*, 2015b) are shown.

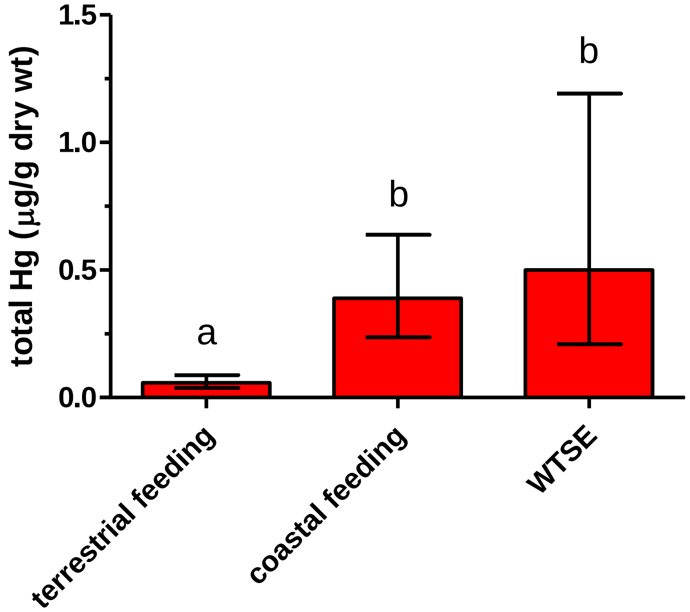

Figure 6. Geometric mean (and 95% confidence interval) total Hg dry weight concentrations in: GE eggs associated with SI signal inferred as indicative of terrestrial [upland] feeding in laying females (n = 11 eggs); (ii) GE eggs associated with SI signal inferred as indicative of coastal feeding (n = 15 eggs); WTSE eggs (n=6). Groups with different letters have significantly different geometric mean Hg concentrations Tukey post-hoc test, P<0.05).

The number of years for which we had data (2009-2014) were limited but there was no obvious trend over time. Although sample numbers per year were also limited, it was possible to generate a Shewhart chart for Hg concentrations in terrestrial feeding golden eagles based on the annual geometric mean Hg concentrations (Fig. 7),and define associated 95% prediction intervals. This chart provides a "baseline" against which the significance of future annual changes in egg Hg concentrations can be evaluated and thereby provides a means a sentinel by which changes in Hg bioaccumulation in northern upland areas of Britain can be tracked.

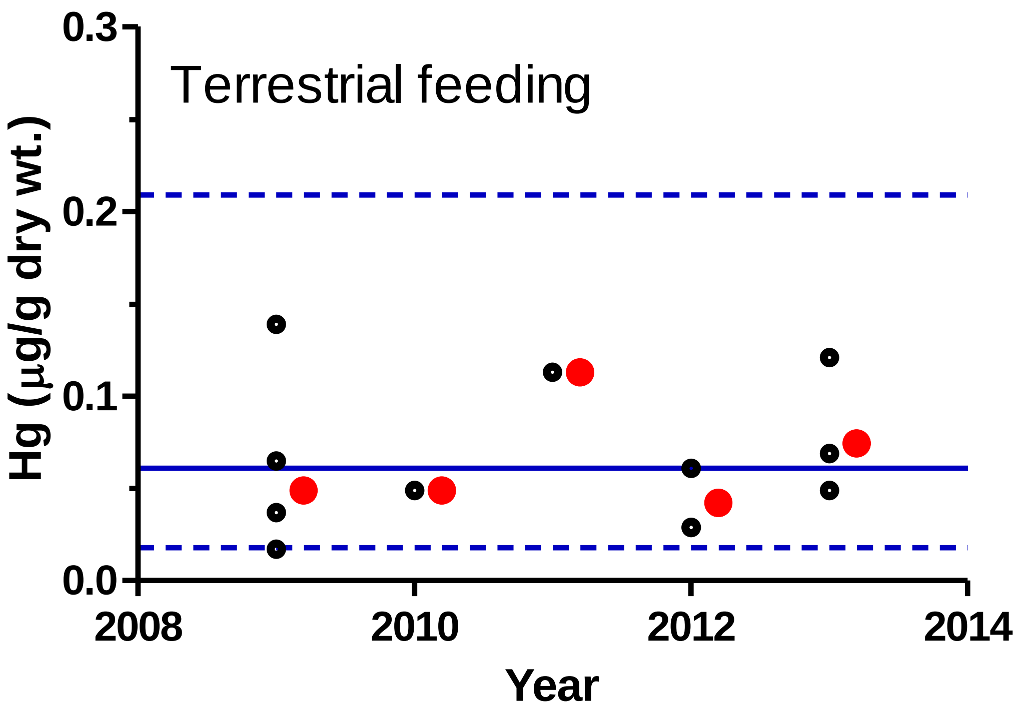

Figure 7. Control Hg charts showing Hg dry weight concentrations (µg/g dry wt.) in eggs laid by terrestrial feeding golden eagles. Chart shows concentrations in individual eggs (black dots), annual geometric mean concentrations (red dots), and overall mean (solid blue line) and 95% prediction intervals (blue dashed lines) derived from the annual geometric mean concentrations.

8. Conclusions

Liver Hg concentrations were relatively low in birds that died in 2013 and 2014. Age and sex were significant factors in determining the magnitude of liver residues. None of the birds that died in 2013 and 2014 had liver Hg residues higher than that proposed to be generally indicative of mortality. Three birds had liver Hg concentrations above the concentration that may be generally indicative of potential impacts on reproduction.

Our analysis of data for liver Hg concentrations in sparrowhawks over the last 25 years (1990-2014), investigation of Hg concentrations in different tissues in sparrowhawks, and comparison of historic (1980-1989) trends in liver Hg concentrations in sparrowhawks and kestrels have demonstrated that:

(i) although there has been significant between year variation in liver Hg concentrations in sparrowhawks over the last 25 years (1990-2014), there has been no consistent upward or downward trend.

(ii) using the 1990-2014 data, it is possible to define “baseline” liver Hg concentrations for sparrowhawks against which future years with unusually high or low annual concentrations, and consistent time trends, can be objectively and rapidly assessed.

(iii) there are close relationships between total Hg concentrations in the liver, kidney and brain and so it is possible to transfer long-term monitoring of Hg in sparrowhawks (including retrospective estimation of baseline concentrations and associated Shewhart charts) so that it is based on either kidney or brain concentrations. Such a switch would preserve liver samples for other analyses.

(iv) there was close correspondence in declines in liver Hg between sparrowhawks and kestrels for the period 1980-1998. These data suggest that, although sparrowhawk predominantly feed on more mobile prey than kestrels, they as likely as kestrels to be representative of changes in environmental exposure to, and subsequent bioaccumulation of, Hg.

Finally, the conclusion of our studies on Hg concentrations in golden eagle eggs have demonstrated that combined analyses using SI signatures and sensitive fluorescent Hg analysis can be used to generate control charts for Hg concentrations in eggs laid by females that are likely to be predominantly feeding on terrestrial prey. These charts can be used to rapidly identify changes in exposure and associated bioaccumulation of Hg into eggs and act as a sentinel of change in Hg bioavailability in upland habitats in northern Britain.

Overall, adoption of similar approaches when analysing PBMS data on Hg concentrations in otters tissues and gannet eggs can provide integrated assessment of future changes in bioaccumulation in sentinel top predators in lowland and upland terrestrial systems, freshwater and marine systems. Such increases or decreases are one evidence strand to assess long term changes in environmental Hg exposure and the possible effectiveness of the Minamata Convention.

9. Acknowledgements

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