Predatory Bird Monitoring Scheme
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Lead (Pb) and Mercury (Hg) concentrations in predatory bird livers 2012: a Predatory Bird Monitoring Scheme (PBMS) report

L.A. Walker, J.S. Chaplow, A.J. Lawlor, M.G. Pereira, E.D. Potter, A. W. Sainsbury[*], & R.F. Shore[1]

Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK
[*]Institute of Zoology, Zoological Society of London, Regents Park, London NW1 4RY, UK

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[1]Corresponding author: RF Shore, Centre for Ecology and Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK. E-mail: rfs@ceh.ac.uk. Website: http://www.ceh.ac.uk/

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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 contaminant monitoring and surveillance work. By monitoring sentinel avian predators, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife.

The present study summarizes the results of monitoring to quantify the scale of exposure to [and associated risk from] two toxic metals, lead (Pb) and mercury (Hg) in predatory birds.

A major concern over Pb is that birds of prey may be exposed to Pb shot and ammunition fragments when they prey on or scavenge game animals. The aim of the current Pb monitoring was to quantify the extent of exposure [assessed from liver residues] in four predatory bird species, the barn owl (*Tyto alba*), common buzzard *(Buteo buteo*), red kite (*Mivus milvus*) and sparrowhawk (*Accipiter nisus*). The red kite and buzzard are scavengers and, as such, are particularly at risk from consumption of ammunition and shot in unretrieved game. In contrast, barn owls and sparrowhawks predominantly take prey [live small mammals and passerine birds, respectively] that are unlikely or less likely to have been shot. Pb levels in barn owls and sparrowhawks may therefore give an indication of "background" Pb contamination in birds of prey against which to judge any elevated levels in red kites and buzzards.

Mercury is a neurotoxin and there has been global concern over its impact of humans and wildlife. It has been predicted that global Hg emissions may rise 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 means of assessing this is to monitor exposure of sentinel wildlife species. We report Hg residues in birds of prey with the aim of providing baseline data against which to assess future change in Hg environmental concentrations and associated wildlife exposure. We did this in two ways: (i) by examining liver Hg concentrations in those species that we analysed for Pb, thereby providing a current measure of accumulation in different species, and (ii) by combining new liver Hg data for sparrowhawk with those obtained in previous years to determine current trends in exposure and assess key factors that may influence accumulation in this sentinel species.

Median liver Pb concentrations in the four species studied were between 0.03 and 0.6 µg/g dry wt., lower than levels associated with clinical and sub-clinical adverse effects in Falconiformes. Barn owls and sparrowhawks generally had lower Pb concentration than those measured in buzzards and red kites. Female sparrowhawks had higher liver Pb concentrations than males, but there were no differences between males and females for the other species.

Median liver total Hg concentrations ranged between 0.22 and 1.3 µg/g dry wt. in the four species studied and all birds had residues below those suggested to be potentially indicative of mortality or effects on reproduction. Red kites had lower Hg residues than sparrowhawks and buzzards and barn owls had lower residues than sparrowhawks. In sparrowhawks, Hg residues were higher in adults than juveniles and in starved than non-starved birds. Between 1990 and 2012, there was no significant temporal change in liver total Hg concentrations in sparrowhawks and 9.4% of female sparrowhawks had liver Hg concentrations greater than those potentially associated with effects on reproduction.

1. 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 the scientific evidence needed to determine 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 decisions about sustainable use of chemicals (for example, the *EU Directive on the Sustainable Use of Pesticides*, *Biocides Directive 98/8EC, UNEP Convention on migratory species: Minimizing the risk of poisoning to migratory birds; UNEP/CMS 10.26)*. In addition, the outcomes from the monitoring are used to assess 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 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 Lead (Pb) and mercury (Hg) in predatory birds

Lead (Pb) is a highly toxic metal that is a non-specific poison that affects all body systems and has no known biological requirement (Franson and Pain, 2011). The major sources of Pb in the environment arise from lead mining, the refining and smelting of Pb and other metals, and the manufacture and use of alkyl lead fuel additives (WHO, 1995), although the use of these fuel additives has been banned in the European Union since 2000[2] with production of tetra ethyl lead for use outside Europe continuing within the U.K.. Natural releases of Pb into the environment arise from volcanic emissions, geochemical weathering and emissions from sea spray. The consequence of anthropogenic and natural releases is that Pb is ubiquitous in air, water and soil.

The main route of exposure to lead in vertebrate wildlife is thought to be through inhalation and ingestion. Once Pb is absorbed into the blood stream, it is distributed among body tissues, primarily the blood, liver, kidney and bone (Franson and Pain, 2011). Exposure to even low levels of Pb can result in some measurable physiological effects, including inhibition of delta-amino levulinic acid dehydratase (ALAD) activity (WHO, 1989). Exposure to higher concentrations can adversely affect the central nervous system, peripheral nerves, kidney, haematopoietic system, reproduction, juvenile growth and, ultimately, survival.

The concentrations of Pb in the wider environment are not generally thought to directly affect the survival of most wildlife (Franson and Pain, 2011). However, significant numbers of wild birds may be exposed to large amounts of lead through the ingestion of spent lead from ammunition (shotgun pellets and bullets). Ingestion of fragmented lead ammunition is also a cause of dietary exposure and mortality in scavenging birds (Pain *et al.*, 2009, Green *et al.*, 2009). Ingestion can occur in birds that feed in hunting areas when they mistake lead shot for grit or food particles. Raptors, owls and scavenging birds can ingest Pb from ammunition and shot lodged in the carcasses of wildlife that have been killed but remain unretrieved or that are shot but survive. They may also ingest Pb residues that have been accumulated in the soft tissues of their prey. All predatory and scavenging birds that feed on game species are likely to be exposed to shot, especially if they feed in areas where game species are hunted (Pain *et al.*, 1995).

The risk of Pb ingestion by waterfowl is well recognized and has been mitigated against by banning shooting with Pb shot over water bodies. However, there is currently no restriction on the use of Pb shot and ammunition in terrestrial systems, and this has led to concerns over the potential risks of ingesting Pb for both humans and wildlife (Pain *et al.*, 2009, Pain *et al.*, 2007,

[2] the only exception is aviation fuel for piston engine-powered aircraft

Pain *et al.*, 2010, Pain *et al.*, 2005, Pain *et al.*, 1995).

1.3 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. 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 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 Minimata 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.

1.4 Aims of the current study

As far as we are aware, there has not been any recent measurement of monitoring of Pb and Hg concentrations in predatory birds in Britain.

We conducted a monitoring exercise for Pb in selected birds of prey over three years and combined these analyses with some additional data collected by the PBMS in 2008 and 2009. The aim was to quantify the extent of exposure to lead [as assessed from liver residues] in four predatory bird species, the barn owl (*Tyto alba*), the common buzzard (*Buteo buteo*), red kite (*Mivus milvus*) and the sparrowhawk (*Accipiter nisus*). The red kite and common buzzard are scavengers and, as such, are particularly at risk from consumption of Pb ammunition and shot in unretrieved game. Barn owls and sparrowhawks feed predominantly upon prey [live small mammals and passerine birds, respectively] that are unlikely to be shot in the UK. These two species are likely to be suitable environmental sentinels of any changes in diffuse Pb contamination and act as a “background controls” against which spatial and temporal variation in Pb concentrations in red kites and buzzards can be assessed, although some exposure to lead ammunition may occur as sparrowhawks occasionally scavenge for food or prey upon wood pigeons *Columba palumbus* that have been shot. If exposure to lead shot and ammunition in scavengers occurs to a significant degree in the UK, we would predict that Pb residues in buzzards and red kites will be greater than in barn owls [and to a lesser extent sparrowhawks].

We also conducted analysis of Hg residues in birds of prey, the aim of which was to provide baseline data against which to assess future change in Hg environmental concentrations and associated wildlife exposure. This was done in two ways. First, we examined liver Hg concentrations in those species that we analysed for Pb, as Hg and Pb data were collected simultaneously by the same method. Second, we combined the data for sparrowhawks with those obtained by the PBMS for the same species in previous years to determine current trends in exposure and assess key factors that may influence accumulation in this sentinel species.

2. Methods

Between 2008 and 2012 dead barn owl, sparrowhawk, buzzard and red kite carcasses were collected as part of the Predatory Bird Monitoring Scheme and the Disease Risk Analysis and Health Surveillance Programme operated by the Centre for Ecology & Hydrology and the Institute of Zoology, respectively. Collaboration between these two schemes is fostered through the WILDCOMS network (www.wildcoms.org.uk**).** Both schemes rely on citizen science in that members of the public submit to the scheme dead birds that they find. A post-mortem examination is undertaken on all carcasses and, as part of this, the age class and sex of each bird was determined. Various tissue samples, including liver, were excised and stored at -20ºC prior to analysis. For the purposes of this study, juvenile birds are classed as individuals that hatched in the current or previous year to that in which they were found dead.

A total of 30 barn owls, 87 sparrowhawk, 60 buzzards and 38 red kite livers were analyzed for liver total Pb and Hg concentrations (Table 2.1). The birds had died from a variety of causes. 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 then made up to an initial digest volume of 25ml using ultrapure water (Millipore, MilliQ). Samples were further diluted ten-fold using ultrapure water immediately prior to analysis by inductively couple plasma mass spectrometry (ICPMS) using a Perkin Elmer DRCII ICPMS. Lead concentrations were measured using the ICPMS 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 of the analysed sample and the moisture content of the sub-sample.

The instrumental limit of detection (LoD) for Pb (0.06 µg/L) and 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 0.05 µg/g dry weight (dry wt.) and 0.09 µg/g dry wt. For Pb and Hg, respectively. 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 lead from samples of Tort 2 and Dolt 4 were 88.7% and 95.4%, respectively, while the mean recoveries for total mercury were 109.6% and 106.4%, respectively.

The results from this study was combined with previously reported data (Walker *et al.*, 2011) for long-term trend analysis of mercury concentrations sparrowhawks that died between 1990 and 2012.

Lead concentrations in the liver were not normally distributed. As a result, data for sparrowhawks, buzzards and red kites were Johnson-transformed prior to general linear model analysis. Data transformation was unsuccessful in normalising the liver Pb residues for barn owls and these data was analysed untransformed by non-parametric Kruskall-Wallis test with birds divided into separate age/sex classes (adult male, adult female, juvenile male, juvenile female). Inter-species comparisons were investigated using the Kruskall-Wallis test.

Liver mercury concentrations for all four species were Johnson-transformed prior to general linear model analysis.

Unless otherwise stated, concentration are presented here as µg/g dry wt. 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.).

Table 2.1 Summary of livers analysed from each year in which they were found for barn owl, buzzard, red kite and sparrowhawk.

	Year collected					
Species	2008	2009	2010	2011	2012	Total
barn owl	-	-	10	10	10	30
buzzard	10	20	10	10	10	60
red kite	-	-	22	13	3	38
sparrowhawk	-	-	31	30	26	87

3. Results and Discussion

3.1 Total lead concentrations in livers of predatory birds

Birds with no history of lead poisoning usually have liver Pb concentrations of <2 µg/g wet weight and frequently of <1 µg /g wet weight (wet wt.) (Franson and Pain, 2011). These "background" values are equivalent to dry wt. Pb concentrations of <7.1 and <3.6 µg /g dry wt. respectively in the predatory bird livers that were analyzed.

The median liver Pb concentrations in the four species studied were between 0.03 and 0.6 µg/g dry wt. (Figure 3.1).

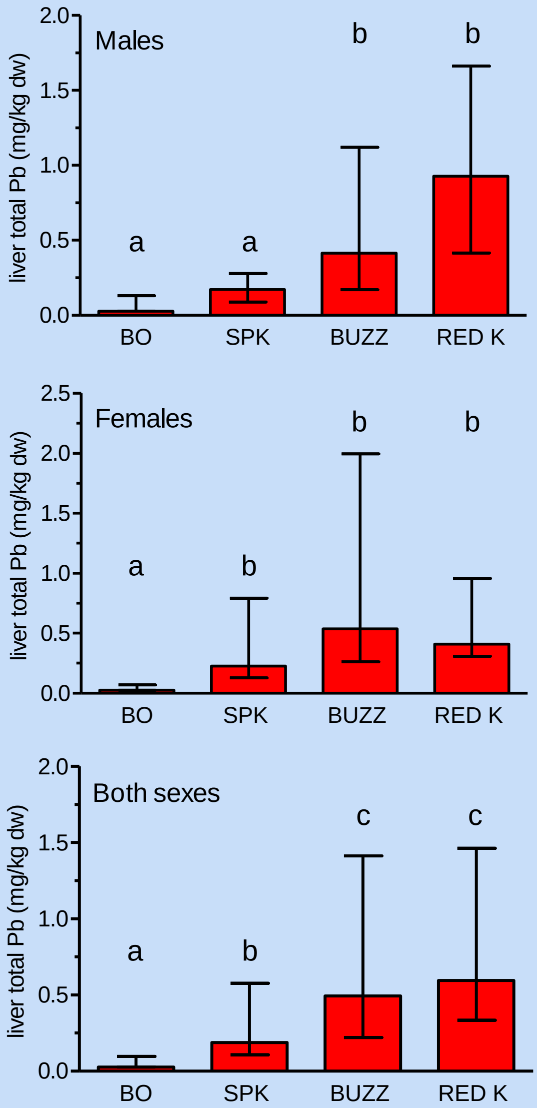

Figure 3.1. Median (± Inter Quartile range) liver lead concentrations in barn owl (BO), sparrowhawks (SPK) buzzard (BUZZ) and red kite (REDK). Within each graph, significant (P<0.05) differences between species are indicated by different letters. Sample sizes (male: female: sexes combined) were 14:14:30 (BO), 37:46:87 (SPK), 33:25:60 (BUZZ) and 18:14:38 (REDK); information on sex was unavailable for some individuals.

Only four birds from any year had a liver residue greater than "background" concentrations. These were adult female sparrowhawk and three juvenile male buzzards. The maximum residue measured was 13.2 µg/g dry wt. (equivalent to 3.79 µg/g wet wt.) in one of the buzzards. The sparrowhawk and two of the buzzards were found with injuries consistent with a collision while the third buzzard was in a starved condition. For Falconiforms, sub-clinical poisoning has been reported in birds with liver concentrations of between 2 and 6 µg/g wet wt., although the associated physiological effects are insufficient to severely impair normal biological functioning and do not result in external signs of poisoning (Custer *et al.*, 1984, Henny *et al.*, 1991, Kramer and Redig, 1997).

Age, sex, body condition and year of death were included as factors in the General Linear Models that were run to assess differences in liver Pb for each of buzzard, red kites and sparrowhawks. Female sparrowhawks had significantly higher liver Pb concentrations than males ($F_{1,75}$=9.20, P=0.003) but there was no effect of sex in the other two species and no significant effect of age, body condition and year in all three species. In barn owls, there was no significant difference between different age/sex groups when tested using a Kruskall-Wallis test (KW=0.499, P=0.91). Because residues varied with sex in sparrowhawks, inter-species comparisons of liver Pb were conducted for males and females separately, as well as for both sexes combined. However, differences between species were broadly similar in all three analyses, with barn owls and sparrowhawks generally having lower median Pb concentrations compared to buzzards and red kites (Figure 3.1).

Liver Pb concentrations have been measured in red kites from the United Kingdom previously. The median (inter-quartile range) liver Pb concentration in 20 red kites that had died between 1994 and 1999 was found to be 0.99 (<1.13-2.24) µg /g dry wt. (Shore *et al.*, 2000), similar to the levels found in the current study. However, another study of 44 birds that had been found dead between 1995 and 2003 found that seven of 44 red kites had elevated (>6 µg/g dry wt.) liver Pb concentrations, and six (14% of total sample) had concentrations greater than 15 µg/g dry wt., compatible with fatal Pb poisoning (Pain *et al.*, 2007).

The Pb concentrations detected in the present sample of sparrowhawks, are similar to those reported for birds that died between 1982 and 1992 (median: 0.55 µg/g dry wt., maximum 12.3 µg/g dry wt; (Pain *et al.*, 1995)), suggesting that there has been little change in Pb contamination in sparrowhawks over the last 20 years. Similarly the Pb concentrations in buzzards from this study are similar to those reported by Pain et al. (1995). This contrasts with declines of over 70% in bone Pb concentrations in a freshwater vertebrate sentinel, the otter *Lutra lutra* over a similar time period (Chadwick *et al.*, 2011). The differences in temporal trends in sparrowhawks and buzzard compared to otters may reflect exposure to different sources of lead and differences between habitats in the extent to which Pb is distributed and accumulated.

3.2 Total mercury concentrations in livers of predatory birds

The concentrations of Hg in the livers of the four species studied were generally low. The proposed indicative liver Hg concentrations 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 the species reported in the present study. All the birds from this current study that died between 2008 and 2012 had liver concentrations below those associated with either mortality or effects on reproduction.

Following Johnson transformation of the Hg liver residues general linear model analysis indicated that there were significant difference among species ($F_{3,182}$=15.39, P<0.001). Neither age or sex were significant factors determining liver Hg residues ($F_{1,182}$≤1.87, P≥0.173) and there were no significant interaction terms within the model. Red kites had the lowest average liver Hg concentration while sparrowhawks had the highest (Figure 3.2).

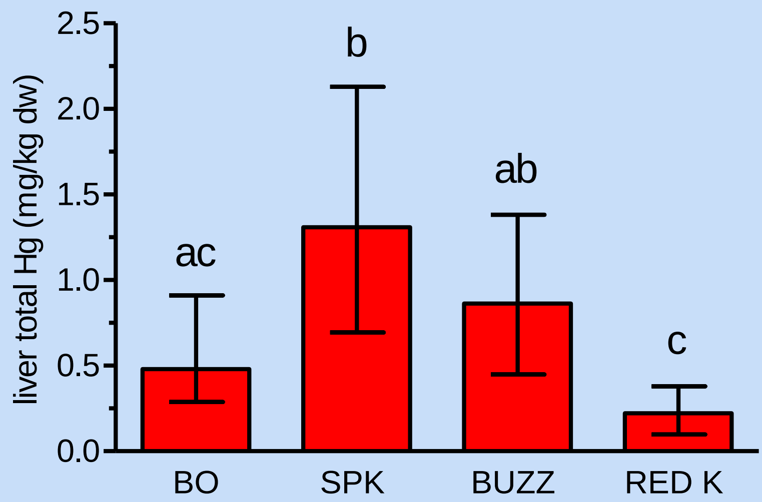

Figure 3.2. Median (± Inter Quatrile range) liver mercury concentrations in barn owl (BO), sparrowhawk (SPK) buzzard (BUZZ) and red kite (RED K). Significant (P<0.05) differences between species are indicated by different letters.Sample sizes were 30 (BO), 87 (SPK), 60 (BUZZ) and 38 (RED K).

3.3 Long- term trend analysis of total mercury concentrations in livers of predatory birds

The liver mercury concentrations in sparrowhawk livers described above were combined with those from a previous PBMS report (Walker *et al.*, 2011). The Walker et al. (2011) report demonstrated that there had been a significant decline in average Hg residues between the 1970s and 1990 (data not shown), but there had been little change in liver total Hg residues since then. Here, we analysed liver total Hg concentrations in sparrowhawks between 1990 and 2012 (Figure 3.3) and there was no evidence of a significant change over time

($F_{1,1163}$=1.20, P=0.274). However, liver Hg concentrations were significantly higher in adults than juveniles ($F_{1,1163}$=25.9, P<0.001) and in starved than non-starved birds ($F_{1,1163}$=80.7, P<0.001). Mercury residues also tended to be a higher in males than females although this difference were not statistically significantly ($F_{1,1163}$=2.97, P=0.087).

For the period 1990 to 2012, none of the sparrowhawks livers analyzed exceeded liver Hg concentrations mercury associated with mortality. However, 9.4% of female birds had residues higher than those associated with effects on reproduction (Shore *et al.*, 2011).

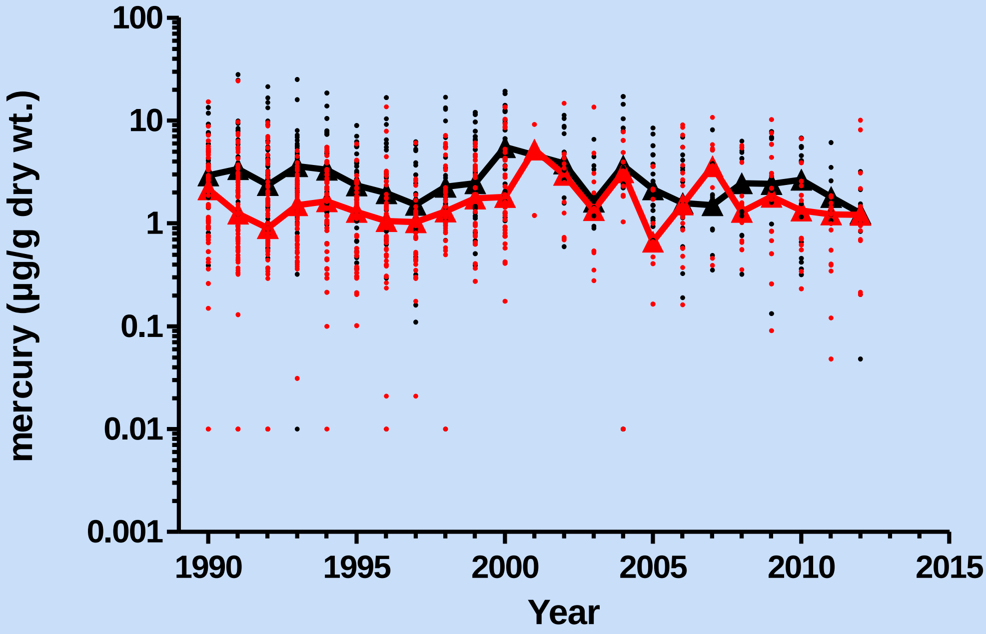

Figure 3.3 Long-term trends in liver Hg concentrations in starved (black symbols) and non-starved (red symbols) Eurasian sparrowhawk (Accipiter nisus). Dots are individual liver concentrations, and the triangles are annual median values with a connecting line.

4. Conclusions

Analysis of liver Pb concentrations demonstrated that buzzards and red kites generally had higher Pb concentrations than barn owls sparrowhawks. This was consistent with the concept that these species are at greater risk from exposure to Pb, likely because of exposure to shot and ammunition in game animals. Although median concentrations in red kites and buzzards were approximately 3 and 20 fold higher than sparrowhawks and barn owls respectively, all the birds examined in this study had liver Pb concentrations below those thought to cause clinical and sub-clinical adverse effects in Falconiforme species.

The data on Hg residues provided in this report provides a baseline set of data against which to evaluate future changes in environmental concentrations and/or bioavailability of Hg. The largest dataset available is for sparrowhawks and they generally had higher liver Hg concentrations than the other species examined in the present study. We did not detect any significant change in liver Hg concentrations in sparrowhawks between 1990 and 2012 and, during this period, 9.4% of female sparrowhawks had liver Hg concentrations in excess of concentrations suggested as indicative of potential adverse effects on reproduction in birds generally.

Body condition, age, and possibly sex, are all factors that influence the magnitude of liver total Hg residues and need to be taken into account if sparrowhawks are to be used as sentinels for detecting change in Hg bioaccumulation at a national scale.

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