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## Short Note

## Invisible threats to native mammals — mercury levels in three Eurasian red squirrel populations

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## Abstract

Understanding how biodiversity, ecosystem function and species conservation are affected by factors such as climate or land use change, disease and pollution is an important aspect of species conservation and a key focus of the field of Conservation Medicine. Mammals such as squirrels can be valuable biological sentinels for environmental pollution and the intention of this pilot project was to test a new, rapid technique that allows direct detection of mercury (Hg) in hair samples. Our aim was to establish, for the first time, if red squirrels in Europe show any indication of mercury pollution; and to compare levels from red squirrels in two rural UK (Arran and Brownsea Island) and one Polish city population (Warsaw). The latter is exposed to higher levels of Hg from coal-fired power plants. Total mercury levels ranged from 11.1 to 801.95  $\mu\text{g}/\text{kg}$ . Contrary to our expectations, females from the Island of Arran had significantly higher Hg values than either males or females from the two other sites. Although the Isle of Arran was the only site where the difference between females and males was significant, our findings for both Poland and the UK suggest that mercury does not only accumulate in marine food chains and the arctic ecosystem, but is present in urban ecosystems and terrestrial woodlands. Data on trends to determine if Hg values are accumulating in arboreal mammals are completely lacking, and point to a need for a monitoring strategy of mercury levels and associated, potential health impacts in endangered species.

Mercury (Hg) is an extraordinary heavy metal. It is the only one that is liquid at ambient temperature and its compounds are highly toxic and cause disease in both humans and other animals (Syversen and Kaur, 2012). Mercury is used in several industrial processes from gold production, to seed treatment, dental fillings and excipients for drugs. It (Hg) is also an undesired by-product of several industrial processes such as the burning of fossil fuels, metallurgical processes, gold and cement production. As a result, Hg is found in water bodies, including drinking water, as well as in the air we breathe, and the food we and other animals consume (Wren, 1986; Day et al., 2007; Poissant et al., 2008). Once released into the environment by natural or anthropogenic processes, Hg changes form (e.g. to methylmercury  $\text{CH}_3\text{HG}^+$ , a highly toxic form of mercury), cycles between environmental reservoirs and concentrates in the food chain (Boening, 2000; Selin, 2009). For humans, there are indications that methylmercury in low doses can affect cognition. Furthermore, prenatal exposure with higher doses can interfere with brain development (Carpenter, 2001). Another aspect of mercury toxicity is the damage it can cause to mitochondria (Wu et al., 2016). In wild animals reported mercury impacts, for example in birds, are associated with effects on the immune system, kidney and cardiovascular function, as well as behavioural changes (e.g. Boening, 2000; see also Day et al., 2007). Similarly, mercury is reported to affect renal function in rats, as well as female caribou (*Ranger tarandus groenlandicus*) body weight and thus adversely affect body condition (Akgül et al., 2016; Gamberg et al., 2016). Mercury is also associated with or-

gan lesions and may act as a co-factor causing additive effects as part of a pollution cocktail in infections (e.g. Sonne et al., 2007). Geological and biological chemical timescales in Hg cycles are quite long, and despite international commitments to reduce mercury pollution since the 1970s (Selin, 2009), significant emissions continue. For example, a recent parliamentary report in Germany (Tebert, 2015) highlighted emissions by coal power plants in Europe with highest levels being emitted in Poland, Greece and Germany. There is an urgent need for monitoring and information on how mercury not only affects ecosystems and their function, but also interacts with diseases and detrimentally affects mammal and bird population dynamics. Mammals such as squirrels can be valuable biological sentinels for environmental pollution (Jenkins et al., 1980; Wren, 1986; Gersternberger et al., 2006) and the intention of this pilot project was to (i) test a new, rapid technique that allows direct detection of mercury without the need for chemical digestion in hair samples; (ii) to establish if red squirrels (*Sciurus vulgaris*) in Europe show any indication of mercury pollution; and (iii) to compare potential mercury pollution levels in an urban Polish population (Warsaw, with several nearby coal power stations) with a rural Scottish population (Arran, an island west of Glasgow, Scotland) whose main economy relies on tourism, farming and forestry, and a rural English population (Brownsea, an island), which contains a nature reserve and relies on tourism.

The samples were collected from the coat of the animals, either from the body or tail or from both, using gloved fingers and placed in small, individually-labelled, clear plastic bags. On the Scottish Island of Arran (55°34'36" N, 5°9'4" W) samples were collected as part of an opportunistic health surveillance project in which volunteers collect red

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squirrels that are killed on the island's roads (samples from spring and summer 2016). On Brownsea Island (50°41'30" N, 1°58'42" W), a small wooded island in Poole Harbour, Southern England with a remnant red squirrel population, hair samples were collected during a wildlife disease-screening project in September 2016. Animals were live-trapped under licence (Natural England Ref: 2016-24517-SCI-SCI) using 21 single-catch cage traps baited with peanuts, anaesthetised, screened and released. Two of the 20 squirrels caught on Arran were young squirrels and 4 of the 26 squirrels on Brownsea. Samples from Poland were collected from live-trapped animals, caught in three locations in Warsaw, Poland's capital and largest city, with almost 2 million inhabitants. Hair was plucked from the back of the squirrel using gloved fingers and placed in a plastic container (Eppendorf) and stored. Samples were collected throughout the year (2012–2013: spring – 8, summer – 5, autumn – 4, winter – 1 samples). Five of the animals trapped were young, all others were adults. The first location was Łazienki Park (52°12'51.06" N, 21°1'58.27" E), placed approx. 2 km from the city centre (9 samples collected there). Łazienki Park covers approx. 76 ha and was founded in the 17<sup>th</sup> century, and is among the biggest and oldest parks in Warsaw. Skarpa Ursynowska Reserve (4 samples collected) (52°9'56.04" N, 21°3'2.30" E) is located approx. 7 km from the city centre. It encompasses 33 ha and protects the scarp on the edge of the Vistula valley. The reserve includes a multi-species stand of broadleaved woodland (30–40% of the protected area), meadows and ruderal plant associations. The scarp runs to the south of the city and connects Skarpa Ursynowska Reserve with the third location: Natolin Forest Reserve (5 samples collected) (52°8'32.30" N, 21°4'43.83" E). It is located approx. 10 km from the city centre. It covers 105 ha of the land that was formerly an area of parkland and thanks to a spontaneous regeneration of woodland is now covered with stands of oak more than 250 years old. The three sampling sites were 3–4 km distant from a power plant, the biggest in Poland and the second biggest in Europe, Siewerki Power Station. The other big power plant (Żerań Power Station) was located 9 to 17 km from the study sites. Poland has one of the biggest anthropogenic mercury emissions in Europe. The main source is coal-combustion in coal-fired power plants (Głodek and Pacyna, 2009). Both main power plants, as do the other smaller ones both in Warsaw and in its neighbourhood, operate mainly on black coal and power plants located in Warsaw and its vicinity are characterised by a relatively low emissions of mercury (as compared to others located in other parts of the country that use mainly brown coal; Zysk et al., 2011). However, geochemical studies of soils in Warsaw parks (including Łazienki Park, one of the sampling areas in this study) showed soil enrichment in mercury (as well as other heavy metals; Dusza-Dobek, 2012).

In order to detect mercury, we adapted the approach detailed by Rüdél et al. (2011) (see also Jiménez and Castano, 2012). For the analytic measurements a direct mercury analyser equipped with tri-cell cuvette (DMA-80 III) was used, manufactured by Mikrowellen-Technologie (MWT) in Heerbrugg, Switzerland. The unique optical path spectrophotometer within this analyser achieves a detection limit of 0.0015 ng Hg and can detect contents up to 1500 ng reliably. With this approach, the samples need no preparation such as digestion or extraction. It is possible to detect Hg in solid, liquid or gaseous samples. With an attached analytical balance the hair samples were weighed in quartz boats and placed on the autosampler, which can be loaded with up to 40 boats in parallel. Samples are dried and thermally decomposed in a furnace under an oxygen atmosphere. Mercury and other combustion products are released from the sample and pass over the catalyst. Nitrogen oxides, sulphur oxides, halogens and other interfering compounds are eliminated. Mercury is trapped in a gold-containing amalgamator. In the last step the mercury is terminally released and measured using an optical spectrometer. Each run takes approx. 5 minutes. The method detects total mercury levels (organic and inorganic combined). Where possible and sufficient hair was available, seven replicate samples were measured per individual, and the mean used. Previous testing of the approach indicated that the results of multiple replicate samples from the same specimen show some variation, but the mean is useable, as has

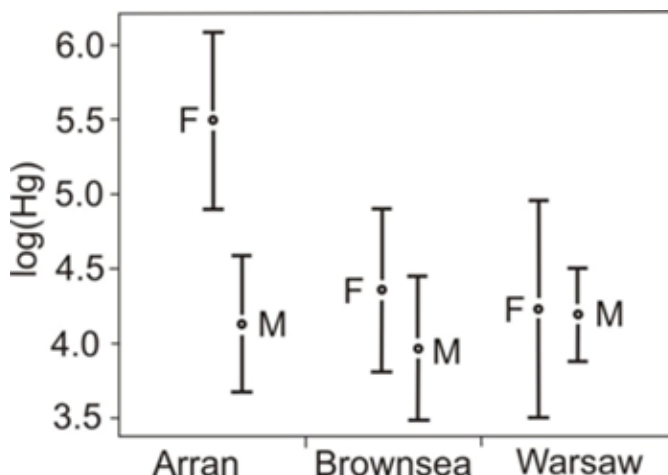
**Table 1** – Summary of general linear model explaining differences in Hg values (log-transformed) found in hair samples of red squirrels. Females from the Island of Arran were a reference category.

Predictor	Estimate (SE)	t-value	p-value
Intercept	5.49 (0.24)	22.73	0.0000
sex: male	-1.37 (0.34)	-4.00	0.0002
site: Brownsea	-1.13 (0.32)	-3.57	0.0007
site: Warsaw	-1.27 (0.38)	-3.37	0.0014
sex: male * site: Brownsea	0.98 (0.45)	2.15	0.0358
sex: male * site: Warsaw	1.33 (0.50)	2.64	0.0107

been shown with Hg contaminated soil samples (Heller, 2016). The samples therefore do not need to be homogenized. The relationship between Hg values in red squirrel hair samples and sex of animals and site of collection was tested using a general linear model (GLM). Interactions between sex and site of collection were investigated by incorporation as fixed effects. As data were not normally distributed, we used log-transformed data for the analysis.

Levels were very high in some female red squirrels on Arran, with one individual showing a mean value of 801.95 µg/kg. Overall females had a mean of 324.18±247.37 µg/kg (N=10, range 71.32–801.95 µg/kg). Males on Arran showed significantly lower values from 21.20–185.39 µg/kg (N=10; mean 74.08±47.70 µg/kg; Tab. 1). In contrast, in the samples from Warsaw, mercury levels in females (N=7; mean 91.99±83.39 µg/kg, range 31.5–256.28 µg/kg) were similar to males (N=11; mean 72.54±32.92 µg/kg, range 29.38–120.55 µg/kg), and peak levels of mercury in the hair samples were less extreme. On Brownsea Island values were comparable to Warsaw, but with a tendency for females (N=14; mean 107.43±78.06 µg/kg, range 11.1–283.32 µg/kg) to have higher values than males (N=12; mean 64.61±32.47 µg/kg, range 7.57–133.86 µg/kg). Overall, females from the Isle of Arran had significantly higher Hg values than either males or females from the two other sites. Also, Arran was the only site where difference between females and males was significant (Tab. 1 and Fig. 1).

Understanding how biodiversity, ecosystem function and species conservation are affected by factors such as climate or land use change, disease and pollution is an important aspect of species conservation and a key focus of the field of Conservation Medicine (Aquirre and Tabor, 2008). Threats to species and ecosystems are often multifactorial and contributing factors to species declines can be subtle, and act in concert with disease. Furthermore, pollutants may also negatively affect the health of endangered species (e.g. marine mammals; Lahay et al., 2007). Mercury pollution in particular, has the ability to impair the immune system, cause organ lesions, and act as a potential co-factor negatively affecting a species health status (e.g. Carpenter, 2001; Day



**Figure 1** – Differences in mean Hg values from hair samples of female (F) and male (M) red squirrels from three sites.

**Table 2** – Examples of published mercury levels in different mammal species for comparison. Samples are difficult to compare as they vary in time, location, method and tissue type and concentrations of Hg (or MeHg<sup>+</sup>) that cause deleterious effects will vary with species. Note that all data were converted to mg/kg for comparison.

Species	Sample type	Hg mg/kg	Source
Arran red squirrels	Hair	0.02–0.80	Current pilot project data
Brownsea red squirrels	Hair	0.08–0.28	Current pilot project data
Warsaw red squirrels	Hair	0.02–0.25	Current pilot project data
Arctic Fox juveniles ( <i>Vulpes lagopus</i> )	Hair	0.69–13.5	Bocharova et al. 2013
Arctic Fox adults ( <i>Vulpes lagopus</i> )	Hair	0.57–31.2	Bocharova et al. 2013
Bank vole ( <i>Myodes glareolus</i> )	Hair	0.18, 0.91	Bull et al. 1977
Wood mouse ( <i>Apodemus sylvaticus</i> )	Hair	0.12, 0.78	Bull et al. 1977
Caribou ( <i>Rangifer tarandus groenlandicus</i> )	Liver	0.24–0.46	Gamberg et al. 2016
Greater White-toothed Shrew adults	Bone	0.55–3.99	Sánchez-Chardi et al. 2007

et al., 2007; Sonne et al., 2007). Red squirrels in the UK have been declining and are considered threatened as a result of disease-mediated competition by introduced North American grey squirrels (*S. carolinensis*; Gurnell et al., 2004, 2014; White et al., 2014). Disease-mediated competition may also be a significant factor in red squirrel replacement in Italy (Romeo et al., 2014), and other disease outbreaks had marked and worrying effects on local population viability in the UK and Germany (Everest et al., 2008; Bosch et al., in press). Heavy metal pollution with mercury can have significant health impacts and influence disease dynamics. There is currently a complete lack of information on heavy metal pollution in arboreal species such as squirrels and a critical first step is to determine if this is a factor that can affect the endangered Eurasian red squirrel. We investigated if red squirrels show any levels of mercury pollution and if the city population of Warsaw, which has large coal-fired power plants known to emit mercury, are potentially more affected. The findings of our pilot study indicated that red squirrels indeed show elevated levels of mercury and that some females on Arran have relatively high values (see Tab. 2 for a comparison with other tested species, Gersternberger et al., 2006). The mercury levels measured in the latter would certainly exceed recommended levels for food products. For example, threshold values for mercury and mercury compounds for non-predatory fish and shellfish published by the government in Germany are 500 µg/kg (Schadstoffverordnung, 1988) and three Arran individuals had values of 544, 604 and 801 µg/kg. In the urban population in Poland mercury levels in both sexes were similar while the rural population in Scotland (Isle of Arran) showed significant differences between males and females. Values for red squirrels on Brownsea Island were more comparable to Warsaw than Arran and both provide a baseline of levels of mercury in hair. However, it was the finding for the squirrels from Arran that was the most surprising. In contrast to red squirrels from Warsaw, with known sources such as its coal power plants that emit mercury (Tebert, 2015), Arran has no industry except tourism (Wikipedia, 2017), and its main exports currently are whisky, beer, cheese and timber. We did not expect to find these relatively high levels of mercury pollution in red squirrel hair. The observed peak values of 544, 604 and 801 µg/kg are similar to elevated values of mercury in bank voles (*Myodes glareolus*) and woodmice (*Apodemus sylvaticus*) measured near a chlor-alkali works where mercury is used in the process (Bull et al., 1977, Tab. 2). They are also similar to low values for arctic foxes (*Vulpes lagopus*), where fluctuations in Hg depend on their geographic location (inland versus coastal) and feeding strategies and the resulting proportion of marine food types in their diet (Bocharova et al., 2013, Tab. 2). Whilst a study on insectivorous shrews (White-toothed shrew, *Crocidora russula*) exposed to mercury in the Ebro Delta, Spain found peak levels of up to 3990 µg/kg in adults, our values are within the lower observed range of 550 µg/kg.

Based on the fact that only three females of the 20 squirrels tested on Arran had high values, we suggest that the most likely route is not via air pollution but diet. The three female red squirrels found dead on roads on Arran were all collected in spring and summer, the breeding period for this species. In this period, females are known to gnaw on bones or consume soil to obtain minerals (e.g. calcium) to satisfy the increased demand during lactation (e.g. Bosch and Lurz, 2012). Most

woodlands on Arran are not far from the shore, but there is no information in the literature on red squirrels foraging on the beach for fish or other seafood remains, but it cannot be ruled out. Our findings do indicate that similar to what has been reported for arctic foxes (Bocharova et al., 2013), feeding behaviour and female preferences in red squirrels caused by dietary requirements during pregnancy and lactation may be critical in terms of detected mercury. In this context it is interesting to note that location (home range and region) and individual body condition may matter. While values on Brownsea were lower than on Arran, there were marked variations between individuals with many females having higher values than males. Of the three females with the lowest Hg values on Brownsea, two were reproductively inactive. Detailed and extensive tissue analyses were beyond the scope and resources of this pilot study. However, our findings for both Poland and the UK suggest that mercury does not only accumulate in marine food chains and the arctic ecosystem (Sonne et al., 2007), but is present in terrestrial woodland and urban ecosystems. Data on trends to determine if Hg values are accumulating are completely lacking, and point to a need for a monitoring strategy on mercury levels and associated, potential health impacts in endangered species. The continued release of mercury through industrial and other processes (e.g. coal power generation; Tebert, 2015) has the capacity to lead to higher and steadily increasing levels. This equates to a slow poisoning of our environment with currently unknown future impacts on food production, ecosystem function, animal and human health. ☞

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