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Persistent pollutants exceed toxic thresholds in a freshwater top predator decades after legislative control

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Abstract

Declining emissions of persistent organic pollutants (POPs), subject to international control under the Stockholm convention, are not consistently reflected in biotic samples. To assess spatial and temporal variation in organochlorine pesticides and PCBs in UK freshwaters, we analysed tissues of a sentinel predator, the Eurasian otter, *Lutra lutra* between 1992 and 2009. Past declines in otter populations have been linked to POPs and it is unclear whether otter recovery is hampered in any areas by their persistence. PCBs, DDT (and derivatives), dieldrin and HCB were detected in over 80% of 755 otter livers sampled. Concentrations of ∑PCB, ∑DDT and dieldrin in otter livers declined across the UK, but there was no significant time trend for ∑PCB-TEQ (WHO toxic equivalency, Van den Berg, 2006) or HCB. In general, higher concentrations were found in the midlands and eastern regions, and lowest concentrations in western regions. Concentrations of PCBs and HCB in otters increased near the coast, potentially reflecting higher pollutant levels in estuarine systems. Decades after legislative controls, concentrations of these legacy pollutants still pose a risk to otters and other freshwater predators, with spatially widespread exceedance of thresholds above which reproduction or survival has been reduced in related species.

Capsule:

Dieldrin and DDT have declined in UK otters since the early 1990s, but HCB has not; PCBs frequently exceed toxic thresholds associated with reproduction in a related species.

Introduction

Chemical pollution of the environment has had major impacts on biodiversity, ecosystem function and services throughout the world (Rockström et al., 2009). Persistent organic pollutants (POPs) such as organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs), which have been widely used in agriculture and industry, are resistant to environmental degradation, bioaccumulate in animal tissues and biomagnify up food chains. Due to biomagnification, contaminant concentrations in biota may be at levels significant to animal (including human) health, while contemporaneous abiotic samples have low concentrations. Therefore, abiotic POPs concentrations do not necessarily reflect, nor can be used to predict, biotic concentrations. Biomonitoring is the most effective way to determine actual exposure and therefore risk to wildlife (Gomez-Ramirez et al., 2014).

POPs are known to have affected wildlife species at both the individual and the population level. They can impair individual survival (Blackmore, 1963), reproduction (Reijnders, 1986), development (Morrisey et al., 2014) and immune function (De Swart et al., 1996). At the population level, such impacts have been associated with population crashes and regional extinctions in species such as predatory birds (reviewed by Walker, 2014). In freshwater systems specifically, declines in Eurasian otter *Lutra lutra* populations across Europe were linked to exposure to organochlorine (OC) insecticides and PCBs. There remains debate as to which of these two groups may have been the major driver (Chanin and Jefferies, 1978; Jefferies and Hanson, 2000; Macdonald, 1991; Mason 1998; Mason and Wren, 2001).

A series of legislative controls have limited or stopped the use of many POPs across most of the developed world (e.g. the Stockholm Convention, 2001). There is some evidence linking declining emissions of PCBs and DDTs to a decline in accumulation in some wild species, with concomitant improvements in their reproduction and population size (Roos et al., 2012). In the UK however, although there have been well-documented declines in OC pesticides in terrestrial predatory birds (Newton et al., 1993) the evidence for long-term PCB declines in the same species is lacking (Walker et al. 2011). In marine systems, there is also a lack of consistent temporal trends in PCB contamination, for example in gannet (*Morus bassanus)* eggs (Pereira et al., 2009). In marine mammalian predators, initial declines of PCB concentrations have now ceased, and remaining concentrations are associated with long-term population declines, low or zero reproduction and, in some species, population collapse (Bachman et al., 2014; Jepson et al., 2016; Desforges et al. 2018).

Rather surprisingly, there appear to be few published long term studies on POPs in freshwater predators (but see Roos et al., 2001, 2012; Rigét et al., 2019) and this makes it difficult to elucidate large-scale temporal and spatial trends of POPs in freshwater wildlife (Yamaguchi et al., 2003). POPs measurements in fish and birds indicate that rivers have higher PCB burdens in urban than rural areas, that rivers in agricultural regions or near pesticide factories have higher burdens of pesticides such as DDT (Elliot et al., 2015; Lu et al., 2017; Nyberg et al., 2014; Yamaguchi, 2003) and that contaminant accumulation by fish is greater in tidal areas than further upstream (Jurgens et al., 2015). We are not aware of any published long-term, large-scale studies of POP contamination in freshwater wildlife in Britain.

Here, we use the archive of data and samples held by the Cardiff University Otter Project (www.cardiff.ac.uk/otter-project). The otter is a top predator, has a diverse but primarily freshwater aquatic diet (Moorhouse-Gann et al., 2020) and ranges widely (up to 40km, Green et al., 1984); it thereby acts as an integrative indicator of pollutant levels in aquatic ecosystems. The availability of both contaminant data and associated post mortem data provides a powerful means of controlling for variation in POP accumulation that is driven by factors such as sex, age and nutritional condition (Clarke & Shore, 2001; Saxena et al., 1981; Wolkers et al., 1998; Yordy et al. 2010). Our specific objectives were to quantify long-term temporal and large-scale spatial variation in the concentration of POPs in UK freshwater systems, and to examine whether there have been changes in the proportion of individual otters exceeding relevant toxic thresholds (defined for related species such as mink: Bleavins et. al., 1984, Zwiernik et al., 2011). We also evaluated the evidence for any change in reproductive activity in otters over time, during the period when pollutants were monitored (1992-2009) and since (up to 2019). We hypothesised that there would be declines in pollutant concentrations with time, reflecting legislative controls, and that concentrations would vary spatially dependent on regional variation in chemical usage, and with proximity to the coast. Furthermore, we hypothesised that if toxic thresholds were exceeded, then spatial and temporal variation in reproductive activity would reflect POP burdens.

Methods

Sample collection and post-mortem examination

Otter carcasses found in England and Wales between 1992 and 2009 were collected and stored at -20°C prior to post-mortem examination. The provenance of each carcass was assigned to a region based on Environment Agency (EA) and Natural Resources Wales (NRW) boundaries, which are based on river catchments (EA and NRW are UK public bodies responsible to UK and Wales governments). Variables determined during post-mortem examination were sex, age class, body length (nose to tail tip in mm), and cause of death. Length and weight were used to derive condition, using Kruuk et al.'s (1987) condition index. Age was categorised as juvenile (females <2.1kg, males <3kg), subadult (females ≥2.1kg with no sign of reproductive activity, males ≥3kg with a baculum <60mm in length) or adult (females with signs of reproductive activity, males with baculum ≥60mm). Cause of death was categorised as "acute physical trauma" (including road traffic or rail accident, shooting, fatal dog attack, drowning, snared, n=695) or "other" (e.g. disease, infection, or starvation, n=60). The distance from the coast was measured along rivers (rather than straight line), using RivEX (Hornby, 2017) with 1:50,000 Watercourse Network layer (Centre for Ecology & Hydrology) in ESRI ArcMap (version 10.2.2). Of a total 1508 otters examined between 1992-2009, only those with sufficient intact liver showing little signs of autolysis were used for pollutant analysis (n = 755, of which 280 were adult male, 154 adult female, 143 subadult male, 138 subadult female, 23 juvenile male and 17 juvenile female). Liver samples were retained, wrapped in aluminium foil and stored at -20°C prior to chemical analysis.

Chemical analysis

Liver samples were analysed for 38 determinands, including a range of persistent organic pollutants, listed in full (with frequency of detection) in Table 1, SI1. Many of these determinands were infrequently detected, providing insufficient data for further analysis. Further data analysis focused on the PCB congeners, DDT and derivatives, dieldrin and hexacholorobenzene (HCB), and details of further data treatment of these groups is provided below (see *Data analysis*). Standardised methodologies were followed at Environment Agency National Laboratory Service (NLS) laboratories, in nine consecutive batches spanning a seventeen year period (1993-2010). Advances in analytical methodology (e.g. instrument used) over this period are controlled for statistically (see below). All NLS labs are accredited to ISO17025 (UKAS group accreditation number 0754) and where applicable to the MCERTs standard for analytical testing. Below we report the analytical methods used most recently; earlier analytical methods are given by Simpson et al., 2000).

Approximately 20g sample of liver tissue was removed from each otter and homogenised. Hydromatrix and surrogate standards were added (D6 – alpha HCH, D8 – p,p'-DDT, and PCB 155) to a 2g sub-sample and samples were extracted into a mix of dichloromethane, iso hexane and acetone using Accelerated Solvent Extraction (ASE). Gel Permeation Chromatography (GPC) followed by cartridge column chromatography were used to clean up the extract. Following concentration, extracts were injected into a Gas Chromatograph interfaced with a Mass Selective detector (Agilent 6890-5973) operating in the selected ion monitoring mode. A minimum of two extracted blanks and two extracted Quality Control Samples were analysed every 20 samples. Quality control samples (used for recovery and precision) were prepared by adding 250µl purchased standard solutions to 2g of cod liver oil made up to 10ml with dichloromethane. All pollutants were measured in µg kg^{-1} wet weight. Detection

limits were 1.0 µg kg^{-1} wet weight in earlier batches, and although methodological improvements led to lower detection limits for some pollutants in later years, for consistency across time, values below this threshold were treated as below detection limit in all cases. In a small number of cases, limited availability of liver tissue led to non-detection where detection limits were >1; those values were removed from the dataset. Recovery rates varied from 80-120% and the reported data were not recovery-corrected.

Data analysis

Due to the *ad hoc* nature of carcass collection, data were unbalanced with regards year of collection, region, sex and age, therefore, general linear mixed effect models (with a Gaussian error family and identity link function) were employed. Changes in analytical methods over 25 years were controlled for by including batch number as a random term in each model. Pollutants were modelled separately using the lmer function in the lme4 package (Bates et al., 2015) in R (R Core Team, 2019) with the RStudio interface (RStudio Team, 2019). Models were fit by REML, refined using stepwise deletions of insignificant terms, and model fit assessed via examination of residuals for normality, homoscedasticity and absence of leverage.

We modelled the concentrations of **∑PCB** based on nine congeners that were both consistently measured and frequently detected (each found in > 92% of samples, all other congeners were detected in <40% of samples). These were congeners 105, 118, 128, 138, 153, 156, 170, 180, 187. Only otters where all nine congeners were quantified were included (subsequent modelling excluded two individuals for which extreme values prevented the fitting of an adequate model; final n =573). We also

187 calculated **ΣPCB-TEQ**, as the sum of dioxin-like congeners with published toxic
188 equivalency factors (TEFs), these were congeners 77, 105, 118, 126, 156 and 169
189 (TEF 0.0001, 0.00003, 0.00003, 0.1, 0.00003 and 0.03 respectively, Van den Berg et
190 al., 2006). The remaining dioxin-like congeners identified by the WHO were not
191 detected. Congeners 77, 126 and 169 were not quantified in many of the samples
192 analysed between 1992-1999, therefore analysis of ΣPCB-TEQ is restricted to data
193 from later years (2000-2009) when all 6 congeners were consistently measured. We
194 also modelled **ΣDDT** (where the dependent variable was the sum concentration of
195 op'DDE, pp'DDE, op'DDT, pp'DDT, op'TDE, and pp'TDE), **dieldrin** and
196 **hexachlorobenzene** (HCB).

197 Each model tested for change over time and spatial variation, while controlling for
198 biotic variation (Table 1). To test for variation in temporal trends between regions the
199 interaction between region and year was included in each starting model. The
200 interaction between age and sex was included in each model to control for potential
201 differences in the sex effect between age groups (e.g. placental and mammary transfer
202 by adult females). Prior to statistical analyses, non-detected concentrations were
203 assigned a value of half the detection limit, except for the calculations for PCB-TEQs
204 which were counted as zero. This more conservative approach was taken because the
205 high TEF value for PCB congener 126 (0.1) meant that concentrations below the
206 detection limit, if replaced with 0.5 (i.e. half detection limit) inflated the value of TEQ
207 above the threshold for harm. Pollutant groups were normalised by log transformation
208 (following preliminary examination of model fit).

209 For each pollutant, the best fitting model was used to derive model predictions, while
210 controlling for other significant variables as follows: adult male otters (the most

common group in the dataset), Wales (the most numerous group, and likely to return conservative estimates of pollutant concentrations), year 2008 (to reflect relatively recent concentrations, with good sample size), “acute physical trauma” cause of death (the most common category), the mean adult male otter body length in the current dataset (1131cm) and the mean distance to coast (25206m). Predictions were back transformed to original scale and are therefore geometric means. Year predictions were extended beyond the period of available data to forecast concentrations up until 2020. Post hoc tests were conducted to test for differences between regions using Bonferroni-Holm correction of all pairwise comparison using the glht command in the multcomp package.

Reproductive status was determined for all adult female otters collected between 1992 and 2019 (i.e. not limited to those with pollutant data), excluding those too damaged to assess. Female otters were categorised as either 1=currently showing evidence of reproductive activity (pregnant or lactating, n = 340), or 0= not (quiescent or never reproduced, n = 303). A chi-squared test was used to test whether there were differences in reproductive activity between regions. Temporal change was tested by calculating the percentage of female otters with signs of recent reproduction for pooled years (pooled into pairs of years due to low sample size in some years and excluding 1992 and 1993 as the only pair where $n<10$), and Kendall’s rank correlation applied to test for any association. Regions with insufficient data (Southern, Thames) or temporally imbalanced data (South West) were excluded; attempts to fit a binomial GLM to simultaneously test region, year, and their interaction failed to meet model assumptions.

Results

The most frequently detected pollutants (>detection limit in 80-100% of samples where results were returned) were dieldrin, ppDDE, ppTDE, HCB and PCB congeners 105,118,128,138,153,156,170,180,187. Measured levels of these frequently detected individual pollutants ranged from < detection limit (of 1 μg kg^{-1}) to 7660 μg kg^{-1} (wet weight); this highest value was of pp-DDE. ΣPCB values ranged from 15.2 to 7868.6 μg kg^{-1}, and ΣPCB-TEQ values ranged from 0.00003 to 44.5 TEQs/kg liver wet weight (Table 1). A further 25 pollutants (hexachlorocyclohexane, aldrin, isodrin, endrin and additional PCB congeners) were detected less frequently (<40% of samples) and are detailed in Table SI 1.

Temporal and spatial trends

Between 1992 and 2009 there were significant declines over time in otter liver concentrations of dieldrin, ΣDDT and ΣPCB (Table 1 and Figure 1), which we forecast would continue to average values in 2020 of 45.76, 56.14 and 186.76 μg kg^{-1} (wet weight) respectively. Likewise, congeners making up ∑PCB declined (Figure 1). The highest annual concentrations were for ΣDDT, which also exhibited the steepest decline over time. There was no significant trend with time in liver HCB concentrations, nor for liver ∑PCB-TEQ concentrations (although there was a shorter time series for ∑PCB-TEQ, which was restricted to 2000-2009 due to lack of testing of some congeners in earlier years).

Although temporal trends were consistent across all regions (i.e. the interaction term Region:Year was not significant), there was significant spatial variation in pollutant

concentrations. Region was a highly significant term in all models (Table 1 and Figure 2). ∑PCB and dieldrin had significantly higher concentrations in the Midlands region, ∑PCB-TEQ had significantly lower concentrations in the South-West region, HCB had significantly lower concentrations in the Wales region, and ΣDDT was significantly higher in the Midlands and eastern (Anglian, North-East) regions than western (South-west, Wales, North-West) regions. ∑PCB was significantly higher in the Midlands region than Southern region but note small sample size in Southern (n=9).

Concentrations of HCB, ∑PCB and ∑PCB-TEQ were significantly higher in the livers of otter carcasses found nearer the coast (HCB: $Chisq_{1,18}=6.87$, p=0.009; ∑PCB-TEQ: $Chisq_{1,18}=12.39$ p<0.001; ∑PCB: $Chisq_{1,19}=33.56$, p<0.001). The models estimated that for every10km progression inland, there was on average a 0.0006 TEQ/kg reduction in ∑PCB-TEQ, a 0.62 μg kg^{-1}(wet weight) reduction in HCB and a 28.6 μg kg^{-1}(wet weight) reduction in ∑PCB. Dieldrin and ∑DDTs were not associated with distance from coast.

Biological predictors of tissue pollutant concentrations

The interaction between sex and age was a significant term in most models (Table 1). Juvenile female otters had the highest pollutant concentrations (∑DDTs, HCB, ∑PCB and ∑PCB-TEQ), followed by juvenile males (Figure 3). Adult male otters had higher concentrations than sub-adults of both sexes, and adult female otters had the lowest concentrations (Figure 3). For dieldrin, only age class was significant ($Chisq_{2,13}$ =9.47, p=0.009), juveniles again had the highest liver concentrations, adults were intermediate, and sub-adults had the lowest liver concentrations. With age class

controlled in the model, liver concentrations of all pollutant groups except dieldrin were significantly positively correlated with otter body length (Table 1). Higher concentrations of ΣPCB, ΣPCB-TEQ, ΣDDT and HCB were found in otters that died of disease, infection or starvation ("other") compared to those that died of acute physical trauma, whereas for dieldrin there was no significant association between concentration and cause of death.

Potential health effects: toxic thresholds and reproductive status

Toxic thresholds based on ecologically relevant endpoints were selected from the literature. Levels of toxicity for otters have not been tested experimentally (and protected species legislation would preclude this) therefore it was necessary to use those from closely related species. The PCBs threshold used was 77ng TEQs/kg liver wet weight, suggested by Zwiernik et al. (2011) based on mink (*Neovison vison)* kit survivability in three maternal feeding experiments. In the current study, only 19 of the otters collected between 1992 and 1999 were analysed for congeners 77, 169 and 126 and so calculation of TEQ concentrations based on the six dioxin-like PCB congeners (77, 105, 118, 126, 156, 169) was not possible for most otters collected in this time period. However, of those 19 otters, six (32%) exceeded the published threshold based on American mink (Zwiernik et al., 2011). Between 2000-2009, all six congeners were measured in 464 individuals and 178 (38%) exceeded the TEQ threshold. There was no significant temporal trend in ∑PCB-TEQ, and individuals exceeding toxic threshold were found across years (Figure 1). The distribution of otters exceeding the PCB-TEQ toxicity threshold was spatially widespread, with cases in every region (Figure 2 and 4).

The fox (*Vulpes vulpes*) is thought to be one of the more sensitive mammals to dieldrin (Jefferies, 1969; Jefferies and Hanson, 2000), and lethal dieldrin liver residues in this species are 1 µg/g ww (Blackmore 1963). During the current study five otters exceeded this level, two in 1996, one in 2002 and two in 2008, originating from the Anglian, Wales and Midlands regions. No obvious signs of pathology were noted at post mortem examination, although the two found in 2008 were severely emaciated. A dieldrin concentration associated with retinal dysplasia in otters (339 µg/kg wet weight liver; Williams et al., 2004) was exceeded by 54 otters (7%) found in most years and regions (Figures 1 and 2), however, the eyes of the otters in the current study were not examined. DDT liver residues of 1300 µg/kg in female mink have been associated with physiological effects such as increased embryonic loss and altered kit sex ratio (Gilbert, 1969); this threshold was exceeded in 31 otters (4%) which were found across most years (1993-2008) and from the Midlands, Anglian, North East and Wales regions (Figures 1 and 2). There are no published relevant toxic thresholds for hexachlorobenzene. The proportion of adult females showing signs of reproduction was 31.6%, it was highly variable, and varied widely between years (minimum 22.5%, 9/40 in 2014-2015; maximum 50%, 6/12 in 1998-1999 [though note low n]). There was no evidence for a significant trend over time between 1994 and 2019 (Kendall's tau = -0.21, p 0.37). The highest proportion of reproductive activity was recorded in Wales (35.75%, n = 74/133), followed by Anglian (32.88%, n = 24/49), North East (30%, n=15/35), North West (28%, n = 14/36) and Midlands (20.63%, n = 13/50), but differences between regions were not statistically significant (Chi-squared 5.57, df 4, p 0.23).

Discussion

Overall, temporal trends suggest an ongoing decline in average concentration of many PCBs, DDTs and dieldrin in UK otter liver tissues, that is consistent across regions, and is in continuation of declines reported previously (Jefferies & Hanson, 2000; Mason, 1998). Legislation to ban or limit use is likely to be the major driver of these declines. Indeed, the domination of pp'DDE rather than DDT, as in fish (Jurgens et al., 2016) and otters elsewhere (Lemarchand et al., 2010), indicates little or no recent exposure to DDT. Temporal trends of POPs found here in UK otters are similar to those found in otters elsewhere in Europe (Mason & Wren, 2001; Roos et al., 2012), in the UK atmosphere (Schuster et al. 2010a) and in eels (Macgregor et al., 2010), a favoured prey item of otters. It is likely that the decline in otter exposure to POPs has been accelerated by the concurrent decline in eel populations (a long-lived, fat-rich prey species) (Bevacqua et al. 2015) and replacement in otter diet by smaller prey with shorter life spans (Moorhouse-Gann et al., 2020), characteristics that are linked to lower pollutant burdens. Unfortunately, spatially widespread testing of otter prey species has not been carried out so it is not possible to assess any correlations between prey and otter exposure.

Declines in POPs are not universal, however. Despite a clear decline in ∑PCB and the most frequently detected congeners, PCB-TEQ did not show a consistent time trend This reflects a high degree of between year variability, and lack of overall decline, in the non-*ortho* congeners 77, 126 and 169 which have much higher toxic equivalency factors (TEFs (3.33, 3333.33 and 1000 times higher) than the more frequently detected mono-*ortho* congeners 105, 118 and 156. Even small variations in their

354 frequency of occurrence therefore exert a disproportionately large effect on TEQ.
355 Similarly, in a worldwide review of human blood levels during the same time period
356 (1989-2010), no significant decline in non-*ortho* PCBs were found (Consonni et al.,
357 2012). Historical production is likely to be the major source of PCBs in these otters
358 with minor contribution from current activities such as waste incineration (Weber et
359 al., 2008). We advocate future evaluation of the localised distribution of these non-
360 *ortho* PCBs. We found no significant decline in HCB concentrations in otters, despite
361 its ban as a fungicide in 1975. Similarly, at a global level although HCB levels in
362 abiotic matrices have declined, time trends in biota are less clear (reviewed by Barber
363 et al. 2005).

364 Although temporal trends were consistent across regions, there is some variation
365 between regions in total concentrations, largely reflecting historic usage patterns of
366 pollutants. Higher concentrations of dieldrin, ∑DDT and HCB in the midlands and
367 east of England, also observed in predatory birds (Newton et al., 1993; Pereira et al.,
368 2009), are likely to reflect the historic higher pesticide and fungicide usage in these
369 more arable areas (Morton et al., 2011). For PCBs, human population is a suitable
370 proxy for diffuse primary emissions (Schuster et al. 2010b); denser populations in
371 central and south eastern England than in Wales and the south west of England are
372 broadly reflected in otter liver PCB concentrations. PCB levels in otters have also
373 been linked to areas of industrialisation (Macdonald, 1991), which makes the lower
374 than average PCB concentrations in the northwest perhaps surprising given the
375 industrialised nature of much of the region. Most of the samples collected from this
376 region were, however, clustered within a more rural part (Cumbria, 44/52 of the
377 northwest samples). In the marine system, POP concentrations in UK harbour
378 porpoises show a different spatial pattern, with higher levels in Wales and the west of

England, and the authors suggest this reflects legacy from past production sites (Williams et al., 2020). Comparative analysis of sources, and flows, into terrestrial and marine systems are needed, with a focus at a finer level of spatial resolution, exploring the potential impacts of landscape, land use and historic sites of manufacture in more detail.

Higher concentrations of HCB and PCBs found in otters closer to the coast is congruent with levels reported in other species found in or near to marine environments, including fish (Jurgens et al., 2015), birds (Walker et al., 2011), porpoises (Law et al., 2010) and other marine mammals (Jepson et al., 2016). River flow washes pollutants downstream, resulting in higher exposure. Simultaneously, high sediment load in estuarine habitat acts as a sink from which POPs can be resuspended (Achman et al., 1996). The impact of these higher pollutant levels could be exacerbated in otters by their feeding on more fat rich and longer lived prey than further inland (Moorhouse-Gann et al., 2020). Indeed, the high pollutant levels found in estuarine compared to upstream eels were highly correlated with lipid content (Jurgens et al., 2015).

The DDTs, PCBs and HCB concentrations in otter livers all showed a positive association with body length which we assume represents accumulation with age. Lower concentrations in adult females and higher concentrations in juvenile otters are typical of maternal transfer (e.g. Saxena et al., 1981). Mobilization of lipids in sick or starving animals (Clarke & Shore, 2001; Yordy et al., 2010) might explain the higher DDTs, PCBs and HCB concentrations found in infected and/or emaciated otters, but it is also possible that this association is indicative of health impacts. Higher PCB concentrations recently measured in UK porpoises were associated with increased risk of infectious disease mortality, after controlling for nutritional status (Williams et al.,

2020). We found too few diseased otters to test whether a similar association occurs in otters, but did find higher concentrations of pollutants in otters that died of 'other' causes (pooled) compared to those which died of acute physical trauma. It is important to note that a bias toward finding otters as roadkill means that we likely underestimate POPs contamination and associated health impacts on the population as a whole. Only dieldrin did not show a significant increase in pollutant load in infected or emaciated otters. Overall, the dieldrin model explained less variation in concentrations than other pollutant models, and did not show any indication of maternal transfer; this, along with the weakly significant temporal decline ($p=0.055$), and concentrations generally well below those likely to cause acute toxicity, possibly indicates the decline in dieldrin is stabilising at low levels as in other species (Harris et al., 2005).

The pollutants recorded here were not the cause of death for these study animals. Sub-lethal effects, however, are possible, based on exceedance of a range of indicative thresholds for DDT, dieldrin and PCBs across the study period. It is important to note that although average concentrations declined (for most pollutants), upper extremes remained high. Such a high percentage exceeding thresholds for harm, particularly of PCBs, suggest either 1) otters are at continued risk from POPs, or 2) that the extant population has adapted to survive and reproduce at such POPs levels or 3) these thresholds are not appropriate for *Lutra lutra*. Evidence of otter reproduction (pregnant or lactating females) did not show significant temporal or spatial trends, and our hypothesis that spatial and temporal variation in reproductive activity would reflect pollutant burden therefore cannot be accepted. However, nor have we seen a clear increase in signs of otter reproduction in the UK (as has been described in Sweden following declines in pollutant concentrations there, Roos et al., 2012). A

simple binomial analysis (signs of reproduction, or not) is a relatively weak indicator, and it is interesting to note that reproductive activity is highest in Wales where most contaminants were low, and lowest in the Midlands where most contaminants were high. More detailed field monitoring of reproductive rate and numbers of young are needed.

Otter populations in the UK have largely recovered in recent decades, from small, isolated fragments in the periphery of the UK in the 1970s, to a current status where otters are recorded in every county (Crawford, 2010; Strachan, 2010; Findlay et al., 2015). Regional trends in liver POPs concentrations have to some extent mirrored the recovery of UK otter populations, with earlier and more comprehensive recovery from remnant populations in Wales and the south west (Crawford, 2010; Strachan, 2010) where pollutant concentrations are generally lower. Population recovery remains particularly slow in the south east of England, but the small number of carcasses recovered from this region prevents local assessment of POPs concentrations and reproduction. It is also difficult to separate the potential impacts of contaminant load from those of small starting population size. The potential for re-circulation of pollutants (e.g. Barber et al., 2005) with changes in climate (Noyes et al., 2009) or river management practices (e.g. increased dredging) may exacerbate pollutant risk.

Conclusion

Our data demonstrates the utility of the otter as a sentinel for contaminants that enter water courses. Declines in POPs in otter tissues in the UK were similar to those found elsewhere within the global distribution of *Lutra lutra*. DDT and dieldrin are unlikely to be of continued threat to otters in the UK, however frequent exceedance of PCB thresholds indicative of harm, and an absence of a clear decline in ∑PCB-TEQ and

HCB, highlight a need for continued investigation and surveillance. Attention should be paid to the recorded upper values of legacy pollutants, rather than focusing exclusively on average values, particularly in areas where vulnerable species or ecosystems may be affected. We suggest that current monitoring based on abiotic, invertebrate or fish samples, cannot achieve the thorough risk assessment that is possible when including higher trophic levels. We therefore advocate the use of top predator sampling to complement surveillance of current use, emerging and legacy contaminants, as an indicator of chemical threats to the wider freshwater ecosystem.

CRediT author statement

Eleanor Kean: Formal analysis, data curation, writing – original draft. **Graham Scholey:** Investigation, resources, funding acquisition. **Richard Shore:** Conceptualization, Supervision, writing – review and editing. **Rob Strachan:** Investigation, resources, funding acquisition. **Liz Chadwick:** Conceptualization, investigation, data curation, writing – review and editing, visualisation, project administration

Declaration of competing interest

No conflict of interest in this article.

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Tables

Table 1. Fixed effect terms in linear mixed effect models explaining persistent organic pollutants in otter livers. Test statistic (chisq) and significance (*p<0.05, **p<0.01, ***p<0.001, "."p=0.055) are provided for each significant variable; 'int' indicates a significant interaction, model statistics are presented for the interaction rather than individual single variables. NS indicates variables included in the starting model that were not significant in the final model. Cause of death was categorised as binomial: acute physical trauma (road traffic accident, rail accident, shooting, fatal dog attack, drowning, snared) or 'other' (e.g. death by disease, infection, or starvation). Batch number (for laboratory analyses) was also included as a random effect in all models. [a] Two outliers (6632.21 and 7868.62 μg kg^{-1} ww) were removed from the full ΣPCB dataset prior to statistical analysis, [b] 0.5 = half detection limit.

Dependent variables:	ΣPCB	ΣPCB-TEQ	ΣDDT	Dieldrin	HCB
Descriptive statistics for the modelled data					
n	573	483	751	744	672
Min-max μg (or TEQ) kg^{-1} ww otter liver	15.2- 5283.7[a]	0.00003-44.5	2.5-7662.5	0.5[b]-1710	0.5[b]-479
Overall model statistics					
Conditional R-sq	0.27	0.34	0.23	0.09	0.27
Marginal R-sq	0.27	0.13	0.23	0.09	0.14
Test statistics (chisq) for each biotic independent variable					
Length (nose to tail, continuous)	8.97**	8.50**	4.59*	NS	13.48***
Cause of death (trauma or other, binomial)	44.60***	12.00***	6.05*	NS	4.12*
Condition (index value, continuous)	NS	NS	NS	NS	NS
Age (juv, subadult, adult – categorical)	*int*	*int*	*int*	9.47**	*int*
Sex (male, female – categorical)	*int*	*int*	*int*	NS	*int*
Sex:Age (interaction)	25.09***	5.99*	14.88***	NS	19.90***
Test statistics (chisq) for each abiotic independent variable					
Year (1992-2009, continuous)	5.07*	NS	11.12***	3.67.	NS
Region (8 regions, categorical)	43.81***	31.65***	83.22***	44.73***	30.77***
Distance from coast (m, continuous)	33.56***	12.39***	NS	NS	6.87**
Region:Year (interaction)	NS	NS	NS	NS	NS

Figures

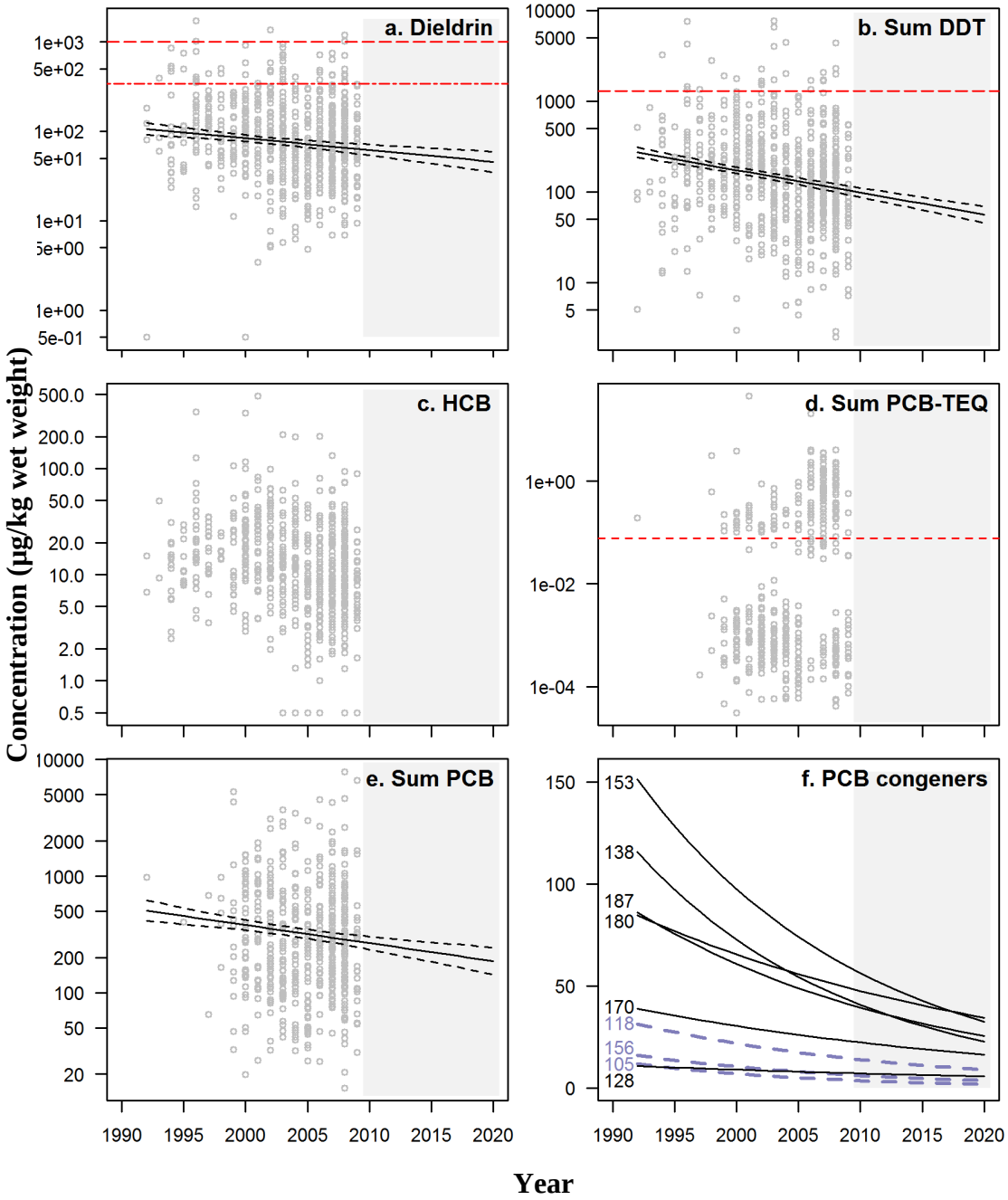
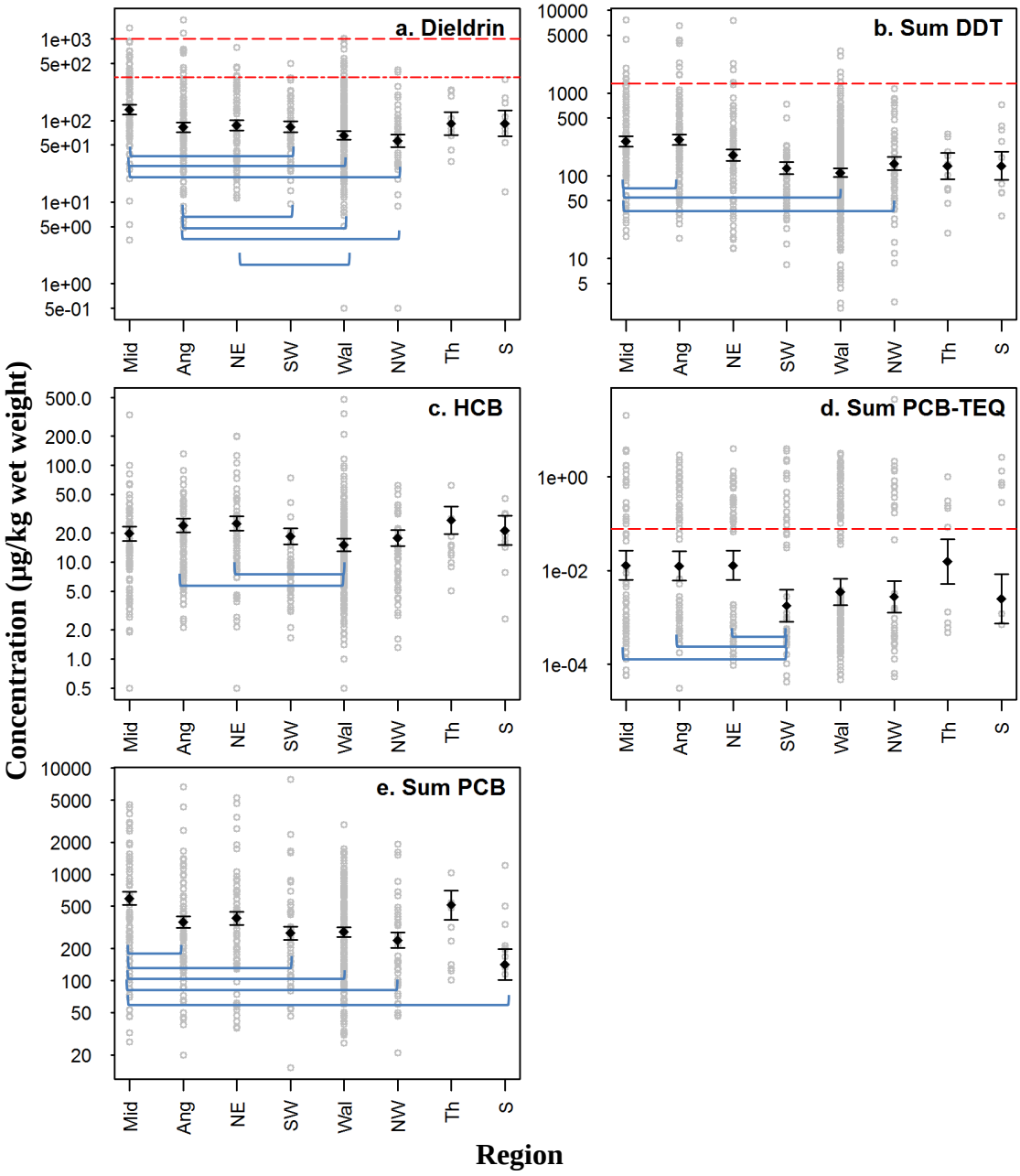


Figure 1. Change over time in liver POP concentrations in otters (note log scale plots a-e). Model predicted annual concentrations (black lines, ± SE) are based on measured concentrations 1992-2009 (grey symbols) and are forecast to date (grey shading). Other variables in the model are controlled where relevant (see statistical methods). Red lines indicate potentially relevant toxic thresholds (see text). Note that the split in (d) data distribution is caused by presence/absence of non-*ortho* congeners 77, 126 and 169 which have much higher toxic equivalency factors (3.33, 3333.33 and 1000 times higher) than the mono-*ortho* congeners 105, 118 and 156. Panel (e) represents the sum of 9 frequently occurring and consistently measured congeners, shown individually in (f), in which those in blue are also included in Sum PCB-TEQ.



753 **Figure 2. Spatial variation in liver POP concentrations in otters** (note log scale all
754 plots). Model predicted concentrations (black symbols, ± SE) are based on measured
755 concentrations (grey symbols) in individuals categorised by region (in Midlands,
756 Anglian, North East, South West, Wales, North West, Thames and Southern;
757 predictions for Thames and Southern may not be robust due to small sample size
758 (n=8-10, depending on pollutant; sample size for other regions was >45 in all cases).
759 Other variables in the model are controlled where relevant (see statistical methods).
760 Red lines indicate potentially relevant toxic thresholds (see text). Blue brackets
761 indicate significant differences between pairs ($p<0.05$). Note that the split in (d) data
762 distribution is caused by presence/absence of non-*ortho* congeners 77, 126 and 169
763 which have much higher toxic equivalency factors (3.33, 3333.33 and 1000 times
764 higher) than the mono-*ortho* congeners 105, 118 and 156. In (f), in which those in
765 blue are also included in Sum PCB-TEQ.
766

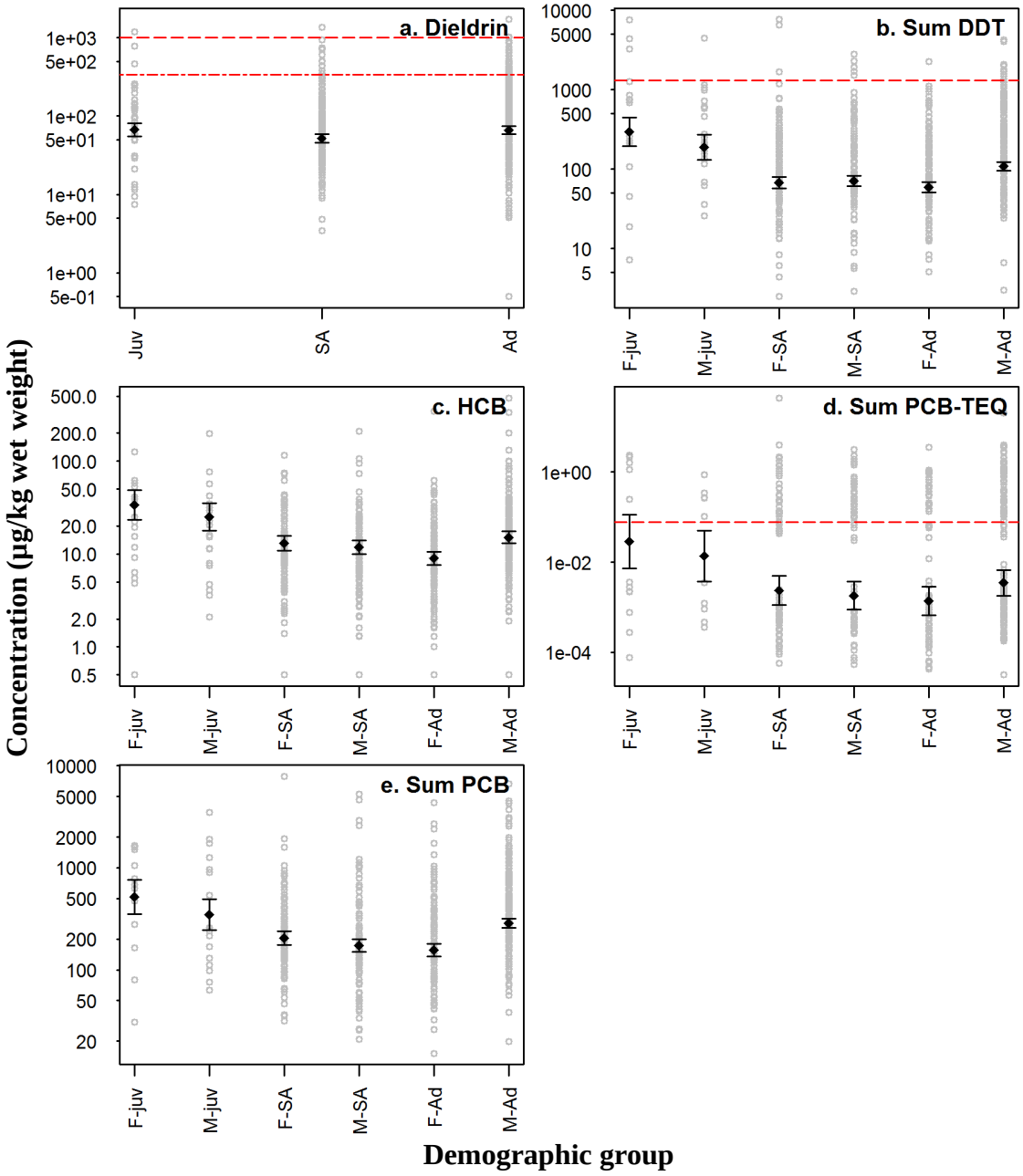


Figure 3. Biotic variation in liver POP concentrations in otters (note log scale all plots). Model predicted concentrations (black symbols, ± SE) are based on measured concentrations (grey symbols) in individuals categorised by age-class only (a. dieldrin), or by sex and age-class (all other pollutants)(juvenile, Sub-Adult, and Adult otters, Males and Females). Other variables in the model are controlled where relevant (see statistical methods). Red lines indicate potentially relevant toxic thresholds (see text). Note that the split in (d) data distribution is caused by presence/absence of non-*ortho* congeners 77, 126 and 169 which have much higher toxic equivalency factors (3.33, 3333.33 and 1000 times higher) than the mono-*ortho* congeners 105, 118 and 156.

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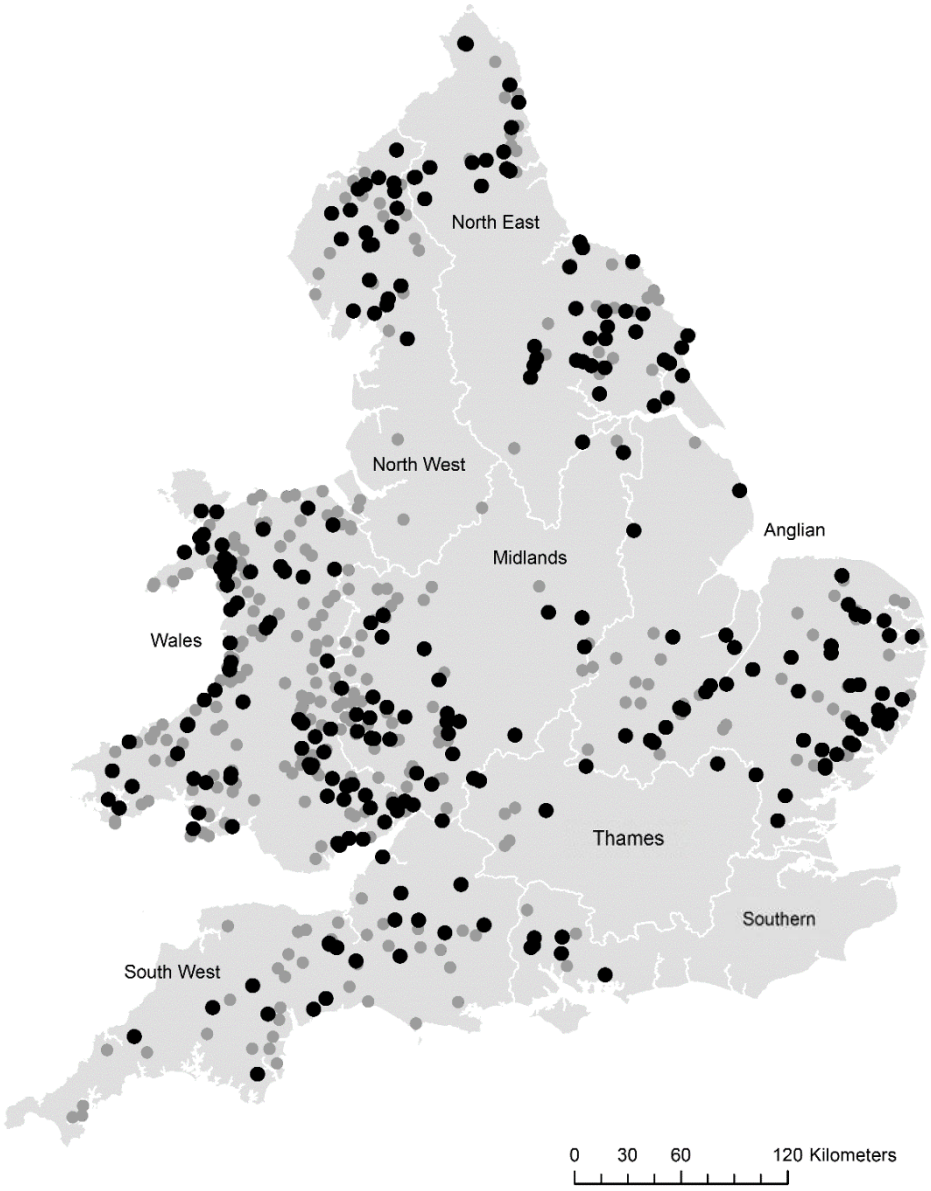

782 **Figure 4. Distribution of otters in which a toxicity threshold for dioxin-like PCB**
783 **congeners was exceeded (2000-2009).** The total TEQ value for PCB congeners 77,
784 105, 118, 126, 156 and 169 was summed. Individuals in which the sum was greater
785 than published toxicity threshold of 0.077µg TEQs/kg liver wet weight (Zwiernik,
786 Vermeulen and Bursian, 2011) are shown in black, those below threshold in grey.