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1 Long-term increase in secondary exposure to anticoagulant  
2 rodenticides in European polecats *Mustela putorius* in Great Britain

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13

14 **ABSTRACT**

15 As a result of legal protection and population recovery in Great Britain, European polecats  
16 (*Mustela putorius*) are expanding into areas associated with greater usage of second-  
17 generation anticoagulant rodenticides (SGARs). We analysed polecat livers collected from  
18 road casualties from 2013 to 2016 for residues of five SGARs. We related variation in  
19 residues to polecat traits and potential exposure pathways, by analysing stable isotopes of  
20 carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) in their whiskers. In all, 54 of 68 (79%) polecats had  
21 detectable residues of at least one SGAR. Bromadiolone (71%) was the most commonly  
22 detected compound, followed by difenacoum (53%) and brodifacoum (35%). Applying  
23 historical limits of detection to allow comparison between these new data and previous  
24 assessments, we show that in the 25 years from 1992 to 2016 inclusive, the rate of detection  
25 of SGARs in polecats in Britain has increased by a factor of 1.7. The probability of SGAR  
26 detection was positively related to increasing values of  $\delta^{15}\text{N}$ , suggesting that polecats feeding  
27 at a higher trophic level were more likely to be exposed. Total concentrations of SGARs in  
28 polecats with detectable residues were higher in polecats collected in arable compared to  
29 pastoral habitats, and in the west compared to the east of Britain. The number of compounds  
30 detected and total concentrations of SGARs increased with polecat age. There was no  
31 evidence of regional or seasonal variation in the probability of detecting SGARs, suggesting  
32 that the current risk of exposure to SGARs does not vary seasonally and has increased (from  
33 that in the 1990s) throughout the polecat's range. We recommend quantification of current  
34 practices in rodenticide usage, particularly in the light of recent regulatory changes, to enable  
35 assessment and mitigation of the risks of secondary exposure to rodenticides in non-target  
36 wildlife.

37

38 **Keywords:** rodenticides; polecat; secondary exposure; bromadiolone; difenacoum;

39 brodifacoum

40 **Capsule:**

41 79% of polecats in Great Britain were found to have been exposed to rodenticides from 2013

42 to 2016 and exposure has increased by a factor of 1.7 since the 1990s.

43

## 44 INTRODUCTION

45 Rodents, primarily brown rats (*Rattus norvegicus*), are estimated to cost the UK economy  
46 between £60 and £200 million a year, arising primarily from spoiling of food and from  
47 disease transmission (Battersby, 2004). Anticoagulant rodenticides dispensed in baits are the  
48 primary means of reducing this damage. They function by interrupting the blood clotting  
49 mechanism by inhibiting the action of Vitamin K epoxide reductase (Watt et al., 2005) and  
50 lethal exposure leads to death by internal haemorrhaging (Watt et al., 2005; Rattner et al.,  
51 2014). In response to the emergence of resistance in rats to warfarin and other first generation  
52 rodenticides, second generation anticoagulant rodenticides (SGARs) with higher acute  
53 toxicity were developed (Buckle et al., 1994; WHO, 1995) and are now used routinely  
54 worldwide to control rodent infestations (Stone et al., 2003; Buckle and Smith, 2015).

55 The extensive use of SGARs has led to secondary exposure in a range of mustelids including  
56 stoats (*Mustela erminea*) and weasels (*Mustela nivalis*) (McDonald et al., 1998; Elmeros et  
57 al., 2011), European polecats (*Mustela putorius*) (Shore et al., 2003; Elmeros et al., 2018),  
58 American mink (*Neovison vison*) (Ruiz-Suárez et al., 2016), stone martens (*Martes foina*)  
59 (Elmeros et al., 2018) and fishers (*Pekania pennanti*) (Gabriel et al., 2012; Thompson et al.,  
60 2014). There is also evidence of widespread exposure in other predators such as red foxes  
61 (*Vulpes vulpes*) (Tosh et al., 2011; Geduhn et al., 2015), San Joaquin kit foxes (*Vulpes*  
62 *macrotis mutica*) (Cypher et al., 2014), mountain lions (*Puma concolor*) and bobcats (*Lynx*  
63 *rufus*) (Riley et al., 2007; Serieys et al., 2015), barn owls (*Tyto alba*) (Geduhn et al., 2016;  
64 Shore et al., 2016), sparrowhawks (*Accipiter nisus*) (Hughes et al., 2013; Hughes et al., 2014;  
65 Walker et al., 2015) tawny owls (*Strix aluco*) (Walker et al., 2008) and red kites (*Milvus*  
66 *milvus*) (Walker et al., 2017). Secondary exposure occurs via the consumption of exposed  
67 prey (Smith et al., 1990; Smith et al., 2007; Rattner et al. 2014). These may be target species  
68 that are the subject of control measures, such as the brown rat and house mouse (*Mus*

69 *domesticus*), or non-target species that feed on bait and are inadvertently contaminated during  
70 control campaigns targeted at commensal rodents (Tosh et al., 2012; Elliott et al., 2014). The  
71 scale of secondary exposure in predators can vary with habitat (Geduhn et al., 2014; Nogueira  
72 et al., 2015), sex (McDonald et al., 1998) and time of year (Shore et al., 2003). In some  
73 species the magnitude of residues is greater in older animals (Ruiz-Suárez et al., 2016),  
74 arising from the cumulative effect of multiple sub-lethal exposures and the relatively long  
75 tissue half-lives of these compounds (Vandenbroucke et al., 2008; EPA, 2008).

76 There is concern that secondary exposure may lead to significant impacts on predators, many  
77 of which are species of conservation interest. The extent of any mortality is likely to be  
78 species-dependent as tolerance varies by several orders of magnitude (WHO, 1995; Erickson  
79 and Urban, 2004; Thomas et al., 2011; Berny et al., 2010). Relatively few poisoned animals  
80 are reported in national surveillance schemes, when compared to the numbers known to be  
81 exposed (e.g. Barnett et al., 2004; Barnett et al., 2005). The likelihood that exposed  
82 individuals die out of sight (Newton et al., 1999), combined with limited external signs of  
83 toxicosis (Murray, 2011) and difficulties with using liver residues as a diagnostic of mortality  
84 (Thomas et al., 2011), mean that the true extent of secondary poisoning may be  
85 underestimated. There may also be sub-lethal effects such as increased susceptibility to  
86 natural and anthropogenic stressors (Albert et al., 2010), reduced body condition (Elmeros et  
87 al., 2011) and less resistance to pathogens mediated through impairment of the immune  
88 system (Riley et al., 2007; Serieys et al., 2015). However, the mechanisms by which any sub-  
89 lethal effects occur and their possible impacts on long-term survival and reproductive output  
90 remain unclear.

91 Species that consume rats and other target species may be at particular risk of secondary  
92 exposure and poisoning by SGARs (Eason and Spurr, 1995; Brakes and Smith, 2005). The  
93 European polecat, a medium-sized carnivore that occurs across Europe, is one such species.

94 It is protected in England and Wales under the Wildlife and Countryside Act (1981) and is  
95 currently expanding its distribution, having been extirpated (through predator control) from  
96 most of its range in Great Britain during the nineteenth century (Birks, 2015; Croose, 2016).  
97 Although the polecat is a generalist feeder with a diverse diet that varies across its European  
98 range (Blandford, 1987; Lodé, 1996, 1997; Birks and Kitchener, 1999; Baghli et al., 2002;  
99 Hammershøj et al., 2004; Rysava-Novakova and Koubek, 2009; Santos et al., 2009; Malecha  
100 and Antczak, 2013), in England and Wales rabbits (*Oryctolagus cuniculus*) and rats are the  
101 primary prey (Birks and Kitchener, 1999).

102 A study of rodenticide residues in polecats in Great Britain that died between 1992 and 1999  
103 established that 31 out of a sample of 100 animals had detectable residues of at least one  
104 SGAR (Shore et al., 2003). Detection rates were slightly higher (40%) in animals that died in  
105 the first half of the year. It was speculated that this may have been a result of the  
106 predominance of rats in the diet during the winter, since rats may comprise up to 65% of  
107 polecat diet in the winter months (Birks, 1998). However, SGAR exposure in polecats has not  
108 specifically been linked to any contemporary dietary analysis. Stable isotope analysis offers  
109 the opportunity to explore such links.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  are measures of the ratio of heavier to  
110 lighter stable isotopes of nitrogen ( $^{15}\text{N}$  to  $^{14}\text{N}$ ) and carbon ( $^{13}\text{C}$  to  $^{12}\text{C}$ ) relative to a standard  
111 (DeNiro and Epstein, 1981). As the lighter  $^{14}\text{N}$  is preferentially excreted during metabolic  
112 processes,  $^{15}\text{N}$  enrichment from prey item to predator occurs (DeNiro and Epstein, 1981).  
113 Variation in  $\delta^{13}\text{C}$  reflects diversity in basal resources consumed, e.g. between marine and  
114 terrestrial, and plants with C3 or C4 photosynthetic pathways (Smith and Epstein, 1971,  
115 DeNiro and Epstein, 1978). Analysis of  $\delta^{15}\text{N}$  has been widely used for developing  
116 understanding of biomagnification of contaminants with increasing trophic level in fresh-  
117 water and marine environments (Spies et al., 1989; Cabana and Rasmussen, 1994; Kidd et al.,  
118 1995; Jarman et al., 1996; Bearhop et al., 2000; Hobson et al., 2002), and can be applied to

119 examine secondary exposure to rodenticides. Rats are omnivorous opportunistic feeders and  
120 their diets vary with location (Major et al., 2007; Dammhahn et al., 2017), so polecats feeding  
121 on rats might be expected to have enriched  $\delta^{15}\text{N}$  signatures compared to those eating a greater  
122 proportion of rabbits, which are herbivorous (Southern, 1940). If rats are the main trophic  
123 pathway through which polecats are secondarily exposed to SGARs, it would be expected  
124 that there might be a positive association between liver SGARs and enriched  $\delta^{15}\text{N}$  signatures.

125 In the 20-25 years since the last quantification of the exposure of polecats in Great Britain to  
126 SGARs (Shore et al., 2003), populations of this species have undergone a substantial  
127 recovery and have expanded their range into areas of the country associated with higher  
128 usage of SGARs (Packer and Birks, 1999; Birks, 2000; Dawson et al., 2003; Dawson and  
129 Garthwaite, 2004). It might therefore be predicted that overall exposure in the polecat  
130 population is likely to have increased, if animals in newly recolonised areas subject to greater  
131 SGAR usage also feed on rats. Furthermore, the methods of chemical analysis for  
132 rodenticides have become more sensitive (lower limits of detection) and so earlier studies in  
133 any case are likely to have underestimated levels of exposure (Dowding et al., 2010). The  
134 current extent of exposure of polecats to SGARs, and how and why this varies between  
135 individuals, is therefore unknown. Using polecat carcasses collected from across their range  
136 in Great Britain between 2013 and 2016, our aims in the present study were to: (i) determine  
137 the current extent of SGAR exposure in polecats (via measurement of liver residues) and  
138 whether this has changed over the last 20-25 years; (ii) identify any spatial and temporal  
139 patterns in exposure; (iii) elucidate trophic correlates of exposure through stable isotope  
140 analysis of whiskers, and (iv) explore the effect of age on rodenticide accumulation in  
141 polecats, a factor not examined by Shore et al. (2003), but recently found to be important in  
142 other mustelids (Ruiz-Suárez et al., 2016).

143



144 **METHODS**

145 *Carcass collection and sample preparation*

146 Polecat carcasses were collected as part of a national monitoring survey carried out by The  
147 Vincent Wildlife Trust between December 2013 and March 2016 (Croose, 2016). Sixty-eight  
148 carcasses were selected for rodenticide analysis, based on stratification by sex, location and  
149 collection date. Of the animals selected, 82% (n = 56) were road traffic casualties; the  
150 remainder were found dead in fields, killed by dogs, trapped or the cause of death was  
151 unknown.

152 Collection date and location were recorded for all carcasses, which were stored frozen until  
153 necropsy examination at the National Museum of Scotland. The poor condition of the  
154 majority of the carcasses precluded assessment of clinical signs of exposure to rodenticides.  
155 Where carcass condition allowed, gross necropsy examination included recording of sex,  
156 head and body length (nose to tip of tail), mass and internal fat, scored on a five-point scale  
157 (McDonald et al., 1998). A body condition score (e.g. Schulte-Hostedde et al., 2005) was not  
158 calculated because many carcasses were too damaged or incomplete. Teeth (for ageing),  
159 whiskers (for stable isotope analysis) and liver tissue (for rodenticide analysis) were  
160 collected. Liver samples were frozen and transferred to the Centre for Ecology & Hydrology  
161 (CEH) for rodenticide analysis. Whiskers were prepared for analysis at the University of  
162 Exeter and analysed at Elementex, UK and teeth were sent to Matson's Lab LLC, USA for  
163 aging by analysis of cementum layers.

164 *Determination of rodenticides in liver using liquid chromatography tandem mass*  
165 *spectrometry*

166 Concentrations of the five SGARs licensed for use in Great Britain (bromadiolone,  
167 difenacoum, brodifacoum, flocoumafen and difethialone) were determined in the polecat  
168 livers. The analytical method is summarised here. A detailed description is available in  
169 Walker et al. (2017). A 0.25 g sub-sample of each liver was thawed, weighed accurately,  
170 ground and dried with anhydrous sodium sulphate. Labelled standard ( $d^5$ - Bromadiolone,  
171 QMx) was added to each sample for quality control purposes and determination of analyte  
172 recovery. Each liver sub-sample was solvent-extracted and then cleaned-up using size  
173 exclusion chromatography followed by elution through solid-phase cartridges. Extraction was  
174 carried out twice with clean solvent. Each extraction involved vortex mixing of the sample  
175 with 1:1 v/v chloroform:acetone, mechanical shaking and centrifugation. The resultant  
176 supernatants from the two extraction runs were combined, solvent-exchanged into (1:1; v/v)  
177 chloroform:acetone, filtered (0.2 mm PTFE filter), subjected to a further solvent exchange  
178 into (1:23; v/v) acetone:DCM, filtered again, and cleaned-up by size-exclusion  
179 chromatography (Agilent 1200 HPLC). The cleaned extract was solvent-exchanged into  
180 1:1:8; v/v. chloroform:acetone:acetonitrile and underwent a second clean-up using solid  
181 phase, methanol-washed, acetonitrile-activated extraction cartridges (ISOLUTE® SI 500 mg,  
182 6 ml). The cartridges were eluted with the same solvent and the eluate exchanged for the  
183 mobile phase.

184 Liver SGAR residues were quantified by HPLC linked to a triple quadrupole mass  
185 spectrometer interfaced with an ion max source in Atmospheric Pressure Chemical Ionisation  
186 mode (APCI) with negative polarity. Full details of the operational parameters used are as  
187 given by Walker et al. (2017). All rodenticide standards (Dr Ehrenstorfer) were matrix  
188 matched and linear calibration curves were defined such that  $R^2 > 0.99$ . A blank was run with  
189 each batch of unknowns. The mean method limit of detection (LOD) across batches for each  
190 compound was 0.0014  $\mu\text{g/g}$ , except for difethialone which was 0.0022  $\mu\text{g/g}$ . The mean ( $\pm$

191 SE) recovery for the total procedure was calculated from the labelled bromadiolone standard  
192 applied to each sample and was  $68.0 \pm 2.1\%$ . Liver SGAR concentrations were not recovery  
193 corrected and are expressed on a wet weight basis. Summed ( $\Sigma$ ) SGAR liver concentrations in  
194 individual animals were calculated by summing the concentrations of the five different  
195 SGARs, a zero concentration being assigned to individual compounds that were not detected.

#### 196 *Stable isotope analysis*

197 Whiskers were gently rinsed in distilled water and then freeze dried for 24 hours. One  
198 whisker per animal was cut into ~1mm segments using a scalpel, starting at the proximal end  
199 of the whisker. Consecutive segments were pooled until the summed sample weight was ~0.7  
200 mg (mean  $\pm$  SE sample weight  $0.68 \pm 0.01$  mg). The sample was enclosed in a tin cup and put  
201 into a tray for analysis. The next segment was prepared in the same way and the process was  
202 further repeated until either the whole whisker was used, or less than 0.2 mg was remaining.  
203 Samples were analysed on a Thermoquest EA1110 elemental analyser linked to a Europa  
204 Scientific 2020 isotope ratio mass spectrometer at Elementex Ltd (Cornwall, UK) for  $\delta^{15}\text{N}$  and  
205  $\delta^{13}\text{C}$ .  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  abundance are reported as  $\delta$ -values and expressed as a per mil (‰)  
206 deviation from the international reference standards (PDB for carbon and AIR for nitrogen)  
207 (Mariotti, 1983):

$$208 \quad \delta^{15}\text{X}\text{‰} = \left[ \frac{(^{15}\text{X}/^{14}\text{X})_{\text{sample}}}{(^{15}\text{X}/^{14}\text{X})_{\text{standard}} - 1} \right] \times 1,000$$

209 Replicate analysis of standards (USGS 40, USGS 41 and an in-house bovine liver standard)  
210 yielded standard deviations of 0.05 – 0.29 for  $\delta^{15}\text{N}$  and 0.05 – 0.22 for  $\delta^{13}\text{C}$ .

#### 211 *Cementum aging*

212 Cementum ageing was undertaken by Matson's Lab LLC (Manhattan, MT, USA) following a  
213 standard protocol (Matson et al., 1993). In brief, after decalcification in a weak hydrochloric  
214 acid solution, teeth were sectioned sagittally and mounted on glass slides. The sections were  
215 stained to allow visual differentiation of annual cementum growth layers. These layers  
216 (annuli) were examined microscopically for age estimation at time of death. Birth date was  
217 set to 1 May for the purpose of estimating age in months.

#### 218 *Data analysis*

219 All data were analysed using R [version 3.4.1] and R Studio [version number 0.99.896].  
220 Generalised linear models were built using a) the 2013-16 data (henceforth "new data") and  
221 b) a combination of new data and the historical polecat rodenticides data from Shore et al.  
222 (2003) (henceforth "combined data"). Combination of new and historical data involved  
223 applying the limits of detection (LOD) for each compound from Shore et al. (2003), which  
224 were higher than those in the present study, to eliminate biases caused by changes in  
225 analytical sensitivity.

226 We modelled exposure in three ways: i) probability of detecting at least one SGAR; ii)  
227 number of SGARs detected; and iii) of those polecats with detectable residues, total  
228 concentration levels of all SGARs detected. Total SGAR concentration data were log-  
229 transformed before building models so that they were normally distributed. Polecats with no  
230 SGARs detected were excluded from the total SGAR concentration models to allow us to  
231 explore the variables related to differences in concentration levels.

232 Explanatory variables included in the three "new data" models were: age (months), sex (male  
233 / female), half of year in which the carcass was collected (first / second), region (North /  
234 South / East / West), land class (arable / pastoral), fat score,  $\delta^{13}\text{C}$  (‰) and  $\delta^{15}\text{N}$  (‰).

235 Carcasses collected between January– June were categorised as "first" half of the year, those

236 collected between July – December were categorised as “second”. Regions were defined  
237 using U.K. Government Office Regions. North comprised North East, North West, Yorkshire  
238 and the Humber; South comprised London, South East and South West; East comprised  
239 Eastern and East Midlands and West comprised Wales and West Midlands. No animals were  
240 analysed from Scotland. Quantum GIS [version 2.12.3] was used to generate land class  
241 classifications. Carcass collection locations were overlaid onto the CEH Land Cover map  
242 (2007 <https://www.ceh.ac.uk/services/land-cover-map-2007>), 1 km buffers were applied  
243 around each carcass coordinate and the majority land class calculated for each point, for  
244 whichever was largest between “arable” or “pastoral”, i.e. improved grasslands. Models  
245 included the mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for each whisker. We also modelled the maximum  $\delta^{15}\text{N}$   
246 value for each whisker in place of the mean  $\delta^{15}\text{N}$ , as it was considered that it may only take  
247 one contaminated meal to cause secondary exposure and maximum  $\delta^{15}\text{N}$  might better reflect  
248 such episodic incidents than the mean value for the whole whisker. However models with the  
249 maximum  $\delta^{15}\text{N}$  did not differ markedly from the models with the mean  $\delta^{15}\text{N}$  and hence  
250 analysis of maximum values is not reported.

251 The “combined” data models, adjusted for limits of detection, included two categorical  
252 explanatory variables: collection period (1992 – 1995, 1996 – 1999, 2013 – 2016) and  
253 location (in or outside of the 1990s polecat range as determined by Birks & Kitchener  
254 (1999)). The first two carcass collection periods were 1992 – 1995 and 1996 – 1999, and  
255 represent an approximately even split (in calendar years and numbers) of the 100 polecats  
256 analysed by Shore et al. (2003). The third collection period was the “new data” carcasses  
257 collected in 2013 – 2016. Location was included with the aim of assessing whether polecat  
258 expansion into new areas where SGAR use may have been greater was a factor that might  
259 enhance SGAR exposure.

260 Models were built using lme4, MuMIn and car packages in R. Models were checked for  
261 collinearity (none was evident). Model fit was assessed using QQ plots. Models were mean  
262 centred and standardised using two standard deviations to facilitate comparisons between  
263 effect sizes (Gelman, 2008). Top models were then selected using Akaike's Information  
264 Criterion (AIC) values less than two different from the best model. Averaged models were  
265 created using the top models as none of the top models was weighted >0.9 (Grueber et al.,  
266 2011). Interaction effects between parameters were not significant and did not appear in any  
267 of the top models when added, and so were removed for simplicity. Standardised conditional  
268 average model outputs were summarised. Model predictions were drawn using the ggplot2  
269 package in R.

## 270 **RESULTS**

271 The 68 polecats analysed for SGARs came from throughout England and Wales (Figure 1);  
272 29 were female, 38 male and the sex of one could not be determined. The age of the polecats  
273 in our sample ranged from one month to six years. The youngest polecats with detectable  
274 residues of SGARs were two months old while the oldest polecat without detectable SGARs  
275 was three years old. Mean  $\delta^{15}\text{N}$  values for polecat whiskers ranged between 7.2 and 13.2‰.  
276 Mean  $\delta^{13}\text{C}$  values ranged from -27.98 to -21.41‰. In all, 54 (79%) polecats had detectable  
277 liver residues of at least one SGAR compound (Table 1). The number of polecats with one,  
278 two, three or four compounds in the liver were 19 (27.9%), 16 (23.5%), 16 (23.5%) and 3  
279 (4.4%) respectively. The median number of compounds detected in polecat livers was 2.

280 The rate of detection of liver SGARs differed significantly between compounds ( $\chi^2 = 77.5$ ,  $df$   
281  $= 4$ ,  $p < 0.0001$ ), with bromadiolone most frequently detected, followed by difenacoum and  
282 brodifacoum (Table 1). Difethialone was only detected in livers that contained residues of all  
283 three commonly detected SGARs. Flocoumafen was never detected. There was no significant

284 difference between compounds in the median concentrations of residues in those animals  
285 with detected residues (KW = 2, df = 2, p = 0.37).

286 *Probability of detecting at least one SGAR in the liver*

287 The probability of detecting liver SGAR residues could be explained by set of top models  
288 that included age,  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ , fat score and land class; age and  $\delta^{15}\text{N}$  appeared in all the top  
289 models (Table 2a). In the resultant average model (Table 2b), there was a positive effect of  
290 enriched  $\delta^{15}\text{N}$  signatures on the likelihood of SGAR detection in liver residues. The model  
291 predicted that at the mean level of  $\delta^{15}\text{N}$  (9.9 ‰), the probability of detecting SGARs was  
292 89% (95% confidence limits: 68% - 97%, Figure 2). Although age,  $\delta^{13}\text{C}$ , fat score and land  
293 class also featured in the average model, the confidence intervals for the effects of these  
294 parameters overlapped 0, indicating that they had no significant effect on the probability of  
295 detecting liver SGAR residues.

296 *Number of SGARs detected in the liver*

297 Age,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and half of year were selected in the top models of the number of liver  
298 SGARs detected in individuals (Table 2a). Age appeared in all of the top models and, in the  
299 average model (Table 2b), was positively associated with the number of compounds detected.  
300 The effects of  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and time of year were also included in the average model but had no  
301 clear effect on the number of SGARs detected. Overall, the model predicted that by thirty-six  
302 months old, polecats will on average have accumulated detectable concentrations of 2.1  
303 SGARs (95% confidence limits: 1.5 - 2.7) in their livers, assuming mean  $\delta^{15}\text{N}$ , mean  $\delta^{13}\text{C}$   
304 and first half of year values.

305 *Total SGAR concentrations*

306 There were five top models for total SGAR concentrations and these contained age, land  
307 class, region,  $\delta^{13}\text{C}$  and fat score as variables (Table 2a). Age was positively associated with  
308 total SGAR concentrations in the average model. Total SGAR concentrations were also  
309 significantly higher in polecats collected from arable compared with pastoral landscapes and  
310 in animals in the west compared with those in the east (Table 2b). There was no clear effect  
311 of  $\delta^{13}\text{C}$  or fat score on total SGAR concentrations.

#### 312 *Comparison of exposure in polecats from 1992-9 and from 2013-16*

313 When historical limits of detection (0.027, 0.010 and 0.005  $\mu\text{g/g}$  for bromadiolone,  
314 difenacoum, and brodifacoum respectively) from less sensitive analytical techniques as used  
315 in the earlier study by Shore *et al.* (2003) were applied to our “new data” for animals that  
316 died in 2013-16, the rates of detection in the “new data” were reduced to 40%  
317 (bromadiolone), 35% (difenacoum), 21% (brodifacoum) and 54% (any SGAR). As  
318 flocoumafen was not detected in any animals in either study and difethialone was not tested  
319 for in the 1990s, these compounds were excluded from this part of the analysis. These  
320 compare to detection rates of 12%, 22%, 3% and 31% respectively in Shore *et al.* (2003). The  
321 change in prevalence from 31% to 54% of polecats with one or more SGAR detected equates  
322 to an increase in the rate of detection by a factor of 1.7 between the two studies. A greater  
323 proportion of animals in the “new data” had two (24%) and three compounds (9%) than those  
324 recorded by Shore *et al.* (2003), who found that only 2% of polecats had liver residues of two  
325 compounds and a further 2% had detectable liver residues of three compounds.

326 Survey period and location appeared in all top model sets (Table 3a). In the average models  
327 of the probability of detecting SGARs residues and the number of SGARs detected, the  
328 period 2013 – 2016 was associated with higher rates of detection of rodenticides than the  
329 period 1992 – 1995 (Table 3b). There was also an increase in the rate of detection between



330 the 2013 – 2016 when compared to polecats collected in the period 1996 – 1999, but this was  
331 a smaller effect. The number of compounds detected in the most recent survey was higher in  
332 the most recent survey period than both of the previous collection periods. Survey period did  
333 not have a consistent effect on the total concentrations of SGARs detected. Location (animals  
334 in 1990s range vs animals in areas colonised post 1990s) did not have a consistent effect in  
335 any of the average models.

336

## 337 **DISCUSSION**

338 The detection of SGARs in 79% of the polecats collected 2013-16 was comparable with the  
339 findings of recent studies of other mustelids from elsewhere. Detection rates of ~79% were  
340 reported for American mink in Scotland (Ruiz-Suárez et al., 2016), 78% were reported for  
341 fishers in California (Gabriel et al., 2012) and 95% for stoats and weasels in Denmark  
342 (Elmeros et al., 2011). A recent study of exposure of polecats and stone marten (*Martes*  
343 *foina*) in Denmark detected SGARs in 94% and 99% of animals respectively (Elmeros et al.,  
344 2018). Similarly high prevalence of residues has been found in birds of prey in Britain, with  
345 94% of barn owls (a generalist small mammal predator) with detectable residues of one or  
346 more SGARs (Shore et al., 2016) and 100% of a sample of 18 red kites, a scavenger that  
347 often feeds on rats, with detectable liver SGAR residues (Walker et al., 2017).

348 Overall, the prevalence of residues in the present study is greater than that reported for  
349 polecats that were collected in the 1990s in Britain (Shore et al., 2003). This is in part due to  
350 improvements in analytical sensitivity, but even when this methodological difference is  
351 accounted for (by applying common limits of detection), we identified an increase by a factor  
352 of 1.7 in the prevalence of SGAR residues over the 25 years from 1992 to 2016 inclusive. We  
353 found no evidence of differences in rates of detection between polecats within and beyond the

354 limits of their 1990s range, suggesting that the increase in exposure over time has occurred  
355 throughout the polecat's current range in Britain, and has not been caused simply by  
356 expansion into areas where SGAR use has traditionally been considered to be higher  
357 (Dawson et al., 2003; Dawson and Garthwaite, 2004).

358 SGAR detection in polecats may have increased owing to more widespread use of SGARs  
359 and / or changes in polecat diet. There is some evidence of an increase over time in SGAR  
360 usage. In a nationwide survey of rodenticide usage, Dawson et al. (2003) found that between  
361 1992 and 2000 the proportion of farms in Britain using SGARs changed from 74% to 89%.  
362 Furthermore, rabbit populations have declined since 1995 (Aebischer et al., 2011; Battersby,  
363 2005), which may have increased the reliance of polecats on rats and other rodents as prey. In  
364 our study, the increased prevalence of brodifacoum from 3% in Shore et al. (2003) to 35% in  
365 our most recent survey (21% using historical LODs) was particularly notable and may reflect  
366 growing resistance in rats to bromadiolone and difenacoum in England and Wales (Buckle,  
367 2013) and a consequent attempt to control resistant populations through use of brodifacoum.  
368 The proportion of American mink in Scotland recently found with liver residues of  
369 brodifacoum and flocoumafen was only 10% (Ruiz-Suárez et al., 2016) but resistance to  
370 bromadiolone and difenacoum is not widely documented in Scotland (Buckle and Prescott,  
371 2012) and so there may be less pressure to use compounds, such as brodifacoum, when there  
372 is no or little known resistance in rats.

373 The positive relationship between more enriched values of  $\delta^{15}\text{N}$  and the presence of  
374 rodenticide residues (Figure 2) was consistent with our hypothesis that polecats would be  
375 more likely to be exposed to SGARs due to their consumption of contaminated target prey,  
376 primarily rats, which are likely to have higher  $\delta^{15}\text{N}$  signatures than herbivorous rabbits. Other  
377 studies have found that detection of SGAR residues in predators varies with available food  
378 sources (Hegdal and Blaskiewicz, 1984; Tosh et al., 2011; Geduhn et al., 2016) and while it

379 seems most likely that the elevated  $\delta^{15}\text{N}$  signatures reflect polecats feeding at higher trophic  
380 level, we cannot be certain whether the sources of contamination are rats as the target species,  
381 or other non-target omnivorous rodents. Alternatively, enriched  $\delta^{15}\text{N}$  signatures might  
382 distinguish polecats that had been living and feeding in landscapes exposed to anthropogenic  
383 enrichment of soil  $^{15}\text{N}$ , perhaps associated with practices associated with agricultural  
384 intensification (Rubenstein and Hobson, 2004; Crawford et al., 2008). It was notable that  
385 there was no significant relationship between  $\delta^{15}\text{N}$  and total SGAR concentrations and this  
386 suggests that dietary preferences may have the greatest effect on whether exposure takes  
387 place at all, rather than influencing the magnitude of exposure. The frequency of exposure  
388 and resultant residue accumulation is likely to be driven more by patterns that influence the  
389 extent of exposure in the prey and the numbers of those prey that are eaten over time.

390 Age was positively related to number of SGARs detected in the liver and to total SGAR  
391 concentrations in polecats that died between 2013 and 2016. This reflects the greater time  
392 period over which older polecats can encounter and eat contaminated prey, together with the  
393 persistence of SGAR residues in liver tissues. Similar positive associations between age and  
394 exposure have been found in birds (Christensen et al., 2012; Walker et al., 2015) and  
395 mustelids (Gabriel et al., 2012; Ruiz-Suárez et al., 2016).

396 We found that total SGAR concentrations in the 2013-16 polecats varied with the  
397 predominant land-use in the area in which they died. Geduhn et al. (2015) found a significant  
398 difference in contamination between urban areas and areas with high livestock density. Total  
399 SGAR concentrations were higher in polecats from arable than pastoral areas, which may  
400 indicate heavier SGAR usage on arable farms. This is in line with findings from previous  
401 national rodenticide usage surveys on arable farms compared to farms growing grass and  
402 fodder (De'Ath et al., 1999; Garthwaite et al., 1999). The higher total SGAR concentrations  
403 in polecats collected in the west compared to the east was surprising, as we might have

404 expected rodenticide usage to be higher in the east of England where there is a greater density  
405 of arable farms (Dawson et al., 2003). However, this finding is consistent with those of Shore  
406 et al. (2003), in which bromadiolone residues were higher in polecats in Wales, Midlands and  
407 West England than in animals in the East and the South-East of England, and difenacoum  
408 residues were higher in Wales than in the East and South-East of England. We did not detect  
409 significant variation between exposure at different times of year in the polecats that died in  
410 2013-16, contrary to the earlier polecat surveys (Shore et al., 1999; Shore et al., 2003). Thus  
411 we have no evidence that current exposure in polecats is greatest in the autumn and winter, as  
412 previously thought, and may indicate that exposure is now similar year-round.

413 In conclusion, we have determined that SGAR contamination in polecats in Britain is likely  
414 to be greatest in older animals that eat rodents, live in the west of the country and inhabit  
415 arable areas; these individuals may therefore be at greater risk of adverse effects. We have  
416 also demonstrated that exposure has increased in scale (proportion of animals exposed,  
417 number of residues accumulated) since the 1990s and that this increase appears to have  
418 occurred throughout the polecat's range. The implications for polecats arising from this  
419 widespread exposure to SGARs is a key question arising from this study. Diagnosis of  
420 mortality caused by rodenticides would ideally draw upon ante-mortem observations, post-  
421 mortem detection of non-trauma related haemorrhaging and quantification of liver AR  
422 residues (Murray, 2018). Although liver concentrations  $>0.2 \mu\text{g/g}$  wet weight have elsewhere  
423 been considered to be potentially lethal (in barn owls; Newton et al. 1999), liver residues  
424 alone cannot be used as clear indicators of lethal poisoning, as the relationship between  
425 residue magnitude and likelihood of mortality is variable (Thomas, 2011). We have identified  
426 high liver SGAR residues in some polecats but all of these animals were killed on the road  
427 and the resultant trauma precluded clinical detection of any rodenticide-related  
428 haemorrhaging. It is nevertheless conceivable that SGAR exposure may have contributed to

429 their mortality if such exposure affected the likelihood of animals being run-over and/or if it  
430 exacerbated trauma. It is also possible that these animals may ultimately have succumbed to  
431 SGAR poisoning, had they not been run-over. We did not find any evidence of sub-lethal  
432 effects, such as reduced kidney fat levels, in animals with detectable liver residues, which  
433 may have been expected given that reduced body condition has been observed in other  
434 studies of secondary exposure in mustelids (Elmeros et al. 2011). Overall, whilst we have  
435 shown that the rate of detection of SGARs and the number of compounds detected per animal  
436 have both increased over time, polecats have continued to recolonise Great Britain over the  
437 same period (Birks and Kitchener, 1999; Birks, 2008; Croose, 2016). They are now  
438 widespread in central, eastern and southern England, but are yet to re-establish themselves in  
439 parts of northern England and Scotland. Research exploring polecat survival and productivity  
440 in relation to varying degrees of exposure to SGARs would help inform our understanding of  
441 the impacts that SGARs may have on polecat populations and rates of recolonisation.

442 The regulatory framework concerning SGAR deployment in Britain changed in July 2016,  
443 with a relaxation of restrictions on the use brodifacoum, flocoumafen and difethialone, but  
444 there has been a concomitant introduction of a stewardship scheme designed to promote best  
445 practice in use and thereby reduce non-target primary and secondary exposure  
446 (<http://www.thinkwildlife.org/stewardship-regime/Stewardship>). The effect of these  
447 regulatory changes for primary consumers of SGAR target species, such as polecats, is  
448 uncertain. The outcome could be less prolonged use of difenacoum and bromadiolone in  
449 areas where resistance in rats to these two compounds is a problem, while at the same time  
450 there may be an increase in the use of more acutely toxic, “resistance-busting” SGARs, such  
451 as brodifacoum and flocoumafen.

452 One of the biggest gaps in our understanding of the risk posed by SGARs to polecats and  
453 other non-target wildlife, concerns usage patterns and rodent control practices. There is a

454 need to determine how much and how frequently SGARs are used and how usage varies  
455 between different types of landowners in different parts of the country. Contemporary  
456 research into predator diets, including fine-scale application of stable isotope approaches to  
457 predators and their prey, will also improve understanding of pathways of exposure. Exploring  
458 user practices and how these may change following the introduction of stewardship is critical  
459 to inform our understanding of the current and likely future scale of the risks presented to  
460 non-target wildlife by anticoagulant rodenticides.

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725 **TABLES AND FIGURES**

726 Table 1. Prevalence and concentrations of residues of second generation anticoagulant  
727 rodenticides (SGARs) in the livers of 68 polecats collected in England and Wales, 2013-  
728 2016. Totals are the prevalence of residues of any SGAR and the median of the summed  
729 SGAR concentrations.

| <b>Compound</b> | <b>Number (% of total sample) of polecats with detected residues</b> | <b>Median (range) concentration (µg/g wet weight)</b> |
|-----------------|--|---|
| Bromadiolone    | 48 (71%)   | 0.0581 (0.0014 – 3.0833)                              |
| Difenacoum      | 36 (53%)   | 0.0587 (0.0021 – 0.5125)                              |
| Brodifacoum     | 24 (35%)   | 0.0080 (0.0016 – 0.7298)                              |
| Difethialone    | 3 (4%)   | 0.0193 (0.0035 – 0.0505)                              |
| Flocoumafen     | 0 (0%)   | N/A   |
| Total           | 54 (79%)   | 0.1204 (0.0014 – 3.1628)                              |

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Table 2a: Summary of statistical models of variation in second generation anticoagulant rodenticide (SGAR) residues in polecat livers collected from 2013 to 2016. Top models are from analyses of i) probability of detecting residues ii) number of compounds for which residues were detected and iii) total concentrations. AIC is Akaike's Information Criterion and  $\Delta$ AIC is the difference in AIC from the best model. Only models with  $\Delta$ AIC < 2 are included in the top model set. Weight is the weighting given to that model when the average model is calculated. Sample sizes vary because of missing variables and the exclusion of animals with no residues detected in models of total concentrations.

| Model  | Covariates   | df | Log likelihood | AIC    | $\Delta$ AIC | Weight |
|--|--|----|----------------|--------|--------------|--------|
| <b>i) Probability of detecting <math>\geq 1</math> liver SGAR residue (n = 59)</b> |  |    |                |        |              |        |
| 1  | Age + $\delta^{15}\text{N}$  | 3  | -24.72         | 55.87  | 0.00         | 0.24   |
| 2  | Age + $\delta^{15}\text{N}$ + land class                           | 4  | -23.76         | 56.26  | 0.39         | 0.20   |
| 3  | Age + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + land class   | 5  | -22.70         | 56.53  | 0.66         | 0.17   |
| 4  | Age + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$                | 4  | -24.04         | 56.83  | 0.96         | 0.15   |
| 5  | Age + $\delta^{15}\text{N}$ + fat score + land class               | 5  | -23.04         | 57.21  | 1.34         | 0.12   |
| 6  | Age + $\delta^{15}\text{N}$ + fat score                            | 4  | -24.34         | 57.41  | 1.54         | 0.11   |
| <b>ii) Number of SGARs detected (n = 59)</b>                                       |  |    |                |        |              |        |
| 1  | Age + $\delta^{13}\text{C}$ + $\delta^{15}\text{N}$                | 4  | -85.54         | 179.82 | 0.00         | 0.27   |
| 2  | Age  | 2  | -88.31         | 180.82 | 1.01         | 0.16   |
| 3  | Age + $\delta^{13}\text{C}$  | 3  | -87.24         | 180.92 | 1.10         | 0.15   |
| 4  | Age + $\delta^{15}\text{N}$  | 3  | -87.33         | 181.10 | 1.28         | 0.14   |
| 5  | Age + half of year + $\delta^{13}\text{C}$                         | 4  | -86.20         | 181.14 | 1.33         | 0.14   |
| 6  | Age + half of year + $\delta^{13}\text{C}$ + $\delta^{15}\text{N}$ | 5  | -85.04         | 181.21 | 1.40         | 0.13   |
| <b>iii) Total SGAR concentration (n = 46)</b>                                      |  |    |                |        |              |        |
| 1  | Age + land class + region  | 7  | -87.51         | 191.97 | 0.00         | 0.33   |
| 2  | Age + land class   | 4  | -92.11         | 193.19 | 1.22         | 0.18   |
| 3  | Age + land class + $\delta^{13}\text{C}$                           | 5  | -90.86         | 193.22 | 1.25         | 0.18   |
| 4  | Age + land class + $\delta^{13}\text{C}$ + region                  | 8  | -86.71         | 193.31 | 1.34         | 0.17   |
| 5  | Age + land class + region + fat score                              | 8  | -86.92         | 193.72 | 1.75         | 0.14   |

Table 2b: Standardised conditional average model coefficients and relative importance of variables include in top model sets (AIC < 2) of variation in second generation anticoagulant rodenticide residues in polecat livers for i) probability of detecting residues; ii) number of compounds for which rodenticides were detected; and iii) total concentrations. Parameter names with brackets show the effect of that parameter category against the reference category (half of year = first, land class = arable, region = east). Parameters highlighted in bold are those where the confidence intervals do not span zero on the model scale, indicating a consistent directional effect. Coefficient estimates, standard errors and confidence intervals are presented on the model scales. Importance reflects the number of models that the parameter appears in and its importance to the average model.

| Parameter   | Coefficient estimate | SE          | 2.5% CI      | 97.5% CI     | Importance      |
|---|----------------------|-------------|--------------|--------------|-----------------|
| <b>i) Probability of detecting <math>\geq 1</math> liver SGAR residue (binomial regression, logistic scale)</b> |                      |             |              |              |                 |
| (intercept)   | 1.54                 | 0.55        | 0.44         | 2.65         | -               |
| Age   | 2.20                 | 1.18        | -0.17        | 4.57         | 1.00 (6)        |
| $\delta^{15}\text{N}$   | <b>2.53</b>          | <b>0.92</b> | <b>0.68</b>  | <b>4.37</b>  | <b>1.00 (6)</b> |
| Land class (pastoral)   | 1.16                 | 0.80        | -0.43        | 2.76         | 0.50 (3)        |
| $\delta^{13}\text{C}$   | 1.10                 | 0.88        | -0.66        | 2.86         | 0.32 (2)        |
| Fat score   | -0.78                | 0.78        | -2.34        | 0.78         | 0.24 (2)        |
| <b>ii) Number of SGARs detected (Poisson regression, log scale)</b>   |                      |             |              |              |                 |
| (intercept)   | 0.46                 | 0.13        | 0.20         | 0.73         | -               |
| <b>Age</b>  | <b>0.47</b>          | <b>0.17</b> | <b>0.13</b>  | <b>0.81</b>  | <b>1.00 (6)</b> |
| $\delta^{13}\text{C}$   | 0.40                 | 0.22        | -0.05        | 0.84         | 0.70 (4)        |
| $\delta^{15}\text{N}$   | 0.36                 | 0.22        | -0.09        | 0.81         | 0.54 (3)        |
| Half of year (second)   | -0.28                | 0.24        | -0.76        | 0.19         | 0.27 (2)        |
| <b>iii) Total SGAR concentration (linear regression, log scale)</b>   |                      |             |              |              |                 |
| (intercept)   | -1.97                | 0.52        | -3.03        | -0.92        | -               |
| <b>Age</b>  | <b>1.44</b>          | <b>0.56</b> | <b>0.30</b>  | <b>2.57</b>  | <b>1.00 (5)</b> |
| <b>Land class (pastoral)</b>  | <b>-1.98</b>         | <b>0.67</b> | <b>-3.33</b> | <b>-0.62</b> | <b>1.00 (5)</b> |
| Region (north)  | 0.29                 | 0.97        | -1.67        | 2.25         | 0.64 (3)        |
| Region (south)  | 0.37                 | 0.79        | -1.22        | 1.97         | 0.64 (3)        |
| <b>Region (west)</b>  | <b>1.97</b>          | <b>0.82</b> | <b>0.32</b>  | <b>3.63</b>  | <b>0.64 (3)</b> |
| $\delta^{13}\text{C}$   | 0.74                 | 0.55        | -0.38        | 1.85         | 0.35 (2)        |
| Fat score   | 0.56                 | 0.56        | -0.56        | 1.69         | 0.14 (1)        |

Table 3a: Summary of statistical models of variations in second generation anticoagulant rodenticide (SGAR) residues in polecat livers. Top models from analysis of i) probability of detecting residues; ii) number of rodenticides detected and iii) total concentrations using “combined” Shore et al. (2003) and new rodenticide data. AIC is Akaike’s Information Criterion and  $\Delta$ AIC is the difference in AIC from the best model. Only models with  $\Delta$ AIC < 2 are included in the top model set. Weight is the weighting given to that model when the average model is calculated. Sample sizes vary because of the exclusion of animals with no residues detected in models of total concentrations.

| <b>Model rank</b>   | <b>Covariates</b> | <b>df</b> | <b>Log likelihood</b> | <b>AIC</b> | <b><math>\Delta</math>AIC</b> | <b>Weight</b> |
|---|-------------------|-----------|-----------------------|------------|-------------------------------|---------------|
| <b>i) Probability of detecting <math>\geq 1</math> liver SGAR residue (n = 168)</b> |                   |           |                       |            |                               |               |
| 1   | Survey            | 3         | -107.70               | 221.55     | 0.00                          | 0.72          |
| 2   | Survey + location | 4         | -107.59               | 223.43     | 1.88                          | 0.28          |
| <b>ii) Number of SGARs detected (n = 168)</b>                                       |                   |           |                       |            |                               |               |
| 1   | Survey            | 3         | -168.05               | 342.26     | 0.00                          | 0.52          |
| 2   | Survey + location | 4         | -167.10               | 342.45     | 0.19                          | 0.48          |
| <b>iii) Total SGAR concentrations (n = 68)</b>                                      |                   |           |                       |            |                               |               |
| 1   | Null              | 2         | -104.13               | 212.44     | 0.00                          | 0.43          |
| 2   | Location          | 3         | -103.26               | 212.90     | 0.46                          | 0.34          |
| 3   | Survey            | 4         | -102.53               | 213.69     | 1.25                          | 0.23          |

Table 3b: Standardised conditional average model coefficients and relative importance of variables include in top model sets ( $AIC < 2$ ) of variation in second generation anticoagulant rodenticide (SGAR) residues in polecat livers for i) probability of detecting residues; ii) number of compounds for which rodenticides were detected; and iii) total concentrations using “combined” Shore et al. (2003) and new rodenticide data. Parameter names with brackets show the effect of that parameter category against the reference category (survey = “2013-2016”, location = “inside 1990s range”). Parameters highlighted in bold are those where the confidence intervals do not span zero on the model scale, indicating a consistent directional effect. Coefficient estimates, standard errors and confidence intervals are presented on the model scales. Importance reflects the number of models that the parameter appears in and its importance to the average model.

| Parameter   | Coefficient estimate | SE          | 2.5% CI      | 97.5% CI     | Importance      |
|---|----------------------|-------------|--------------|--------------|-----------------|
| <b>i) Probability of detecting <math>\geq 1</math> liver SGAR residue (binomial regression, logistic scale)</b> |                      |             |              |              |                 |
| (intercept)   | 0.21                 | 0.28        | -0.34        | 0.76         | -               |
| <b>Survey (1992 - 1995)</b>   | <b>-1.40</b>         | <b>0.46</b> | <b>-2.30</b> | <b>-0.50</b> | <b>1.00 (2)</b> |
| Survey (1996 - 1999)  | -0.75                | 0.39        | -1.52        | 0.03         | 1.00 (2)        |
| Location (outside 1990s range)  | -0.23                | 0.49        | -1.19        | 0.74         | 0.28 (1)        |
| <b>ii) Number of SGARs detected (Poisson regression, log scale)</b>   |                      |             |              |              |                 |
| (intercept)   | 0.03                 | 0.16        | -0.29        | 0.35         | -               |
| <b>Survey (1992 - 1995)</b>   | <b>-1.22</b>         | <b>0.32</b> | <b>-1.86</b> | <b>-0.59</b> | <b>1.00 (2)</b> |
| <b>Survey (1996 - 1999)</b>   | <b>-0.89</b>         | <b>0.26</b> | <b>-1.41</b> | <b>-0.38</b> | <b>1.00 (2)</b> |
| Location (outside 1990s range)  | -0.35                | 0.25        | -0.85        | 0.15         | 0.48 (1)        |
| <b>iii) Total SGAR concentrations (linear regression, log scale)</b>  |                      |             |              |              |                 |
| (intercept)   | -1.93                | 0.20        | -2.32        | -1.54        | -               |
| Survey (1992 - 1995)  | -0.49                | 0.40        | -1.28        | 0.31         | 0.23 (1)        |
| Survey (1996 - 1999)  | -0.48                | 0.31        | -1.09        | 0.13         | 0.23 (1)        |
| Location (outside 1990s range)  | 0.41                 | 0.31        | -0.22        | 1.04         | 0.34 (1)        |

## Figure captions

Figure 1: Collection locations of polecat carcasses used for analysis of second generation anticoagulant rodenticides. Black points are carcasses collected and analysed in this survey while white points are carcasses collected and analysed in Shore *et al.* (2003).

Figure 2: Predictions based on output of the averaged model for the probability of detecting second generation anticoagulant rodenticide residues in polecat livers. Figure 2a shows the probability of detecting SGARs at different levels of  $\delta^{15}\text{N}$  in pastoral landscapes, when polecat age,  $\delta^{13}\text{C}$  and fat score are kept constant at their mean values (16.2 months, -25.54 ‰ and 2.6, respectively).



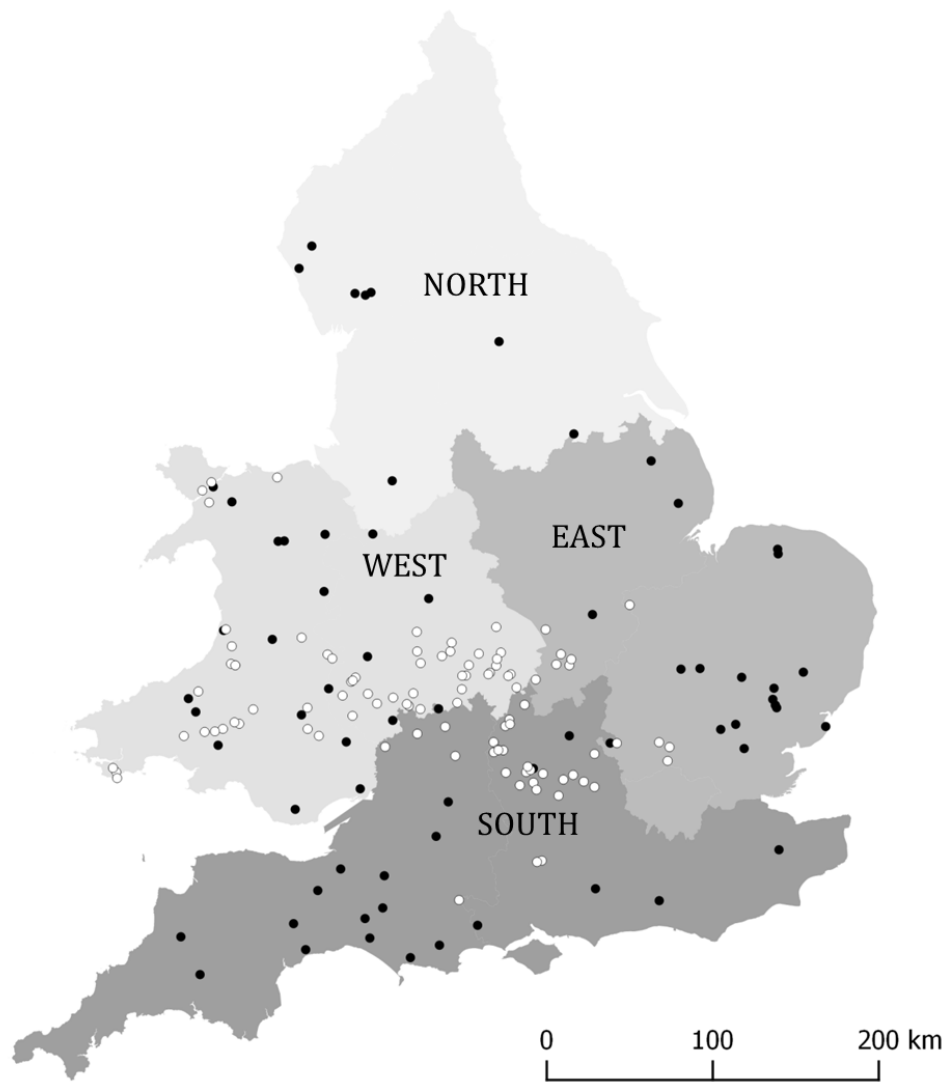


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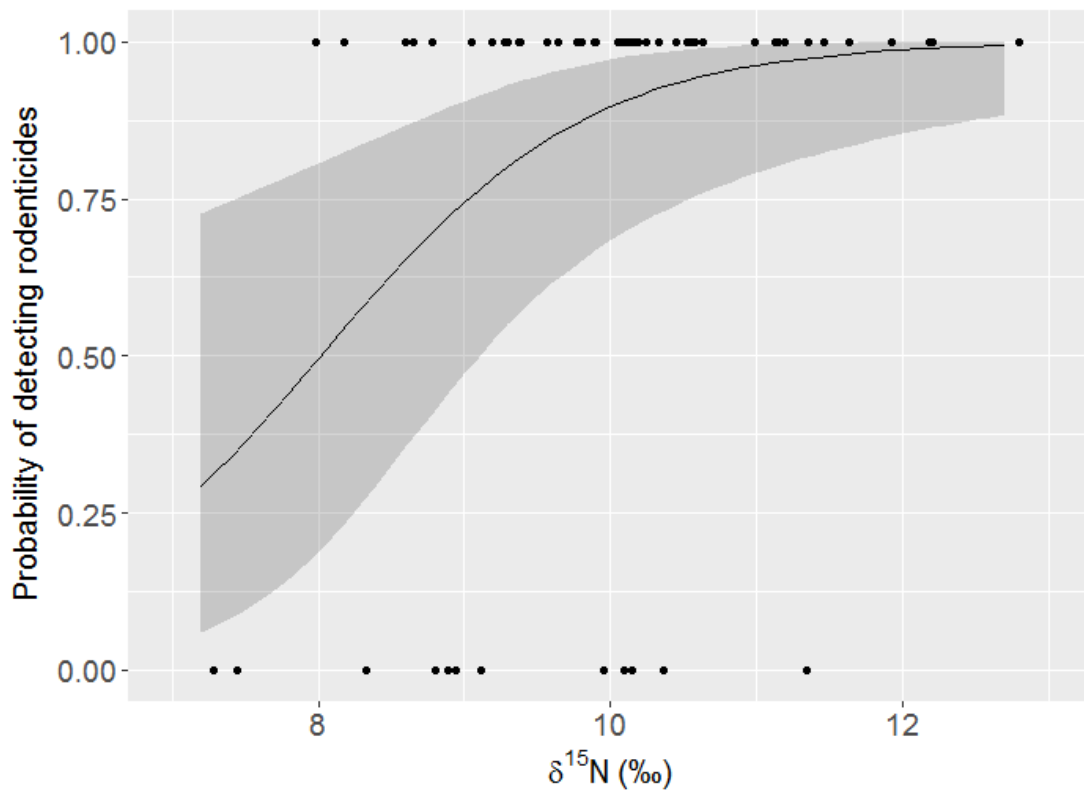


Figure 2: Predictions based on output of the averaged model for the probability of detecting second generation anticoagulant rodenticide residues in polecat livers. Figure 2a shows the probability of detecting SGARs at different levels of  $\delta^{15}\text{N}$  in pastoral landscapes, when polecat age,  $\delta^{13}\text{C}$  and fat score are kept constant at their mean values (16.2 months, -25.54 ‰ and 2.6, respectively).